6th World Congress on Leishmaniasis

ABSTRACTS BOOK

16th - 20th May 2017

6 TOLEDO CONFERENCE CENTER
TOLEDO-SPAIN

DNDi
Drugs for Neglected Diseases *initiative*

Instituto de Salud Carlos III

In collaboration with
PLOS | COLLECTIONS

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INTRODUCTION

THE ORGANIZERS AND COMMITTEES

WorldLeish 6 was organized by the Instituto de Salud Carlos III (ISCIII), Spain and Drugs for Neglected Diseases *initiative* (DNDi), Switzerland.

The Organizing Committee (OC) comprised five members of the two organizing institutions, with Jorge Alvar (DNDi) and Javier Moreno (ISCIII) as chair and co-chair of the congress, respectively. In addition, the OC identified a coordinator of each of the four scientific committees, the WorldLeish Committee (K.P. Chang), the *Ad hoc* Committee (C. Bern), the Spanish Committee (L. Rivas) and the Organizing Institutions Committee (J. Moreno and G. Bilbe).

Marca España is the highest reputed mark of the image of Spain at various levels, including events. In December 2016, a comprehensive concept note on WorldLeish6 was sent to the High Commissioner Office for *Marca España* applying for support from this office belonging to the President´s office and the Minister of External Affairs. This note described the outline of the congress, the general and specific objectives, the different processes in progress, current and expected outcomes and a detailed description of the organizing institutions. In February 2017 the OC received the confirmation of this support and since then the *Marca España* logo has accompanied core documents.

HOW THE SCIENTIFIC PROGRAMME WAS ORGANIZED

The OC decided to follow a programme timeline where 80% of the scientific programme would be outlined 6 months before the conference, to leave enough time for rearrangements, breakthrough communications and operational procedures immediately before the conference. To this end, a two-step programme preparation was conducted.

The representatives of all committees identified a number of hot topics on leishmaniasis that were grouped into the 15 core topics and then organized into three main parallel tracks (basic science; diagnostics and clinical aspects; epidemiology, entomology and public health) each with subsequent parallel sessions. Four of the topics were selected to constitute the set of plenary sessions.

Invitations to chairs and speakers and modifications of these parallel sessions followed during autumn 2016 and by the end of the year more than 90% of the programmed parallel and plenary sessions were confirmed.

The second step was a bottom to top approach that included the incorporation in early 2017 of the communications drawn from the abstract process. Again, selected abstracts were organized into sessions keeping the 15 WorldLeish core topics.

Finally, there was a progressive incorporation throughout the project of the symposia proposed by sponsors as sponsorship agreements were closed and of non-sponsored symposia organized by DNDi and ISCIII.

Abstracts. The abstract submission process ended on December 16th with 1526 abstracts received, grouped into the 15 WorldLeish core topics. The OC prepared a list of reviewers, distributed by topic. Each reviewer was invited to participate in the process and received between 10-20 abstracts so that every abstract was evaluated by a minimum of 2 reviewers. After this anonymous peer review process by independent scientists, the OC selected 231 abstracts to be delivered orally and 1191 to be delivered as posters.

Sessions. The scientific programme was finally composed of:

- 36 parallel session
- 4 plenary sessions
- 10 symposia
- 2 consensus symposia
- 27 oral communications sessions
- 4 poster sessions

A total of 256 speakers, chair and co-chairs participated in these sessions (35% women).

Registrations. Regular registrations reached 1473 as of 12 May 2017. Participants represented over 70 countries. Spain, with 164 participants, and Brazil, with 152 participants, were the most represented, followed by the UK, the US, India, France, Colombia, Belgium, Switzerland, Italy, Canada, Iran, Israel, Germany, Tunisia, Argentina, Portugal, Turkey, Peru, Sudan, Bangladesh and Ethiopia.

The "100 students initiative". Aiming to facilitate attendance to students and young scientists, the OC elaborated an open scholarship programme to provide travel and accommodation grants to candidates from all countries and areas of expertise. This fundraising opportunity was included in the negotiations with sponsors and also published on the web, aiming to cover partial or full expenses of 100 students.

SATELLITE ACTIVITIES

In addition to the congress programme, four activities were organized in parallel with the aim to bring greater awareness of leishmaniasis to the general public:

1. The **WorldLeish6 PLoS Collection** was an initiative established in May 2016. The main objectives were to share research and accelerate open access publications related to the main topics of the congress. Inclusion of manuscripts in the collection followed the regular publication process for *PLOS One*, *PLOS NTDs* and *PLOS Pathogens* as per PLoS guidelines, and a second validation and distribution into categories by the chair and co-chair of the congress, curators of the collection. PLoS provided a special collection publication fee for authors and DNDi announced that 10 papers will be awarded prizes to help fund the publication of future research in this field. The call for papers launched on May 2016 resulted in 51 papers already published in the collection by May 2017. The collection finally also included some papers from other open access journals.

2. A **Photography Contest and Exhibition** was displayed at the Cultural Center 'San Marcos', in Toledo, from May 17th - May 25th, 2017 aiming to approach leishmaniasis to the general public. In addition, a book featuring these images was printed and distributed to all those attending the congress.

3. A **Special Session at the Spanish Royal Academy of Medicine (RANM) on "Violence and Leishmaniasis"** was organized in Madrid on May 22nd, 2017. This Special Session addressed the epidemics in South Sudan, Colombia, Middle East, and Syrian refugee camps in the context of war and civil unrest.

4. An **Exhibition on Leishmaniasis at the National Natural History Museum** in Madrid was inaugurated after the congress, displaying simple diagrams, photographs, and 3D models to explain the biology of *Leishmania* and the sandfly. The exhibition explained the biological life cycle of leishmaniasis and an informative article on "Leishmaniasis beyond your pet" published in *NaturalMente*, the museum's online, open access journal.

Books. Participants received two books as part of the congress documents:

- "Leishmaniasis, the hardship of a disease of poverty", the compilation of the photographs exposed in the San Marcos Cultural Centre. Edited by J. Alvar, C. Jiménez and J. Moreno, as congress organizers.
- "Methods in sandfly research". Edited by R. Molina, M. Jiménez and J. Alvar, from the Unit of Medical Entomology at the ISCIII and DNDi.

Finally the **"Battle of Organs concert" in the Cathedral of Toledo,** featuring renowned international organists playing up to 7 out of the 10 organs in the Cathedral, was a remarkable way to transmit the participants on the historical richness of Toledo.

APPENDIX: PLOS COLLECTION WINNERS

Section	Manuscript	First author
Understanding the parasite	Transcriptional Profiling in Experimental Visceral Leishmaniasis Reveals a Broad Splenic Inflammatory Environment that Conditions Macrophages toward a Disease-Promoting Phenotype	Fanping Kong
	Leishmania HASP and SHERP Genes Are Required for In Vivo Differentiation, Parasite Transmission and Virulence Attenuation in the Host	Johannes S.P. Doehl
	Leishmaniavirus-Dependent Metastatic	Marie-

	Leishmaniasis Is Prevented by Blocking IL-17A	Anne Hartley
	Exacerbated Leishmaniasis Caused by a Viral Endosymbiont can be Prevented by Immunization with Its Viral Capsid	Patrick Castiglioni
Developing new tools for treatment and diagnosis	Single locus genotyping to track *Leishmania donovani* in the Indian subcontinent: Application in Nepal	Keshav Rai
Clinical and Experimental Aspects of Disease and Treatment/Prevention	Immune Activation and Bacterial Translocation: A Link between Impaired Immune Recovery and Frequent Visceral Leishmaniasis Relapses in HIV-Infected Patients	Maria Luciana Silva-Freitas
	Apoptosis-like cell death in *Leishmania donovani* treated with KalsomeTM10, a new liposomal amphotericin B	Md. Shadab
Epidemiology and Control	Compartmentalized Immune Response in Leishmaniasis: Changing Patterns throughout the Disease	Alhelí Rodríguez Cortez
	Experimental infection and transmission of *Leishmania* by *Lutzomyia cruzi* (Diptera: *Psychodidae*): Aspects of the ecology of parasite-vector interactions	Everton Falcao de Oliveira
Public Health and Social Sciences	Cost-effectiveness of meglumine antimoniate versus miltefosine caregiver DOT for the treatment of pediatric cutaneous leishmaniasis	Brandon A. Berger

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Honour Preseidency

H.M. The Queen

Committee

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Manuel Cuenca-Estrella, General Deputy Director of Applied Services, Education and Investifgation of ISCIII

CONGRESS COMMITTEES

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ABSTRACTS BOOK

PARALLEL SESSIONS

Tuesday, May 16th

(1) Expression and signal transduction in leishmaniasis

1.1 TRANSCRIPTOME PROFILING OF LEISHMANIA MEXICANA LIFE CYCLE STAGES

Michael Fiebig, Tom Beneke, Ross Madden, Steve Kelly, ***Eva Gluenz***

University of Oxford, UK

To gain insight into the differences between Leishmania mexicana life cycle stages, we used Illumina sequencing of poly-A selected RNA to profile the transcriptome of promastigotes (PRO), axenic amastigotes (AXA) and amastigotes (AMA) in mouse bone marrow derived macrophages (BMDM). These data enabled genome-wide mapping of trans-splice sites and poly-A addition sites, producing the first transcriptome evidence-based annotation of gene models for this species. The revised genome annotation encompassed 9169 protein-coding genes including 936 novel genes as well as modifications to previously existing gene models. Differential expression testing identified 1290 transcripts that showed at least a 2-fold change in abundance between PRO and AMA. Genome-wide distribution analysis of the differentially expressed genes revealed that the tetraploid chromosome 30 is highly enriched for AMA-upregulated genes, suggesting a possible link between this whole chromosome duplication event and adaptation to the vertebrate host. To facilitate functional interrogation of large transcriptomics datasets such as this, we developed a CRISPR-Cas9 toolkit that allows rapid tagging and gene knockout in kinetoplastids. A set of plasmids allows easy and scalable generation of DNA constructs by PCR for transfections in just a few hours. The single-guide RNA is transcribed in vivo by T7 RNA polymerase from PCR-generated DNA templates and our online resource (LeishGEdit.net) facilitates automated primer design. Focusing on genes associated with motile flagella, which comprise a large proportion of PRO-upregulated genes, we show how these tools enable for the first time rapid and large-scale knockout screens to study gene function in Leishmania.

1.2 NEW INSIGHTS INTO LEISHMANIA DONOVANI STAGE-SPECIFIC EXPRESSION AND PHOSPHORYLATION USING A SYSTEMS-LEVEL APPROACH

Pascale Pescher[1], ***Spaeth Gerald***[2]

Institut Pasteur Paris France

Full text

Modern high-throughput technologies combined with computational biology approaches provide a powerful experimental platform to gain systems-level insight into *Leishmania* biology. Here we used physiologically relevant amastigotes obtained from infected hamster spleens and corresponding culture-derived promastigotes at early passage to investigate stage-specific expression patterns and regulatory mechanisms applying RNAseq, label-free quantitative proteomics, and phospho-proteomics analyses. Stage-specific differences only poorly correlated between RNA and protein levels with a three-fold higher dynamic range of abundance observed in the proteome compared to the transcriptome, suggesting that translational and post-translational mechanisms outweigh RNA turn-over in regulating stage differentiation. Using a pharmacological approach we defined a crucial role for the *Leishmania* proteasome in post-translational regulation of protein abundance essential for the amastigote-to-promastigote developmental transition. Phospho-proteomic analysis revealed the proteasome as a major target for promastigote-specific signal transduction. Together our data link environmentally-induced *Leishmania* differentiation to phospho-regulated protein degradation and confirm the proteasome as an interesting target for anti-parasitic intervention.

1.4 INTEGRATED ANALYSIS OF THE TRANSCRIPTOME OF LEISHMANIA DONOVANI AND MACROPHAGE HOST CELL UPON INFECTION WITH ANTIMONY-RESISTANT AND ANTIMONY-SENSITIVE STRAINS

Geraldine De Muylder[1], *Bart Cuypers*[1], *Hideo Imamura*[1], *Malgorzata Domagalska*[1], *Suman Rijal*[2], *Syamal Roy*[3], *Jean-Claude Dujardin*[1]

[1]*Institute of Tropical Medicine Antwerp Belgium*
[2]*DNDi India*
[3]*Indian Institute of Chemical Biology India*

1 Background

Antimonials like sodium stibogluconate (SSG) have been widely used to treat leishmaniasis worldwide, but the emergence of drug resistance has limited their use in the Indian subcontinent (ISC). It has previously been demonstrated that antimony-resistant (SSG-R) *Leishmania donovani* from ISC show a higher infectivity and a general better fitness than sensitive strains. This has been linked, among others, to the occurrence of specific glycans on the surface of SSG-R parasites that activate a cascade of signaling events ultimately leading to increased levels of IL-10 and decreased production of reactive oxygen and nitrogen intermediates. In order to understand the influence of the SSG susceptibility background on both the parasite's survival skills and the host cell response, we have initiated the first integrated study of gene expression patterns of parasites and macrophages infected with SSG-R or SSG-S *L.donovani*.

2 Methods

Macrophage and parasites transcriptomes were analysed in parallel, by RNAseq of mouse peritoneal macrophages *in vitro* infected with two sets of SSG-R and SSG-S strains. A specific protocol was applied for the amplification of parasite transcriptomes from infected host cells, based on the detection of mini-exon spliced transcripts. These experiments were carried out in the absence of drug pressure.

3 Results

First experiments confirmed upregulation of IL-10 in SSG-R infected cells, while type I interferon (IFN) signaling and IFN regulatory factors were downregulated, as compared to SSG-S infected cells. Remarkably, the same transcriptional pattern was observed after infection with two different SSG-R strains. Analysis of parasite transcriptomes showed upregulation in SSG-R parasites of, among others, amastins, GP63 and galactosyltransferase, the enzyme responsible for addition of the SSG-R-specific galactose residue. Integrated transcriptome analyses in presence of SSG are currently being carried out.

4 Conclusions

These data suggest that SSG-R parasites acquired different abilities than their SSG-S counterparts to avoid the SSG-induced immune activation, and that these abilities remain in the absence of drug pressure. SSG-R parasites differentially modulate the host response ultimately leading to a better infection.

(2) Diagnostic methodology in practice and under development

Biomarkers in leishmaniasis

2.2 SPECIAL FORMS OF POST-KALA-AZAR DERMAL LEISHMANIASIS

Ramesh Venkatachari

Safdarjung hospital, New Delhi, India

Full text

Post-kala-azar dermal leishmaniasis (PKDL) is a generalized dermatosis with a wide spectrum of skin lesions which may be monomorphic or polymorphic in presentation. The polymorphic forms that have been emphasized in most books showing the centrofacial papulonodules, and erythematous and hypopigmented patches distributed over rest of the body. The lack of symptoms, slow progression, misdiagnosis, difficulty in demonstration of amastigotes in slit-skin smears and poor treatment options have led to grotesque forms of PKDL in both the monomorphic and polymorphic presentations. In addition some unusual forms like the annular, fibroid, tumorous and erythrodermic manifestations have expanded the clinical picture. PKDL is an immunologically mediated condition characterized by the change in cytokine profile which can be well understood under Th1/Th2 dichotomy. In PKDL Th2 response shows the persistence of IL-10 in skin and systemically the production of IFN-g as Th1 response. Immunological studies done so far reveal differences in the immunoglobulin and cytokine levels in the various clinical presentations of PKDL. Measurement of anti-leishmanial antibodies IgG show significantly higher levels as compared to the macular variant. Amongst the subclasses IgG1 and IgG3 possess high levels of complement fixing and opsonising activities which potentially accounts for parasite persistence and can be implicated in disease chronicity. Studies in Indian PKDL have proposed Ig, IgG, IgG1 & IgG3 to be potential prognostic serological markers for polymorphic PKDL. When stimulated with leishmania antigen, polymorphic group has higher proliferative capacity, granzyme secretion, TNF-α and IFN-g secretion than macular PKDL and also a higher parasite load. Stronger cell mediated immunity (CMI) is seen in acute PKDL than in the chronic type where there is slow and continuous extension over the skin giving rise to the various morphological forms. In the macular type the CMI is good with low antibody levels and fewer parasites. Production of IL-10 and TGF-β along with expansion of CD4+ CD25+ cells play critical role in aggravation of PKDL. In Sudanese PKDL the presence of CD4+ cells and enhanced effector memory (EM) T-cell response contributes to its self healing nature. Occurrence of severe forms with ulceration and mucosal affection is seen in the Sudanese variety.

The diverse manifestations, geographical differences and varying drug response has made PKDL a multifaceted disease where the role of immunity in maintaining chronicity needs to be further studied.

2.3 ADVANCES IN UNDERSTANDING THE IMMUNOBIOLOGY OF POST KALA AZAR DERMAL LEISHMANIASIS (PKDL)

Poonam Salotra

Indian Council of Medical Research, New Delhi, India

Full text

Post kala azar dermal leishmaniasis (PKDL) is a dermal sequel of visceral leishmaniasis (VL), that occurs after an apparent cure from VL. The disease is mainly confined to two distinct geographical regions i.e. South Asia (India, Nepal and Bangladesh) and East Africa (primarily in Sudan) with an incidence of 5-15% and 50-60% of VL cases, respectively.

The precise understanding of immunopathogenesis of PKDL is still obscure. Moreover, there are marked variations between the Sudanese and the Indian PKDL. The disparities include the preponderance of CD8+ T-cells in Indian PKDL in contrast to the Sudanese variant where CD4+ predominate. The expression of IL-10 from keratinocytes is considered a key factor and predictor in development of Sudanese PKDL. Moreover Sudanese variant is reported to demonstrate genetic polymorphism in IFN-gR1 gene. The enhanced effector memory T- cell response along with CD4+ cells leads to spontaneous self healing in Sudanese PKDL.

Studies in Indian PKDL have shown mixed Th1 and Th2 responses, as reflected by higher expression of cytokines in PKDL lesions, compared with normal skin tissue. Other investigations have demonstrated the upregulated lesional expression of Th17 markers (IL-17 and IL-23). Further, elevated mRNA levels of nTreg markers (Foxp3, CD25 and CTLA-4) is observed, that directly correlates with parasite load in lesions tissues, with higher levels in nodular lesions compared to macules/papules. Increase in IL-10-expressing CD3+ CD8+ T lymphocytes has been found in circulation which gets restored to normal post treatment. Tissue inhibitors of matrix metalloproteinases (TIMP-1 and TIMP-3) have also been implicated in the pathogenesis of PKDL.

Recent reports have elucidated the role of CD26, a co-stimulatory molecule essential for T-cell signal activation There is a significant decrease in the frequency and expression of CD26 on CD3+CD8+ T-cells, along with plasma levels of sCD26, which leads to attenuated Th1 response. The elevated levels of both activated CD4+ and CD8+ T cells along with increased granzyme B secretion upon *in vitro* stimulation with TSLA advocates the role of cytotoxic cells in resistance to *L.donovani* infection in polymorphic PKDL. Further, evaluation of immunogenicity of vaccine candidates in peripheral blood cells of PKDL patients revealed induction of Th1 cytokines, suggesting the presence of memory immune response.

(3)Leishmaniasis epidemiology and control: biological inputs

3.1 WHY BIOLOGICAL UNDERSTANDING OF RESERVOIR AND VECTOR IS ESSENTIAL TO UNDERSTANDING EPIDEMIOLOGY AND DEVELOPING CONTROL STRATEGIES

Caryn Bern[1], *Orin Courtenay*[2]

[1]*Epidemiology & Biostatistics, UCSF School of Medicine USA*
[2]*University of Warwick UK*

1 Background

The epidemiology of leishmaniasis involves complex interactions among parasite, vector and host that are incompletely understood. Experimental data can elucidate the biological processes underlying epidemiological phenomena that are difficult to study in humans.

2 Methods

Literature review

3 Results

In the Indian subcontinent, visceral leishmaniasis epidemic decades occur every 15-20 years (peaks in 1940s, 1970s, early 1990s and mid-2000s) with intervening periods of low incidence. Similar patterns are reported in Brazil and data suggest that cycles occur in Africa as well. The 1950s-1960s in India, when VL transmission was extremely low, coincided with blanket insecticide spraying under the malaria eradication programme; this was followed by an explosive resurgence in the 1970s. Strong spatial clustering of VL has been observed, especially during first half of an epidemic decade. Data on human infectiousness are sparse, but longitudinal data in dogs with *Leishmania infantum* suggest marked heterogeneity, with <20% of dogs contributing >80% of infections (superspreaders). If the distribution of human infectiousness is similar, this heterogeneity could help explain spatial clustering. The cyclic epidemic patterns have historically been attributed to growing community level immunity that results in transmission decline until births (and/or in-migration) replenish the susceptible population sufficiently to fuel resurgence. The best hope for long-term elimination and control may be a vaccine that mimics naturally acquired immunity, which appears to depend on the presence of live organisms. Although limited data demonstrate the infectiousness of kala-azar and post-kala-azar dermal leishmaniasis (PKDL) patients to sand flies, the potential for transmission from asymptomatically infected individuals is an unanswered question. The occurrence of rare cases in areas with proven or presumed *L. donovani* (Bhutan, hilly areas of Nepal) calls into question the assumption that kala-azar or PKDL patients must be present to sustain transmission over time, raising the possibility that the reservoir might consist of asymptomatically infected people. Animal models can help to define the dynamics of transmission from hosts with high (symptomatic) vs low (asymptomatic) parasite loads.

4 Conclusions

This session will address findings from experimental models that help to clarify determinants and dynamics of leishmanial transmission and pathogenesis, and relate these to the epidemiology of the disease.

3.2 LEISHMANIA IN THE SAND FLY AND DETERMINANTS OF INFECTIVITY OF SAND FLY BY BITE AND SUBSEQUENT PATHOLOGY.

Matthew Rogers

London School of Hygiene and Tropical Medicine London UK

1 Background

Infected sand flies differ greatly from needle-based infections and a growing body of evidence shows that sand flies are very efficient at transmitting *Leishmania*. However, large gaps in our understanding remain, not least: how does transmission work, how does it change during the course of the sand fly infection and how are infections acquired by sand flies?

2 Methods

Quantitation of parasite transcripts from bitten mouse ears by real-time PCR (RTqPCR) has provided the most physiologically relevant data on transmission to date and has highlighted its variability. This approach has shown that the majority of infected flies transmit low doses of parasites. However, roughly a quarter of infected flies transmitted significantly higher doses (≥1,000 parasites) and this was associated with transmission of a high proportion of the parasite load in the midgut. This begs the questions – what is the contribution of these different transmissions, how are they generated and are there superspreading sand flies?

3 Results

We have previously shown that the accumulation of the glycan-rich gel, the promastigote secretory gel (PSG), is likely to direct this as it defines the blocked fly hypothesis of transmission, originally proposed by Shortt and Swaminath in 1928. PSG is a consistent feature of efficient transmission, and our work has revealed that it manipulates sand fly bloodfeeding behaviour and the innate immune system of the host following transmission to promote infection. Recently, we have developed and validated a metacyclic-specific RTqPCR to measure, for the first time, the quality of the infected dose delivered to skin from individual sand flies. This has revealed considerable variability in the proportion of transmitted metacyclics, which has led two further observations – one, the ability of PSG to selectively enrich the bite for metacyclics, and the second, the relationship between sand fly infection and optimal transmission in terms of dose quality (% metacyclics) and quantity (number of metacyclics). This has led to some surprising results, which challenge our current thinking about transmission and infection.

4 Conclusions

As a result, we now have the ability to interrogate transmission more closely and incorporate this mode of transmission into models of Leishmania infection. This talk will focus on these new aspects of transmission, both to and from the vector, and how this may inform current models of epidemiology.

(4) Gene regulation and new methods for gene and genome editing

4.1 RNA-BINDING PROTEINS AS MAJOR REGULATORS OF THE LEISHMANIA RESPONSE TO INTRACELLULAR STRESS AND AMASTIGOTE DIFFERENTIATION

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1 Background

RNA-binding proteins (RBPs) are key regulators of post-transcriptional control. Not only regulate mRNA processing, stability, and translational efficiency but they also play major roles in the cellular response to environmental changes, cell differentiation, and cell death. In *Leishmania*, we have recently identified a DEAD-box RNA helicase of the DDX3 subfamily that together with its interacting partners monitor the parasite response to intracellular stress. DDX3 is a highly conserved ATP-dependent DEAD-box RNA helicase with multiple roles in all steps of RNA metabolism and cellular signaling.

2 Results

We showed that *L. major* and *L. infantum* DDX3$^{(-/-)}$ knockout parasites become highly susceptible to various stress stimuli encountered in macrophages and are thus unable to undergo amastigote differentiation, to cope with oxidative burst, and to promote disease in an experimental mouse model. Genetic inactivation of DDX3 leads to increased accumulation of reactive oxygen species (ROS), which upon stress results in mitochondrial membrane potential loss, mitochondrial fragmentation, and cell death. Furthermore, in the absence of DDX3 the unfolded protein response (UPS) is activated and poly-ubiquitinated proteins accumulate both in the cytosol and the mitochondrion, as an indicator of protein damage. Increased proteotoxicity in DDX3$^{(-/-)}$ parasites leads to translation inhibition due to a defect in the elongation process. Complementation studies using DDX3 mutants lacking conserved functional motifs highlighted the essential role of the DDX3 ATPase activity in this phenotype. Consistent with these findings, co-immunoprecipitation and mass-spectrometry studies revealed potential interactions of DDX3 with key components of the cellular stress response, particularly the antioxidant response, the UPS, and essential components of the protein quality control. These studies also revealed additional interactions of DDX3 with other RBPs that seem to negatively regulate the stress response.

3 Conclusions

Taken together, these findings provide for the first time insights into the central role DDX3 RNA helicase and its interacting partners play in monitoring the *Leishmania* response to stress stimuli inside macrophages by preventing ROS-mediated protein damage. A comprehensive understanding of how DDX3 regulates protein quality control upon stress would possibly open new avenues for the development of therapeutic strategies not only against parasitic but also other human diseases.

4.3 RIBOSOME PROFILING OF TRYPANOSOMATID PARASITES

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Full text

The recent availability of (almost) complete genome sequences for many trypanosomatid parasites has allowed the researchers unprecedented opportunities to interrogate changes in gene expression between different lifecycle stages and growth conditions. However, while technologies (such as RNA-seq) for measuring transcript abundance are well-developed, those (such as LC-MS/MS) that assess protein abundance generally do not measure low abundance and/or poorly soluble proteins. Moreover, for many genes, mRNA levels are not good surrogates of protein abundance, thereby leaving a substantial gap in our ability to identify all the changes in protein abundance under different conditions. Ribosome profiling provides a more reliable means of elucidating global protein production byusing RNA-seq methodology to measure the small mRNA fragments protected by ribosomes assembled on actively translated mRNAs. This powerful approach has now been applied to trypanosomatid parasites. We have ribosome profiling to identify new protein coding sequences (CDSs) and clarify the boundaries of previously annotated CDSs in *Trypanosoma brucei* and *Leishmania donovani*; as well as demonstrating that translation efficiency varies widely between genes and across stages. We have shown that more than 2500 *Leishmania* genes undergo at least 2-fold changes in translation level during axenic promastigote-to-amastigote differentiation. In addition, ribosomal proteins and translation factors themselves are subjected to substantial translational control, suggesting a means of reinforcing global translational regulation under different growth conditions.

4.4 DIRECT SEQUENCING OF LEISHMANIA GENOME IN BIOLOGICAL SAMPLES

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1 Background

Until now, studies on *Leishmania* genome diversity were performed using DNA of cultured isolates. However, these results may not fully reflect the situation *in vivo*, as i) there might be selection bias introduced by isolation/cultivation, and ii) we observed drastic changes in aneuploidy pattern of a given strain in different environments (*in vitro* promastigotes vs *in vivo* amastigotes). Understanding the dynamics of *Leishmania* in a natural context requires direct analyses of parasite genomes in host tissues.

2 Methods

The greatest challenge in direct sequencing of biological samples is the low parasite/host DNA ratio. To overcome this problem, we developed a *Leishmania* genome capture method using Agilent Sureselect technology. It is based on beads coated with 218,904 RNA probes (120 bp length), designed against the JPCM5 *L. infantum* reference genome, which are used to pull-down the parasite DNA from a complex sample. After the enrichment step, the parasite's whole genome sequence is obtained using the Illumina platform.

3 Results

We first tested our new capture method using simulated biological samples, where the ratio of *L. donovani*/human DNA was between 1/1500 (=0.06% of *Leishmania* DNA) to 1/150000 (=0.0006% of *Leishmania* DNA). We observed the enrichment of *Leishmania* DNA in the range of 400x-1000x with (i) all 36 chromosomes covered proportionally to their expected somy in samples with two highest proportions of *Leishmania* DNA, and (ii) genome coverage ranging from 87% in the most concentrated sample to 7.5% in the most diluted one. In the next step, we verified the method with real biological samples where DNA was extracted from i) bone marrow or spleen of visceral leishmaniasis patients in Nepal, ii) dogs infected with *L. infantum*, iii) bone marrow, liver and spleen from experimental animals (Syrian golden hamsters infected with *L. donovani*). These analyses are in progress and results will be available for WL6. Wherever possible, we will perform pairwise comparisons between the genome of intracellular amastigotes (biological sample) with the genome of *in vitro* cultured promastigotes derived from the same samples.

4 Conclusions

We have developed a method allowing whole genome analysis of *Leishmania* directly in biological samples. This breakthrough will open new perspectives for revisiting genome diversity in the natural context of infection and/or disease, in humans, animal reservoir, and potentially in insect vectors.

(5) Drug development: conventional and alternative. Clinical and experimental therapy in cutaneous and visceral leishmaniasis

5.3 NEW INSIGHTS INTO THE EXPOSURE-RESPONSE RELATIONSHIPS FOR MILTEFOSINE IN THE TREATMENT FOR VL

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Full text

An effective, safe, oral-based field-adapted treatment is urgently needed for visceral leishmaniasis (VL), particularly in Eastern Africa. Miltefosine, currently the sole oral treatment available for VL, is considered the only option for combination with existing drugs or future oral candidate compounds. Understanding miltefosine's pharmacokinetics and dose-exposure-response (PK-PD) relationships is therefore of critical importance to guide a rationale for future development of combination treatments containing miltefosine.

Only few studies have been performed worldwide on the pharmacokinetics of miltefosine, more recently also in Eastern Africa. Children were under-exposed to miltefosine when given the conventional 2.5 mg/kg/day dosing, which has been associated with decreased efficacy in this age group in Kenya and Sudan. Increasing the mg/kg dose in children (i.e. an allometric dose) was recently found to be tolerable and increased exposure to some extent towards that of adults, although unexpected non-linearities in the pharmacokinetics were observed, possibly related to absorption of the drug. Besides pediatric under-exposure, extensive inter-regional differences have been detected not only in terms of miltefosine therapeutic response, but also in terms of drug exposure. Overall miltefosine exposure was ~30% lower in Eastern African patients, compared to Indian VL patients and Colombian CL patients, which could be mechanistically modelled and explained as reduced bioavailability of the drug in the first week of treatment – possibly related to disease-specific factors and/or malnutrition. Achieving sufficient miltefosine exposure appeared clinically highly relevant: both in Nepal as well as in studies in Eastern Africa, PK-PD relationships were established between the time period miltefosine concentrations were above 10xEC50 or EC90 values and probability of treatment failure as well as time to relapse (relapse hazard) in a time-to-event analysis. Pragmatic clinical pharmacokinetic targets were established for Eastern Africa, that could be used for future dose and treatment duration optimization.

The identified inter-regional pharmacokinetic differences illustrate the heterogeneity of VL patient populations and the importance of host differences in (oral) drug-specific absorption, distribution, metabolism and excretion and possible effects of disease and malnutrition on these pharmacokinetic processes. These findings are particularly relevant for strategies of future dose-finding studies in VL for novel oral drugs and drug combinations.

5.4 PERSPECTIVES FOR IMPROVED TREATMENTS ON VISCERAL LEISHMANIASIS

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Over the last decade, some improvements have been reached on the treatment of visceral leishmaniasis (VL). In Asia, one single administration of liposomal amphotericin B and combination treatments such as miltefosine and paromomycin for 10 days have proven to be highly efficacious and have been deployed successfully in the elimination program. In Eastern Africa (EA), the development of the combination of sodium stibogluconate (SSG) with paromomycin has reduced the time of SSG monotherapy treatment from 30 to 17 days, and has been adopted as 1st line treatment in the region. In Latin America, liposomal amphotericin B is a safer option for the treatment of VL patients.

The experience of clinical trials performed in the different regions has shown that evidence cannot be extrapolated between regions, and clinical research needs to be adapted to the local epidemiological context of the disease. Comparing outcome of clinical trials with various treatment regimens in Asia vs Africa, in general the efficacy in Africa is much lower than in Asia, requiring higher doses or longer duration to obtain satisfactory efficacy rates. EA bears now the highest burden of VL worldwide, so the focus is to identify a new treatment for this region, followed by Latin America.

New combination treatments based on liposomal amphotericin B and the new treatment fexinidazole did not reach sufficient efficacy to justify proceeding to phase III trials. However, data obtained from these trials were relevant to better plan the future development of new treatments. The optimal target profile of the new treatment is a combination of oral drugs that is highly efficacious (> 95%), safe (does not require safety monitoring), adapted to field conditions, with short duration (7-14 days) and affordable.

A new phase III trial to assess the combination of miltefosine with paromomycin in EA will be initiated in 2017, aiming to improve safety by replacing SSG with miltefosine, and reducing time of hospitalization. The real innovation is expected to come from the new chemical entities (NCEs) from DNDi pipeline and partners. Over the next 5-7 years, at least 10 new compounds are expected to progress to pre-clinical development, from which 5-6 should be taken to clinical development. There is high chances that at least one-two compound(s) will be fully developed as a product, to be combined with other NCE or with the currently available oral treatment, miltefosine. This opens the perspective of much improved treatments not only for VL, but also for HIV-VL co-infection, cutaneous leishmaniasis and PKDL patients.

(6)Operational Research

6.1 REACHING THE ELIMINATION TARGET IN THE DISTRICT OF VAISHALI, BIHAR, INDIA

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1 Background

The burden of Kala-azar in central north-eastern India, especially in Bihar continues to be endemic, despite the existence of effective treatment and vector control strategies to control the mortality and morbidity of the disease. During 2011-15, an average 13000 new cases and 27 deaths occur/year. Approx, 66 million people at risk. Hence, in 2015, Government of India (GOI) had entrusted RMRIMS (ICMR, Patna) to develop an integrated control programme as a model for achieving the elimination goal (1 case per-10,000 population at sub-district level) in the endemic region of Bihar. Initially, study was conducted in the second most highly-endemic region Vaishali district (13 highly-endemic blocks, incidence rate ranges 1.07 - 4.82), Bihar.

2 Methods

Vaishali is consisted of 1569 villages distributed in 16 blocks and covered a geographic area of 2036 square kilometer. Total population is 3.49 million. Elimination strategies involve active case detection, vector control using indoor residual spray (IRS) by DDT (50%) and SP (alpha-cypermethrin 5%), awareness campaign (IEC/BCC), training of medical staffs, ASHAs and spray men and the three-tier close monitoring from district to squad level. First time, the insecticide quantification kit (IQK) for quantification of active ingredients of insecticide to maintaining the standard deposition of DDT (1gm / square-meter) on the wall was evaluated. Conventional stirrup pump was replaced by modern compressor pump as per the norm of WHO. Sand fly densities were monitored at 15-days before and 15-days, 1-month and 3-months interval after the spray. Micro-action plan for the IRS was processed and prepared at GIS platform. All the endemic villages of last-three years and the non-endemic villages ranged within the 500m periphery of highly-endemic villages were selected for IRS based on the sand fly flight range.

3 Results

Results showed that number of cases and affected villages were reduced from 664 in 2014 to 155 in 2016 and 327 in 2014 to 138 in 2016, respectively. Percentage of case reduction increases from 19.3% in 2014 to 60.7% in 2016. All the 16 blocks were achieved the elimination target. Incidences rate ranges from 0.17 to 0.86 per-ten thousand population.

4 Conclusions

Based on the results of Vaishali success story it has been decided to scale-up the model in all the endemic districts of Bihar, India.

6.3 COST-EFFECTIVENESS ANALYSIS OF NEW DIAGNOSTIC TOOLS FOR CUTANEOUS LEISHMANIASIS IN AFGHANISTAN

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Full text

Context: Cutaneous leishmaniasis (CL) is the most common form of leishmaniasis. Although not fatal, CL is responsible for chronic and disfiguring skin lesions resulting in morbidity and social stigma. The currently available diagnostic tools for CL are microscopy and molecular techniques, but these present associated drawbacks which inhibit their use as routine diagnostic in endemic countries. Microscopy has a low and variable sensitivity and requires trained personnel, whilst molecular techniques such as PCR and qPCR are costly and technically complex. Accordingly, there is a crucial need for diagnostic tools that are highly accurate (i.e. high sensitivity and specificity), easy to use, field-amenable and inexpensive. These would enable us to (i) lower the risk of relapses; (ii) accelerate the healing of the skin; (iii) decrease local morbidity caused by large or persistent skin lesions; and (iv) diminish the reservoir of infection in given geographic areas. The objective of this study is to evaluate the cost-effectiveness of two new point-of-care (POC) diagnostic tools: Loopamp ™ *Leishmania* Detection Kit (Eiken Chemical Co, Japan) and CL Detect ™ Rapid Test (InBios International Inc., USA), using a combination of microscopy and PCR as a reference.
Methods: Data were collected at the National Malaria and Leishmaniasis Control Program (NMLCP) health facility in Afghanistan. Information on costs related to the diagnostic tools was taken both from the health system and patient perspectives using tailor-made questionnaires. The effectiveness of the test was assessed through two channels: the specificity and sensitivity of the tools and an indicator of disease burden – the disability-adjusted life years (DALYs). The natural history of CL was represented through a Markov model and designed in TreeAge. Probabilistic analyses (Monte Carlo simulations) were run to account for parameters' uncertainty.
Results: Under the conservative approach (only one test is done at a time with Loopamp), preliminary results from Monte Carlo simulations suggest a domination of CL Detect ™ over Loopamp ™ Leishmania Detection Kit Rapid Test. CL Detect ™ has an associated cost of $68 USD [95% CI: 64.16, 72.26] and an effectiveness of 192 [CI 95%: 189.11, 196.84] DALYs, whereas Loopamp ™ Leishmania Detection Kit Rapid Test has an associated cost of $89 USD [CI 95%: 82.98, 94.67] and an effectiveness of 194 [CI 95%: 190.22, 198.06] DALYs. However, when relaxing the conservative approach and taking full advantage of the diagnostic capacity of Loopamp (12 tests are done at a time) then this tool becomes more cost-effective than CL Detect ™.

6.4 CROSS-SECTIONAL SURVEYS IN BANGLADESH, INDIA, ETHIOPIA & SUDAN TO UNDERSTAND VISCERAL LEISHMANIASIS TREATMENT SEEKING, DIAGNOSIS, TREATMENT AND HOUSEHOLD ECONOMIC BURDEN.

*Lucy Paintian[1], MercË Herrero[2], **Vikas Aggarwal[3]**, Mark Pearson[4], Tansy Edwards[1], Atia Alatiaby[5], Dinesh Mondal[6], Kingsuk Misra[7], Cherinet Adera[2], M Prakash[8], Mahmudur Rahman[7], Shewaye Belay Tessema[9], Bewketu Mengesha Khalid Gamal[10], Yassen Hamed[11], Mosab Alhag[12], Toby Leslie[4], Jose Ruiz-Postigo[13], Daniel Argaw[13], Margriet van den Boer[14], Sakib Burza[15], Jayne Webster[1]*

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1 Background

The KalaCORE project is a DFID-funded initiative to eliminate and control visceral leishmaniasis (VL) in Bangladesh, India, Nepal, Ethiopia, South Sudan and Sudan. The goal is to reduce the health impact and economic burden of VL by ensuring access to early diagnosis and complete treatment.

2 Methods

Cross-sectional surveys of VL patients treated at 46 VL treatment facilities in endemic areas of Bangladesh, India, Ethiopia and Sudan were conducted between February and September 2016. A structured questionnaire was used to collect data on patient and household characteristics, the treatment pathway and the cost to the household of the recent VL illness episode. The surveys provide a baseline to identify where support from KalaCORE to the VL control programmes can be targeted to improve patient management and access to care.

3 Results

A total of 909 VL patients were interviewed across the four countries. Patient demographics varied considerably by country: in Bangladesh and India 60% of patients were male; most were young adults and males had a median age 7 years older than females. In Ethiopia, almost all patients were young males of working age; just over half were migrant workers (pre-determined by sampling strategy in Ethiopia). In Sudan, the median age of patients was 13 years; almost 70% were male. The shortest time between onset of symptoms and receiving VL treatment was found in Sudan with a median of 21 days. This compared to 40 days in Ethiopia, 52 days in Bangladesh and 54 days in India. Patients in South Asia visited more providers during their illness than patients in East Africa. Total financial costs exceeded 20% of annual household expenditure for between 13.7% (India) and 31.4% (Ethiopia) of patients. In all countries, diagnostic procedures and treatments received before a correct VL diagnosis incurred substantial financial loss for the patient. Including reported loss of earnings for the patient and any caretakers resulted in economic costs of illness approximately double the financial costs.

4 Conclusions

VL results in substantial economic burden for patients and their households, largely due to financial costs incurred before receiving a VL diagnosis and loss of income to patients and/or their caretakers. The KalaCORE

project will contribute to the VL control programmes in Bangladesh, India, Ethiopia and Sudan to reduce the time between onset of symptoms and start of treatment through patient- and provider-side interventions.

Wednesday, May 17th

(7) Molecular retooling in macrophage-parasite interactions

7.1 THE SIGNALING PATHWAY THAT INITIATE LEISHMANIA DIFFERENTIATION

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Full text

Protozoan parasites of the genus *Leishmania* cycle between promastigotes in the sand fly mid-gut and amastigotes in the phagolysosome of mammalian macrophages. While much of the molecular mechanism of development inside macrophages remains a mystery, development of a host-free system that simulates phagolysosome conditions (37ºC and pH 5.5) has provided new insight into these processes. Using high coverage transcriptomic and proteomic approaches to interrogate changes in gene expression during promastigote-to-amastigote differentiation of *Leishmania donovani* have shown that differentiation is well-regulated, with ordered and coordinated changes in mRNA and protein abundance resulting in the parasites re-tooling their metabolic pathways for life in the new host environment. Phosphoproteomic analyses also revealed substantial changes in protein phosphorylation within minutes of exposure to the differentiation signal. Null mutants of early-activated protein kinases revealed that protein kinase A (PKA)-mediated signaling pathway plays a critical role in initiating the transition between lifecycle stages. PKA is ubiquitous in eukaryotic cells. Activity of the catalytic subunit(s) (PKAC) is controlled by assembly with the regulatory subunit(s) (PKAR) into holoenzyme complex that is inactive in the absence of cAMP. Cyclic nucleotide binding domains (cNBDs) in the PKAR bind cAMP, resulting in a conformational change, dissociation of the PKAC-PKAR complex and activation of PKAC. Our analysis indicated that, in *L. donovani*, changes in the interaction between PKAC3 and PKAR′ are crucial for the response to the temperature and pH signals, as well as the timing of the differentiation process. Null mutants of PKAR' differentiate at a rate slower than wild type and die during the last phase of differentiation. Time-course analysis of PKAC3 phosphorylation during phase I supports a critical role in *L. donovani* promastigote-to-amastigote differentiation. Overall, our work provides the first insight into the molecular mechanism of *Leishmania* differentiation by identifying the signaling pathway that initiates this process.

7.2 PHAGOSOME REMODELLING BY LEISHMANIA

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Full text

Successful vacuolar pathogens have developed sophisticated strategies to hijack the endomembrane system of host cells and evade antimicrobial responses. The protozoan parasite *Leishmania*, the causative agent of leishmaniases in humans, is particularly adept at transforming the macrophage into a hospitable host cell. As they establish within phagocytes, *Leishmania* promastigotes release molecules that sabotage host cell microbicidal and immune functions. Lipophosphoglycan (LPG) and GP63 are two virulence factors involved in this process. Hence, we previously established that insertion of LPG from *L. donovani* promastigotes into host cell lipid microdomains causes remodeling of the parasitophorous vacuole, delays its maturation into a highly microbicidal phagolysosome, and prevents recruitment of the v-ATPase. We also discovered that the surface metalloprotease GP63 enables *Leishmania* promastigotes to target the macrophage membrane fusion machinery, to create an intracellular compartment favorable to the establishment of infection and to manipulate host immune responses. To achieve this, LPG and GP63 must traffic from the parasitophorous vacuole to the cytoplasm. We obtained evidence that the mechanism associated to this trafficking process involves the host cell endoplasmic reticulum-Golgi intermediate compartment. Collectively, these studies provide insights into the mechanisms of *Leishmania* pathogenesis.

Supported by the Canadian Institutes of Health Research

7.3 IRON HOMEOSTASIS AND ROS GENERATION REGULATE VIRULENCE IN LEISHMANIA AMAZONENSIS

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Iron is an essential element but is toxic to cells when in excess. Therefore, iron import and export have to be tightly regulated. Until recently, very little was known about iron and heme homeostasis mechanisms in Leishmania parasites. Studies from our laboratory and collaborators showed that the availability of iron in the culture medium regulates expression of several genes in L. amazonensis. Functional characterization of iron-regulated genes led us to identify the membrane proteins LFR1 (ferric iron reductase), LIT1 (ferrous iron importer), LHR1 (heme importer) and LIR1 (transporter involved in iron export). Our evidence indicates that these proteins act in concert with each other to regulate iron and heme homeostasis, and are required for the development of virulence in L. amazonensis. Surprisingly, we also found that exposure to iron-depleted medium triggers the differentiation of L. amazonensis promastigotes into amastigotes, independently of the classical temperature and pH differentiation signals. This iron-dependent differentiation process requires expression of the iron transporter LIT1 and involves generation of reactive oxygen species (ROS). Investigating this process, we found that iron uptake into mitochondria through the transporter LMIT1 activates the iron-dependent superoxide dismutase FeSODA, which converts ROS generated within mitochondria into the diffusible signaling molecule H2O2. Promastigote to amastigote differentiation was achieved by directly exposing L. amazonensis promastigotes to H2O2, consistent with evidence from other systems showing that H2O2 can regulate the transition between cellular proliferation and terminal differentiation. Collectively, our results provide important new insight into the roles of iron and mitochondrial ROS in regulating the development of virulence in Leishmania

7.4 LEISHMANIA USES MINCLE TO TARGET AN INHIBITORY ITAM SIGNALING PATHWAY IN DENDRITIC CELLS THAT DAMPENS ADAPTIVE IMMUNITY TO INFECTION

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1 Background

Leishmania can be detected by several Pattern Recognition Receptors, which trigger activating or inhibitory pathways. *L. major* is a poor inducer of dendritic cell (DC) activation. Moreover, this parasite inhibits DC migration to draining lymph nodes (dLN) and consequently, priming of T helper 1 (Th1) cells and macrophage microbicidal activity. The mechanisms underlying this early *L. major*-induced DC suppression remain ill-defined.

Mincle is an FcR gamma-chain-coupled myeloid-inducible C-type lectin receptor (CLR) for endogenous SAP-130 exposed and released by dead cells and for glycolipids on the cell walls of bacteria and fungi. Binding of these ligands to Mincle triggers phosphorylation of immunoreceptor tyrosine-based activation motif (ITAM) tyrosine residues in the FcR gamma-chain, followed by the recruitment and activation of the kinase Syk, which generates an activating signal that boosts immunity to infections and inflammation. We hypothesized that *Leishmania* infection is sensed by Mincle.

2 Methods

We have used Mincle-deficient mice or mice with depletion of SHP1 or Syk in the CD11c^{+} compartment. Mice were infected intradermally into the ear pinnae with *L. major.* Next, we evaluated pathology, parasite load and immune responses in the skin and dLN. We also generated bone marrow derived DC cultures from these mice to further characterize DC-parasite interaction.

3 Results

We have found that human and mouse Mincle bind to a soluble ligand released by *Leishmania*. Mincle-deficient mice had milder dermal pathology and a tenth of the parasite burden compared to wild-type mice after *Leishmania major* infection. Mincle deficiency enhanced adaptive immunity against the parasite, correlating with increased activation, migration and priming of a Th1 cell response to parasite antigens by Mincle-deficient dendritic cells (DCs). *Leishmania* triggered a Mincle-dependent inhibitory axis characterized by SHP1 coupling to the FcR gamma-chain. Selective loss of SHP1 in CD11c+ cells phenocopies enhanced adaptive immunity to *Leishmania* observed in the absence of mincle.

4 Conclusions

Leishmania shifts Mincle to an inhibitory ITAM (ITAMi) configuration that impairs DC activation. Thus, ITAMi can be exploited for immune evasion by a pathogen to delay sterilizing immunity in vertebrate hosts. and may represent a paradigm for ITAM-coupled receptors sensing self and non-self. The low effectiveness of vaccines based on whole *Leishmania extracts* could partially rely on the presence of a ligand for Mincle.

(8) Non-invasive field adapted tolos (and biomarkers) for VL diagnosis and test of cure

8.1 CHALLENGES AND PROGRESS TOWARDS A SIMPLE POINT OF CARE DIAGNOSTIC TEST FOR VISCERAL LEISHMANIASIS IN EAST AFRICA

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Full text

Back ground: Visceral Leishmaniasis (VL/ Kala-Azar) is an increasing health problem caused by the *kinetoplast* parasite of the genus *Leishmania.* The annual global burden of VL is 500,000 cases and the yearly incidence of VL in East Africa is between 29,400 and 56,700 cases, accounting for15 % of the global cases. The disease was recorded in Sudan, South Sudan, Ethiopia, Kenya, Uganda and Somalia with new cases described from Eritrea. Kala-Azar epidemics were recorded in Sudan and South Sudan resulted in hundred of thousands fatalities. VL in eastern Africa is caused by *Leishmania donovani* complex parasites and is deadly if not treated. Diagnosis of VL in eastern Africa is still problematic due to the endemicity of the disease in remote regions not accessible during the rainy season, the inadequate health service, the civil unrest, population movement and displacement, poverty, lack of political commitment of health authorities, and the inadequacy of human and laboratory resources.

Methods and conclusions: Parasitology is the gold standard diagnostic procedure, and it requires invasive sampling for microscopic detection of Leishmania amastigotes. Bone marrow, spleen or lymph node aspiration are used to demonstrate Leishmania amastigotes in Giemsa stained smears. The sensitivity of microscopy varies between 40 and 95% depending on the aspirated organ and the experience of the microscopist. Parasite culture raises the sensitivity of tissue aspiration but it requires tissue culture resources, not attainable in most endemic regions. Several serological tests were evaluated for diagnosis of VL and they suffer from the failure to distinguish between old, present and asymptomatic infections and influenced by coinfection with HIV. The Direct Agglutination Test (DAT) was used as a screening test with variable sensitivities and patch to patch variation, and it requires cold chain storage and laboratory setting for pipetting and long incubation. Rapid test presented a strong promise for bed side diagnosis. Development of rK39 based chromatographic test provided a simple rapid diagnostic test (RDT) format suitable for VL diagnosis in remote endemic areas. The performance of the test in eastern Africa was dissatisfactory with sensitivity <80%, lower than it's performance in the Indian sub-content. The new development of rK28 - a fusion polyprotein of rK39, rK9 and rK26 proteins–by IDRI increased the sensitivity of the test to 94% and the specificity to 99% when evaluated in Sudan providing a new tool for accurate bed side diagnosis that needs to be further evaluated in the region. Molecular diagnosis using PCR was hopeful but requires advanced laboratory setting not economical to set up in remote endemic regions. Recent advancements in developing a Loop Mediated Isothermal Amplification (LAMP) showed very promising performance in pilot evaluation in eastern Africa. The test is under evaluation in different endemic regions. Detection of Leishmania antigen is a potential approach that will identify current infection and used as prognostic test. Several Urine excreted Leishmania antigen have been identified and characterized and are currently evaluated in for diagnostic and prognostic value in East Africa.

8.3 DEVELOPMENT OF PROGNOSTIC SEROLOGICAL BIOMARKERS FOR VISCERAL LEISHMANIASIS, AND THEIR RELEVANCE TO DISEASE CONTROL

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Background
Visceral leishmaniasis (VL) is a major scourge that warrants intense research and strenuous international efforts at disease control. Only a proportion of individuals exposed to Leishmania donovani or Leishmania infantum infected sand flies succumb to symptomatic VL, dependent on the vulnerability of local human populations. Diagnosis of VL is problematic. To investigate distinct regional efficacies of the rK39 diagnostic test we have analysed genetic diversity of the kinesin antigens and compared levels of antibody response to local Leishmania strains during active VL in India and in East Africa. There are currently no reliable biomarkers to predict a) progression of serologically positive (rK39 or DAT) asymptomatic carriers to active VL b) post-chemotherapeutic relapse.
Methods
By ELISA we have profiled subclass antibody responses to Leishmania in patients with different clinical status. Based on the findings, we have developed a clinically discriminative, point-of-care (POC) lateral flow rapid diagnostic test incorporating Leishmania lysate (RDT; VL Sero K-SeT). We are also applying mass spectrometry and in silico comparative genomics to define specific antigens for this RDT.
Results
IgG1 specific antibody levels are elevated in active VL and in post-chemotherapeutic relapse but decreased in cure. A strong positive VL Sero K-SeT in asymptomatics predicts progression to active VL. Strong or sustained RDT positivity at 6 months follow up after chemotherapy predicts that patients will subsequently relapse, whereas negative RDT is indicative of cure. In parallel, we have shown that comparative genomics of progeny from experimental genetic crosses of L. donovani provides insight into the evolution and thus realistic potential of candidate biomarkers.
Conclusions
The predictive test is an important non-invasive and low cost adjunct to clinical diagnosis and prognosis. Furthermore, VL Sero K-SeT has applications in epidemiological research and in enhancing elimination of VL. Whilst VL Sero K-SeT positive asymptomatic carriers may not be treated with drugs that have side effects, they may be followed up and their dwellings sprayed with insecticide to limit the spread of infection. Furthermore, follow up of VL Sero K-SeT positive patients beyond 6 months will be life-saving and contribute to elimination of VL.
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8.4 THE DIAGNOSTIC CHALLENGES AND NEEDS IN THE KALA-AZAR ELIMINATION PROGRAMME IN SOUTH ASIA

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DNDi India

Full text

Kala-azar Elimination Program (KAEP) was launched in 2005 in Bangladesh, India, and Nepal to achieve elimination of kala-azar as a public health problem by 2017. Visceral Leishmaniosis (VL) or kala-azar, in this region affects the populations of the lowest socio-economic strata most of whom have limited access to proper health care. Untreated, kala-azar is almost always fatal. The drugs currently in use are quite toxic, thus confirmation of diagnosis before starting therapy is crucial. Also a key strategy of the KAEP is early diagnosis and appropriate treatment. Decentralization of VL care to primary health centers, has become possible with the availability of rapid diagnostic tests, rk39 ICT, which have shown to be highly sensitive and specific. The main limitations are in the diagnosis of relapse cases and to test for cure. Also parasitological diagnosis is limited to referral hospitals and specialized VL treatment which may not be easy to access. In addition to VL, PKDL has also been implicated to be a reservoir for sustaining transmission. Diagnosis of PKDL is currently difficult as there are no easy to use and accurate tests that can be deployed in the peripheral health centres. The proper implementation of the diagnostic strategies within the VL elimination program involves not only the procurement, training, and supervision of staff, but also quality control both before and after deployment in the field. The logistical requirements are enormous, and therefore standardized guidelines for procurement and quality control must be established.

(9) Tools to understand the epidemiology of leishmaniasis and asymptomatic infection

9.1 BIOMARKERS OF INFECTION AND DISEASE

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Full text

The key control measures against leishmaniasis mainly rely on early case detection and chemotherapy. The identification and management of asymptomatic carriers has become a new and increasingly important challenge for visceral leishmaniasis control programs. Suitable and validated biomarkers are desirable in order to accurately distinguish asymptomatic infection from disease progression, and to predict relapses from cure after treatment.They should be cost-effective, accurate, noninvasive and easily translatable to the field.
Biomarkers for the outcome of leishmaniasis treatment are most urgently needed both for clinical decision making and to facilitate the shortening of clinical trials of new drugs or regimens. Most clinical trials confirm initial cure for visceral leishmaniasis with a *Leishmania*-negative spleen or bone marrow aspirate confirmed by microscopy; and for cutaneous leishmaniasis is much less clear, cure is associated with the absence of inflammatory signs and complete scarring or reepithelialisation of ulcerative lesions. Subjects with active leishmaniasis can be monitored with a combination of unspecific biomarkers, as metabolites and inflammatory mediators, and highly specific direct biomarkers, as DNA/RNA with a good correlation with clinical outcome. Although the parasite loads decrease with clinical cure, they are undetectable before clinical cure can be established. In the search of new biomarkers for confirming early cure after treatment, cell-mediated immunity assays against *Leishmania* appear to be a promising strategy. Cytokine/chemokine quantification after the whole blood assay (WBA) and cytokine production after positive cell proliferative response (CPA) demonstrated their utility for following up solid organ transplant (SOT) recipients treated for visceral leishmaniasis, further they could help confirm cure and prevent relapses. Recently, the positive cell proliferation response, in addition to CD4count, has been described as a good predictive marker of non-appearance of relapses in *Leishmania*/HIV+ co-infected patients.
To estimate the asymptomatic *Leishmania* infection, a combination of pathogen-specific assays is recommended to avoid underestimation, such as a serological test, polymerase chain reaction (PCR)/ loop-mediated isothermal amplification (LAMP), and leishmanin skin test (LST). Recently, cytokine/chemokine quantification after WBA has emerged to replace LST assessing cell-mediated immunity in field assays. *Leishmania*-specific IL-2 secretion after WBA has been proposed as a sensitive biomarker of asymptomatic *Leishmania infantum* infection in immunocompetent subjects and in those receiving immunosuppressive therapy. But before considering it as alternative or adjunct biomarker to identify asymptomatic subjects, other cohorts as malnourished, co-infected with helminths, etc. of exposed people must be tested in several endemic areas.
As described, promising biomarkers and biomarker signatures are being discovered in leishmaniasis. But efficient validation and qualification process are requested before their evaluation in lengthy and costly trials. Currently, there is a lack of validated biomarkers of infection and disease in leishmaniasis.Further intensified research efforts are required for ongoing biomarker discovery, evaluation and test development.

9.3 LEISHMANIN SKIN TESTING VERSUS INTERFERON GAMMA RELEASE ASSAYS IN LEISH

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Full text

The leishmania skin test (LST) has a high sensitivity and predictive value for the diagnosis of American tegumentary leishmaniasis (ATL) in patients with disease caused by *Leishmania (Viannia) braziliensis*. The LST has also been used to detect exposure to leishmania. High IFN-γ levels in supernatants of lymphocyte cultures stimulated with soluble leishmania antigen (SLA) is observed in up to 95% of patients with cutaneous leishmaniasis (CL) caused by *L. braziliensis*. Here the LST and IFN-γ were used to determine exposure to *L. braziliensis* in subjects living in an endemic area and to evaluate immune response in cured of CL subjects. Participants were 308 household contacts (HC) of CL patients without previous history of CL, and 20 CL patients evaluated before and after therapy. In the 308 HC the LST and IFN-γ were positive in 30(9,7%). Only a positive LST was observed in 23(7,4%) and 65 (21,2%) produced only IFN-γ. The determination IFN-γ increased 1,7 foldthe sensitivity to detect exposure to *Leishmania*. Of the 308 HC followed for 5 years 45 (24,6%) developed CL. The ability to control leishmania was associated with IFN-γ production but not with a positive LST. In cured CL patients IFN-γ production and/or antibodies anti LSA were observed in 50 and 90% of the cases respectively**.** The sources of IFN-γ in cured CL were effector memory and central memory T cells. In 7 of 8 cured CL the LST revealed a mixed hypersensitivity type 3 and type 4 reaction with a small indurated area and a large painful erythematosus lesion. In conclusion while production of IFN-γ and a positive LST has a high concordance in patients with active CL, but the concordance of these tests is only moderate in individuals with SC *L. braziliensis* infection, and weak in subjects cured of CL. IFN-γ production was associated with ability to control *L. braziliensis* in HC of CL patients. After CL therapy antibodies remain positive but *in vitro* T cell response decreased or was not detected. The exuberant type 3 and type 4 reaction in patients cured of CL even in the absence of IFN-γ production indicate that likely skin resident T cells and antibodies against SLA were responsible for the maintenance of a positive LST.

9.4 ASYMPTOMATIC INFECTION WITH THE PROTOZOAN LEISHMANIA INFANTUM: THE RELATIONSHIP OF THE LEISHMANIA SKIN TEST AND TYPE 1 PERIPHERAL BLOOD MONONUCLEAR RESPONSES

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Manifestations of Leishmania infantum infection range from asymptomatic to fatal visceral leishmaniasis (VL). Cure or protection against disease requires antigen-specific Type 1 immunity. The intradermal Leishmanin skin test (LST) is a delayed type hypersensitive test that has been interpreted as a marker of protective immunity. However, it has not been shown that LST correlates with a Type 1 response, or whether LST and Type 1 immunity are maintained long-term. These questions were examined in an 8-year longitudinal nested case study of LST status and peripheral blood mononuclear cell (PBMC) antigen-specific cytokine responses within a Brazilian family cohort. Some subjects maintained long term positive LSTs, whereas others retroconverted without developing visceral leishmaniasis.The prevalence of positive LST and the size increased with age ($p=0.0021$). The size of LST correlated with PBMC antigen-induced IFN-g secretion ($r=0.6186$, $p<0.0001$). IL-6 was released at higher levels by PBMCs of subjects who retro-converted from positive to negative LST than subjects with stable LST status ($p=0.005$). These data suggest that differences in innate immune responses may affect whether individuals maintain a positive LST response and that PBMC IFN- g might suffice as a better surrogate marker for protective immunity.

Support: National Institutes of Health, CNPq and CAPES

(10) Myeloid subsets in the macrophage-Leishmania outcomes

10.1 NEUTROPHIL FUNCTIONS IN LEISHMANIASIS

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Full text

Leishmania are inoculated in the dermis into a blood pool formed during its insect vector feeding and promastigotes may interact with blood and tissue cells and with extracellular matrix (ECM) proteins as well. Among the blood cells, neutrophils are the most abundant leukocytes and play an important role in the innate immune response. They are endowed with powerful antimicrobial mechanisms, including phagocytosis, degranulation and netosis. This last mechanism occurs with the release of a scaffold of chromatin associated with different granular and intracellular proteins, named neutrophil extracellular traps (NETs). We have shown that *Leishmania* interaction with neutrophils trigger the release of NETs which are composed of decondensed chromatin associated with granular and cytosolic proteins. Netosis, the NET release process, may occur through classical and early/rapid mechanisms. *Leishmania* promastigotes trigger the classical netosis, by promoting redox imbalance, which is mediated by NADPH-oxidase and nitric oxide synthase derived ROS/RNS, respectively. This mechanism is dependent on PI3K, ERK, intracellular calcium mobilization, peptidyl arginine deiminase (PAD)-4 and elastase activity. Furthermore, promastigotes promoted the early/rapid, ROS-independent NET formation occurring only 10 minutes after neutrophil-parasite interaction, which is dependent of elastase, but not on PAD4.

NETs trap and kill the promastigotes, which can escape NET-mediated killing by their 3`-nucleotidase/nuclease activity, by the lipophosphoglycan expressed by some species on the promastigote membrane and also by an endonuclease present in the vector saliva, which is inoculated into the host together with the parasite. NET released at the inoculation site may also modulate the function of different cells, such as the monocytes. Monocyte-derived dendritic cells are essential for the development of a Th1 protective immune response against Leishmania parasites. We are showing that NETs interfere with the monocytes differentiation into dendritic cells in response to IL-4 and GM-CSF, reprogramming the formation of monocytes-derived dendritic cells and stimulating a pro-inflammatory macrophage differentiation. Thus, NETs could not only ensnare and kill the parasites, but also affect host cells differentiation, skewing the monocytes to differentiate into macrophages with an anti-inflammatory profile. This ultimately allows *Leishmania* to grow inside the cells, increasing their infectivity, and ascribing a new role for the neutrophil traps in leishmaniasis.

10.3 DENDRITIC CELL SUBSETS IN LEISHMANIASIS

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Full text

Dendritic cells in the skin are part of the innate immune system residing in tissue as resident cells and upon activation are able to induce and modify resulting adaptive immunity against e.g. invading pathogens. Recently, more than 5 subsets of dendritic cells have been identified in skin and draining lymph nodes. It was found that epidermal Langerhans cells primarily induce regulatory T cells against Leishmana spp. which promote parasite persistence and prevent healing. Recent evicence suggests that the dendritic cell subsets that are responsible for inducing protection against the pahtogen either reside in the dermis or in the draining lymph node.

10.4 INFLAMMATORY MONOCYTES PLAY A CRITICAL ROLE IN PATHOGENESIS OF VISCERAL LEISHMANIASIS

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1 Background

Monocyte recruitment to the infection site is one of the earliest steps of the immune response and it is generally associated with bacterial and parasitic elimination. During visceral leishmaniasis the role of inflammatory monocytes is completely unknown. In this study we investigated the role of inflammatory monocytes in *Leishmania donovani* infection.

2 Methods

Age and sex matched BALB/c mice were infected with 10^7 DS-Red-*L. donovani* (LV39) or *L. donovani* amastigotes and monocyte populations in the liver and spleen were characterized by imaging flow cytometry. Ly6c^{hi} monocytes were isolated from the infected livers and spleen and profiled using microarray and flow cytometry. CCR2 antagonist was used to block influx of Ly6c^{hi} monocytes into organs and its impact on T cell response and disease progression was analyzed.

3 Results

Infected mice showed preferential and continuous recruitment of Ly6Chi monocytes into the spleen and liver. Inflammatory monocytes harbored parasites during early infection and displayed an unconventional activation state. Interestingly, blockade of inflammatory monocyte recruitment using a CCR2 antagonist during acute or chronic infection reduced parasitic loads in infected organs and reduced the frequency of IFNy/IL10 double-producing CD4+ T cells.

4 Conclusions

Our findings reveal an unexpected role for inflammatory monocytes in promoting parasite survival and open the possibility of targeting this cell population during visceral leishmaniasis.

(11) Immunosuppression and leishmaniasis

11.3 SOLID ORGAN TRANSPLANTS AND LEISHMANIASIS

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Full text

Immunosuppression represents the main individual risk factor for developing visceral leishmaniasis in areas with active transmission of the parasite. Further, immunosuppression may alter both the clinical presentation of the disease and the response to treatment. Currently, non-HIV related immunosuppressive conditions as those induced in transplant medicine, rheumatology, hematology and oncology are becoming more important and are emerging as a major risk factor associated with leishmaniasis. Specifically, the worldwide number of cases of VL in recipients of solid organ transplants (SOT) has quadrupled since the 1990s. Most of these clinical cases has been reported in the Mediterranean basin, particularly in Spain, which is among the foremost in performing SOTs, and also endemic for L. infantum transmission.
During the recent outbreak of leishmaniasis in Fuenlabrada (Southwest Madrid), eight out of the total 130 SOT recipients residing in the area developed visceral leishmaniasis (6.15%), which allow us to calculate that these patients have a risk of developing leishmaniasis more than 100 fold greater than immunocompetent individuals. Immunossupresor treatment to avoid graft rejection probably affects T-cell lymphocytes altering the mechanisms of defense against intracellular microorganisms as Leishmania, but the precise mechanism behind the VL risk has not been well defined. Despite this higher risk to develop clinical leishmaniasis, asymptomatic infection has also been described in SOT recipients from endemic areas, indicating that parasite infection does not always lead to overt disease. Treatment response in SOT patients with visceral leishmaniasis is not as good as in immunocompetent population but it is better than in HIV-infected individuals. Relapse rate in these patients is also low and secondary prophylaxis is not usually prescribed.

In order to assess the prevalence of asymptomatic Leishmania-infection in SOT recipients from endemic areas and the risk to develop leishmaniasis, we have studied a cohort of 57 SOT recipients without previous history of leishmaniasis, all of then living in Fuenlabrada. Further, we have also studied 6 SOT recipients already treated for visceral leishmaniasis. Different serological, parasitological and immunological analyses carried out in these patients demonstrated that 12 of 57 patients were asymptomatic and showed specific lymphoproliferation and IFN-g production in response to the parasite antigens, cell immunity that persisted 6 months later. On the contrary, one subject that was serologically positive and showed no lymphoproliferative response to leishmanial antigens developed VL within 2 weeks of the blood sampling. Such cell immunity was also confirmed in 5 out 6 SOT recipients patients after VL treatment, all these five patients remained relapse-free during the follow up, while the subject who showed nor lymphoproliferation neither IFNg production after invitro cell stimulation, relapsed four months after blood sampling.
These results highlight the need to assess previous exposure to the parasite in patients subjected to induced immunosuppression, and to follow up SOT recipients in areas with endemic leishmaniasis. Furthermore, the measurement of the cell-mediated immune response in SOT recipients is critical to confirm recovery following VL treatment, allowing to estimate the risk of relapse and the prescription of secondary prophylaxis.

(12) Sand fly- Leishmania interactions

12.1 SAND FLY MIDGUT MICROBIOTA AUGMENTS VIRULENCE OF LEISHMANIASIS

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Full text

It has been well established that transmission of *Leishmania* parasites by bites of vector sand flies enhances disease virulence. This had been attributed to immuno-modulation by a complex multi-component infectious inoculum, and associated to a sustained recruitment of neutrophils to bite sites where they protect captured parasites early after transmission. The mechanism underlying this phenomenon remains unknown. Here, we establish that microbiota residing in the sand fly gut are egested during infected bites where it triggers inflammasome activation and drives neutrophilic persistence. We also provide evidence of its importance in the dissemination of *L. donovani*, the causative agent of visceral leishmaniasis. The significance of egested vector microbiota may transcend sand flies and leishmaniasis and have analogous ramifications for other vector-borne diseases.

12.2 NEW PARASITES AND NEW VECTORS

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Full text

Leishmania enriettii was first described in 1948 and for many years remained an enigmatic species, never quite fitting into the prevailing classification of the day, but until quite recently generally considered to be a member of the subgenus *Leishmania* (*Leishmania*). However, recent work has provided evidence for a new phylogenetic grouping of *Leishmania* parasites, initially termed the *Leishmania enriettii* complex but now raised to subgenus level as *Leishmania* (*Mundinia*), to add to the existing subgenera *Leishmania*, *Viannia* and *Sauroleishmania*. Members of *Leishmania* (*Mundinia*) include *L. enriettii* itself (from Brazil), *L. martiniquensis* (originally described from Martinique, a Caribbean island but now known to have a wider distribution), and unnamed *Leishmania* species from Australia, Namibia, Ghana and Thailand, potentially six species in total. Some of these parasites are known human pathogens, whilst others appear to be confined to wild animals. The very wide geographical spread of the *Leishmania* (*Mundinia*), more widely distributed than any of the existing subgenera, together with their branching from the base of the *Leishmania* phylogenetic tree, both suggest an early branching and ancient lineage. The insect vectors of the *Leishmania* (*Mundinia*) have not been established with certainty for any member. It has been speculated that *L. enriettii* has a phlebotomine sand fly vector, however, this has not been proven, and *L. enriettii* is not able to establish mature infections in *Lutzomyia longipalpis*, a widely used permissive sand fly host. In the case of the *Leishmania* from Australia evidence to date supports the vectors to be day-biting midges of the genus *Forcipmyia*, although transmission by midge-bite has not been demonstrated. *Leishmania* from Ghana, Thailand and *L. enriettii* are able to develop better in experimental midge vectors (*Culicoides*) than in *Lu. longipalpis* under laboratory conditions, and some species of *Sergentomyia* have been found PCR-positive in Ghana and Thailand. Therefore, the current evidence indicates non-conventional vectors, i.e. likely not *Lutzomyia* or *Phlebotomus* species. It appears that some, or maybe even all, of the *Leishmania* (*Mundinia*) may have non-sand fly vectors. This speculation remains to be proven or disproven, with efforts currently underway in both Ghana and Thailand to identify potential vectors. However, if true, this would require a re-definition of the genus *Leishmania* to include non-sand fly transmission or the creation of a new genus to accomodate the non-sand fly transmitted parasites.

12.3 SAND FLY TRANSMITTED LEISHMANIA NANOBIOME: IMPACT ON CUTANEOUS LEISHMANIASIS

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Full text

Sand fly-transmitted *Leishmania* parasites promote macrophage (MØ) dysfunction by abrogating its ability to mount an effective immune response, favouring persistent infection. For instance, we firmly established the critical role of *Leishmania* metalloprotease GP63 enriched in exosomes (*Exo*) extracellular vesicles, as a key virulence factor. GP63 hijacks MØ signalling, microbicidal functions and innate inflammatory response by activating MØ negative regulatory mechanisms (Protein Tyrosine Phosphatases (PTP)). Additionally, we reported in a seminal study that *Leishmania Exo* (*Leish Exo*) are released in the gut of its sand fly vector and co-inoculated with *Leishmania* during blood meals. Co-egested *Leish Exo* exacerbates cutaneous leishmaniasis (CL) skin lesions by overproducing inflammatory cytokines (IL-23/IL-17) in Balb/c mice. Interestingly, we recently obtained evidence that *Phleboviruses*, as part of the vector "nanobiome" were found to be co-egested during blood meal. This finding established a new paradigm for transmission of vector-borne diseases, as this co-inoculation process could be critical for CL progression. Collectively, these different topics will be covered during my presentation as well as new findings in regard to *Leishmania Exo* content and their impact on CL progression.

(13) Microbiome and nanobiome in relation to Leishmania

13.1 MICROBIOME, ANTIMICROBIAL PEPTIDES AND LEISHMANIA INSIDE THE SANDFLY

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Antimicrobial peptides (AMPs) are abundant in nature, are found in animals, invertebrates and plants, and are part of an ancestral innate immune response repertoire. They can be active against virus, bacteria and parasites. Insects depend on AMPs for defense and for the control of their own microbiota. The first information on sand fly AMPS comes from studies in *Phlebotomus duboscqi*, after challenges with bacteria and *Leishmania major*. The identified defensin had activity against *Leishmania*, thus having a potential role in infection.

We have identified AMP genes in *Lutzomyia longipalpis*, and we are presently characterizing the putative role of these molecules in *L. longipalpis* microbiota homeostasis and responses to *Leishmania* and other pathogen infections.

We have identified four *L. longipalpis* defensin genes and have investigated the expression of these genes in male and female insects fed on sugar. These genes had different expression levels both along time after eclosion at different days after sugar-feeding, as well as when males and females were compared. This indicates a potential functional difference for these four different defensins. Interestingly the peak of expression of most of them coincided with an increase in microbiota in the gut of the insect, suggesting a possible role on microbiota control for these AMPs. Both a cecropin and an attacin gene showed similar expression pattern. Males had higher expression of most immune molecules, which might be related to their exclusive sugar diet, which results in a faster growth of the microbiota, which might be controlled by increased levels of AMPs. Our group has also found a differential modulation of one defensin gene in relation to *L. longipalpis* infection with different bacteria and *Leishmania*, and different routes of infection. We have also found that upon *Leishmania* infection, there is an increase in the expression of different AMPs at approximately three days after infection, when the parasites have contact with the insect gut. Coincidently, at this point there is a decrease in parasite load in the gut. To confirm the role of AMPs in the control of parasite abundance, we are presently silencing the AMP genes previous to infections.

Alternative ways to control infective diseases, among them leishmaniasis, are necessary. AMPs have a potential role in *Leishmania* and microbiota control in *L. longipalpis*, and are thus good candidates for the development of these alternative actions.

13.3 THE LEISHMANIA VIROME AND ITS ROLE IN DISEASE SEVERITY

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Full text

Leishmania in South America often bear the dsRNA *Leishmaniavirus* (LRV1). Like most Totiviruses, LRV1 is neither shed nor infectious, and thus may be viewed as a persistent endobiont. Perspectives on the importance of protozoal viruses changed upon discovery that *L. guyanensis* LRV1 is associated with hypervirulence and increased metastasis in animal models, the latter being a hallmark of the more severe forms of leishmaniasis (Ives *et al. Science* 2011). We have been pursuing this observation intensively as a new paradigm of protozoal virulence. For *Leishmania* we developed RNA interference tools for reproducibly generating isogenic lines lacking LRV1s (Brettmann *et al PNAS* 2016). This has allowed extension of findings with *L.guyanensis* to *L. braziliensis*, the predominant agent of mucocutaneous leishmaniasis (MCL). An important question is the contribution of LRV1 with *Leishmania* pathogenicity in human infections, where disease manifestations differ greatly from those seen in murine models, which is complicated by several factors. Recently we showed that the presence of LRV1 was associated with increased relapse and/or treatment failures in human *L.braziliensis*-infected patients treated with pentavalent antimonials in Peru and Bolivia, as well as in *L.guyanensis* infections treated with pentamidine (Adauai *et al* & Bourreau *et al. J. Inf. Dis* 2016). The association of LRV1 with clinical drug treatment failure could serve to guide more effective treatment of tegumentary disease caused by *Leishmania* sp.
We have embarked on a systematic survey of known and new viruses in Leishmania as well as their monoxenous insect trypanosomatid relatives, using a wide range of methods including next-gen RNA sequencing. The properties and role of these in various aspects of parasite biology will be investigated in future studies.

Acknowledgements
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13.4 LEISHMANIA, INNATE IMMUNITY AND MICROBIOME INTERFACE AS LEITMOTIV FOR LEISHMANICIDAL COMPOUNDS

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Full text

Until recently, the interaction of *Leishmania* with other cells was mostly circumscribed to its relationship with the macrophage and with the immune cells polarizing the macrophage response. The rising awareness of the importance of the microbiome in the final outcome of *Leishmania* parasitism starts to be unveiled. In this upper level of complexity of cellular interplay within the triad microbiome-host-*Leishmania*, the role of membrane active peptides from the host starts to be unveiled, but knowlege about the leishmanicidal peptides produced by the microbiome is nil.

In this talk, the feasibility of membrane-active peptides as a valuable source of new leishmanicidal hits will be addressed under a three-pronged approach: i) The leishmanicidal mechanism of phenol-soluble modulins, a set of amphipatic cationic peptides produced by skin pathogens and commensals belonging to genus *Staphylococcus,* and their interactions with antimicrobial peptides from the skin. Ii).- The leishmanicidal activity of the circular bacteriocin AS-48 produced from *Enterococcus faecalis*, as a proof-of-concept of bacterial peptide structures, nowadays mostly unattainable by chemical synthesis, and finally,iii) How peptide chemistry can harness natural antimicrobial peptides with weak leishmanicidal activity, as the insect cecropin A, into powerful leishmanicidal agents.

In all, leishmanicidal peptides from the microbiome add a new factor in the complexity of *Leishmania* infection, specially relevant for the cutaneous and mucocutaneous form of the disease, and how these peptides may constitute a new and extremely diversified source of new leishmanicidal structures, amenable for chemotherapeutic development.

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(14) VL Drug discovery

14.1 THE A TO Z OF DRUG DISCOVERY FOR VISCERAL LEISHMANIASIS

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Full text

The Drug Discovery Unit at the University of Dundee and the Kinetoplastid Disease Performance Unit, GlaxoSmithKline, Tres Cantos, Spain have, with support from the Wellcome Trust, formed a ten year partnership to conduct kinetoplastid disease drug discovery, with a focus on visceral leishmaniasis and Chagas disease. This collaboration has made significant progress in its first 5 years, highlighted by the delivery in 2016 of two novel, potent, oral preclinical development candidates for visceral leishmaniasis (VL) with differing mode of action. With a current limited pipeline and the current standard of care for VL suffering from multiple issues (lack of efficacy, poor safety, drug resistance, high cost, parenteral administration only), these novel preclinical candidates offer a significant step forward towards delivery of new oral drug treatments for VL.
Here I will present the drug discovery process we have put in place to identify and then rapidly move forward screening hit series to deliver candidate compounds for entry into preclinical development, highlighting some of the key learnings along the way.

14.2 KINETOBOXES: THE BEGINNING OF A BEAUTIFUL FRIENDSHIP

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Full text

Three of the Neglected Tropical Diseases (NTDs) declared by the WHO are caused by kinetoplastid parasites. They are human African trypanosomiasis, which is caused by subspecies of *Trypanosoma brucei;* Chagas disease, which is caused by the infection with *Trypanosoma cruzi*; and various clinical manifestations of leishmaniasis, which are caused by more than 20 species of *Leishmania*. All NTDs have been categorized as "tool ready," yet also "tool deficient" because many of these tools (i.e. drugs and diagnostics) and implementation strategies are inadequate to achieve the desired goals. New effective, safe, and affordable drugs, preferably oral, are needed.

As part of the fight against the neglected tropical diseases, recently GlaxoSmithKline has published three anti-kinetoplastid chemical boxes of around 200 compounds each as an open source for future lead discovery or chemical biology research.[1] The GSK 1.8 million compounds diverse collection has been phenotypically screened against their causative parasites, respectively *Leishmania donovani*, *Trypanosoma cruzi* and *Trypanosma brucei*, using the state-of-the-art methodologies available in high throughput screening.

Secondary confirmatory and orthogonal assays have been applied in order to confirm anti-parasitic activity and to identify potential cytotoxicity activity. Hit compounds have been chemically-clustered and triaged for suitable physicochemical properties. As a result of this effort, three anti-kinetoplastidal boxes of approximately 200 compounds each have been assembled, which represent all the chemical and biological diversity identified and are intended to serve as an open source of starting points for further lead discovery programs, as well as to address important research questions.

[1] Imanol Peña *et al. Scientific Reports***Volume:5**,8771Year published:, (2015). DOI:doi:10.1038/srep08771

14.3 NOVEL ANTILEISHMANIAL AGENTS: AMINO-PYRAZOLE UREAS WITH POTENT IN VITRO AND IN VIVO ACTIVITY

Paul Glossop[1], *Yuichiro Akao*[2], *Stephanie Braillard*[3], *Alan Brown*[4], *Yafeng Cao*[5], *Min Chen*[5], *Nobuo Cho*[2], *Simon Croft*[6], *Pim-Bart Feijens*[7], *Mark Gardner*[8], *Karl Gibson*[1], *Wen Hua*[5], *Masahiro Kamaura*[2], *Louis Maes*[7], *An Matheeussen*[7], *James Mills*[1], *Garreth Morgans*[9], *Charles Mowbray*[3], *Mitsuyuki Shimada*[2], *William Speed*[10], *Katrien Vanbocxlaer*[7], *Gavin Whitlock*[1], *Vanessa Yardley*[7]

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[10]*Drugs for Neglected Diseases initiative) UK*

Full text

Background: Visceral leishmaniasis (VL) is a devastating neglected disease; current treatment options suffer from significant toxicity, efficacy and administration issues. Left untreated, VL is invariably fatal and caused approximately 48,000 deaths in 2012 in affected regions of the world. Only one approved oral therapy currently exists, and safe new oral treatments are urgently needed. The overall goal of this project is to deliver a short-course oral treatment for VL that meets the published DND*i* Target Product Profile (TPP).

Methods: A high-throughput screening campaign led to the identification of a weakly active amino-pyrazole hit compound that was structurally distinct from previously known antileishmanials. A preclinical drug discovery project was subsequently run to optimise the *in vitro* potency, *in vivo* efficacy and pharmacokinetic properties of this series.

Results: Excellent *in vitro* potency against *L. infantum* and *L. donovani* was achieved. In addition, examples from this series show high levels of *in vivo* efficacy (>99%) against *L. infantum* in a hamster model of VL and against *L. donovani* in a mouse model of VL. Furthermore, potent *in vitro* and *in vivo* activity was observed against species of *Leishmania* parasites that cause cutaneous leishmaniasis (CL). Compounds from the series demonstrate excellent *in vivo* pharmacokinetics and metabolic stability in human microsomes, predictive of oral doses in human that are in line with the TPP criteria.

Conclusions: This novel series of antileishmanial compounds has the potential to deliver a breakthrough in the search for safe and effective oral agents for the treatment of VL.

14.4 DISCOVERY AND OPTIMISATION OF A NEW CLASS OF POTENT ANTILEISHMANIAL COMPOUNDS AND DETERMINATION OF THEIR MECHANISM OF ACTION

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1 Full text

Antiparasitic drug discovery is often complicated by the lack of validated targets. To identify new preclinical candidates for treatment of visceral leishmaniasis, we performed a high throughput screen on a 3 million compound library to discover inhibitors of *Leishmania donovani* growth. One HTS hit, GNF5343, was optimized by medicinal chemistry to improve its *Leishmania* growth inhibition potency, selectivity on mammalian cell lines and pharmacokinetic properties. Optimization involved the design and synthesis of ~3,000 compounds, and yielded the preclinical candidate GNF6702 which potently cleared parasites from mice infected with *Leishmania donovani* or *Leishmania major*. To identify the molecular target of GNF6702, we evolved mutant *Trypanosoma cruzi* cell lines resistant to GNF6702, and through whole genome sequencing, we discovered that the resistance-conferring mutations are located in the beta 4 subunit of the parasite proteasome. We further showed that GNF6702 selectively inhibits chymotrypsin-like activity of the kinetoplastid proteasome and does not inhibit the mammalian protesome or growth of mammalian cells. Our data provide genetic and chemical validation of the *Leishmania* proteasome as a promising therapeutic target for treatment of leishmaniasis. Furthermore, GNF6702 did not show activity in panels of human receptor, enzyme and ion channel assays, and has potential to yield a new treatment for visceral and cutaneous leishmaniasis.

(15) Outbreak assessment & surveillance

15.1 NEW ESTIMATES OF LEISHMANIASIS INCIDENCE IN SELECTED HIGH BURDEN COUNTRIES

Josè Antonio Ruiz-Postigo, *Lise Grout, Mona Osman, Mar Velarde, Daniel Argaw*

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1 Background

In 2007, the World Health Assembly resolution 60.13 on control of leishmaniasis requested the Director-General of the World Health Organization "to monitor progress in the control of leishmaniasis…".
An essential component in assessing the progress in leishmaniasis control is the estimation of the underreported cases.
WHO previously published global estimates of leishmaniasis incidence in 2012. This paper provides new estimates calculated in 2016 for cases reported up to 2014 for selected high burden countries.

2 Methods

The Delphi method was used to approach 53 experts on 18 high burden countries, 14 mainly endemic for visceral leishmaniasis (VL), three for cutaneous (CL) and one for both forms through a structured questionnaire. A second round was arranged for experts representing the same country but with different responses, allowing them to revise their answers after being provided a summary of the previous round. In addition to reported cases, recent literatures and reports were reviewed.

3 Results

A total of 33 responses were received during the first round with at least one response from 17 countries (Algeria, Bangladesh, Brazil, Colombia, Ethiopia, India, Kenya, Morocco, Nepal, Paraguay, Peru, Somalia, South Sudan, Spain, Sudan, The Syrian Arab Republic and Tunisia) and 17 answers during the second round.
Quantitative answers on the underreporting factor were received for 69% (11/16) of the countries, two mainly endemic for CL: Algeria (2.6-4.6) and Tunisia (1.5-4.6) and nine for VL: Bangladesh (1.05-1.8), Ethiopia (1.2-1.8), India (1-1.8), Kenya (2-4), Nepal (1.2-1.8), Somalia (2-4), South Sudan (1.2-1.8), Spain (1-3) and Sudan (1.2-1.8).
For five countries, Brazil, Colombia, Paraguay, Peru and the Syrian Arab Republic, experts proposed that estimates be calculated through surveys to accurately determine underreporting. No feedback was received from Afghanistan.
Upon applying the underreporting factor to the number of cases reported in 2014 from each country, the total estimate for the nine VL countries ranges from 29 969 to 51 008 and from 19 152 to 40 439 for the two CL countries.

4 Conclusions

The new global estimates for leishmaniasis represent a 84%-86% decrease for VL and 83%-87% decrease for CL compared to the last WHO estimates published in 2012, a significant decrease which may be mainly attributed to intensified control measures.
Prevalence surveys would be needed to confirm these estimates.

Thursday, May 18th

(16) T cells response in the protection and pathology of leishmaniasis

16.3 IDENTIFICATION OF PROTECTIVE LEISHMANIA-SPECIFIC CD4+ T CELLS: IMPLICATIONS FOR VACCINE DESIGN AND VACCINATION STRATEGIES AGAINST LEISHMANIASIS

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Full text

Parasitic diseases are among one of the major causes of mortality and morbidity around the world, particularly in developing countries. Human leishmaniasis are a complex spectrum of diseases affecting over 12 million people with an estimated 350 million people at risk of becoming infected globally. Manifestations of the disease range from small self-healing skin ulcers and lesions to large disfiguring scars or even death, depending on the species of Leishmania involved. Despite this significant health and societal burden, there is currently no effective vaccine against the disease. Worse still, the existing treatments either cause major side effects or are only effective in specific circumstances. The lack of effective vaccine is mostly attributed to lack of understanding of factors that regulate the development, maintenance and loss of secondary immunity. Interestingly, humans and animals that recover from infection remain immune for life, suggesting that memory cells able to protect against secondary infection develop following recovery from primary infection. Using reverse immunology and proteomics techniques, we identified a highly conserved protein that provides striking protection in vaccinated mice. With the help of collaborators around the globe, we demonstrated this protein also induces strong immune response in human patients. These studies subsequently led to the development of first Leishmania-specific reagent called tetramers, which is capable of identifying Leishmania-specific T cells at single cell level over an entire course of infection. It also helped to show that majority of the immune response is directed towards this protein. This unique reagent helped us to generate transgenic mice whose T cells all express receptor for this particular protein. We are now poised to use these animals to address fundamental questions regarding the factors that regulate antigen-specific memory response in leishmaniasis. Understanding these factors is critical to developing effective vaccine and vaccination strategies against leishmaniasis.

16.4 IL-17 IS ASSOCIATED WITH PROTECTION IN HUMAN VISCERAL LEISHMANIASIS

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1 Background

Visceral leishmaniasis (VL) is a potentially fatal parasitic disease that impose a huge health problem on the vulnerable population claiming the lives of 0.2- 0.4 million worldwide. Better understandings of disease pathogenesis that dampen the immune response are critical for controlling the disease. We had previously shown the role of IL-27 and IL-21 in differentiation and expansion of antigen-specific IL-10 producing T cells and inhibition of Th17 differentiation in VL patients. In the present study, we investigated the IL-17 association with protection in human VL.

2 Methods

We measured the circulating plasma levels and Ag specific response of IFN-γ, IL-12p40, IL-10, IL-22, IL-23, IL-17A and TNF-α in VL patients before and after treatment. Whole blood IFN-γ and IL-10 neutralization with monoclonal antibodies were peroformed in active VL patients and cured subjects. Monocyte-derived macrophages from normal donors were treated with (IFN-γ, IL-17, IL-22, their combination/or medium alone) for mRNA expression analysis. Effects of recombinant human IFNγ and IL-27 on induction of IDO in monocytes derived macrophage were also investigated.

3 Results

We found elevated mRNA expression of indolamine 3-3-di-oxygenase in VL patients, the enzyme that negatively regulates T-cell effector function during infection. No up-regulation of IL-17 or RORγT mRNA expression in spleens cells and plasma IL-17 levels were detected in active VL, however significantly elevated mRNA levels of both subunits of IL-23 were detected in post treated VL splenic biopsies compared to active patients. IFN-γ or/and IL-10 neutralization does not affect IL-17 levels in whole blood cells of active VL and 6 months cured VL. However, plasma IL-17 and IFN-γ levels were elevated in 6 month cured VL which were further enhanced with SLA stimulation in whole blood. Analysis of Th17/Th1 cytokines response in subjects from a cohort of endemic healthy individuals who were protected against VL showed that IL-17 is strongly associated with protection against VL. These findings were further supported by enhance parasite clearance with recombinant IL-17 (rhIL-17) and rhIFN-γ by inducing TNF-α mRNA and down regulating IL-10 in monocyte derived macrophages infected with *L. donovani* amastigote

4 Conclusions

Findings of the study suggests that along with Th1 cytokines, IL-17 play complementary role in human protection, and that a defect in Th17 induction may increase the risk of VL.

(17) Diagnosis of dermal leishmaniasis, limitations and challenges

17.1 EXPLOITING THE HUMAN ANTI-ALPHA-GAL RESPONSE FOR THE DIAGNOSIS OF OLD WORLD CUTANEOUS LEISHMANIASIS

Krishanthi Subramaniam[1], Victoria Austin[1], Waleed Al-Salem[2], Mina Nesri[1], Igor Almeida[3], Katja Michael[3], ***Alvaro Acosta-Serrano[1]***

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Old World cutaneous leishmaniasis (CL) is caused primarily by Leishmania major and L. tropica parasites. The number of CL cases has drastically increased as a result of the current conflicts and refugee crisis in the Middle East. Screening for CL is not straightforward since several other cutaneous diseases can have similar clinical features. Therefore, there is an urgent need to identify biomarkers specific to CL in order to create a more sensitive and affordable screening tool. The surface of Leishmania sp. is covered with immunogenic glycoconjugates that may or not contain immunogenic alpha-galactosyl residues, which are strongly recognized by the human immune system. Previously we found that CL patients from Saudi Arabia, infected with either L. major or L. tropica, develop a strong antibody response to alpha-galactosyl epitopes (W. Al-Salem et al. 2014, Parasitology). However, the specific glycan epitopes recognized by these antibodies are still unknown. In this lecture I will present recent results from our group on the screening of three different cohorts from Saudi Arabia (patients infected with L. major, cured individuals and patients with other dermatological skin disorders that resemble CL) using a panel of synthetic glycoproteins (neoglycoproteins; NGPs). Our data show that patients with active CL have significantly higher IgG titres to specific alpha-galactosyl neoglycoproteins as compared to the other groups. Our results also demonstrate that several alpha-galactosyl NGPs can act as potential biomarkers for CL diagnosis.

17.2 ROLE OF MOLECULAR TOOLS IN CLINICAL MANAGEMENT OF CUTANEOUS LEISHMANIASIS: DIAGNOSIS, SPECIES IDENTIFICATION AND PARASITE QUANTIFICATION

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Full text

Cutaneous Leishmaniasis is spread worldwide and is the most common manifestation of leishmaniasis. The diagnosis is performed by combining clinical and epidemiological features, and by detecting *Leishmania* parasites (or DNA) in tissue specimens or trough parasite isolation in culture medium. Once the parasite is isolated in culture medium, several methods can be applied to identify the etiological agent. Molecular approaches have the great advantage to be applied directly from the clinical sample, avoiding the isolation and cultivation of the parasite and allowing parasite quantification and species identification. Molecular identification of *Leishmania* species, particularly with PCR-based methods, is the most sensitive approach. Therefore, these methods can be used to monitor parasite load during treatment, improving the clinical management of the disease. This is also useful for optimizing treatment due to the association of certain species of *Leishmania* with the severity of the disease and treatment outcome.

Leishmania DNA quantification has been pointed out as a relevant approach for monitoring the response to treatment, although there are controversial results on this matter. It is expected that positive response to treatment is related to a sustained decrease in parasite load as well as correlation between parasite load before treatment and the response, but some studies show that parasite load was lower in poor compared to good responders to antimonials, while others demonstrated that initial parasite load did nor predict therapy outcome. The question then remains as to the validity of the method not only for diagnostic purposes but also for monitoring the parasite load in patients during treatment and follow-up as a way to assess or predict the outcome of therapy. The detection of parasite in asymptomatic carriers indicates qPCR as a tool for epidemiological surveillance, but following-up studies are needed to confirm that.

It is impossible to predict thespecies responsible for CL clinically,and the morphology of both amastigotes and promastigotes it is not a useful parameter as well. The species can be assumed accordingly to the area of infection, but many studies are constantly reporting species that had never been reported in several regions, a result of new epidemiological scenario with spread of some *Leishmania* species, or the use of better methods for species identification. Different methods exist for identification of the infecting *Leishmania* species and different levels of identification are achieved (e.g. *Leishmania* subgenus, species complex, species). Additionally, the statement of species for *Leishmania* parasites is not a trivial task.

However, since PCR-based methods started to be employed for *Leishmania* detection, identification and parasite load determination, several methodologies were tested without consensus concerned to protocols and molecular targets. As a result each laboratory uses its own preferred assay, impairing data comparison.

Lack of consensus protocol is also observed for sample collection. The frequently used methodologies for harvesting samples include scraping or brushing the ulcer, aspirating lesions, and skin biopsy. But, it was already demonstrated that the method of collection would influence the PCR result.

There is an urgent need for conceptual definitions and the establishment of consensus protocols that will result in an appropriated description of the role of molecular tools in clinical management of cutaneous leishmaniasis. To define the best typing method for species identification it is crucial to start by the definition of species for *Leishmania* parasites. Multicenter studies should be conducted aiming to define standardized protocols for molecular diagnoses, parasite quantification and species identification, as well as sample collection.

The standardization of a real time PCR assay for quantification of parasite load from lesion samples of patients with cutaneous leishmaniasis in Americas is being conducted with the support of PAHO/WHO and DNDi (see abstract by Moreira et al), involving different researcher groups from Argentina, Bolivia, Brazil, Colombia, Costa Rica, Mexico, Panama and Peru. In parallel, studies are being conducted concerned to *Leishmania* typing method. The results obtained so far indicate the sequence analysis of Cytochrome oxidase I region as the best typing method, which associated with hsp70 can distinguish among all described *Leishmania* species. Furthermore, efforts are being made concerned to the definition of the most appropriated method for species identification, comparing to other methods, aiming to use in a future multicenter and inter-laboratory study.

17.4 MINIMALLY INVASIVE MICROBIOPSIES: A NOVEL SAMPLING METHOD FOR IDENTIFYING ASYMPTOMATIC, POTENTIALLY INFECTIOUS CARRIERS OF LEISHMANIA DONOVANI

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1 Background

Visceral Leishmaniasis (VL) is a potentially lethal, sand fly-borne disease caused by protozoan parasites of the *Leishmania donovani* species complex. Although there are several adequate methods for diagnosing VL, the majority of infected individuals remain asymptomatic, comprising potential parasite reservoirs for transmission of the disease. The gold-standard for assessing host-infectiousness to biting vector insects is xenodiagnosis (i.e. scoring infection rates among insectary-reared insects that had fed on suspect individuals). However, when it comes to sand flies and leishmaniasis, xenodiagnosis is an intricate operation burdened by logistical hurdles and ethical concerns that prevent its effective application for mass screening of widely-dispersed communities, particularly in rural regions of underdeveloped countries.

2 Methods

Minimally-invasive microbiopsy (MB) devices were designed to penetrate the skin to a depth of ~200μm and absorb blood as well as skin-cell lysates, mimicking the mode by which phlebotomine sand flies acquire blood-meals. MBs were analyzed by *Leishmania*-specific quantitative PCR.

3 Results

MBs taken from 137 of 262 volunteers living in endemic VL foci in Ethiopia, detected *Leishmania* parasites that could potentially be imbibed by feeding sand flies. Although the volume of MBs was tenfold smaller than finger-prick blood samples, *Leishmania* DNA detection rates were significantly higher, implying that skin, more often than blood, was the source of parasites.

4 Conclusions

Minimally invasive, painless MBs should be considered for reliably and efficiently evaluating, both *L. donovani* infection rates among large numbers of asymptomatic carriers and their infectiousness to blood-feeding sand flies.

(18) New vector control measures

18.1 EXPERIMENTAL STRATEGIES FOR INTERCEPTING SAND FLIES TO PREVENT THEM FROM REACHING AND ENTERING HOMES

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1 Background

Cutaneous leishmaniasis (CL) transmitted by *Phlebotomus sergenti* (*Leishmania tropica*) and *Ph. papatasi* (*L. major*) is a fast emerging public health problem in Israel. The current review summarizes studies designed to curtail sand fly biting and CL transmission in and around houses.

2 Methods

Fine mesh, insecticide-laced vertical nets were used to intercept flies attempting to enter inhabited areas. Suction and/or sticky traps were used to assess the flight height and study the behavior of sand flies as they attempt to enter houses through windows. Diffusible insecticides and permethrin-impregnated wall-lining were tested inside rooms.

3 Results

Sand flies tend to fly close to the ground (87% were captured at ground level, 11% at 1m and only 2% at 2m above ground). Encountering vertical obstacles, sand flies proceed upwards (57% were captured at ground level 27% at 1m and 16% at 2m). In one study, a 60m-long, 2m-high insecticide-impregnated net barrier intercepted 60% of the sand flies (*Ph.sergenti*) approaching houses from their natural habitats. In a second study a similar net, 400m long intercepted 85% of the sand flies (*Ph. papatasi)* approaching houses from surrounding fields. Using sticky traps below and around the windows of an experimental house (EH), we demonstrated that sand flies entered windows having landed previously on the wall below or on either side of the window. Shelves with sticky surfaces constructed underneath windows significantly reduced the number of sand flies entering the EH. Electric vaporizers with 1.5% prallethrin were effective in protecting people from sand fly bites (*Ph. papatasi*) inside rooms. Similarly, deltamethrin-impregnated wall lining increased the mortality rates of sand flies (*Ph.sergenti*) by 300% compared with control, non-treated rooms.

4 Conclusions

When they encounter a vertical obstacle, sand flies proceed upwards close to the obstacle with intermittent stops. Therefore, insecticide-sprayed walls or vertical nets should be effective for preventing sand flies from reaching homes. Shelves under windows sprayed with insecticide and insecticide sprayed on walls around windows, should reduce the numbers of sand flies entering rooms. Finally, diffusible insecticides or insecticide-impregnated wall lining should reduce the biting burden in doors.

18.3 NOVEL APPROACHES FOR CONTROL OF PHLEBOTOMUS ORIENTALIS, THE PRINCIPAL VECTOR OF VISCERAL LEISHMANIASIS IN EAST AFRICA

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[6]*KalaCORE Program, Mott MacDonald, UK;*
[7]*Warwick University, UK;*
[8]*DNDI, Geneva, Switzerland;*

Background: The sylvatic nature of Phlebotomus orientalis, the principal vector of visceral leishmaniasis throughout East Africa, pauses a tremendous challenge to control programs attempting to halt the transmission of the disease. As a component of KalaCORE program in E. Africa, the aim of this study is to understand the vector bionomics and evaluate cost-effective measures that can be used to suppress the transmission of the disease. Here, we present and discuss results of an extensive study on the distribution of the vector in relation to VL control. We also discuss how insecticide spraying of outdoor fences, insecticide treatment of animals and use of personal protection measures can impact the man biting rates of the vector.

Methods: The study was conducted in two main study areas; one among resident populations in 6 VL endemic villages in eastern Sudan and the other in agricultural farms in NW Ethiopia where seasonal and migrant populations contract the disease. The vector distribution was investigated using CDC light traps and sticky-paper traps set at fixed positions at indoor, outdoor, peridomestic and sylvatic sites of the villages and different microhabitats of the agricultural farms. A small trial was also conducted in 4 clusters of houses in a village in eastern Sudan to determine impact of deltamethrin spraying of courtyard fences in reducing the vector abundance.

Results: Our results demonstrate that the vector is rarely found at indoor sites and most transmission takes place in the outdoor sites of the courtyard, and the surrounding peridomestic and sylvatic habitats. The data indicates that the fences are already acting as a barrier against the vector resulting in highly significant reduction ($P<0.001$) of its abundance in peri-domestic habitats and the outdoor courtyard sites. Spraying of these fences with deltamethrin insecticide resulted in further reduction of P. orientalis both at outdoor and peri-domestic sites.

Conclusions: Our results show that the traditional methods of residual spraying of indoor sites has no impact on the vector. Development of improved house fences and spraying them with environmentally friendly pyrethroid insecticides may provide a better alternative to indoor IRS. Preliminary results also indicate that currently used wide-mesh LLIN have little impact on the vector and their use for VL control in Gedaref state should be reconsidered.

18.4 INTEGRATED VECTOR MANAGEMENT FOR THE CONTROL OF WILD SAND FLY POPULATIONS ASSOCIATED WITH ANIMAL FACILITIES

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1 Background

Phlebotomine sand flies [Diptera: Psychodidae] are primary vectors of canine and human leishmaniasis. The World Health Organization (WHO) estimates that 310 million people worldwide are at risk of acquiring leishmaniasis, and that annually 1.3 million new cases are recorded. Current measures for sand fly control include mostly indoor interventions, such as residual spraying of dwellings (IRS) to target endophilic sand fly species with very limited number of vector control tools for outdoor interventions against exophilic sand flies. Research is required to develop additional tools to control sand flies in outdoors settings in order to more effectively mitigate the risk of leishmania transmission.

2 Methods

In 2011 a sand fly management research program was initiated in Greece, in areas of increased canine leishmania transmission, in order to investigate the behavior of local sand fly species that are major vectors of leishmania and assess the efficacy of novel sand fly management practices (Ultra Low volume space sprays - ULV, attractive toxic sugar baits – ATSB). Animal facilities were identified as areas with highest sand fly activity and were used as study units for all testing. Flight activity patterns, flight height preferences and resting locations of the sand flies were investigated within study sites prior to any treatments. This information is important in maximizing the effectiveness of chemical control practices, such as ULV and ATSB, by targeting the sand flies during periods and locations of maximum insect activity. Populations of sand flies were monitored pre and post treatments and were compared in parallel with control/untreated populations.

3 Results

Both ULV and ATSB treatments were effective in reducing sand fly populations. ULV treatments provided with a pronounced and rapid decrease of sand fly populations (<95% population reduction), however, very little residual effect was observed. ATSB treatments, on the other hand, provided with good residual efficacy of up to 20 days but were very sensitive to rainfall events.

4 Conclusions

The outcomes of this research program will contribute in the development of practical vector management programs through the integration of effective control practices that can be adjusted to other ecological conditions such as in the Middle East and Southwest Asia in order to assist in control of the much larger problem with *Leishmaniasis* in those regions.

(19) Molecular basis on virulence and tropism

19.1 PROBING THE STRATEGIES OF ANTIMONY RESISTANT LEISHMANIA DONOVANI TO INDUCE AGGRESSIVE INFECTION

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1 Background

Kala azar patients harbouring antimony resistant *Leishmania donovani* (LD) parasites show higher parasite burden and greater IL-10 compared to those harbouring sensitive parasites. Also, there are reports of expression of autophagy proteins in infection. The study aims to understand the molecular mechanism how these two categories of parasites are being 'scrutinized' differently by the host cells to explain clinical disease outcome.

2 Methods

Mouse peritoneal macrophages (MØ), infected with antimony resistant (LD-R) or surface glycan knockdown antimony resistant LD or sensitive LD and the resulting cytokines were assayed. Important interactions were confirmed by the use of knockout mice (TLR2$^{-/-}$, IL-10$^{-/-}$, IL-12$^{-/-}$). MyD88-specific shRNA cassette containing self-inactivating lentiviral vector pCRI.LV was injected into spleen of mice for generating MyD88 knockdown (KD) mice. Autophagy and apoptosis were studied by TEM and by flowcytometry respectively.

3 Results

As compared to the sensitive counterpart, the LD-R displays unique surface glycans, induces autophagy markers in MØ, and gives rise to aggressive infection in experimental models. The glycan of LD-R interacts with TLR2 and increases IL-10/IL-12 in NFkB dependent manner. MyD88 acts downstream of most of the TLR dependent signaling. We now show that infection of MyD88$^{-/-}$MØ with LD-R results in higher intracellular parasite as compared to wild type MØ. Such attribute is not seen with sensitive parasites. LD-R up-regulates miR-466i, which binds to 3'-UTR leading to down regulation of MyD88 post-transcriptionally. We also show that upon infection with LD-R, MyD88$^{-/-}$ mice harbour higher organ parasites as compared to wild type mice. Infected MØ with Sb-R but not with sensitive counterpart show markers of autophagy at the early stage of infection followed by apoptosis.

4 Conclusions

Thus LD-R adopts multiple strategies like increasing the disease promoting cytokines and inducing a switch from autophagy to apoptosis in the infected host cell. Such switch may favour parasites to egress from apoptotic cells to infect adjacent normal cells in the organs. Thus LD-R may exploit a multitude of mechanisms to induce aggressive infection.

19.2 METASTATIC LEISHMANIASIS

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1 Background

Viruses can offer their microbial host incentives of fitness in exchange for the use of their metabolic machinery. In the last few years, our groups have described this notion as a fundamental factor in the severity and dissemination of leishmaniasis including the most severe pathological forms which are often subject to relapses after a chemotherapy. We reported that the dsRNA of the *Leishmania* RNA virus (LRV1), a viral endosymbiont residing inside the *Leishmania* human protozoan parasites, enhances survival of infected macrophages via expression of miR-155 and phosphorylation of the AKT pro-survival kinase. In addition, we demonstrated that LRV1 induces type-1 IFNs which in turn promote inflammatory cytokines and chemokines and IL-17 that is relevant for the dissemination of the infection to secondary sites in the absence of IFN-g. More recently, we investigated the role of other innate pattern recognition receptors capable of reacting to dsRNA or contributing to LRV1-mediated inflammatory pathology. We found no role for RIG-like receptors (RLRs) or inflammasome-dependent Nod-like-receptors (NLRs) in the pathology of *L. guyanensis* infection irrespective of its LRV1-status. The crucial exacerbating role of type-1 interferons suggests that exogenous viral infection could substitute for endogenous LRV1.

2 Methods

To this end, we infected footpads with *L. guyanensis* parasites lacking LRV (*Lgy LRV1-*) together with LCMV, which induces an acute infection and used specific deficient macrophages and knock-out mice to determine the impact of LCMV on *Leishmania* pathology.

3 Results

We demonstrated that LCMV participated in the exacerbation of the infection and could induce metastasis in our mouse model. Interestingly, after healing of the footpad lesions caused by *Lgy LRV1-*, intraperitoneal injection of LCMV reactivated footpad lesion development. This suggested that not only the LRV endosymbiont but also other viruses could influence the outcome of the disease either concomitantly or subsequently. Exacerbation and reactivation of inflammation were dependent on IFN-β since in both cases, IFN type-1 receptor deficient mice (IFN-AR-/-) did not show any exacerbation or reactivation of the infection.

4 Conclusions

Our metastatic model of dissemination of infection to secondary sites and our co-infection approaches are instrumental in defining parameters important in terms of understanding the pathology of inflammatory diseases, co-infections, reactivation of inflammation and relapses.

19.3 THE GENETIC BASIS FOR THE NATURAL ATTENUATION OF LEISHMANIA DONOVANI.

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1 Background

One of the central questions in *Leishmania* research is why some species cause cutaneous infections but others such as *Leishmania donovani* cause fatal visceral disease. Interestingly, *L. donovani* causes both visceral and cutaneous leishmaniasis in Sri Lanka.

2 Methods

L. donovani clinical isolates from Sri Lanka were isolated from a cutaneous leishmaniasis (CL) and from a visceral leishmaniasis (VL) patient and subjected to genome sequencing to identify genetic determinants associated with *Leishmania* tropism. A biological approach was also taken by performing Gain-of-Function (GOF) selection experiments where the attenuated CLstrain was repeatedly passaged through the spleen of mice over several months to selected forstrains with GOF for survival in the spleen. The regions of genetic change in the CL, VL and GOF strain were compared to identify sequences associated with survival in visceral organs. CRISPR- Cas was then used to perform functional genomic analysis to investigate the biological significance of the identified genetic differences.

3 Results

The CL isolate was severely attenuated in visceral infection in experimentally infected mice, while the VL isolate was highly virulent, demonstrating that these isolates were phenotypically different. Genome sequencing and transcriptome analysis revealed that these *L. donovani* strains were different, but were more similar to each other than to the reference *L. donovani* strain from Nepal. There were no gene deletions or pseudogenes specific to the CL isolate. Among the identified genetic differences, the A2 gene family had a higher copy number in the VL than the CL isolate and there were numerous SNPs. Experimentally increasing A2 expression in the CL isolate promoted parasite survival in visceral organs and removal of A2 genes using CRISPR-Cas resulted in a severe attenuation in visceral organs but not growth in culture. Among the SNPs identified, an amino acid change in the active site of the ras like RagC GTPase (R231C) enzyme in the TOR pathway also appears to have contributed to the attenuation of the CL parasite. Further analysis of the GOF strain will be presented.

4 Conclusions

Comparison of these closely related *L. donovani* parasites is an effective way to identify proteins required for visceral disease and also identifies mechanisms associated with attenuation through genetic change. This has far-reaching implications understanding parasite virulence and possibly for the development of attenuated vaccine strains.

(20) CL treatment and pipeline drugs

20.1 CURRENT RECOMMENDATIONS FOR TREATING CUTANEOUS LEISHMANIASIS (CL) WORLDWIDE

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INTRODUCTION: Physicians dealing with cutaneous leishmaniasis (CL) patients have limited treatment alternatives. Accessibility to available drugs can be difficult and may cause a significant economic burden. Treatment regimens are still not standardized and depend on physician's preferences rather than based on valid and multiple evaluations of different alternatives.

METHODS: A literature review was performed to analyse the different recommendations and standards of care for cutaneous leishmaniasis in old and new world CL. A particular emphasis was made on the World Health recommendations that resulted from experts' consensus worldwide.

RESULTS: Pentavalent antimonials, despite their side effects and cost, remained the standard of care of CL worldwide. Other local treatments such as cryotherapy, thermotherapy and paromomycin ointments might be suitable alternatives particularly when antimonials are contra indicated or uncomplicated CL cases. In complex situations, the decision must be discussed on an individual basis. The following treatments were proposed following appropriate regimens and indications. Fluconazole, revealed a variable efficacy when precribed orally against L. major cutaneous leishmaniasis . Oral miltefosine and IV liposomal amphotericin B have been used at tertiary care level. According to the World Health Organization (WHO), drugs used to treat CL should not induce life-threatening complications; and the treatment decision is based on the risk–benefit ratio of the intervention for each specific situation. The treatment strategy depends on the clinical features of CL (size, number, location , evolution of lesions and degree of dissemination) ; the parasite specie and the immunological and health status of the patient (immunocompromised , diabetes, heart, liver or chronic kidney failure). CL case management algorythms and treatment standardized procedures will be presented and discussed.

CONCLUSION: The physician tool box for the treatment of CL is limited. Further clinical research is needed to optimize CL case management. The WHO recommendations are an excellent starting point if applied.

20.4 HARMONISED CLINICAL TRIAL METHODOLOGIES FOR CUTANEOUS LEISHMANIASIS

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1 Background

Today we have few treatment options for cutaneous leishmaniasis (CL) and limited confidence that they work. To correct this, we need not only to step up investments in R&D but also to promote improved standardized methodologies to assess treatment effects. Cochrane systematic reviews of old-world and new-world CL (OWCL and NWCL) yield only weak evidence from clinical studies because of weaknesses in trial design and inconsistent methodologies.

2 Methods

Standardised clinical trial methods were generated via a collaborative agreement-seeking process. The background situation analysis was derived from Cochrane systematic reviews. An expert consultation produced a guidance paper on CL treatment trial methodology (Olliaro et al, PLoS NTD, 2013). This was used as the reference document at two stakeholder's consultations involving OWCL (7 countries, 10 trial sites) and NWCL (7 countries, 11 trial sites) triallists with special focus on core eligibility criteria and outcome measures.

3 Results

To enrol a patient into a CL clinical trial, diagnosis must be parasitologically-confirmed (species identification only for data analysis) and baseline safety tests should be done dependent on risks associated with the treatment (phase of development, route of administration, age of subjects).
As for efficacy assessment, the main efficacy parameter should be re-epithelisation for ulcerated lesions (and absence of induration) and flattening for non-ulcerated lesions. Initial cure should be assessed at Day 90-100 (Day 1 being the first day of treatment) and also at Day 42-45 for OWCL. Final cure should be assessed at Day 180 (6 months after initiating treatment). Failure should be defined at Day 42-63 as < 50% re-epithelisation or flattening; and at Day 90-100 and 180 as <100% re-epithelisation or flattening. The presence and grading of scars should be standardised. Areas of further research to improve the robustness of these criteria were also identified.

4 Conclusions

The agreement reached allows standardisation of future clinical research across OWCL and NWCL sites. Adopting common standards is expected to optimize research outcomes and use of resources.The expertise present between the two sets of clinical sites provides the basis for a powerful consortium with potential for extensive, standardized assessment of interventions for CL.

(21) What´s new on reservoirs

21.2 THE ROLE OF BATS AS RESERVOIRS OF LEISHMANIASIS

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Full text

The relationship between bats and human diseases have been known for years, especially after Carini[1] has observed the presence of rabies *Lyssavirus* in vampire bats. Currently, bats have been incriminated as potential reservoir for many zoonotic infections such as Ebola virus, Hendra and Nipah virus[2], and several trypanosomatid species mainly from the *Trypanosoma* genus, including *Trypanosoma cruzi*, the etiologic agent of Chagas disease[3]. Despite their role as reservoir of zoonotic diseases, bats are generally extremely important from the ecological point of view, with species acting as seed and pollen dispersers[4], and others that regulate insect's populations[5].

Human leishmaniasis is caused by more than 20 species[6] with different infection patterns and clinical diseases and, the role that mammals' species may play as a host of *Leishmania* spp. may differ from place to place and along time periods[8].

Many mammals' species, including wild animals, can be infected by *Leishmania* spp., and some play a role in the maintenance of leishmaniasis acting as reservoirs, such as carnivorous, rodents, primates and marsupials[8,9].

The definition of a reservoir has changed over time and the identification of species naturally infected by *Leishmania* spp., although may activate the hypothesis of being a reservoir, it is not enough to fulfill its participation as a reservoir, that is, a system where one or more mammalian species are responsible for maintaining the parasitein nature[8].

It is quite difficult to objectively incriminate a mammal host as a reservoir, especially for wild species, mainly due to the necessity of demonstrating the parasite's long-term maintenance[10]. Thus, the World Health Organization (2010) has proposed criteria to base the evidences. Here we comment some of these criteria and studies that have contributed to improve our knowledge on the role of bats as potential reservoir of leishmaniasis.

The report made by Lampo et al.[11] showing that the New World sandfly vector *Lutzomyia longipalpis* could feed on bats stimulated researchers to find evidences for implicating them as reservoirs. Bats live in caves where there are ideal conditions of temperature and humidity for sandflies. Additionally, bats usually live in colonies which can provide significant food source for sandflies and close host–sandfly contact necessary to allow the parasite transmission among them[12]. Furthermore, bats live long, are highly abundant and are able to adapt to different environments, including the synanthropic ones[8,13]. For instances, about one fourth of the 180 Brazilian species have already been recorded in urban or suburban areas[14,5].

The majority of the studies have focused on evaluating the presence of *Leishmania* spp. in bats. Nevertheless, detecting *Leishmania* in bats has not always been successful as reported by Rotureau et al.[15], which analyzed 216 bats from forests in French Guiana, where cases of cutaneous leishmaniasis occurred. Millán et al.[12] also tried to amplify *Leishmania* spp. kDNA from blood samples of 35 bats captures in Northern Spain and did not find positivity. Besides the sandflies blood meals preferences, difference in susceptibilities between bats species may exist[12] and have to be taken into account while detecting this parasite in bats.

However, some other researchers have shown the presence of some *Leishmania* species in different places. In the Old World, Mutinga[16] showed the presence of *Leishmania donovani* bodies in bats from Kennya, and Morsy et al.[17] demonstrated the presence of anti-*Leishmania* antibodies in bats from Egypt. Later, Kassahun et al.[18] could detect, for the first time, *Leishmania* DNA from bats in the Ethiopia. They could amplify *L. major* and *L. tropica* DNA, both causative agents of cutaneous leishmaniasis, from spleen samples of two species of bats.

In the New World, De Lima et al.[19] isolated *L. infantum* (syn. *L. chagasi*) from the blood of a *Carollia perspicillata* bat in Venezuela, after inoculation in mice and PCR-RFLP identification.

In Brazil, the presence of *L. amazonensis* and *L. infantum* DNA in bats was first reported by Savani et al.[20] in blood and/or spleen samples of 21 out of 659 bats from non-urban areas surrounding the city of São Paulo, a preserved forest area where rare cases of American cutaneous leishmaniasis and no cases of visceral leishmaniasis had been registered.

Leishmania braziliensis DNA was reported in bats for the first time by Shapiro et al.[21] at Mato Grosso do Sul state, Brazil. In this case, one of the bats presented cutaneous lesions, which brought attention to the researchers that were able to amplify *L. braziliensis* DNA from the skin lesions and from liver sample. Although the criterion that a reservoir must not show symptoms could not be fulfilled in this case, it may reinforce the role of bats as reservoirs because host-parasite relationship may not always be harmonic, as already mentioned by Roque and Jansen[8].

Berzunza-Cruz et al.[13] observed the presence of *Leishmania* (L.) *mexicana* in the skin and liver/spleen of 13 species of bats (frugivorous, insectivorous and nectarivorous) from Mexico. In this study, two experimentally infected *Tadarida braziliensis* showed no evidence of lesions after 30 days and the cultured parasites were viable and infective to BALB/c mice, suggesting the feasibility of transmission although bats competence to infect sandflies was not tested by the authors.

Despite these examples, studies on bats from urban areas had not been published until Oliveira et al.[22] had reported, for the first time, the detection of *Leishmania* spp. kDNA in bats that were captured strictly in urban areas. Prevalence was high (23.9%) and although punctual, this finding may fulfills the criterion that a considerable proportion of individuals have to be infected in the reservoir population[10].

The majority of the bats were positive for *L. amazonensis,* a causative agent of cutaneous leishmaniasis, which is sporadic in the municipalities where bats were captured[22] but had been reported by Tolezano et al.[23] in a dog. In this study, *L. infantum* and *L. amazonensis* were also detected for the first time in the hematophagous bat *Desmodus rotundus*. This bat species was previously investigated for the presence of *Leishmania* DNA by Cunha et al.[24] with no detection in the analyzed samples.

The findings of *Leishmania* spp. in different bats species, with diverse alimentary habits, and the fact that bats, with their colony living habits and wide spreading distribution, even in urban areas, can ensure the maintenance and transmission of the parasites, reinforces their role as potential reservoirs of leishmaniasis in certain ecological niches. Nevertheless, studies including *Leishmania* spp. isolation/identification and xenodiagnosis are still necessary to accumulate more evidences to incriminate bats as reservoir of leishmaniasis.

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21.3 POTENTIAL RESERVOIRS IN MEDITERRANEAN LEISHMANIASIS

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Full text

Dogs are considered the main reservoir of *Leishmania infantum* infection in endemic areas, however the role of other wild vertebrate reservoirs as reservoir of infection has been demonstrated/proposed and could be, in some areas, as a possible cause of the lack of success of control measures. *L. infantum* infection has been reported in many wildlife genera such as carnivores, lagomorphs, and rodents. Even if the red fox *Vulpes vulpes* has received most attention, probably because it is the most abundant wild carnivore in Europe also other wild carnivores often have high prevalences of infection but their infectiveness to the sandfly vector has never been demonstrated. Regarding rodents, xenodiagnosis has demonstrated that black rats, *Rattus rattus*, could infect sandflies and the finding of infected rats have on an island where dogs are not present suggests that rats could act as reservoirs of *L. infantum* infection. Recently, the Iberian hare, *Lepus granatensis*, has been recognized as the origin of a leishmaniosis outbreak in humans in Spain and xenodiagnosis has shown that this species is also able to infect sandflies. Infection has been reported in many lagomorphs population in many southern European countries. Even if the role of wildlife in *L.infantum* epidemiology still shows many unsolved question, it seems to be clear that wildlife role must be better investigated as environmental changes could increase the risk of overlapping of domestic and wild cycle. The comparison of parasite strains circulating among humans, dogs and wildlife populations and xenodiagnosis studies carried out on selected wild vertebrate species could, in the coming future, allow us to better clarify the role of wild animals in the epidemiology of leishmaniosis.

21.4 IS TARIM HARE A RESERVOIR OF DESERT-TYPE VISCERAL LEISHMANIASIS IN TAKLAMAKAN BASIN, XINJIANG, CHINA ?

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1 Background

The desert type zoonotic visceral leishmaniasis (DT-ZVL) has long been reported in the oases surrounding the Taklamakan basin in the northwest Xijiang autonomous region of China. *Phlebotomus wui* is the known vector for transmitting visceral *Leishmania* to cause this disease, but the reservoir animal has not been identified. Work reported here suggests that Tarim hare may serve in that capacity.

2 Methods

Since 2004, wild desert animals, e.g. Tarim hare(*Lepus yarkandensi*), *M. meridianas*, *D. sagitta* and *S. crassicada* have been trapped mainly in the shrub habitats of *diversifolious poplar* andtamarisk from fall to winter for examination of *Leishmania* infection, including seropositivity with rK39 and microscopy of splenic and other tissues. Positive samples were cultured for promastigotes and inoculated into susceptible animals, e.g. *Lagurus lagurus*. *P. wui* vectors were collected during the sand fly season in the summer for laboratory studies, including cultivation of *Leishmania*. Promastigotes grown from patients, *L lagurus* and *P. wui* were studied by gene sequencing to determine their relatedness.

3 Results

A total 1,284 wild animals were collected and examined, representing 16 different species, of which only Tarim hare (47/485) and dog (23/28) were rK39-positive. Positive cultures of *Leishmania* were obtained from 3 seropositive hares (3/44) and 6 infected *P. wui* (6/762), but none from the dogs. *P. wui* was shown to feed only on tarim hare and *Hemiechinus auritus*, the biting rate being 9.5% and 3.3%, respectively.*Leishmania* isolated from patients, *P. wui* and Tarim hare were equally infective to cause splenomegaly in *L. lagurus.* All *Leishmania* from the three sources are identical in the sequence of their *its1, hsp70* and *nagt*, which segregate with reference species of *L donovani/infantum* complex into the same clade by phylogenetic analysis.

4 Conclusions

The data presented suggest that Tarim hare is a potential candidate of animal reservoirs for the DT-ZVL. Work is on-going to verify this proposal by fulfilling the experimental criteria for reservoir identification.

(22) Advances in Immunopathology

22.1 PATHWAYS INVOLVED IN THE OUTCOME OF LEISHMANIA INFANTUM INFECTION

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Background. Visceral leishmaniasis (VL), caused by *Leishmania infantum* or *L. donovani* has been associated with loss of peripheral blood mononuclear cell type 1 (IFN-gamma) responses to antigen and loss of cutaneous DTH responses. We undertook a study of transcript abundance in peripheral blood or splenic leukocytes from dogs, in an endemic region for *L. infantum* infection in northeast Brazil.

Methods: RNA seq was performed in samples isolated from blood and spleen of dogs exposed to *L. infantum*. The reads were filtered and aligned against *Canis familiaris* genome using Bowtie. Differential gene expression was analyzed using the Cufflinks workflow. The genes were considered as differentially expressed when showed a q-values ≤ 0.05.

Results: A marked difference in gene expression profile in both spleen and peripheral blood of dogs with asymptomatic versus symptomatic was observed. Approximately, 2,700 genes were upregulated in the spleens of symptomatic animals, including CTLA4 (q=0.0082; Log FC=2.264), CCR7 (q=0.007; Log FC=2.083) and NLRP3 (q=0.00019; Log FC=1.838). Approximately 2,800 genes were downregulated in the same group of animals, including *SKALP, C1QB* and *C1QC*. The cytoarchitecture of the spleens of symptomatic dogs were drastically altered, with partial loss of white and red pulps margin. Intense inflammatory infiltrates were observed in the liver, gut and skin of these animals. Confirming the RNA-Seq findings, immunofluorescence of tissue samples showed increase in CTLA4, CCR7 and NLRP3 in symptomatic dogs, while SKALP, an elastase inhibitor, was decreased in dogs with VL, but increased in asymptomatic dogs.

Conclusion: The increased expression of NLRP3, IL1B and CCR7 suggests that there is persistent inflammation during VL, which in part could be involved in the pathogenesis of canine leishmaniasis.

Support: National Institutes of Health, CNPq and CAPES

22.3 NEUTROPHIL PHENOTYPES IN HUMAN LEISHMANIASIS

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1 Full text

Leishmaniasis is a group of vector-borne parasitic diseases caused by the obligate intracellular *Leishmania* spp. protozoa. Leishmania are most abundant in macrophages throughout infection, but murine models show neutrophils (PMNs) are the first to internalize the parasite at the site of infection. PMNs are found at infected sites throughout chronic infection of both humans and mice, but their role is undefined. Study of Brazilian patients with cutaneous leishmaniasis (CL) caused by *Leishmania braziliensis* revealed that peripheral blood neutrophils were more prone to become activated as defined decreased surface CD62L and increased surface localization of the granule content marker CD66b, than health control subjects. Observations of total blood neutrophils from both Brazilian subjects with CL and Indian patients with visceral leishmaniasis (VL) caused by *L. donovani* revealed an expanded population of neutrophils that expressed markers of antigen presentation (HLA-DR, CD80, CD86) in peripheral blood. In patients with CL, HLA-DR+ PMNs were highest in neutrophils derived from the site of the cutaneous lesion. This subset was enriched in low-density PMNs, a neutrophil subpopulation recently observed in the blood of VL subjects from Ethiopia. HLA-DR+ PMNs morphologically resembled conventional PMNs, but they exhibited increased activation, degranulation, oxidant generation and phagocytosis of parasites and zymosan particles suggesting they might be primed. There was no evidence they were immature cells, differentiating them from descriptions of granulocytic myeloid derived suppressor cells (G-MDSCs). Normal subjects also contained HLA-DR+ PMNs at low abundance, and HLA-DR could be induced on healthy subject PMNs by incubation in TNF, LPS or plasma from patients with active VL or CL. This suggests there may be a connection between neutrophil "priming" and upregulation of HLA-DR, and possibly relate to mediators in the environment. Importantly, HLA-DR positive neutrophils of VL subjects did not enhance T cell proliferation, but they did express higher PDL1 than other neutrophils, corresponding to increased levels of surface PD1 on lymphocytes of the same subjects. These findings suggest that HLA-DR+ PMNs may not act as antigen presenting cells, but they do expressed markers implicating a potential role in T cell exhaustion.

22.4 LEISHMANIA RNA VIRUS-1 IS EQUALLY DISTRIBUTED AMONG SEVERE AND NON-SEVERE PHENOTYPES IN A PROSPECTIVE COHORT OF AMERICAN TEGUMENTARY LEISHMANIASIS

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1 Background

American tegumentary leishmaniasis (ATL) is a neglected disease of South America where metastatic mucosal lesions (ML) can be seen in up to 5-20% of *Leishmania Viannia* complex infections. The *Leishmania* RNA virus-1 (LRV1) has been implicated as a possible virulent agent in ATL since disseminated disease (DisL) was induced by high LRV1-expressing amastigotes in an animal model, and human cases of DisL have been reportedly infected with LRV1-containing *Leishmania* parasites. None of these studies clearly supported its role in human disease.

2 Methods

Subjects with confirmed ATL were assessed clinically and parasitologically, and followed out to 24-months post-treatment. Lesion biopsy specimens were processed for detection and quantification of *Leishmania (Viannia)* DNA by a quantitative real-time PCR (qPCR) assay targeting kinetoplast DNA (kDNA) minicircles. *Leishmania (Viannia)* species were identified by PCR targeting the *mpi* gene and PCR-RFLP assays targeting *cpb* and *hsp70* genes, and/or Sanger sequencing. LRV1 RNA detection was performed using reverse transcription (RT)-qPCR targeting a conserved viral capsid sequence.

3 Results

Fifty-six enrolled subjects were clinically classified as follows: a) Localized cutaneous leishmaniasis (LCL) (n=25, 44.6%); b) ML (n=28, 50%), and c) DisL (n=3, 5.4%). Species identification by clinical category was: a) LCL: *L. (V.) braziliensis* (52%), *L. (V.) guyanensis* (28%), and *L. (V.) peruviana* (8%); b) ML: *L. (V.) braziliensis* (50%), *L. (V.) peruviana* (11%), and *L. (V.) guyanensis* (4%); and c) DisL: *L. (V.) guyanensis*. LRV1 was detected in 18% of all specimens (n=10). Rate of LRV1 detection by phenotype was 14.3% in ML (4/28), 24% in LCL (6/25) and 0% in DisL. All 8 patients (28.6%) with relapsing ML were infected with strains of *Leishmania* that were LRV1-negative. Parasite load in ML/DisL and LCL was comparable (37.8 vs. 86.4 parasites per 10^6 human cells, p=0.52). There were no clinical or parasitological differences between parasites infected or not infected by LRV1.

(23) Physical methods and natural (phyto) products for CL treatment

23.1 THERMOTHERAPY IN THE TREATMENT OF CUTANEOUS LEISHMANIASIS

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1 Background

Dermal leishmaniasis species were found to be heat sensitive. Using various devices, the application of heat to active leishmaniasis skin lesions has been reported to have good efficacy as a treatment modality. In addition, thermotherapy is well tolerated.

2 Methods

In this review talk, the relevant medical literature is reviewed. The role of thermotherapy for leishmaniasis treatment, as recommended in various clinical practice guidelines, is discussed. Using examples from our clinical trial of thermotherapy compared to intravenous sodium stibogluconate, practical matters of application, adverse events, and time to healing will be addressed.

3 Results

Thermotherapy tends to have reasonable efficacy in the treatment of several species of cutaneous leishmaniasis. In addition, heat treatment is well tolerated with few adverse effects.

4 Conclusions

Physical methods to treat cutaneous leishmaniasis are inexpensive; administered properly they can be effective and well tolerated. Drug resistance is not applicable so they should have wide effectiveness across species; a major scientific question is the role of thermotherapy in clinical situations concerning for metastatic infection (including subcutaneous nodules, mucosal associated species).

23.3 EVALUATION OF THE ANTILEISHMANIAL EFFECTS OF NATURAL PRODUCTS (PLANTS)

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1 Background

Infections caused by protozoa of the genus *Leishmania* are a major worldwide health problem, with high endemicity in developing countries. In the absence of a vaccine, there is an urgent need for effective drugs to replace/supplement those in current use. Treatment of the leishmaniasis is difficult due to a limited number of drugs capable to eliminate the intracellular parasite form. The drugs for conventional treatment require parenteral administration and side effects frequently occur. Natural products are a promising source of bioactive as alternative treatment of leishmaniasis. The present work constitutes the results of experimental tests with plant extracts (*Libidibia*) and fractions of natural origin showing antileishmanial activity.

2 Methods

Fractions of methanolic extract of fruits from L. ferrea were obtained using hexane (Hex), methylene chloride (DCM), ethyl acetate (AcOEt) and butyl alcohol (BuOH). Macrophages J774 infected with L. amazonensis or L. guyanensis were treated with extracts and fractions for 24 hours after parasitic infection and maintained in RPMI 1640 medium for 48 hours. Cytotoxicity with trypan blue was undertaken in macrophages J774. Was evaluated the number of infected macrophages and the number of amastigotes per macrophage. Pentamidine was used as a positive control and DMSO (3% v/v) was negative control. Hamsters (Mesocricetus auratus) were treated with topical application, as a model for pre-clinical studies.

3 Results

The DCM fraction was the most promising among the fractions evaluated in amastigotes with EC50 of 64.26 ± 5.39 µg/mL against *L. amazonensis* and 134.74 ± 9.16 µg/mL against *L. guyanensis.* Fraction AcOEt was second in activity, with an EC50219.53 ± 0.26 in *L. amazonensis.* Reported studies showed increased resistance of *L. guyanensis* with natural and synthetic substances.The DCM fraction showed low cytotoxic activity (493.02 ± 8.34 µg/mL) in uninfected macrophages. The animals lesions reduced with the crems application, mainly with the DCM fraction.

4 Conclusions

These results indicate that DCM fraction is promising for future chemical and pharmacological studies.

23.4 CLINICAL EXPERIENCE USING HERBAL MEDICINE FOR CUTANEOUS LEISHMANIASIS

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1 Background

Treatment of patients with cutaneous leishmaniasis (CL) is quite a difficult task. Although, the gold standard for treatment remains the pentavalent antimonials other treatment modalities such as pentamidine, liposomal amphotericin B, oral miltefosine, various topical and oral antibiotics and antifungals have also been used with variable success. In addition to these treatments, alternative methods, namely physical methods, such as thermotherapy and cryotherapy have also been employed. Despite all the discoveries and advances made in this field, many patients still suffer form long-lasting disease and experience resistance to treatment. In this light, many patients have turned to traditional medicine and natural products to alleviate their symptoms and to seek cure.

2 Methods

Great interest in phytotherapy and the isolation and purification of active compounds in many natural and herbal products has opened a new horizon in evaluating some of these compounds for their anti- leishmania effect. This surge of interest in plant derivatives, with possible anti-leishmania efficacy, has led to extensive research on this subject both *in vitro* as well as in animal models.

3 Results

To date, many new compounds have been isolated, however, clinical trials using these compounds have been very limited and reported mostly form the Old World. The use of the mixture of pure extracts of *Althaea rosa*, *Althaea officinalis* and members of the families Leguminosae, Faliaceae, Malvaceae, and Lythraceae by Zerehsaz et al. (1999), 5% garlic cream by Gholami et al (2000), topical honey application by Nilforoushzadeh et al (2007), the use of *Morinda Citrifolia* (Noni) stem extract by Sattar et al (2012), topical MJ1 (a non-synthetic dairy origin agent) by Janghorbani et al (2015), and *Juniperus excelsa* M. Bieb by Parvizi, Handjani et al, unpublished data (2016) have been documented. One of the major problems with conducting clinical trials with these products is ethical considerations and the inability to use these compounds alone in many circumstances.

4 Conclusions

In conclusion, natural products are good candidates for the ideal cheap, less toxic and easily available treatment modality for CL. However, more well designed clinical trials with larger sample sizes are needed to prove the efficacy of these compounds in CL patients.

(24) WHO control Programmes

24.1 STRUCTURE, ACHIEVEMENTS AND CHALLENGES IN LEISHMANIASIS CONTROL IN THE WHO AFRICAN REGION

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[5]*WHO Switzerland*

1 Background

Leishmaniasis remains a major NTD in this region. About 14% of the global burden is found in 27 countries. Visceral Leishmaniasis (VL) is endemic in 21 countries mainly East African (EA) countries. Cutaneous leishmaniasis (CL) is endemic in 24 countries and 15 countries are endemic for both VL and CL (with Cameroon, CAR and the Gambia requiring confirmation). There is no structured regional programme for leishmaniasis in the WHO African Region. Very little is known about CL in this region. The epidemiology of VL is better known especially in East Africa. There is major overlap in the endemicity of leishmaniasis with other case-management neglected tropical disease (NTDs) in the African region which calls for integrated interventions.

2 Methods

The sources of data are reports from the national control programmes, personal communication to national programme managers and literature review.

3 Results

The WHO Regional Committee adopted in 2013 the resolution AFR/RC63.R6 endorsing the *"Regional NTD Strategy" and the "Regional Strategic Plan for NTDs, 2014–2020"*, as means of implementing the WHO Global NTD Roadmap. Most East African countries established national programme and updated their VL treatment manuals. From 2004-2013 more than 60,000 VL cases (in Ethiopia, Kenya, South Sudan and Uganda) and over 125,000 CL cases (in Algeria) were diagnosed/treated. The rollout of combination therapy is successful in Ethiopia and South Sudan. The VL case fatality rate in the past 3 years is below 4% in Ethiopia, South Sudan and Kenya.
The Regional Office developed an "Integrated strategy for the control of Case Management NTDs". Some countries in the region included leishmaniasis in their multi-year NTD strategic plan. Major challenges include: lack of complete epidemiological information on leishmaniasis, poor performance of rapid diagnostic tests, suboptimal efficacy of antileishmanial medicines, inadequate access to treatment and lack of adequate evidence for appropriate vector control measures.

4 Conclusions

The national leishmaniasis programmes in the WHO African Region need to be strengthened for the implementation of the WHO Road map for the control of the disease (prompt case management and IVM). It requires partnership and coordination with improved national ownership. The new agreement: WHO- GILEAD gives new hope to strengthen the VL control programme in the region.

24.3 LEISHMANIASIS CONTROL PROGRAMME IN THE WHO EUROPEAN REGION

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1 Background

The leishmaniases are neglected and poorly reported diseases with underestimated/undetermined incidence in most countries of the WHO European Region. As per the WHO estimates the regional incidence of leishmaniasis is estimated at less than 2 per cent of the global burden. The two main clinical forms of leishmaniasis, VL and CL, are endemic and geographically widespread, while other clinical types occur more rarely and include localized mucosal and lymph node leishmaniasis.
The sole agent of VL throughout the Region is *Leishmania infantum*, which has domestic dogs as the main reservoir host and several phlebotomine species as competent vectors. By contrast, three entities of CL are endemic being caused, respectively, by *L. tropica*, assumed to be anthroponotic in most of its range; *L. major*, a natural parasite of wild rodents; *L. infantum*, frequently detected as genetically different from the typical VL agent.
The main proven or suspected vectors are thought to be *Ph.alexandi, Ph. pemiciosus, Ph. kandelakii, Ph. balcanicus, Ph. sergenti, Ph.smirnovi, Ph.mongolensis, Ph. papatasi, Ph. halepensis, Ph. syriacus, Ph. longiductus, Ph. perfiliewi, Ph. perniciosus, Ph. ariasi, Ph.tobbi, Ph. arabicus, Ph.transcaucasicus,*and *Ph. neglectus.*

2 Methods

Following the World Health Assembly resolution WHA60.13 on leishmaniasis the WHO Regional office for Europe in close collaboration with WHO Headquartes has strenthened regional leishmaniasis programme.

3 Results

In 2014, the "Strategic framework for leishmaniasis control in the WHO European Region, 2014?2020" outlining the regional goal and objectives to be achieved by 2020, and the recommended strategic approaches and priority interventions was developed to help countries in their efforts to control the disease.
To assess a burden of diseases in countries a series of prevalence surveys and studies have been conducted. Countries in need are receiving also support with medicines and diagnostic tests supplies.
The Regional office is finalizing development of the Manual on case management and surveillance of leishmaniasis which will make recommendations on a standardized approach for case management and epidemiological surveillance the leishmaniases across the WHO European Region

4 Conclusions

Leishmaniasis control in the WHO European Region needs to be strenghened in certain countries. Surveillance in endemic countries and in non-endemic ones where imported cases are usually diagnosed needes to be improved.

24.4 EPIDEMIOLOGICAL ANALYSIS OF DEATHS BY VISCERAL LEISHMANIASIS IN THE AMERICAS: CHALLENGES FOR PUBLIC HEALTH

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1 Background

Visceral Leishmaniasis (VL) is the most severe clinical form of leishmaniasis, as it is fatal, if left untreated. Human VL is present in 12 American countries, though 96% of the cases are reported in Brazil. The objective of this study is to describe and analyze the deaths due to VL in Brazil from 2007-2014, mainly focusing on two specific time-period indicators: Time between the onset of symptoms and notification (StoN), and time between notification and death (NtoD).

2 Methods

The source of the data was the Brazilian National Notification System (Sinan), being available since 2007. The system has individual information on VL cases including date of birth, onset of symptoms, notification and death, as well as some demographic and health information; such as gender, area of residence (urban, peri-urban or rural), ethnic group and HIV status. A total of 1,589 deaths due to VL were gathered from Sinan (2007-2014) and studied by descriptive and statistical analysis. The time periods (StoN and NtoD) were modeled as continuous response variables employing multivariable Gaussian generalized linear models, and the available variables of interest (gender, area of residence, year, ethnic group, age at onset of symptoms and HIV status) were included as predictors.

3 Results

The median time between StoN was 25 days, interquartile range (IR:10-60d), and the median for NtoD was 9 days, (IR:4-17d). Patients aged ≥ 50 yearswere at the onset of the symptoms, the greater was the time period of the StoN (estimated coefficient 0.0007, $p<0.05$) and the NtoD (estimated coefficient 0.00015, $p<0.05$) and cases from urban areas had shorter StoN than those from rural areas (estimated coefficient -18.7, $p<0.05$). Regarding ethnic groups, black patients had longer StoN compared to indigenous patients (estimated coefficient 58.81, $p<0.05$). HIV positive patients had longer NtoD compared to the HIV negatives patients (estimated coefficient 9.87, $p<0.05$).

4 Conclusions

Themedian time between notification and death (NtoD) by VL was 9 days and patients aged ≥ 50 yearswere diagnosed posteriorly. The access to early diagnosis and treatment is important to reduce the mortality of VL. Thus, the availability of the rapid test at the primary health care level and prepared professionals to suspect and diagnose this disease is essential. Likewise, adequate clinical management and interventions including secondary prophylaxis may contribute to the better survival of HIV positive patients

Friday, May 19th

(25) Population genetics and molecular epidemiology

25.3 COMPARATIVE ANALYSIS OF GENETICALLY AND PHENOTYPICALLY VARIABLE LEISHMANIA DONOVANI COMPLEX PUTATIVE HYBRIDS ALLOWS TO EXPLORE THEIR CAPACITY FOR ADAPTIVE EVOLUTION

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Full text

Human and canine visceral leishmaniasis due to *Leishmania infantum* remains a considerable public health problem across the Mediterranean. In Cyprus however, the identification of anthroponotic *Leishmania donovani* MON-37 strains revealed the emergence of *L. donovani* in Europe. Microsatellite typing followed by phylogenetic analysis showed that the Cypriot and Turkish strains from Cukurova region (CUK strains), cluster together in a monophyletic group placed between *L. infantum* MON-1 and *L. donovani* genetic populations. Significantly, despite the fact that these strains cluster in a monophyletic group, they result in different types of human disease, cutaneous or visceral leishmaniasis. We hypothesize that the phenotypic variability of the Cypriot strains is most likely due to parasite-related determinants and their capacity to adapt their genome. One approach to investigate genomic adaptability of *Leishmania* parasites is to assess the stability of their genome in cell culture. To test our hypothesis and to characterize in more detail their tropism and virulence phenotype, we conducted *in vivo* experimental murine infections using minimum-passaged MON-37 strains. Finally, we employed a high-throughput whole genome sequence (WGS) analysis aiming to assess and to compare their potential to genetically adapt in culture, and to identify genetic variants with discriminating potential. Assessment of virulence in an *in vivo* murine model of leishmaniasis using selected MON-37 strains isolated from patients with different clinical manifestations, showed significant differences. Moreover, WGS analysis revealed important differences in the genomic stability of these strains as judged by chromosome number variation during cell culture adaptation. The same analysis revealed differences reflecting gene copy number variation in genes associated with tropism or virulence, including the amastin gene. Overall these findings may explain the phenotypic variability of the Cypriot strains. Together our data, highlight the dynamic epidemiology of leishmaniasis in Cyprus and the high capacity of these human isolates to adapt and evolve *in natura*, *in vitro* and *in vivo*. Yet, much work remains to be done to understand the factors behind the evolution of anthroponotic *L. donovani* MON-37 strains in Cyprus, and to assess the risk of potential spreading elsewhere in Europe.

25.4 SYSTEMATICS AND POPULATION GENETICS OF PHLEBOTOMINE SANDFLIES: CONSEQUENCES ON THE EPIDEMIOLOGY OF THE LEISHMANIASES

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Background:

Phlebotomine sandflies are the heart of the epidemiology of the leishmaniases. Their vectorial competence, vectorial capacity, behaviour, and host preferences are essential to allow transmission and to link Man and reservoirs. Sandflies and *Leishmania* share a long co-evolution, starting more than 100 million years ago. Although parasitic captures occurred during this evolutionary process, there is a strong link between *Leishmania* species and sandflies genera/subgenera. Consequently, the systematics of sandflies is very important to understand the epidemiology of leishmaniases.

Methods

A review of the literature has been carried out focusing on Old World and New World leishmaniases and sandflies. Moreover, studies carried out by us complete this analysis.

Results

It is difficult to define a species in general and several kinds of species may be considered: typological, evolutionary and biological species. Regarding Phlebotomine sandflies, we use a conventional definition of the species, as commonly seen in many groups. During a long time, species boundaries were exclusively defined by morphological studies. Since the 1980's, molecular data have also been used in systematics and, more recently, proteomics. These new approaches are very informative especially for closely related species and species groups. Several studies, in groups of medical importance like the genera *Phlebotomus* (subgenera *Larroussius*, *Paraphlebotomus* or *Adlerius*), *Lutzomyia* s. st., *Nyssomyia* or *Psychodopygus*, have been carried out. DNA sequences are required to explore the systematics of species belonging to the genus *Sergentomyia*, sometimes wrongly suspected to act as a vector of leishmaniases in South Eastern Asia.

Conclusions

It is very important to precisely know the systematics and population genetics of Phlebotomine sandflies as they have consequences on our understanding of the epidemiology of leishmaniases. In a recent past, several South-Eastern Asia sandflies misidentifications led to wrong epidemiological conclusions, as revealed by molecular systematics and population genetics (i.e. *Se. gemmea*).

(26) Advances in Immunotherapy

26.1 HOST DIRECTED THERAPY FOR VISCERAL LEISHMANIASIS.

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Full text

Many infectious diseases are associated with potent inflammatory responses. These are required to control infection, but can also damage tissue and cause disease if not appropriately regulated. IL-10 has emerged as an important immune regulator that protects tissues by dampening inflammation. Under conditions of chronic infection, pro-inflammatory $CD4^+$ T cells initiate IL-10 production as a mechanism of self-regulation. We recently identified IL-10 production by IFNγ-producing, T-bet^+ $CD4^+$ T (Th1) cells as a critical immunoregulatory mechanism during visceral leishmaniasis (VL) caused by *Leishmania donovani*. We found that IL-10-producing Th1 (type 1 regulatory; Tr1) cells potently suppressed anti-parasitic immunity and promoted chronic infection. However, IL-10 produced by these same cells also prevented tissue damage caused by the pro-inflammatory cytokines TNF and IFNγ. We have now generated novel data sets from human and mouse $CD4^+$ T cell subsets isolated during clinical VL that allowed us to identify molecular signatures for Tr1 cells, as well as dysfunctional $CD4^+$ T cells present in sites of chronic infection and active disease. We used this data to identify novel immune checkpoint molecules that may be safely targeted for clinical advantage, including improving vaccine efficacy and drug treatment against infectious diseases.

26.3 CPG ODN FOR IMMUNOTHERAPY IN CUTANEOUS LEISHMANIASIS

Daniela Verthelyi

Daniela Vertelyi Silver Spring USA

1 Full text

Cutaneous Leishmaniasis (CL) is a vector-born chronic infectious disease caused by Leishmania parasites that is characterized by the development of skin lesions that range from single self-healing lesions to multiple debilitating chronic sores. The disease is endemic in more than 98 countries and has an annual incidence of about 0.7 million, mostly children. There are no vaccines and treatments are costly and toxic. Further, treatment failure and relapse rates are particularly high in cases of HIV co-infection. CpG ODN type D are synthetic oligonucleotides (ODNs) containing an unmethylated CpG motif in a self- complementary sequence with a poly-G tail. They specifically activate pDC via Toll like receptor 9 (TLR9). Using macaques challenged with *Leishmania (L.) major* or *L. amazonensis* we showed that local intradermal or systemic (IM or SC) treatment with CpG ODN D35 enables parasite control, reduces lesion size and accelerates healing. Importantly the effect is evident in animals that have established lesions as well as in immunocompromised SIV-infected macaques. To better understand the mechanism of action underlying the protective effect of D35 we examined the effect of one or multiple inoculations of D35 on the local skin, draining lymph nodes and peripheral blood of uninfected and infected macaques. Our data shows that in untreated macaques the early response to *L.major* is muted, with only limited upregulation of genes linked to the IFN pathway and selected chemokines. Animals that received D35 showed an immediate increase in expression of S100A8 and S100A9, and chemokines such as CXCL10 and CXCL8. In addition, there was increased local expression of KLRD1, perforin 1 and granzyme B genes that suggest the activation of NK and CD8 T cells, as well as increased antigen presenting cells embedded at the site of infection. The accelerated resolution of lesions in the treated animals correlated with reduced expression of pro-inflammatory genes in response to the parasite (IL-6, TNFα) over time. The protective effect lasted for 21 days. Together these findings show that D35 induces systemic changes to the immune milieu of the skin and identify potential correlates of protection to support the clinical development of CpG ODN D35 as a therapeutic agent for cutaneous leishmaniasis.

26.4 TARGETS FOR IMMUNOTHERAPY OF LEISHMANIASIS

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1 Full text

Background: *Leishmania (Viannia) panamensis* infection primarily causes cutaneous disease characterized by a distinctive hyper-inflammatory response. Current treatment strategies, mainly chemotherapeutic, are suboptimal due to adverse effects, long treatment regimens, and evidence of drug resistance. To address these concerns, alternative approaches using immunotherapy have been studied and several immunomodulatory agents have shown promise in preclinical studies of leishmaniasis.

Methods and Results: We have developed methods to identify new immunomodulators for *L. (V.) panamensis* immunotherapy using ex vivo approaches (human and mouse) in combination with in vivo murine mechanistic studies. Following the identification of markers and optimization of protocols to isolate metacyclic parasites, we developed an in vivo chronic infection murine model that we used to evaluate the therapeutic efficacy of promising immunomodulators. We identified several immunomodulatory treatments (high dose CpG (a TLR9 ligand), nanoparticles containing low dose CpG, and rIL-2-anti-IL-2) that could be administered locally to successfully heal established cutaneous lesions. Critically, disease resolution was linked to the down-regulation of the hyper-inflammatory immune response. Dependent upon the delivery system and/or therapeutic agent, each immunomodulator initially targeted a specific population (Treg, platelets, B cells and MDSCs), although subsequent downstream interactions between these populations likely occur.

Conclusions: Consequently, it is important to understand both the underlying cell populations that sustain inflammation and how to successfully target these populations (through combinations of immunomodulators and drug delivery systems) in order to develop successful immunotherapeutic approaches going forward.

(27) Quantitative studies of Leishmaniasis transmission and control

27.1 LONGITUDINAL STUDY OF TRANSMISSION IN HOUSEHOLDS WITH VISCERAL LEISHMANIASIS, ASYMPTOMATIC INFECTIONS AND PKDL IN BIHAR, INDIA

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1 Background

Visceral Leishmaniasis (VL) is a neglected tropical disease that afflicts some of the poorest populations in the world including people living in the Bihar state of India. The reservoir for transmission remains to be clearly defined since it is unknown what role post kala-azar dermal leishmaniasis (PKDL) and asymptomatic infections play in transmission. This information is vital to establish effective surveillance and monitoring to sustainably eliminate VL.

2 Methods

A longitudinal study was performed to examine VL transmission and seroconversion in households with VL, PKDL and asymptomatic infections in the Saran and Muzaffarpur districts of Bihar. During the initial screening of 5,144 people in 16 highly endemic villages, 195 cases of recently treated VL, 116 healthy rK39 positive cases and 31 PKDL cases were identified. Family members from the VL, PKDL and asymptomatic cases were followed for 18 months to identify new VL cases and seroconversion to rK39-positive.

3 Results

Approximately half of the rK39-positive healthy cases identified during the initial 6-month screening period were from households (HHs) where a VL case had been identified in the initial screening period. During the 18-month follow-up period, seroconversion of family members in the HHs with VL cases, PKDL cases, and rK39-positive individuals was similar to control HHs. Therefore, seroconversion was highest in HHs closest to the time of VL disease of a household member and there was no evidence of higher transmission in households with PKDL or healthy rK39-positive HHs. Moreover, within the PKDL HHs, (the initial 31 PKDL cases plus an additional 66 PKDL cases), there were no cases of VL identified during the initial screen or the 18-month follow-up. Notably, 23% of the PKDL cases had no prior history of VL suggesting that infection resulting directly in PKDL is more common than previously estimated.

4 Conclusions

These observations argue that acute VL cases represent the major reservoir for transmission in these villages and early identification and treatment of VL cases should remain a priority for VL elimination.

27.2 SPATIAL CLUSTERING OF VL AND ASYMPTOMATIC INFECTION IN BANGLADESH

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Background:
Visceral leishmaniasis (VL) has been targeted for elimination in the Indian sub-continent by 2020. However, better understanding of spatial clustering of VL cases and the contribution of asymptomatically infected individuals to transmission is required to achieve and sustain this goal. Existing models of VL fail to account for spatial variation in transmission despite evidence that infection risk increases with proximity to infected individuals, and as a result may overestimate the contribution of asymptomatic individuals.

Methods:
We have developed an individual-based spatiotemporal model of VL transmission and applied it to a detailed epidemiological dataset from Bangladesh to estimate the rate at which infection risk decreases with distance from infected individuals and the contribution of asymptomatic individuals to transmission. The dataset includes longitudinal information on clinical VL incidence (dates of symptom onset and treatment) and cross-sectional rK39 ELISA and leishmanin skin test results for 2494 individuals in 506 geo-located households in 3 villages in Fulbaria upazila, Mymensingh district, Bangladesh from 1999-2004. Spatial heterogeneity in transmission is described in the model via a spatial kernel that scales infection risk with distance from an infected individual. A statistical inference framework that incorporates both the longitudinal incidence data and cross-sectional diagnostic data is used to estimate the spatial kernel, transmission rate, and unknown infection times of clinical cases and asymptomatic individuals.

Results:
The parameter estimates obtained from the model suggest that infection risk decreases relatively quickly with distance from an infected individual (halving within 100m) and that clinical VL cases contribute significantly more to transmission than asymptomatic individuals. This suggests that active case detection and reactive insecticide spraying strategies, in which households within a certain radius of a new VL case are checked for infection and VL and sprayed with insecticide to kill the sandfly vectors, could be effective in limiting transmission. However, case detection and spraying should be performed in a large radius (200-300m) around a new case to be confident of minimising transmission risk, especially given uncertainty in the estimated range of transmission.

Conclusion:
Our analysis highlights the importance of spatial variation in transmission of VL and therefore in the methods used to control it. It also points to the need for additional data to refine estimates of the transmission range of VL and asymptomatic contribution to transmission in the Indian sub-continent.

27.3 COMMUNITY COVERAGE WITH INSECTICIDE-IMPREGNATED DOG COLLARS PROTECTS AGAINST HUMAN CLINICAL VL IN NW IRAN.

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Background
Operational methods to prevent transmission of zoonotic visceral leishmaniasis are largely ineffective or/and lack the appropriate efficacy studies. Insecticide-impregnated collars fitted to the reservoir – domestic dogs – have proven in numerous studies to successfully reduce canine infection incidence, and, from a single study, to reduce human infection incidence (based on seroconversion). The potential impact of dog collars on reducing human clinical infection (VL) has not been tested. Any effective intervention should be sustainable under operational conditions for it to be adopted by a local VL control program.
Here we present the results of a cluster-randomised trial conducted in NW Iran to test the effectiveness of community-wide distribution of collars to reduce incident VL cases, conducted under operational conditions.

Methods
80 villages (clusters) were pair matched based on pre-intervention incidence. Dogs were fitted with collars over 4 transmission seasons in 40 villages. The 40 matched villages were untreated controls. The study was designed to detect a 55% reduction in the confirmed case incidence of VL over 48 months follow-up. Post trial design, the study was conducted within the regional VL control program activities enabling us to monitor operational logistics.

Results
Community-wide collar distribution reduced the incidence of VL in intervention clusters (1.75/1000/yr) compared to in control clusters (0.95/1000/yr) by 50% (95% CI = 17.8–70.0%). These results were obtained by a 66-100% annual collar coverage of community dogs, and under the regional program conditions. Operational logistics (collar distribution, fitting, and replacement) proved highly efficient ensuring the intervention was in place and maintained throughout the 4 month annual transmission season. Protection was not provided to all clusters, but this was not attributable to levels of intervention coverage, implementation logistics, or dog owner compliance.

Conclusion
The is the first trial to demonstrate that community coverage of the canine reservoir population with insecticide-impregnated collars can provide protection against human visceral leishmaniasis. The results also show that such a strategy is likely sustainable with little additional technical training required within the VL control program.

27.4 ELIMINATION OF VISCERAL LEISHMANIASIS ON THE INDIAN SUBCONTINENT: A COMPARISON OF MULTIPLE TRANSMISSION MODELS

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1 Background

On the Indian subcontinent (ISC), visceral leishmaniasis (VL) is targeted for elimination as a public health problem by 2020. The elimination target is defined as an annual VL incidence of <1 per 10,000 capita at (sub-)district level. Interventions focus on vector control, surveillance and on diagnosing and treating VL cases. It is not yet well understood whether and within which timeframe current interventions may achieve this target.

2 Methods

We present multiple mathematical models of VL transmission on the ISC with structural differences regarding the main reservoir of infection, including those with a prominent role of asymptomatic infection, and fit them to recent case data from 8 endemic districts in Bihar, India. Following a rigorous geographical cross-validation of the models, we compare their predictions for achieving the WHO VL elimination targets with ongoing treatment and vector control strategies.

3 Results

All the transmission models suggest that the WHO elimination target will be met in Bihar, India, before or close to 2020 in sub-districts with a pre-control incidence of 10 VL cases per 10,000 people per year or less, when current interventions (60% coverage of indoor residual spraying (IRS) of insecticide and an average delay of about 40 days from onset of symptoms to treatment) are maintained.

4 Conclusions

Increasing the IRS coverage and to a lesser extent reducing the time from onset of symptoms to treatment will decrease the time to elimination. However, in all cases the models suggest there is likely to be ongoing transmission after 2020 and so control measures will have to be kept in place for several years to achieve the longer-term aim of breaking transmission.

(28) Delivery Systems for drugs and vaccines

28.2 ANTI MICROBIAL PEPTIDE BASED IMMUNOTHERAPY: VARIOUS APPROACHES TO CONTROL LEISHMANIASIS

Sima Rafati

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Full text

Leishmaniasis is a vector-borne infectious disease caused by multiple *Leishmania* (*L.*)species with diverse clinical manifestations. There is currently no vaccine against any form of the disease approved in humans, and chemotherapy is the sole approach for treatment. Unfortunately, treatment options are limited to a small number of drugs, partly due to high cost and significant adverse effects. The other obstacle in leishmaniasis treatment is the potential for drug resistance which has been observed in multiple endemic countries. Immunotherapy maybe another important avenue for controlling leishmaniasis and could help patients control the disease. Antimicrobial peptides (AMPs) also known as host defense peptides (HDP), are short and generally positively charged peptides with capacity to kill microbial pathogens directly or indirectly by modulating the host defense system. In previous studies, we have shown the therapeutic effects of human neutrophil peptide 1 (HNP-1) against resolving infection in human neutrophils. Furthermore, recent findings demonstrate the suitability of IP-10, also known as CXCL10 (C-X-C motif chemokine 10), as a potential chemokine candidate for immunotherapy to cure leishmaniasis. This chemokine plays a protective role against leishmaniasis by increasing the induction of nitric oxide (NO) and decreasing the arginase activity. Several studies have shown that oligodeoxynucleotides containing CpG motifs (CpG ODNs) could stimulate Th1 immune response. The efficacy of CpG ODN has been examined alone or in combination with other interventions for treatment of leishmaniasis.

There are various studies in which different components of vaccine materials, including a specific leishmanial component, live and killed parasites, were used as immunotherapeutic tools. Research on animal models of infectious diseases has recently highlighted the application of non-pathogenic organisms as a live vehicle for delivery of therapeutic molecules as a promising approach. *L. tarentolae*, as a nonpathogenic parasite to human, has been proposed as a promising immunization tool against both cutaneous and visceral leishmaniasis. *L. tarentolae* has favorable features such as easiness of genetic manipulation for introducing heterologous genes, minimal nutrition requirement and possibility of large-scale production.

Similar to previous studies where pathogenic strains of *Leishmania* have been utilized as immunotherapeutic agents, here we present some data regarding the use of *L. tarentolae* for this purpose. The capacity to prepare different recombinant forms of the parasite with different genes, such as anti-microbial genes, cytokines, or chemokines, can create different opportunities for further investigation as immunotherapeutic tools by using live nonpathogenic *L. tarentolae*.

The present study shows that *L. tarentolae* expressing either HNP-1 or IP-10 may represent promising therapeutic approaches to control cutaneous leishmaniasis. These findings may lead to the development of a more effective and tolerable alternative to available drugs in the treatment of leishmaniasis.

(29) MCL and DCL

29.1 HOST DETERMINANTS IN THE OUTCOME OF TREATMENT OF DERMAL LEISHMANIASIS

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1 Background

Elimination of *Leishmania* is the goal of anti-leishmanial treatment. However, clinical manifestations of dermal leishmaniasis are the result of parasite-elicited inflammatory responses which progress to immunopathogenically driven disease. The relationship between parasite burden and pathology is not a linear one; most severe manifestations such as chronic non-healing cutaneous lesions and mucosal disease are characterized by a low parasite burden, suggesting that pathology can be maintained and potentially exacerbated by the inflammatory response independently of the parasite load. The outcome of treatment of human cutaneous leishmaniasis (CL) is multifactorial. However, the determinants of the therapeutic response and their interactions are poorly understood.

2 Methods

We have been intrigued by the tripartite interaction between drug, host and parasite factors in determining the outcome of antileishmanial treatment in CL patients in endemic regions of *L. Viannia* transmission in Colombia.

3 Results

Our findings demonstrate 1) the contribution of drug transport within host macrophages in the antimicrobial effect of first-line miltefosine and antimonial drugs, 2) parasite persistence followingclinical cure of CL is the norm rather than the exception, 3) parasite induced modulation of the chemokine network and genomic variants in immune response genes are determinants of the outcome of antimonial treatment, and 4) fundamental pharmacokinetic differences in children compared to adults could contribute to divergent therapeutic responses.

4 Conclusions

Recent findings from our group together with our programmatic experience based on a translational and integrative research platform will be discussed.

29.2 CLINICAL CHARACTERISTICS AND TREATMENT OPTIONS FOR MCL, DCL AND DL PATIENTS

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Full text

Cutaneous leishmaniasis is the most frequent clinical manifestation of tegumentary leishmaniasis, but several other clinical forms like muco-cutaneous leishmaniasis (MCL), anergic diffuse cutaneous leishmaniasis (DCL), and disseminated leishmaniasis (DL) are of upmost relevance due not only to severe clinical manifestations but also to therapeutic challenge.

MCL is associated with intense mucosal inflammation along with few parasites *in situ* whereas DCL is characterized with multiple infiltrated nodules and plaques with a high number of Leishmania. DCL patients usually do not have ulcerated lesions nor mucosal involvment. DL is defined by the presence of more than10 mixed-type lesions (e.g., acneiform, papular, nodular, and/or ulcerated), located in more than two body parts (head, trunk, arms, and legs). Some patients present with hundreds of lesions all over the body, and also nasal mucosal involvment about 40% of cases. There are few parasites in the lesions.

MCL and DL are hard to treat diseases with a high rate of therapeutic failure to pentavalent antimony (up to 76% in DL). A complete cure for DCL is unlikely.Several drugs like miltefosine and liposomal amphotericin B, as well as associated immunotherapy with pentoxyfylline, have been used to improve the cure rates in these severe forms of leishmaniasis. The development of strategies to combine immunotherapy with anti-leishmania drugs or to multidrug therapy interventions are urgent needed.

29.3 THE NATURAL HISTORY OF HUMAN LEISHMANIA VIANNIA INFECTION: METASTASIS AND PERSISTENT INFECTION

Nancy Saravia

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1 Background

Prospective studies in patients and communities in endemic settings have revealed a broad spectrum of outcomes of infection by species of the Viannia subgenus. These outcomes portray the intriguing natural history of infection by these species, including the substantial "invisible" prevalence of asymptomatic infections. Leishmania have been isolated from lymph nodes and peripheral blood of patients with dermal leishmaniasis, having disseminated from the lesion site via lymphatic and hematogenous circulation. Although "metastasis" has been associated with mucosal leishmaniasis, the subclinical presence of Leishmania Viannia in mucosal tissues, peripheral blood and healthy skin, has been shown to be frequent during active cutaneous leishmaniasis.

2 Methods

Guided by findings from prospective epidemiological and clinical investigations of dermal leishmaniasis, molecular amplification of Leishmania DNA and RNA targets and host cell gene expression were used to examine the natural history of infection and disease caused by Leishmania Viannia species in the human host.

3 Results

We have demonstrated the presence and persistence of Leishmania post-healing (after treatment or self-resolution of disease) in peripheral blood and mucosal tissues in a high proportion of skin test positive and negative, asymptomatically or subclinically infected inhabitants of areas of transmission of Leishmania Viannia species. Persistence of infection was detected as long as 55 years after disease resolution. Previous findings of higher incidence of new cases of cutaneous leishmaniasis in skin test positive residents of endemic areas than skin test negative residents supports the potential of asymptomatic infection to contribute to future disease burden.

4 Conclusions

The implementation of molecular genetic technologies and innovative approaches have allowed greater access to and discernment of host and parasite contributions to the symptomatic or asymptomatic outcome, dissemination and persistence of infection by Leishmania Viannia species.

29.4 IMMUNOLOGICAL MECHANISMS OF MCL AND DCL

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1 Background

Cutaneous leishmaniasis presents several forms that cover a clinical, histological and immunological spectrum with different degrees of severity. At one extreme, we observed resistant individuals with localized cutaneous leishmaniasis (LCL) and at the other end susceptible individuals with diffuse cutaneous leishmaniasis (DCL) characterized by multiple lesions, which are disfiguring and difficult to treat. In the center of the spectrum we observed clinical manifestations that affect the mucous membranes or generate aberrant chronic forms, called mucocutaneous leishmaniasis (MCL) and intermediate cutaneous leishmaniasis intermedia (ICL), respectively

2 Methods

The Laboratory has studied the participation of acquired immunity using different technical approaches in cutaneous leishmaniasis lesions demonstrating that the tissue damage observed in cutaneous leishmaniasis is caused by the immune response itself.

3 Results

Our results indicate that CD1a + Langerhans cells are decreased in ICL and DCL when compared to the epidermis of patients with LCL. We have also identified other dendritic cell subtypes: IgE + / FcγRI + inflammatory dendritic cells, activated CD83 + dendritic cells, IL-10 + regulatory dendritic cells and iNOS + dendritic cells. Few CD83 + dendritic cells were observed in the epidermis of patients with ICL and DCL.
The percentage of IL-10 + epidermal dendritic cells in ICL (33.69) calculated from the total population of CD1a + dendritic cells was higher than that observed in LCL (17,45). Few or no dendritic cells IL-12+ or IFN-?+ were identified in the epidermis of LCL and ICL. INOS expression was observed in very few epidermal dendritic cells.
We carry out a study to correlate the activation of myeloid dendritic cells (mDC) and plamocytoid DCs (pDC) with the expression of T cell transcription factors associated with distinct effector T cell functions in peripheral blood of patients with mucocutaneous leishmaniasis (MCL) using flow cytometry.A multiple variance analyses showed that mDCs CD83+ in MCL are associated with ROR-gt, FOXP-3 and GATA-3, whereas pDCs CD86+ is associated with T-bet. These results suggest a higher activation of mDCs than pDCs in MCL patients, which is associated with a Th1/T-bet response rich in IFN- g, whereas, activation of pDCs is mainly associated with Th17- ROR-gt.

4 Conclusions

Our study identified clear immunological features that may explain the course of immunopathology and may open to alternative therapeutic procedure.

(30) Leishmaniasis vaccine pipeline

30.1 KILLED VACCINES

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Full text

Cutaneous leishmaniasis (CL) has been well known for centuries in Latin America and some parts of Asia. As an example AbuBakr Muhammad ibn Zakariya al-Razi (854 CE – 925 CE), has explained the vector born nature of CL lesion. Recovery of CL lesion accompanies with protection against further lesion development. Development of Leishmania vaccine was initiated by using several species of Leishmania. In Brazil, first 5-strain of killed Leishmania braziliensis and mexicana complexes,and later a single strain of L.amazonensis and in Venezuela L. mexicana were tested for prophylaxis and therapy. In Ecuador first a trivalent vaccine and later along with Colombia a single strain of killed L.amazonensis was tested for prophylaxis. In old world single and multiple doses of killed L. major were tested in Iran against zoonotic and anthroponotic CL and in Sudan against visceral leishmaniasis. The results of phase 3 trials of different preparations of killed Leishmania vaccines alone or mixed with mainly BCG (as an adjuvant) which were tested in thousands of volunteers results showed that the vaccines are safe and induced immune responses (shown by in vitro and in vivo). The efficacy rate in different trials varies between -14 to 72%.

Leishmanization (LZ) is an inoculation of L. major at a predetermined site of the body which usually produces a self-healing lesion and upon healing the individual is protected against further CL lesion development. Historically and still LZ is the most effective control measure against CL. In 1980s more than 2 million soldiers and civilians were leishmanized and showed to be highly effective. Challenges and reasons for killed vaccine failure are partly as follow; lack of an appropriate adjuvant, evaluation of killed vaccines in the field is not precise due to nature of the disease. The only way to evaluate Leishmania vaccines is to use live challenge. Global attentions need to solve the problems of live challenge.

30.2 VACCINES THAT PROMOTE CD8+ T CELL RESPONSES

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Full text

Cytokine production by CD4+ T cells plays a significant role in determining the outcome of natural infection with *Leishmania*. Whereas Th1-asssociated cytokine production (notably IFNγ) is associated with protection, Th2 and regulatory cytokines are associated with parasite persistence. Not surprisingly, therefore, the induction of CD4+ Th1 T cell responses has been the focus of first (killed whole organisms) and second (recombinant protein) generation vaccine development. However, a strong literature supports an additional role for CD8+ T cells in host protection, acting either as an additional source of Th1-associated macrophage activating cytokines or through their cytolytic potential. The induction of CD8+ T cell responses by vaccination requires delivery of antigen into MHCI antigen presentation pathways and is most effectively accomplished by "third generation" DNA or viral-vectored vaccines. In this presentation, I will summarise progress in the development of third generation vaccines for leishmaniasis, with an emphasis on the clinical development of ChAd63-KH. ChAd63-KH is a replication defective simian adenovirus expressing a novel synthetic gene (KH) encoding two *Leishmania* proteins KMP-11 and HASPB, the latter engineered to reflect repeat sequence diversity found in clinical isolates of *L. donovani*. Results will be presented from a dose escalation first-in-human Phase I trial in healthy adult volunteers that has shown ChAd63-KH to be safe and immunogenic (ISRCTN 07766359). CD8+ T cells induced by a single intramuscular immunisation of either $1x10^{10}$ or $7.5x10^{10}$ viral particles recognised peptides derived from both KMP-11 and HASPB, with a responder frequency of 85% (by IFNγ ELISpot) and 100% (by intracellular flow cytometry for IFNγ, TNF and / or IL-2). ChAd63-KH is currently being progressed as a therapeutic vaccine for PKDL, with a Phase IIa dose escalation, age de-escalation trial currently underway in Sudan (ISRCTN 11285604). Early safety data from this trial will be presented, and the rationale for taking a therapeutic rather than prophylactic vaccine development path will be discussed.

30.3 FROM MOUSE TO MAN: DEVELOPMENT OF A CANDIDATE VISCERAL LEISHMANIASIS VACCINE

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1 Background

Key antigens of Leishmania species identified in the context of host responses in *Leishmania*-exposed individuals were prioritized for the development of a subunit vaccine against visceral leishmaniasis (VL). Two Leishmania proteins – nucleoside hydrolase (NH) and a sterol 24-c-methyltransferase (SMT), each of which are protective in animal models of VL when properly adjuvanted – were produced as a single recombinant fusion protein NS (LEISH-F3) for ease of antigen production and broad coverage of a heterogeneous MHC population. When formulated with GLA-SE, a TLR4 T_H1 promoting adjuvant, the LEISH-F3 polyprotein induced potent protection against both *L. donovani* and *L. infantum* in mice. A robust immune response to each component of the vaccine with polyfunctional CD4 T_H1 cell responses characterized by production of antigen-specific IFN-g, TNF, and IL-2, and low levels of IL-5 and IL-10 was induced in immunized mice. Based on the sum of pre-clinical data, we prepared GMP materials and performed a Phase 1 clinical study with LEISH-F3 + GLA-SE in healthy, uninfected adults in the United States. The vaccine candidate was shown to be safe and induced a strong antigen-specific immune response, as evidenced by cytokine and immunoglobulin subclass data. These data provide a strong rationale for additional trials in *Leishmania*-endemic countries.

(31) Bioimaging

31.1 A DRUG DISCOVERY PLATFORM AGAINST VISCERAL LEISHMANIASIS, MOVING FORWARD IN VIVO PRECLINICAL MODELS

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Full text

The massive screening of compound libraries by high throughput techniques (HTS) is a powerful methodology to identify new molecules against parasite-borne diseases. Current target-based (biochemical) and non-target (phenotypic) approaches are valid *in vitro* methods to test thousands of compounds but often they are based on hard-to-conciliate paradigms. On the one hand, target-based screenings require a consolidated druggable protein involved in essential biochemical pathways to perform *in silico* studies of the compounds that best interact with the target. Nevertheless, it often occurs that the best-designed compounds are poorly transported, demolished by the xenobiotic-metabolizing enzymes, or have more than one non-identified target. On the other hand, phenotypic screenings are cell-based assays that allow us to assess the effect of compounds on killing or preventing the proliferation of the pathogen, but they provide no evidence about its mechanism of action. Furthermore, it is mandatory that the parasite form responsible of clinical symptoms in the host is culturable. The use of *ex vivo* explants obtained from organs dissected from experimentally infected rodents is a promising method of drug discovery for visceral leishmaniasis. This approach involves real amastigotes infecting spleen macrophages surrounded of the full repertoire of immune cells to test a battery of compounds. This methodology requires of genetically modified parasites to confer a rapid optical readout. Validated examples of this new technology are the hamster-derived spleen explants used to screen a library of several thousands of drugs using a firefly luciferase transfected strain of *L. donovani* or the murine splenic explants from BALB/c mice infected with iRFP-*L. infantum* strain that emits infrared fluorescence. Remarkably, by using this same tool, it has been possible to study parasite dissemination in an *in vivo* chronic model of visceral leishmaniasis based on infrared fluorescence signal. In this communication, we show our results with an iRFP-*L. infantum* amastigote-infecting splenocytes platform ready to perform phenotypic screenings of small molecules and to monitor the progress of *in vivo* visceral leishmaniasis at pre clinical levels.

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31.2 IMAGING TO BETTER UNDERSTAND HOST-PARASITE INTERACTIONS

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1 Full text

Two-photon imaging has become a widely used and powerful tool for the study of the cellular interactions that occur *in vivo* in real-time. We have used this technique to dissect the hepatic immune response to *Leishmania donovani* infection, with a focus on understanding the cellular interactions that initiate, propagate and then control infection in the liver through the development of granulomata. We began by imaging the real-time *in vivo* phagocytosis of *L. donovani* amastigotes by Kupffer cells, the resident macrophages of the liver, and observed rapid uptake, resulting in global Kupffer cell activation. Infiltration of monocytes, and subsequently B cells and T cells matures the reaction resulting in the control of growth and eventually the elimination of the parasites from the liver. We have utilized real-time *in vivo* imaging with a range of transgenic reporter mice to dissect the dynamics of granuloma formation and parasite clearance at a previously unobserved level.

(32) Drug resistance

32.1 TREATMENT FAILURE IS BEYOND DRUG RESISTANCE

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FACTORS DETERMINING TREATMENT OUTCOME

Once the patient has been diagnosed of leishmaniasis, the physician has to decide which the optimal treatment is. The decision is sometimes difficult and depends on several factors, such as the clinical form of leishmaniasis, the geographical area of acquisition, the species of Leishmania involved, the clinical condition of the patient, the availability and affordability of different treatments, health facilities available, etc.

Why do certain patients with the same clinical form of leishmaniasis, coming from the same geographical area, infected by the same Leishmania species and treated with the same drug regimen do not respond to treatment, whereas majority of patients respond to the same medical treatment? Cure of leishmaniasis goes beyond the drug used and other factors play an essential role in clinical cure. -Host factors: as natural resistance and ability to create an effective immune response against the parasite (an example is the lack of cure in patients with cellular immunodeficiency). -Parasite factors: such as the intrinsic virulence of the infecting Leishmania strain, or strains infected by the LRV1 virus, or presence of genetic mutations (primary or acquired) that make the parasite able to survive in the presence of a certain drug. -Drug factors: as the total dose administered to the patient or pharmacokinetic properties of the drug (different in children or in adults), or the adequate concentration of the drug or of its metabolites within the macrophages, or the administered drug quality…

ASSESSMENT OF CURE/FAILURE AFTER TREATMENT.

"To be cured or not to be cured", that is the main issue in clinical practice. The patient must be evaluated after the treatment in order to determine the outcome. Should this evaluation be only clinical or should it also be parasitological? While clinical evaluation is mandatory and easy to perform, parasitic evaluation (TOC) includes aggressive and costly tests (bone marrow biopsy, splenic aspirate, parasite culture, PCR techniques ...).

As relapses usually occur within the first 6-12 months after treatment, sometimes even later, it seems wise to follow the patient at least one year before considering it as cured.

For cutaneous leishmaniasis, the size of the inner and outer borders of lesions, reepithelialization, crustation are used as clinical indicators. Final cure is defined as the absence of all-inflammatory signs and complete scarring or reepithelialization of ulcerative lesions at the 3-month follow-up.

For visceral leishmaniasis normalization of temperature, disappearance of symptoms, decrease in liver and spleen size, rise in peripheral blood leukocyte, hemoglobin, and platelet values, and increased appetite and weight suggest a good clinical response. Failure and relapse are respectively defined as the persistence or return of clinical signs/symptoms in concert with parasitological confirmation

Parasitological cure is defined as the absence of *Leishmania* parasites by microscopy or culture in tissue samples (skin, spleen, bone marrow or lymph nodes). Detection of parasite DNA by PCR in tissues is substantially more sensitive than conventional parasitological techniques, but it may give false positive results when performed too early because of the persistence of non-viable *Leishmania* parasites. Quantitative PCR in peripheral blood can be useful for measuring the initial parasitic load and for monitoring responses to treatment (but these tests are not

standardized and are not widely available for clinical use). Biomarkers, such as cytokines, receptors, acute-phase proteins, and other biomarkers are under evaluation. Serological tests are not useful for follow-up.

In investigational drugs trials TOC have been usually performed one month after the last dose of treatment, which may be too early as residual parasites can be demonstrated in those patients who had a very high parasite load at diagnosis (this may be the case of HIV/AIDS patients). In clinical practice TOC is generally not recommended in patients showing a timely clinical response.

RELATIVE IMPORTANCE OF PARASITE RESISTANCE IN CLINICAL PRACTICE

The correlation between parasite genotype and clinical phenotype is difficult to establish -There are cases of therapeutic failure produced by susceptible strains (typically in immunocompromised patients). -There are cases of therapeutic failure with a given drug that respond to a second cycle of the same drug at the same or at higher doses. -There are cases of failure with the same drug in children and that respond in adults. -There are cases produced by the same species of *Leishmania* that respond to one drug in one geographic area and fail in others. -There are similar clinical cases that respond in some and fail in other patients when the causative Leishmania species are different. -There are cases of resistant strains documented in vitro that respond in vivo to the same drug.

As conclusion, the relative importance of parasite resistance as measured with the techniques we have today is still unclear in clinical practice. Although correlation between in vitro resistance and in vivo response to treatment is well established in certain circumstances, there are other important factors responsible for response to treatment. Sensitivity/resistance studies are experimental and not available in the vast majority of medical clinics/centers where this disease is treated and only requested in particular relapsing cases. Information on drug sensitivity is essential when designing therapeutic guidelines in a given geographical area.

32.2 RESISTANCE TO DRUG COMBINATION THERAPY IN LEISHMANIA

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Full text

Drug combination therapy (combo therapy) has been proposed as a promising therapeutic strategy to overcome drug resistance, improve efficacy of monotherapy regimens and preserve the current drugs against Leishmaniasis. However, the efficacy of combo therapy must be stablished for each *Leishmania* species and must be implemented for all clinical manifestations of leishmaniasis in the different endemic areas. Considering the plasticity of *Leishmania* genome that confers an ability to this protozoan parasite to acquire resistance to single monotherapy, we have explored whether *Leishmania* could develop experimental resistance to drug combinations.

We described the *Leishmania* ability to develop resistance to different drug combinations using promastigotes/ intracellular amastigotes and what combo therapy is more easy and/or difficult to develop resistance. These results are of considerable interest for future prediction of the success of combo therapy. We have studied drug resistance mechanisms in *Leishmania* lines resistant to different drug combinations, including metabolomics analyses, phenotypic adaptations and fitness of combo resistant parasites. Our results clearly suggest the difficulty of *Leishmania* to induce resistance to combo therapy versus monotherapy, and have important clinical relevance for the possible use of some combo therapy and their impact on leishmaniasis control programme.

This work was supported by the Spanish Grant Proyecto de Excelencia, Junta de Andalucía Ref. CTS-7282 (to F.G.), and the Spanish Grants SAF2015-68042-R (to S.C. and F.G.) and SAF2012-34267 (to F.G.).

32.3 MOLECULAR EPIDEMIOLOGY OF LEISHMANIA DONOVANI: THE MANY ROADS TO DRUG RESISTANCE

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1 Background

Drug resistance (DR) represents a challenge for the control of any micro-organism and from the moment any drug arrives in clinical practice, its days are counted, among others because of the adaptive skills of the pathogen. Obviously, DR is not the only actor involved in treatment failure (TF), and factors related to the host or the drug may also play a role. The complex relationship between DR and TF is best illustrated in the case of *L. donovani* in the Indian sub-continent (ISC). After decades of intensive use, antimonials (SSG) were lost because of DR; efficacy of the following drug, Miltefosine (MIL) started to decrease, but this was not (yet) associated with DR.

2 Methods

In this mini-review, I will explain how high-throughput genomic analyses of 204 ISC isolates brought some light on the evolutionary history of *L. donovani* in the region and the emergence and spreading of SSG-resistance (SSG-R). This will be completed with recent experimental work done with fresh clinical isolates. Samples from MIL-TF will be mapped in this phylogenomic context.

3 Results

The main population of *L. donovani* in the ISC likely emerged around 1850 and spread over the Gangetic plain. During this period, the parasite population underwent several bottlenecks, the last one in the 60's, concomitant with the anti-malaria DDT campaign. All strains from that main population show an intra-chromosomal amplification of the H-locus, involving the MRPA gene and associated with a natural lower susceptibility to Sb^{III}. It is unknown which selective pressure drove the emergence of this ancestral character, but it represents a pre-adaptation on which SSG-R has further built, through additional adaptations, like the inactivation of AQP1, the transporter of Sb^{III}. Sexual recombination may also have contributed to the spreading of SSG-R. The molecular adaptations present in ISC parasites did not have an impact so far on the efficacy of MIL, but experimental studies show that they could impact some new lead compounds.

4 Conclusions

Our findings provide a theoretical frame for Health authorities of the region and elsewhere ; they demonstrate the importance of a close monitoring of the parasites, especially in a post-elimination phase. Our results should also be integrated in the R&D for new drugs.

32.4 THE MOLECULAR BASIS OF AMPHOTERICIN B RESISTANCE IN LEISHMANIA.

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Full text

Amphotericin B is used increasingly in the treatment of Leishmaniasis. The drug's specificity is attributed to its binding to ergosterol, and related sterols which are the primary sterol components of the Leishmania membrane. By contrast, mammalian cells use cholesterol as their primary sterol and are hence less sensitive. With increased use of amphotericin B comes increased risk of selection of resistance to this drug. Several reports of treatment failure in different clinical settings have appeared in recent years and a number of studies have shown that resistance can be selected in the laboratory. We have selected several lines of Leishmania mexicana for resistance to amphotericin B. A combination of whole genome sequencing and metabolomics analysis have been used to pinpoint the changes that associate with resistance. In four separate lines we find changes to sterol metabolism, with ergosterol replaced by different intermediates in its biosynthetic pathway. These are accompanied by changes to different genes encoding enzymes of the ergosterol biosynthetic pathway. Only in some cases, however, could resistant parasites transform to macrophage infectious amastigote forms. Overall it appears that resistance to amphotericin B can be selected through a variety of mutations that alter the sterol composition of the Leishmania membrane, and that some mutations are compatible with generation of mammal-infective amastigotes, highlighting the need for caution in assessing the risk of resistance appearing and spreading in the field to this drug.

(33) Canine leishmaniosis: where do we stand

33.1 THE UTILITY OF RAPID DIAGNOSTIC TESTS IN THE CLINIC AND IN THE FIELD

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Full text

The accurate diagnosis of oligosymptomatic or asymptomatic dogs infected with *Leishmania infantum* is a major challenge. Identifying true oligosymptomatic animals is complicated by the poor nutritional condition and co-infection with different pathogens (e.g. *Ehrlichia, Babesia, Dirofilaria*, intestinal parasites) frequently found in dogs from endemic areas. It is now clear that serology requires complementation with more sensitive molecular diagnostic methods that accurately estimate canine infection at the individual or population level. Conventional and real time polymerase amplification methods (PCR) have high sensitivity and specificity ranging between 89-100% and 95-100%, respectively. The high copy number of *Leishmania* kinetoplast (kDNA) minicircles (>10,000) has made this molecular target the basis for most molecular tests. Novel molecular diagnostic methods are being developed for further point of care (POC) implementation. These methods are based on polymerases capable of amplifying DNA at constant temperatures and therefore not requiring sophisticated equipment or high level of expertise. Loop-mediated amplification (LAMP) uses a DNA polymerase with strand-displacement activity and four primers which recognize six different DNA regions, making it highly specific. Previous work by others showed that qualitative LAMP targeting *L. infantum* kDNA has similar sensitivity to conventional PCR when using conjunctival swabs (61% vs. 58.6%, respectively). The results were read with the naked eye by the inclusion of a fluorescent dye. Although in that study LAMP showed limited capacity to amplify *L. infantum* from countries other than China, the method could be optimized by designing additional primers and probe sets. We recently developed a method to identify infected dogs which is based on recombinase polymerase amplification (RPA) and complemented by lateral flow reading (LF) with the naked eye. The RPA mixture contains three proteins: recombinase, single-strand DNA binding protein and strand-displacing polymerase. The RPA-LF utilizes primers and probe that target *L. infantum* kDNA minicircles. It has an analytical sensitivity similar to qPCR (SYBR®green) and is capable of detecting 40 parasites per mL of blood. The evaluation of blood samples preserved in FTA Whatman filter paper from a small group of symptomatic dogs (n=6) showed 100% correlation with lymph node infections. RPA-LF also detected *L. infantum* in the oral mucosa of symptomatic dogs. Screening of 30 clinically normal dogs from an endemic area in Argentina showed that 50% of the animals were subclinically infected as determined by RPA-LF compared with 13.3% that were serologically positive using rK39 test. As opposed to the qualitative tests, quantitative LAMP or RPA require relatively costly equipment and trained personnel unattainable in endemic areas. Therefore, quantitative tests (together with serological and clinical evaluations) should be restricted to assessing individual dog response to treatment and long-term follow up in veterinary clinics. The validation of non-invasive sampling such as skin and mucosal tissues still requires larger filed trials involving asymptomatic dogs. The next step to implement POC molecular tests in resource-limited environments is the optimization of simple, inexpensive and efficient DNA purification methods. This would allow identifying and managing infected dogs at early stages of infection improving the life expectancy and decreasing the epidemiological risk they represent.

33.2 THE IMPACT OF TREATMENT ON DOG INFECTIOUSNESS.

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Full text

Treatment of canine leishmaniosis (CanL) has been limited during decades almost exclusively to the use of antimonials that have demonstrated a good efficacy producing in many cases a "clinical cure" but without a clearance of the infection. Later on, other leishmanicides drugs have been developed, mainly from the group of the alquilphospholipids (miltefosine), and other many drugs already used in veterinary medicine but with certain efficacy in the treatment of the CanL. Currently, the combination of these leishmanicides with allopurinol continues being the recommended treatment.

Nevertheless is always questionable if the drugs used in the treatment of CanL are able to "inhibit" or reduce, at least for a time, the infectiousness of treated dogs. This would be a breakthrough in this zoonosis control. However, the only way to assess this statement is using the technique of direct xenodiagnoses. This procedure is only available in specialised researching centres, which manage experimental colonies of sandflies. The procedure consists in exposing treated dogs (naturally parasitized with *Leishmania infantum/L. chagasi*) to sand flies biting during a set time (1 hour approx.), in order to demonstrate the presence or absence of *Leishmania* infection in the dissected fed female sandflies after few days in the laboratory.

The extreme difficulty and complexity of the process makes a lack of reports, so very few studies have been conducted over the last 25 years. Of these, only the three more recent were conducted on a sufficiently large sample size for valid conclusions to be drawn.

The most relevant is that all show that, after treatment (antimonials alone or in combination with allopurinol, and liposomal formulations), infectiousness is reduced or cancelled during a period of not less than 4 months.

We will review the different reports available and the application of this interesting tool in order to diminishing the epidemiological risks of sick dogs both for human beings and of other healthy dogs.

All the treatment regimens tested significantly reduced the infectivity of dogs towards sand flies, thus Despite not being a simple method, the use of direct xenodiagnostic approach emerged as a useful tool to assess the infection capacity of dogs treated with new drugs and/or new treatment regimens. Despite not being a simple method, the use of direct xenodiagnostic approach emerged as a useful tool to assess the infection capacity of dogs treated with new drugs and/or new treatment regimens.

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33.3 ARE PREVENTION AND CONTROL STRATEGIES WORKING?

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Full text

Canine leishmaniasis is an important disease in veterinary medicine and in public health. From the veterinary standpoint, dogs with leishmaniasis, especially when infected by *Leishmania infantum*, can present mild clinical signs at early stages of the infection, but can also evolve to life-threatening disease. From a public health perspective, dogs may serve as reservoirs of *L. infantum*, which is the causative agent of zoonotic visceral leishmaniasis in many countries around the world. Indeed, zoonotic visceral leishmaniasis is a neglected tropical disease that is still causing considerable morbidity and mortality in affected human populations, especially in rural and suburban areas in Central and South America. Strategies for the control and prevention of leishmaniasis in dogs include: culling of infected dogs, treatment of infected dogs, use of repellents, and vaccination. Culling of infected dogs has been proven ineffective, for several reasons: lack of infrastructure and human resources of in endemic areas, rapid replacement of infected dogs by susceptible ones (infected or not), delay between diagnosis and removal of infected animals, presence of large numbers of stray dogs, low sensitivity and specificity of serological tests used to detect infected dogs, among others. Treatment of infected, sick dogs may reduce parasite load and the infectiousness to phlebotomine sand fly vectors, but should be considered as an individual measure, not as a control strategy from a population perspective. Synthetic pyrethroids (e.g., deltamethrin, permethrin, and flumethrin) have been proven effective in reducing the risk of infection in dogs under field conditions. The repellent products are available mostly as collars and spot-on formulations, and, in some instances, the community-wide application of repellent-impregnated collars have been demonstrated to reduce the risk of infection, not only in dogs, but also in humans. Vaccination has been proposed as strategy for preventing visceral leishmaniasis in dogs, but the available vaccines have limited efficacy and are claimed to reduce the risk of disease development, not infection. Finally, the preventive use domperidone, a dopamine D2 receptor antagonist, was effective in reducing the risk of visceral leishmaniasis development in dogs. It is evident that nowadays veterinarians and dog owners have a large repertoire of products against canine leishmaniasis. Evidence-based medicine supports the use of repellents as the first-line strategy against *L. infantum* infection in dogs. Other strategies such as vaccination may also the used concomitantly, but they should not replace the use of repellents, as they do not prevent dogs from being bitten by an infected phlebotomine sand fly vector.

33.4 NEW MARKER FOR THE DIAGNOSIS OF VISCERAL LEISHMANIASIS IN ASYMPTOMATIC DOGS

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1 Background

Leishmania infantum is the etiological agent of zoonotic canine leishmaniasis (CanL) in Mediterranean basin, Asia, Africa and South America. Domestic dog (*Canis familiaris*) is considered to be the major reservoir of the parasite for humans and other mammalian hosts, making CanL not just a veterinary problem but also a public health issue.The prevalence and incidence of CanL are important epidemiological parameters for controlling transmission, which in turn depends on the reliable identification of infected dogs.More than half of the infected dogs do not show any clinical sign but are still capable of transmitting the parasite to the vector. Thus it is necessary to diagnose CanL as early as possible and give special attention to the asymptomatic infected dogs, to avoid the transmission of *Leishmania* from dogs to humans.

2 Methods

ELISA - based techniques were performed for the detection of antibodies against the recombinant protein *Leishmania infantum* cytosolic tryparedoxin peroxidase (*Lic*TXNPx) and a comparison of the results with those employing soluble *Leishmania* antigens from promastigote or amastigote forms and the homologue recombinant protein *L. infantum* mitochondrial TXNPx (*Lim*TXNPx). Moreover, we evaluated the diagnostic potential of *Lic*TXNPx for CanL in the Portuguese canine population and propose an improvement to the existing ELISA-based serological techniques by combining the *Lic*TXNPx and other antigens as a *Leishmania* antigen mixture

3 Results

The data demonstrated that ELISA based on soluble promastigote or amastigote antigens had generally higher levels of sensitivity (82.9 % and 87.1 % respectively) for detection of antibodies in symptomatic or asymptomatic dogs than for detection of those against isolated recombinant proteins. Nevertheless, the specificities were found to be similar for all target antigens used. Importantly, the protein combinations using ELISA methodology improved the overall sensitivity, maintaining a high overall level of specificity when compare to recombinant antigens using in the diagnosis.

4 Conclusions

Overall, the obtained results showed that the strategy of combining *Lic*TXNPx with other well-defined *Leishmania* antigens, proved to be a sensitive and specific improvement to current serological diagnosis of CanL, being a useful tool for the detection of both clinical and subclinical forms of canine *Leishmania* infection.

(34) Chanllenges on implementation and development of clinical trials

(35) Students and Masters talking out of the box

(36) Platforms for research and control

36.1 THE LEISHMANIASIS EAST AFRICA PLATFORM: CONTRIBUTING TO STRENGTHENING CLINICAL TRIAL CAPACITY, TREATMENT AND CONTROL OF VISCERAL LEISHMANIASIS IN EASTERN AFRICA

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1 Background

Eastern Africa is the epicentre of visceral leishmaniasis (VL) in Sub-Saharan Africa. Countries most affected include Eritrea, Ethiopia, Kenya, Somalia, Sudan, South Sudan, and Uganda. A VL specific platform was created in 2003 by the Drugs for Neglected Disease *initiative* (DND*i*) with local and international partners. The Leishmaniasis East Africa Platform's (LEAP), main objective is to strengthen clinical research capacity, serve as a base for ongoing cooperation in training between countries, and standardize procedures and practices within the region for evaluation, validation, and registration of new treatment regimens.

2 Results

Seven clinical trial sites were identified and developed across the 4 LEAP countries (Ethiopia, Kenya, Sudan and Uganda. To date, 10 clinical trials have been carried out by LEAP and a total of 7,578 VL patients have been treated within and outside clinical trials. An early trial demonstrated the safety and efficacy of a 17-day combination treatment with Sodium Stibogluconate and Paromomycin (SSG&PM), which was recommended as first-line treatment in Africa by WHO in 2010. A pharmacovigilance study of SSG&PM recruited more than 3,150 patients. Additional studies are ongoing. Training was a key component in capacity-building, with approximately 800 staff from 7 clinical trial sites trained in Good Clinical Practice (GCP), Good Clinical Laboratory Practice (GCLP), disease-specific diagnosis and treatment. Over 20 personnel have pursued Masters and PhD degrees. A Data Centre has been developed for centralized clinical data management for the LEAP sites. Capacity for monitoring clinical trials and Data and Safety and Monitoring (DSMB) has been built. LEAP is evolving into LEAP2.0, with a broader mission of clinical research into other forms of Leishmaniasis, Cutaneous and Post Kala-Azar Dermal Leishmaniasis. This changing portfolio will require a revision of LEAP's modus operandi, and establishment of a Strategic Advisory Committee.

3 Conclusions

LEAP has been successful in meeting its objectives to build capacity, support policy change, conduct trials, and contribute to the treatment and control of VL in the region. LEAP is a model of successful collaboration between different partners and countries in conducting trials and treating patients. LEAP's evolution is expected to elevate its profile globally and to streamline its operations in the region.

36.2 LEISHMANIASIS RESEARCH NETWORK (REDELEISH): EXPERIENCE IN LATIN AMERICA

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1 Background

DND*i* aims to strengthen existing clinical research capacity in endemic countries by setting up disease-specific platforms to implement clinical trials which comply with international standards of quality and ethics in research, contributing to the development of new treatments adapted to neglected patients´ needs and fostering North-South and South-South collaborations.

2 Methods

The Leishmaniasis Research Network (redeLEISH) started in 2013, with the identification of reference centers in the treatment of cutaneous leishmaniasis (CL) in Brazil, and was officially launched in September 2014. Since then, it has extended to other endemic countries in Latin America. Its objective is to support the implementation of clinical trials for the evaluation of new therapeutic tools for CL. Coordinated by DND*i* with the support of a technical committee comprised of representatives from partner institutions, the network operates through a web forum, a virtual tool that allows information sharing and discussions between members, together with annual meetings and an annual newsletter.

3 Results

RedeLEISH comprises 90 members (outside DND*i)* working on CL, from 46 institutions across nine countries in Latin America and two in the Old World. Its members include experts, investigators, national control program officers, Pan American Health Organization representatives, international organizations and patients. It plays a key role in: a) promoting exchange of technical and scientific information, b) facilitating consensus on research priorities and harmonization of clinical trial design, c) encouraging debates on research and development challenges in leishmaniasis and strategies to ensure public health impact of the new treatment options developed, and d) encouraging the implementation of collaborative research projects. Three annual meetings have already taken place, allowing for the mapping of clinical research capacities in the region, and for harmonization of criteria for the conduct of clinical trials in CL. In one year, the Web forum compiled more than 150 contributions, mostly scientific publications. The first newsletter was launched in 2016.

4 Conclusions

RedeLEISH acts as a driving force to implement clinical trials and collaborative projects in a coordinated way. This network will play an important role in delivering short duration, safe, efficacious, affordable, and field-adapted treatments for CL in the New World.

36.3 BUILDING A JOINT EFFORT TO OPTIMALLY ASSESS THE EFFICACY OF VISCERAL LEISHMANIASIS THERAPIES: A VL DATA REPOSITORY AND NETWORK

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1 Background

A variety of factors should be taken into account when considering the best therapeutic options to cure VL patients including geographical area, resistance to certain drugs, co-morbidities such as malnutrition or HIV co-infection. The information needed to address these critical questions and reduce the human and economic impact of VL is already available. Data on more than 35,000 patients enrolled to clinical trials internationally in the past 20 years currently exist. This scattered, but rich resource could inform the knowledge gaps in treatment optimisation and improve future research. However, only summary statistics of these trials are available, and details of trial reports lack standardisation and systematic methods, making comparison of efficacy between drugs, regimens and regions almost impossible. Under these conditions, sophisticated meta-analyses that could summarise the clinical trial landscape have not and cannot be achieved.

2 Methods

An improved understanding of the clinical outcomes of VL treatment could be obtained through creation of a central database populated with individual patient data (IPD) from clinical trials. Such a repository would provide a powerful tool to enable the pooling and comparison of the existing, diverse clinical trial datasets and produce the robust clinical evidence needed to optimise treatment across the range of affected regions and populations.

3 Results

The partnership between IDDO and DND*i* has already engaged investigators to plan the design of a VL data repository. An initial pilot project and systematic search of published and unpublished literature has demonstrated the feasibility of a VL data sharing platform to facilitate IPD-based meta-analyses of clinical trial data.

4 Conclusions

Realising the potential impact of the database will require the agreement of the research community to share data, identify relevant scientific questions and analyse the data. The involvement of policy makers is also critical in setting the research agenda and adapting treatment policy to reflect the resulting evidence. By amalgamating and standardising the available data we can maximise the utility of existing resources to address priority questions in VL treatment and create a framework to prospectively assemble future studies, guide optimal data collection and make research progress more efficient.

36.4 KALACORE-BI REGIONAL SUPPORT FOR THE CONTROL AND ELIMINATION OF VISCERAL LEISHMANIASIS IN AFRICA AND ASIA: DEVELOPING AN INTEGRATED APPROACH TO IMPLEMENTATION AND RESEARCH

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1 Background

KalaCORE (KC), a UK DFID funded consortium, aims to reduce the economic and health burden of VL in three countries in Asia (India, Bangladesh, Nepal), where sustained elimination is the goal, and three countries in Africa (Ethiopia, Sudan, South Sudan) where the goal is disease control. KC supports government and state initiatives to achieve these aims.

2 Methods

To (i) roll out training on the use of diagnostics and treatments, (ii) provide quality procurement, increased access to safe and effective drugs and diagnostics, facility upgrading (iii) provide health education, awareness and behavior change activities within communities, (iv) develop outbreak response and surveillance systems, (v) support national programmes (NPs) for Monitoring and Evaluation (M&E), (vi) develop strategies to include private practitioners, (vii) provide technical assistance to NPs to sustain VL control, and (viii) build operational research to fill knowledge gaps.

3 Results

The number of treatment centres in all three countries have been extended, refurbished and supplied with drug, diagnostics and support. As VL requires complex clinical management, KC provides central training, mobile teams for on-site training, bedside and tele-mentoring. Teams investigate suspected outbreaks and new endemic areas with significant results Ethiopia and South Sudan; implementation is following in Sudan. Operational research will provide essential information on access to treatment for groups at risk, on innovative methods for personal protection from sandflies, and will evaluate new methods for vector control. **Results Asia:** Working with state and central governments, KC rolled out AmBisome treatment with logistic support and training across four endemic states. In 2016, 98% of VL patients have been treated with single dose AmBisome through these facilities. IEC and BCC across the endemic districts has sensitized a large proportion of the population at risk to VL and PKDL leading to an increase in earlier case reporting, suspect identification and referral. Comprehensive outbreak response strategies have been developed in Bangladesh and Nepal, where the elimination targets have been reached. These will be critical in the consolidation and maintenance phases of elimination.

4 Conclusions

Implementation of available treatment and control measures is making an impact on VL in six countries; the level of impact is currently being assessed through M & E teams.

SATELLITE SIMPOSIUM

Cutaneous Leishmaniasis prevention, diagnosis and management: existing and future tools

WHO AND PAHO: OVERVIEW OF AVAILABLE TOOLS FOR TRAINING AND EDUCATION

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Background
The World Health Organization (WHO) strengthens country level capacity to improve access to quality health services. Traditional face-to-face workshops and on-the-job training activities are being complemented by interactive or self-learning leishmaniasis on-line courses.
A total of three self-learning courses are currently being offered; two on visceral and cutaneous leishmaniasis in the Americas available at the WHO/PAHO[1] web site and one on post-kala-azar dermal leishmaniasis (PKDL) in the WHO eLearning platform[2]. Additionally, there is an interactive course on case management of cutaneous leishmaniasis in the Old world (with focus on north Africa and Middle East) done through the Open University of Catalonia (*Universitat Oberta de Catalunya*-UOC)[3].

Methods
The three self-learning courses are free of charge and combine slides, videos, pictures and quizzes to guide the student through different modules, mainly on epidemiology, diagnosis and treatment of leishmaniasis.
The interactive course consists of 6 ECTS (European Credit Transfer System) equivalent to 150 study hours. The teaching methodology is asynchronous and participatory where there is interaction with an expert dermatologist who presents real case scenarios to be discussed in the online forums. Weekly assignments are used for continuous assessment alongside a final multiple choice test. The results of the final test are compared with the pre-test done at the beginning of the course to assess the knowledge acquired. This course has a cost of EUR 575 per student and WHO sponsors participants nominated by the ministries of health.

Results
Over the last three years (2014-2016), more than 4000 students enrolled in the self-learning courses offered by WHO/PAHO. In the interactive course offered by the UOC a total of 47 students from seven countries (Afghanistan, Algeria, Chad, Morocco, the Syrian Arab Republic, Tunisia and Yemen) were enrolled.
The drop-out rate in the interactive course was 47% (21/47). From those who completed the course, 92% (24/26) passed the final assessment.
Surveys sent to participants showed high acceptability and satisfaction level among students.
The self-learning course on PKDL was made available in April 2017.

Conclusions
Leishmaniasis on-line training courses are a useful tool to train health professionals. A close follow-up of the students enrolled is needed in order to reduce the dropout rate. Other approaches, such as blended courses where part of the training is done through hands-on activities, could be used as incentive to improve attendance.

[1] https://www.campusvirtualsp.org/?q=en/courses/self-learning
[2] https://extranet.who.int/elearn/
[3] http://www.uoc.edu/portal/en/index.html

BEHAVIOR CHANGE COMMUNICATION THROUGH A COMIC BOOK IN SCHOOLS IN TUNISIAN ENDEMIC AREAS OF ZOONOTIC CUTANEOUS LEISHMANIASIS

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Full text

In Zoonotic Cutaneous Leishmaniasis (ZCL) endemic areas, Behavior Change Communication (BCC) can represent an essential part of a comprehensive ZCL prevention and care program. In fact, effective BCC can increase knowledge, promote appropriate attitudinal changes about perceived personal risk of transmission, reduce stigma and discrimination against CL patients and create a demand for healthcare services. BCC through a dedicated educational tool in schools represents an opportunity to reach thousands of children in endemic areas with appropriate hygiene and health messages. Furthermore, schools can provide an entry point to community as a whole, children taking back to their families' concepts and practices.

Comics combine 2 very rich forms, literature and visual art, that make them effective educational tools particularly in children. Considering this potential for comics, Sanofi's Access to Medicines Department in collaboration with the Pasteur Institute of Tunis developed an educational comic book called "Yamina's secret". Health messages expressed by the story were entirely adapted to the epidemiological realities of ZCL in North Africa within the context of transmission, management and control of the disease in Maghreb countries.

The comic book translated in Arabic was pilot tested in Tunisia for acceptability among 12-13 years old schoolchildren and presented to the National authorities of Health and Education to gain their support. Its effect on schoolchildren ZCL-related Knowledge, Attitude and Practice (KAP) was assessed with the collaboration of the Regional authorities in 11 randomly sampled schools from an endemic region of Central Tunisia. Study was conducted in classroom sitting and concerned 240 pupils from year 6 in the public education system. A pre-/post-reading approach by means of KAP multiple-choice questionnaire showed a significant increase in the overall score after reading the comic book, with significant raises in all ZCL-related KAP items. Baseline scores and magnitude of the increase varied according to the studied item.

Taking into account this positive evaluation, the educational comic book "Yamina's secret" could represent in North Africa a useful tool to improve CL management and control. An adapted version to additional cultural and epidemiological contexts should be considered for other CL endemic countries.

VIDEOS TO RAPIDLY DISSEMINATE PRACTICAL METHODS FOR SENSITIVE DIAGNOSIS AND EFFECTIVE LOCAL THERAPY OF CUTANEOUS LEISHMANIASIS

Oussama Mouri, Sylvie Guérin, Nathalie Chartrel, Isabelle Joly, Benoît Henry, Romain Guéry, Gloria Morizot, Marc Thellier, **Pierre Buffet**

Even with the most sensitive or simple methods for the diagnosis of cutaneous leishmaniasis - like PCR or direct diagnostic tests - sensitivity heavily depends on the quality of sampling. Wide variations in reported sensitivity of diagnostic methods may stem at least in part from this practical parameter. Short versions of videos will show how to perform painless, cell-rich scrapings, how to stain and read smears and how to aspirate lesions and use the collected material for culture and PCR without leaving scars.
A rapidly increasing proportion of patients with cutaneous leishmaniasis now benefits from local treatment rather than from potentially toxic or expensive systemic therapy. Videos will show how to perform the well-validated sequential treatment with superficial cryotherapy followed by intralesional injections of antimony, and how this compares with the local application of cream like aminoglycoside-containing ointments, the efficacy of which has been validated by exploratory and pivotal clinical trials.
While no diagnostic method and no treatment option fit all clinical situations and all local logistical contraints, videos are a powerful tool to rapidly and accurately teach existing approaches and open fruitfull discussions between experts to further optimise the management of leishmaniasis.

SATELLITE SIMPOSIUM

LeishVet

BENEFITS OF A ONE HEALTH APPROACH TO LEISHMANIOSIS

Michael J. Day

Chairman, WSAVA One Health Committee

'One Health' may be defined simply as co-ordination of human and veterinary healthcare in the context of the shared environment in which people and animals live (environmental health). The One Health approach to healthcare offers new insights and practical options for human and animal health and maximizes the effect of increasingly limited healthcare resources. Although the One Health paradigm often focuses on the relationship of human health to diseases of production animals and wildlife, there is an important role for small companion animals within the One Health model. The One Health Committee of the World Small Animal Veterinary Association (WSAVA) was established in 2010 to ensure the prominence of the small companion animal – human interface in the global One Health agenda. The committee works in three areas of One Health related to small companion animals: (1) the social and societal benefits of the human–companion animal bond, (2) comparative and translational clinical research, and (3) zoonotic infectious disease.

There are numerous companion animal zoonoses for which domestic dogs or cats act as a means of transmission or reservoir of infection; or in some instances, as sentinel species for human infection. The domestic dog shares the greatest number of infections with man; and this is proposed to relate to the fact that dogs have been domesticated for much longer than other animal species. Canine zoonoses are significant in both the developed and developing world and the impact of these infections is increased by factors such as the changing role of the dog in family life and the domestic environment, and the large scale international movement of dogs, particularly the movement of dogs that are adopted from the developing world to new owners in developed countries.

The optimum control and prevention of companion animal zoonoses involves a co-ordinated One Health approach. This is certainly true for the two most significant such diseases of global significance: canine rabies virus infection and zoonotic visceral leishmaniosis (ZVL). These are both diseases of poor rural communities in Asia, Africa and Latin America; and both occur against a background of human poverty, poor housing and sanitation, malnutrition, population displacement and climate change. Such diseases are a matter of social justice and reflect the reduced availability to the affected communities of education and healthcare; sometimes compounded by the influence of traditional superstition and healing.

For canine rabies virus infection, the current estimates suggest that a minimum number of 59,000 people die each year from a disease that is entirely preventable by vaccination of dog populations. The majority of these fatalities are children of the rural poor in Asia and Africa. Essentially, one person (child) dies every 10 minutes from canine rabies and the estimated annual economic loss is US$8.6 billion. Global expenditure on canine rabies vaccines is inversely proportional to the geographical distribution of endemic infection.

The One Health approach to control of canine rabies is typified by the very successful Mission Rabies programme that runs in several Asian and African countries (http://www.missionrabies.com/). The programme involves the co-ordination between veterinarians and human infectious disease physicians, educationalists, scientists, epidemiologists, vaccine manufacturers, fundraisers politicians and lobbyists. Mission Rabies has successfully controlled canine and human rabies in target areas through the combined effects of mass vaccination of dogs, public education (specifically of schoolchildren) and veterinary training (in neutering techniques for dog population control). The field vaccination programmes are managed via the Mission Rabies mobile telephone app, which is a powerful tool in disease surveillance and for monitoring vaccination coverage.

Zoonotic visceral leishmaniosis is the second excellent example of a significant canine zoonosis for which a One Health approach would optimise strategies for control and prevention. The most recent estimates for ZVL suggest that there are 200,000 to 400,000 new cases annually; and that these occur mostly in the rural poor of selected Latin American, African and Asian countries (http://www.who.int/leishmaniasis/en/). This is a complex disease involving the canine reservoir, sandfly vector and human patient; occurring on a background of human poverty, climate change and habitat incursion. There is also complexity in the multifaceted control and prevention mechanisms, which include: (1) the medical treatment of human and canine patients; (2) the use of ectoparasite preventives, vaccines or immunotherapy in dogs; (3) dog population control (which should not involve the indiscriminate culling of infected animals); (4) environmental control of sandfly populations by insecticidal spraying; habitat management; disease and vector surveillance and monitoring; (5) scientific research; and (6) public education and awareness.

Given these complexities, the most sensible and economically effective approach to disease control and prevention would involve a co-ordinated One Health strategy. The elements of this should include: (1) co-ordination of veterinarians (private and state), human physicians, public health officials and educationalists working in the field in endemic environments; (2) co-ordination of scientific research (involving parasitologists, microbiologists, immunologists, pathologists, ecologists, epidemiologists and meteorologists) with those involved in the manufacture of ectoparasiticides, drugs, vaccines and diagnostic tests; and (3) co-ordination of politicians, governments, non-governmental organizations, fundraisers and economists.

The ultimate responsibility and co-ordinating role for control programmes for diseases such as canine rabies and ZVL should lie with governments. For this to occur successfully there must be political will (recognition of the significance of the disease and recognition that there are effective means of controlling it), prioritization of disease control and rational allocation of human, animal and environmental healthcare budgets. Such programmes also provide the opportunity for effective public–private partnerships in the veterinary sector, with small companion animal practitioners working collaboratively with state veterinary services.

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Canine leishmaniosis, a vector-borne disease caused by protozoa of the genus Leishmania, is endemic in more than 70 countries from Asia, Africa, Europe and the Americas, but mainly occurs in the Mediterranean region and South America [1]. Dogs have been found infected with at least 12 species of Leishmania, the most important etiological agent of canine leishmaniosis being Leishmania infantum (synonymous with Leishmania chagasi), which also causes visceral and cutaneous disease in humans [2].

In the Old World, canine infections with L. infantum are endemic in many regions of southern Europe, North Africa and Asia, where dogs are the primary reservoir of the parasite for humans and other animals [3]. Seroprevalence in southern Europe ranges from less than 5% to more than 30% depending on the region. Based on serological data from Italy, France, Spain and Portugal, it has been estimated that at least 2.5 million (out of 15 million) dogs are infected in these countries [4]. Domestic dogs have also been shown to suffer from clinical disease associated with infection with Leishmania major and Leishmania tropica, agents of human cutaneous leishmaniosis in the Middle East and North Africa. Nevertheless, these two dermatropic species of Leishmania are considered uncommon causes of canine leishmaniosis in the Old World, as compared to L. infantum [5].

Canine leishmaniosis is widespread in South America, where dogs are exposed to infection with a number of species of Leishmania, such as: Leishmania amazonensis, Leishmania braziliensis, Leishmania colombiensis, Leishmania guyanensis, L. infantum, Leishmania mexicana, Leishmania panamensis, Leishmania peruviana and Leishmania pifanoi. The most widespread species infecting dogs in South America are L. braziliensis and L. infantum. Cases of canine cutaneous leishmaniosis caused by the former have been reported from several countries from the continent. Recent investigations support the hypothesis that L. infantum was brought to the New World from the Mediterranean region, via infected dogs [6].

Phlebotomine sand flies are the vectors of Leishmania. In the Mediterranean basin, nine species of the genus Phlebotomus are closely linked to the transmission of L. infantum to dogs and may be regarded as proven or probable vectors: P. ariasi, P. kandelakii, P. langeroni, P. longicuspis, P. neglectus, P. perfiliewi, P. perniciosus, P. syriacus and P. tobbi [7, 8]. In South America, among several species of Lutzomyia, the only well-established vector of Leishmania to dogs is Lutzomyia longipalpis [9]. The climate conditions in most canine leishmaniosis foci in the New World enable the development of phlebotomine sand flies all year round, while the vector populations in the Mediterranean region have a more defined seasonal pattern, typically from spring to fall [10].

In areas where canine leishmaniosis is endemic, the majority of dogs found infected with L. infantum are apparently healthy. Population studies have shown that only a low percentage of the dogs develop severe disease, while another considerable fraction has persistent subclinical infection. This information is relevant because seropositive but apparently healthy dogs can also serve as a source of infection to phlebotomine sand flies [11, 12].

A northward spreading of canine leishmaniosis in some parts of Europe has been related to changes in vector distribution and abundance, due to the global warming, but also to the increased

movement of infected dogs from the Mediterranean region. In addition, several reports have also shown a significant increase in the numbers of infected dogs in endemic areas [13–15]. Recent scientific evidence also reveals the spread of canine L. infantum infection to some geographic areas previously regarded as non-endemic in southern Brazil [16] and northern Argentina [2, 17].

In addition to dogs, infectiousness to vectors has been confirmed by xenodiagnosis in humans, black rats, crab-eating foxes, opossums, domestic cats and hares, which have the potential to act as reservoirs of L. infantum. These hosts can act as a source of infection to sand flies, but their role as primary or secondary reservoirs requires further study at the population rather than individual level [18, 19]. Since 2009, many human cases of zoonotic visceral leishmaniosis have occurred in the southwest part of Madrid, Spain, along with the identification of a new reservoir, the Iberian hare (Lepus granatensis) [19, 20]. Both in the Old and New Worlds, one notable feature of studies in domestic and wild hosts other than the domestic dog is the low proportion of infections with clinical expression [18].

Insecticides with a repellent effect against phlebotomine sand flies are one fundamental preventative tool to reduce the risk of infection in dogs [21]. The most recent approach available for the prevention of canine leishmaniosis is vaccination. Two vaccines are currently licensed for use in dogs in Europe and one in Brazil [22–24]. The immunostimulant domperidone is also available for prevention [25]. In Europe, dogs with clinical leishmaniosis are routinely treated with first-line drugs, including combinations of allopurinol and meglumine antimoniate or miltefosine [26, 27]. In Brazil, drugs currently used for the treatment of human leishmaniosis cannot be used in dogs [9], but alternative drugs may be adopted for canine leishmaniosis.

Although canine leishmaniosis is more prevalent and more widely distributed than human visceral leishmaniosis, infection in dogs has been suggested as a marker of the transmission risk of L. infantum to the susceptible human population [28–30]. The control of canine leishmaniosis in Brazil is mainly based on the elimination of seropositive dogs, but a recent systematic review concluded that there is no scientific evidence that dog culling could reduce the incidence of the disease in humans [31]. Culling of infected dogs is not mandatory in Europe.

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EMERGENCE OF CANINE LEISHMANIOSIS IN NEW REGIONS; IS DEVELOPMENT IN NON-ENDEMIC AREAS A RISK?

EXAMPLE IN EUROPE

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Introduction

The most important *Leishmania* parasite in dogs is *L infantum*, (= *L chagasi* in south America). This parasite is responsible of a severe and often fatal disease in dogs that in general combines systemic signs to skin lesions. These cutaneous signs often dominate in the initial phase of the disease. In sick dogs clinico-pathological abnormalities are also associated and may even be the only detectable modifications at minor or initial stages of the disease. For this reason infection is not a mainly visceral, like in humans, but a generalized disease (CanGL).

Historically, after the first detection in Tunisia (1908), infection of dogs with *L. infantum* was systematically looked for and described soon after the description of visceral leishmaniasis (VL) in humans in Mediterranean countries. Although suspected long time ago, the infection of dogs due to other *Leishmania* species has been only recently completely validated by molecular identification (i.e. *L. major*, *L. tropica*).

During the last twenty years the knowledge on the disease and infection in dogs considerably changed. Amongst the most important progress was to understand that most of dogs do not declare the disease. As a consequence the population of asymptomatic carriers exceeds by far the visible disease. This fact may be of importance in non-endemic areas where, general condition of dogs being good, relative incidence of clinical cases is low.

Canine leishmaniosis is a typical example of **vectorial disease**. The vector normally drives the epidemiology in each endemic area. Phlebotomine (not all species) have been demonstrated to be the only adapted group of insects regularly involved as biological vectors. Thus the distribution of endemic areas (multiple autochthonous cases) overlaps the distribution of established populations of demonstrated competent species of sand flies. However these vectors are not the reservoir. Every year a new population of uninfected vectors develops then progressively becomes infected after feeding on infected/ infectious mammals.

The concept of **reservoir** has also to be reviewed. The development of new molecular techniques allowed the detection of *Leishmania* in an increasing number of non-domestic asymptomatic animals from many mammal species (black rats, wild rabbits, hares, foxes…)(Millan et al 2014). Moreover the re-discovery of the disease and infection in cats raises the question on the wide circulation of *Leishmania* in multiple animal species and their potential role if such species are infectious to sand flies. It is now clear that zoologically speaking *L. infantum* is not associated to one reservoir host species like dog (important domestic reservoir for Humans) but actively circulates in a multi-species reservoir. The potential role of many wild mammals as reservoirs (long lasting infection ? /infectiousness to sand flies ?) has not still been fully investigated. As an

example the recent outbreak of Human leishmaniasis in Spain (Madrid area) was demonstrated to use hares (*Lepus granatensis*) as reservoir and source for humans and not the dog (Molina 2012, Arce 2013)

CanGL (*L.infantum*) is endemic in more than 70 countries. Being highly prevalent in southern Europe, Africa, Asia, South and Central America. It is now also a concern in many non-endemic countries where imported infected dogs may become a veterinary and public health problem with potential consequences in the future. The other question is the risk and the possible ways of dissemination of canine infection in areas where know vectors are absent.

1 Introduction of *Leishmania* infected dogs in non-endemic areas?

Non endemic areas are characterized by the absence of autochthonous cases and a limited incidence of the disease due to the absence or limited population of competent vectors. In Europe Phlebotomine sand flies have not been found, so far, in many northern and eastern continental countries and British Islands.

It has been proposed that the initial rising incidence of cases of CanGL in non-endemic countries is the consequence of the increased importation of infected dogs (Mencke 2011, Maia C. and Cardoso L. 2015). Since last decades in Europe hundreds of thousands of dogs have freely travelled to endemic areas in Europe and a number of then been infected. Another important aspect is the adoption of dogs originating from endemic areas by owners living in non endemic areas. Due to the reasons of adoption a high proportion of these dogs is infected.
In non-endemic areas, cases of canine leishmaniosis can be misdiagnosed as they are rare and sporadic. Moreover dogs may remains asymptomatic for many years after infection. The number of *Leishmania*-infected dogs is thus much higher than known cases. Most of cases are not documented and very few studies performed: United Kingdom : 257 cases between April 2005 and December 2007 (Shaw et al 2009) ; Germany : 569 positive seras out of 4 681 (Menn et al., 2010).

2 How could *Leishmania* maintain in dogs in non endemic areas ?

Several descriptions have been made of foci of canine Leishmaniosis in non endemic areas with development of autochthonous cases in the absence of sand flies vectors.

Alternative non vectorial routes of transmission have been demonstrated in some circumstances:
Venereal horizontal transmission from males to females (Silva et al 2009, Baskari et al 2013, Silva et al 2014) and vertical transplacental (Rosyzal et al 2005, Boggiatto et al 2011, Naucke et and Lorentz 2012, Basjkari et al 2013, Ben slimane et al 2014). Furthermore direct transmission is possible through direct dog-to-dog, by bite wounds in kennel dogs (Karmako et al 2014, Naucke et al 2016). It can be due to blood exchange and circulation of DNA as it has been proven in dogs as for human blood donors (Ownes et al 2001, De freitas et al 2006). Congenital transplacental transmission is the most likely the route of infection for most of cases. It is not rare in susceptible dog breeds or lineage as shown in Foxhounds in the USA and Shar Pei in France.
Moreover the Vertical transmission may persist for several generations resulting in a long term and stable foci with dissemination of infected dogs.

The role of transmission by various haematophagous arthropods such as fleas or ticks has been also suspected but never really proven in natural condition (Coutinho 2005, 2007). This hypothesis originates long time ago, before the discovery of sand flies as biological vectors. The retrieval of DNA of *Leishmania* in ticks, fleas or any other blood-feeding arthropods (midges) does not imply

that these arthropods can act as vectors at an epidemiological level of significance except in confined population (kennels). Experimental transmission to hamsters by feeding ticks engorged on infected dogs could support the accidental transmission by ingestion.

Known vectors have been sometimes identified outside of the endemic areas: *P. perniciosus*, and *P.ariasi* in non-endemic part of France, *P. perniciosus* in south-western Germany (Naucke and Schmitt, 2004). *P. neglectus* and *P. perfiliewi* in Hungary (Balazs Tanczos et al., 2012).
P. mascittii is found in France, Belgium, Germany and Austria. Its potential role as vector has not been demonstrated.

Role of the breed
The Boxer breed, known since many years to be particularly susceptible to the disease, has a marked genetic predisposition (Miranda et al 2008, Quilez et al 2012). Vertical transmission in non endemic areas was mostly observed in kennels of Boxers : Finland (Karkamo et al. 2014) Germany (Naucke and Lorentz 2012), Czech republic (Svobodova et al 2017).

3 The question of fringe areas

Another major question is the progressive extension of endemicity to contiguous areas by a "wave effect". Several studies have shown the progressive extension of endemicity to new areas: i.e. Italy (Maroli et al 2008), France (Bourdeau et al 2014). This could be due to the evolution of environmental and climatic condition favourable to the increase of population of competent vectors. Such extension of vectors have been demonstrated in the northern of Italy. It is considered an evidence of an increasing risk of establishment by sand fly species, for the Atlantic Coast, inland parts of Germany, Switzerland, and Austria (Joylyon 2014). Climatic changes are favourable to the extension of the sand flies distributions both in latitude and altitude.

However, as the vectors are not reservoir and slowly disseminate, their direct role is unclear. In return the progressive settlement by infected animals from the border of endemic areas could induce the infection of resident sand flies. These animals could be infected dogs but also infected wild fauna as well.

Also the question of other species of sandflies, present in non-endemic areas, that could be candidate vectors is raised. *P. mascitii* is one of these species and its potential role is under consideration.

4 Risk and evolution

Many questions remain in non-endemic areas: exact distribution of potential vectors. number of infected dogs, role of the breed in the risk of vertical or horizontal transmission, optimal strategy for detection.
The reduction of the risk of non-vectorial transmission requires a collaboration between dog owners, associations and veterinarians. Owners of dogs travelling from free areas to endemic areas should be informed and the use topical insecticides strongly recommended
It could recommended also to test every dog before introduction into a non-endemic area. Testing travelling dogs before their re-introduction into a non-endemic area is of limited value because it takes several months for a test to give a positive result after exposure. Dogs born in endemic areas, confirmed to be infected should not be imported from endemic areas into non-endemic areas. The most useful diagnostic should be firstly detection by quantitative serological techniques then if negative demonstration of parasite DNA in tissues (however PCR will detect only one part of

infected dogs). Every infected dogs should be discarded from reproduction. In addition, in case of risk of competent vectors in a free area, the use of insecticide collars in infected dogs in non-endemic areas could be recommended.

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CANINE IMMUNITY TO *LEISHMANIA* INFECTION: MOVING TOWARDS SUCCESSFUL IMMUNOTHERAPY

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Abstract

The elements of cell mediated immunity, including cytokine production that provides protective immunity against Leishmania spp. in dogs is at least partially understood. For protection from progressive clinical CanL there must be high IFNg production by CD4+ T cells, with detectable but low levels of IL-10 and IL-4 production to prevent inflammation-based pathology. This profile can be observed during natural asymptomatic canine infection. Establishing how to produce protective immunity through vaccination has been more elusive. The ability of a vaccine to maintain low parasite load is essential for limiting transmission from dogs to people as an L. infantum reservoir. The cumulative work of many groups has led to multiple lead vaccine/adjuvant combinations potentially capable of producing a cell-mediated immune response against infection by Leishmania. Vaccination as immunotherapy for canine leishmaniosis has had conflicting results. Studies demonstrated success in mounting a balanced Th1-type response from canine cells following TLR agonist supplementation, indicating that vaccination can be successful, but inclusion of a TLR4 agonist within subunit vaccine formulation was not successful in halting disease progression. Perhaps more importantly, healthy yet infected dogs progressing to clinical VL have T-cell exhaustion, which interferes with vaccine/immunotherapeutic responses. CD4+ T lymphocytes from dogs with clinical CanL had significantly reduced proliferation and IFNγ-production to vaccine antigens compared to those from healthy dogs. It is important to consider targeted vaccine formulation able to recover exhausted T cell responses before consideration of any vaccine formulation as an immunotherapy vs. prophylaxis for leishmaniosis.

LESSONS LEARNED REGARDING FELINE LEISHMANIOSIS OVER THE LAST DECADE (INCLUDING GAPS IN KNOWLEDGE)

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General introduction

Feline leishmaniosis caused by *Leishmania infantum* (FeL) is an emergent feline disease more and more frequently reported in endemic areas and sporadically seen also in non endemic areas in rehomed cats (7). Awareness of both the most frequent clinical findings and gaps of knowledge concerning FeL is therefore required to veterinary practitioners worldwide. The aim of this LeishVet presentation is to show recent findings but also to highlight current gaps in knowledge that can affect research or clinical activity concerning FeL.

Etiology and transmission

Leishmania infantum is the species most frequently reported in cats in areas of endemicity such as Mediterranean countries (Italy, Spain, Portugal, France, Greece, Turkey, Cyprus), Iran, Brazil (1, 3, 5, 7) but prevalence of infection is considered lower compared to dogs (7). Other *Leishmania* species are found in the New World (*Leishmania amazonensis, Leishmania braziliensis, Leishmania mexicana, Leishmania venezuelensis*) and recently *Leishmania tropica* and *Leishmania major* have also been molecularly detected in Turkey (3, 6, 7).

Sand fly-transmission of *Leishmania* infection to cats is supported by several studies about the feeding habit of vectors (7) but it has never been investigated as well as have not non-vectorial transmission ways. However, blood transfusion could be a source of infection in cats as it is proven in dogs and humans. In fact in endemic areas healthy cats – similarly to healthy dogs and humans - can be found positive at blood PCR (7).

Pathogenesis and clinical features

No information is available on immune mechanisms involved in pathogenesis of FeL. A first attempt to evaluate the production of specific IFNγ in cats from endemic areas has been recently addressed (10). In endemic areas the feline disease caused by *L. infantum* is less frequent than the canine one and host factors affecting susceptibility of cats may exist. These factors are not definitely established but retroviral infections and immunesuppressive therapies are suspected to play a role by impairing the immunocompetence of hosts (7).

Case reports published in recent years provided information on the most common clinical signs and clinico-pathological abnormalities associated with FeL (7). However, coinfections or comorbidities are frequently detected and they can contribute to a misrepresentation of clinical FeL. Moreover less frequent and less severe clinical presentations could currently be unreported and we are presumably underestimating the clinical relevance of FeL.

Detection of *L. infantum* infection

Parasitological, molecular and serological techniques are effectively used to confirm FeL (7). The main issue in antibody detection is setting the appropriate cut off value for positivity. Validated cut off value of IFAT is established at 1:80 dilution (7). According to the first attempt to compare performances of serological techniques (IFAT, ELISA and WB) for anti-*Leishmania* antibody detection in feline sera, best sensitivity and specificity are offered by WB (9). Most studies using PCR are performed on EDTA blood and very few compared performances of PCR carried out on

different tissues (blood, lymph node, bone marrow, skin) or non invasive samplings (conjunctival or oral swabs) (5, 7) .

Therapy and Prognosis of sick cats

Treatment of cats with clinical FeLV is empirically based (and off label) on the most common drugs used in dogs (allopurinol or meglumine antimoniate and their association) and a clinical cure is usually obtained (2,7). This means that efficay and safety of used protocols have never been evaluated in controlled studies. Therefore cats should be monitored very carefully for adverse effects during treatment and for possible clinical recurrence after stopping therapy. Life expectancy of cats with clinical FeL is usually good unless concurrent conditions or complications occur (8).

One Health

Proven vectors of *L. infantum* (*Phlebotomus perniciosus* and *Lutzomyia longipalpis)* were found infected after feeding on two sick cats with FeL (7). This highlights the importance of improving our knowledge investigating infectivity to sand flies of infected healthy cats and including the cat in the list of mammals playing an epidemiological role as additional reservoir of *L. infantum*.

Prevention of *L. infantum* infection

It is advised to protect individual cats from the risk of developing infection and clinical disease. Protection of feline populations has to be included in a "One Health" strategy for the regional control of *L. infantum* infection. No information is available on preventative strategies specific for cats. Topical insecticides currently available for cats have no demonstrated effect in preventing the bites of sandflies. In fact pyrethroids used for this purpose in dogs are toxic to cats. However, there is scientific evidence that flumethrin collars reduce incidence of *L. infantum* infection in kennelled dogs, and this is currently the only pyrethroid formulation licensed for cats (7). According to current knowledge, testing of blood donors by antibody detection and blood PCR is the only advisable measure for preventing non-vectorial transmission (7).

Conclusion

In last ten years our knowledge about FeL is significantly improved and the paradigm of the cat as an accidental host for *L. infantum* is definitely abandoned. Many important gaps in knowledge however are waiting to be filled and are possibly affecting epidemiological investigations as well as diagnosis, management and prevention of clinical cases.

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UPDATE ON DIAGNOSIS OF CANINE LEISHMANIOSIS IN THE ERA OF VACCINES

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General introduction

The diagnosis of leishmaniosis is very difficult and complex because of the spectrum of clinical signs and clinicopathological abnormalities is broad and unspecific (24), the existence of high prevalence of subclinical infection (1) and the use of vaccines for canine leishmaniosis (CanL) in endemic areas. Two vaccines for CanL (Leish-Tec® and CaniLeish®) are currently marketed in Brazil (20) and Europe (19), respectively. A third vaccine (LetiFend®) has recently been registered in Europe but limited published information is available regarding this vaccine (3, 8). This makes CanL a diagnostic challenge for the veterinary practitioner, clinical pathologist and public health authorities in endemic countries as well as non-endemic regions where imported infection is a concern (23). Moreover, all these different states of infection and clinical conditions have important implications from a diagnostic point of view.
The aim of this LeishVet presentation is to update and discuss the current knowledge on the diagnosis of CanL due to *Leishmania infantum*, focusing on the new challenges raised by the use of vaccines against CanL.

- **What are the various purposes for which *L. infantum* infection diagnosis is performed?**

The main purpose is to confirm disease in canine patients. However, other purposes are to investigate the presence of infection for screening clinically healthy dogs in endemic areas, including blood donors, breeding dogs, dogs prior to vaccination, dogs heading towards disease progression; or for screening clinically healthy dogs in non-endemic areas (travelling dogs), to avoid importation of infected dogs to non-endemic regions and to monitor response to treatment (23, 24).
Therefore, it is important to distinguish between disease, infection and immune responses induced by vaccination (immunogenicity), and to apply different diagnostic techniques accordingly.

- **How clinical CanL is diagnosed?**

Accurate diagnosis of CanL often requires an integrated approach consisting of a clinicopathological assessment and specific laboratory tests. Pertinent clinical history, a thorough physical examination and several routine diagnostic tests such as CBC, biochemical profile, urinalysis, serum electrophoresis and occasionally other diagnostic techniques like abdominal ultrasound can assist in raising the suspicion index for this disease (22).
Numerous diagnostic techniques have been developed to help in the diagnosis of CanL. The detection of *L. infantum* infection in dogs includes parasitological (cytology, histology, immunochemistry and culture of the organism in appropriate medium), molecular (conventional, nested and real-time PCR) and serological methods (qualitative and quantitative antibody tests). In addition, *L. infantum* specific cellular immunity tests have also been developed but they are mainly

used only in research settings. Different diagnostic procedures and interpretations of test results might be used accordingly, depending on the purpose of the diagnostic investigation. It is important to understand the basis of each diagnostic test, the limitations and the appropriate clinical interpretation (23).
Cutaneous lesions, bone marrow, lymph nodes and spleen and less commonly other tissues or body fluids such as joint, cerebrospinal and abdominal fluids are good choice samples to observe *Leishmania* amastigotes in both cytological and histological specimens (23). Parasites should be suspected in macrophagic, neutrophilic, neutrophilic-macrophagic, or lymphoplasmacytic inflammations in different tissues or in reactive hyperplasia in lymphoid organs on cytological or histological preparations (23, 24). Definite histopathological identification of parasites within tissue macrophages may be difficult and an immunohistochemical staining method can be employed to detect or verify the presence of *Leishmania* in the tissue (10). The isolation in culture of parasites from infected tissues is not suitable for rapid diagnosis. Parasite culture is used more often for research purposes (23).

Detection of parasite-specific serum antibodies should preferably be based on quantitative serological techniques, such as IFAT and ELISA due to the variable diagnostic performance of rapid serological tests (15, 23, 24). In non-vaccinated dogs, high antibody levels are usually associated with disease and a high parasite density (7, 21, 25) and, for this reason, they are conclusive of a diagnosis of clinical leishmaniosis. However, the presence of lower antibody levels is not necessarily indicative of patent disease and needs to be confirmed by other diagnostic method such as PCR, cytology or histology (23). The challenges of serology include cross-reactivity with other related pathogens and antibodies elicited by vaccination (17, 18).

- **What about serological testing when *Leishmania* vaccines are available?**

General speaking, the maximum peak of antibodies is found after two weeks of third dose of CaniLeish® vaccine during primary course (17, 18). The maximum peak of antibodies is detected after three weeks of first dose of Leishmune®, after three weeks after second dose of Leish-Tec® during primary course (9) and after two weeks after one dose of LetiFend®(3). Antibodies reactive with *Leishmania* antigen have been reported to persist for 4-12 months in dogs vaccinated with CaniLeish® (18, 26). However, there is a marked decreased of antibody levels during time (9, 18, 26). Interestingly, antibodies elicited by LetiFend® vaccine does not appear to interfere with quantitative or qualitative serological tests (3, 12). Very limited information is available regarding antibodies elicited after annual booster of CanL vaccines (26).

Considerations regarding antibodies elicited by vaccines include:
- Variable immune response to vaccine by dogs might exist (26):
    - Different antigen recognition
    - Differences of duration of antibody response and peak of antibody levels
    - High, low or non-antibody responders
- Diagnostic performance will be different depending on serological test employed and time after vaccination:

- Serological assays based on recombinant proteins are usually less sensitive than the ones based on whole parasite antigen for the recognition of antibodies elicited by vaccination (14, 18, 26).
- Quantitative serological techniques are commonly capable of detecting antibodies elicited by vaccination while rapid serological test are less sensitive (14).

It is important to develop new serological techniques that will discriminate between naturally-occurring antibodies or antibodies elicited by vaccination. For instance, prototype flow cytometry serology test seems to provide a good diagnostic performance with an absence of false-positive results in vaccinated dogs and minor cross-reactivity against other canine pathogens (13).

- **What is known about parasite-specific cellular immunity elicited by CanL vaccines?**

It is well established that a vaccine should elicit a parasite-specific long lasting cellular immunity to be effective against CanL. Classically, a T helper (Th) 1 response correlates with resistance, whereas a Th2 response is associated with susceptibility to infection (increasing parasite burden and a strong but ineffective humoral response). In dogs, a mixed response is required with a predominance of Th1 for protecting and controlling *L. infantum* infection (2, 11). Parasite-specific T cell mediated immunity is assessed in dogs with lymphoproliferation test, leishmanin skin test (delayed-type hypersensitivity, DTH), the production of interferon-γ and tumour necrosis factor-α cytokines by PBMC (4) or whole blood assay (5), and enhanced macrophage leishmanicidal activity via nitric oxide and reactive oxygen substances (18). Past or current marketed vaccines appear to elicit a long lasting parasite-specific cellular immunity such as Leishmune® (6, 16) and CaniLeish® (18).
Considerations about T cell-mediated immunity elicited by vaccination:

- In some vaccines, there are limited studies regarding T-cell mediated immunity as the case of CaniLeish® where the studies were done mainly in small number of Beagle dogs (17, 18). It is likely that in a large scale study including dogs of several breeds, the T-cell mediated immune response to vaccination will not be so homogenous.
- Variable T-cell immune response to vaccine by dogs might exist:
    - Different antigen recognition
    - Differences on kinetics, duration (shorter duration of long-term immunity) and magnitude of T-cell mediated immunity
    - High, low or non-T cell mediated responders
    - Difference of diagnostic performance of T-cell mediated immunity tests

- **How to make a diagnosis of sick dogs with clinical leishmaniosis if previously vaccinated?**

Serology will be less useful if there is a recent history of vaccination

- Diagnostic techniques based on observation of lesion (cytology/histology) and detection of parasite or parasite DNA (PCR) will be employed as first instance.

Serology will be more useful if vaccination has been performed a long time ago

- However, diagnostic techniques based on observation of lesion (cytology/histology) and detection of parasite or parasite DNA (PCR) will be employed as first instance due to the fact that some dogs might have persistent antibodies induced by vaccination.

It is essential to know details on vaccination of evaluated dogs in order to make a correct interpretation of the serological test employed. All evaluated information should be combined together for a comprehensive assessment.

Future research

New diagnostic techniques for the discrimination of immune responses due to vaccination and to natural infection should be potentiated:

- New serological tests to distinguish between naturally-occurring antibodies and antibodies elicited by vaccination.
- New cellular immunity tests to distinguish between vaccination and natural infection.
- Development of less tedious cellular immunity techniques to evaluate response to vaccination and natural infection.

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CURRENT DRUG THERAPY AND RESISTANCE IN CANINE LEISHMANIOSIS

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Canine leishmaniosis (CanL) is a major zoonotic disease endemic in more than 70 countries in the world. It is enzootic in regions of southern Europe, Northern Africa, the Middle East, Central Asia, China, South and Central America and has emerged also in dogs in the USA. CanL is also an important concern in non-endemic countries where imported disease constitutes a veterinary and public health problem. Dogs are the main animal reservoir for human visceral leishmaniosis and the disease is usually fatal if not treated in people (1, 2).
CanL is a good example of a disease in which infection does not equal clinical illness due to the high prevalence of subclinical infection. This makes CanL a diagnostic challenge for the veterinary practitioner, clinical pathologist and public health official in endemic countries as well as non-endemic regions where imported infection is a concern. The typical history reported by owners of dogs with CanL includes the appearance of skin lesions, ocular abnormalities, or epistaxis (1-4). These are commonly accompanied by weight loss, exercise intolerance and lethargy. Dogs from all breeds can be infected with leishmaniosis. The age distribution of the disease is bimodal with a peak of prevalence at 2-4 years and a secondary peak from the age of 7 years. The incubation period prior to the appearance of clinical signs may last 3 months up to several years. On physical examination, the main clinical signs associated with CanL are dermal lesions, lymphadenomegaly, splenomegaly, abnormal nails growth (onychogryposis) and poor body condition. Additional findings include: epistaxis, renal failure, decreased appetite, polyuria and polydypsia, vomiting, melena and lameness (1-4).
The main drugs used for treatment of CanL include:

- The pentavalent antimony meglumine antimoniate (Glucantime®) which selectively inhibits leishmanial glycolysis and fatty acid oxidation,.
- Miltefosine (Milteforan®) which is an alkylphosphocholine drug whose antileishmanial mode of action is not entirely understood.
- Allopurinol which acts by inhibiting protein translation through interfering with RNA synthesis.

Despite the World Health Organization's (WHO) recommendation to reserve anti-leishmanial drugs used for treatment of humans for use in human leishmaniosis and not for veterinary purposes due to potential of drug resistance development (5), pentavalent antimonials and miltefosine are often used for treatment of dogs with CanL. Treatment of CanL usually includes a combined regimen with meglumine antimoniate or miltefosine administered for 4 weeks and allopurinol administered simultaneously and then continuously used for long term therapy (2-4).
Amphothericin B which acts by binding to ergosterol in the parasite's cell membrane and altering its permeability is also effective against *L. infantum* but it is highly nephrotoxic. Some other drugs such as marbofloxacin and aminosidine are sometimes used as second line agents (2).
Anti-leishmanial treatment often achieves clinical improvement in dogs with leishmaniosis but it is frequently not associated with the elimination of the parasite (1-4). Treated dogs may remain carriers of the disease, experience clinical relapses and be infectious to sand flies. The successful treatment of CanL is particularly difficult due to the fact that it is lengthy and often does not result in complete elimination of infection, allowing potential relapse of clinical disease and further transmission of infection from treated animals. A clinical staging system for CanL divides the disease into 4 clinical stages based on clinical signs, clinicopathological abnormalities and level of

anti-leishmanial antibodies (3, 4). This system is helpful for decisions on the therapy most suitable for each patient and for consideration of a prognosis. The clinical stage may change if the dog deteriorates or improves.
Drug resistance has been widely described in human cutaneous and visceral leishmaiasis. Reports on drug resistance in the canine disease are more scarce. Disease relapse in dogs with CanL during allopurinol treatment has recently been described and associated with allopurinol resistance of *L. infantum* isolated from relapsed animals. *Leishmania infantum* strains isolated in culture from relapsed dogs were significantly less susceptible to allopurinol in comparison to isolates from dogs before treatment and those from dogs under treatment with no clinical relapse. Resistance was consistent in three forms of the parasite strains tested including intracellular amastigotes, promastigotes and axenic amastigotes (6). These findings indicate that resistance to allopurinol may develop in dogs experiencing clinical disease relapse which may transmit resistant parasite to other dogs and also enhance the danger of transmission the parasite to humans.

In conclusion, the treatment of CanL would benefit from the development of new effective drugs which would be able to eliminate infection from dogs and would not be used for treatment of humans thus reducing the danger of formation of drug resistant parasites that would be passed on to humans.

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USE OF IMMUNOSUPPRESSIVE DRUGS IN *LEISHMANIA* INFECTED DOGS: HOW TO MINIMIZE RISKS

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In regions where leishmaniosis is endemic, veterinary practitioners frequently have to treat patients that suffer from an allergic or immune-mediated disease and that are also infected with *Leishmania*. Considering that atopic dermatitis affects 15% of dogs and that some immune-mediated diseases as immune-mediated hemolytic anemia, immune-mediated polyarthritis or pemphigus foliaceus are also very prevalent; the co-existence of an allergic or immune-mediated disease with clinical leishmaniosis is not a rare event. It is well known that the immune system plays a key role in the progression of *Leishmania infantum* infection in the dog. Therefore, any drug with a negative impact on the immune system is contraindicated in the management of *Leishmania* infected patients. In many cases, however, the clinical signs are severe and the practitioner needs to prescribe an immunosuppressive treatment. Unfortunately, there are not sound data about the safety or risk of the different drugs and what are the most adequate options. We will review the available data regarding the impact on *Leishmania* infection of most commonly used immune-modulatory and immunosuppressive drugs.

Glucocorticoids

Apart from numerous anecdotal reports and clinical cases, there are some experimental data showing that treatment with glucocorticoids increases the clinical severity of *Leishmania* infection in the dog (Poot et al, 2005). The mechanism is not completely understood, but steroids induce apoptosis of lymphocytes, suppress the function of macrophage and dendritic cells function and inhibit synthesis of IL-2, IFN-γ, IL-4, TNF-α. Steroids will very likely worsen the T-cell exhaustion reported in canine leishmaniasis (Esch et al, 2013). The effect is very probably dose and duration dependent. If absolutely necessary, oral steroids can be given at doses <1mg prednisone /kg/24h or equivalent. Above this dose the risk is too high.

The impact of steroids on a dog with subclinical *Leishmania* infection (controlled by the immune system) is not clear. The current data and the available clinical experience show that at anti-inflammatory doses steroids do not switch the type of immune response and transform a subclinical (Th1) infection in a clinical leishmaniosis (Th2). However, higher steroid doses have been reported to trigger clinical leishmaniosis in both dogs and humans.

Ciclosporin

Ciclosporin has a negative impact on the progression of *Leishmania* infection, although probably less severe than glucocorticoids. The available data come from: (1) observation that immunosuppressed people after kidney transplantation develop leishmaniasis and (2) treatment with ciclosporin increases severity of experimental *L. donovani* infection in mice (Adinolfi & Bonventre, 1990). In most cases, however the dose of ciclosporin was very high (>10 mg/kg/24h). The use of ciclosporin is not recommended in dogs with clinically active leishmaniosis. Nevertheless, if this is the only available option, it is recommended to use the lowest possible dose and to monitorize regularly the blood levels (200-400 ng/dL). Although in most dogs/cats blood serum levels are proportional to dose, there are cases of animals with high absorption and high serum levels despite receiving a reduced dose. There are several studies on going aimed to determine the threshold of immunosuppression in the dog. The measurement of the production of IFN-γ, IL-2 and TGF-β by mononuclear blood cells after stimulation could help to assess the level of immunosuppression of a dog.

Azathioprine

Azathioprine antagonizes purine metabolism thereby inhibiting RNA, DNA synthesis and mitosis, causing immunosuppression. Azathioprine has greater activity on delayed hypersensitivity and cellular immunity than on humoral antibody responses. Clinical response to azathioprine may require up to 6 weeks (treatments are very long). Therefore, it is clearly contraindicated in dogs with leishmaniosis. Furthermore, azathioprine has a drug interaction with allopurinol because both drugs are metabolized through the thiopurine methyltransferase (TPMT). The hepatic metabolism of azathioprine may be decreased by concomitant administration of allopurinol. There are a few case reports of leishmaniasis in patients treated with azathioprine (Hagenah et al, 2007).

Oclacitinib

There are not data available about the impact of oclacitinib on *Leishmania* infection. Based on its mechanism of action and on reported cases of infections in treated patients, its use in dogs with clinical leishmaniasis is not recommended.

Monoclonal anti Il-31 antibody

Due to the high specificity of this treatment, this is probably the best option to treat atopic dogs with clinical leishmaniosis. This monoclonal antibody, as far as we know, only targets IL-31 and does not interfere with other mechanisms of the immune response.

Table 1. Summary of treatments options for dogs with clinical leishmaniosis and concomitant allergic or immune-mediated diseases.

Clinical situation	**Safe treatments**	**Contra-indicated treatments**	**Moderate risk/ Data lacking**
Atopic dermatitis and clinical leishmaniosis	Topical treatments: shampoos, moisturizing creams, EFA spot-ons. Topical tacrolimus. Essential fatty acids. Antihistamines (1st and 2nd generation). Allergen specific immunotherapy. Monoclonal anti IL-31 antibody.	Oral/Injectable steroids. Oral ciclosporin (5 mg/kg/q 24h or higher dose).	Low dose/low duration oral steroids. Oclacitinib (not recommended, but data lacking).

	Low dose/frequency topical steroids.		
Autoimmune or immune-mediated disease (PF, IMHA, IMPA, IMT…) and clinical leishmaniosis	Topical tacrolimus. Topical steroids. Tetracycline – Nicotinamide. Intravenous immunoglobulins.	Azathioprine (allopurinol interaction, thiopurine methyl transferase). Glucocorticoids (at high dose: > 2mg/kg). Ciclosporin (at high dose).	Glucocorticoids (anti-inflammatory dose). Mycophenolate mofetil (1 case report in humans).

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VACCINES AGAINST CANINE LEISHMANIOSIS – ADVANTAGES AND PITFALL

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Protective and effective vaccines constitute an essential tool to prevent any infectious disease and are an important tool for control of leishmaniosis. *Leishmania* prevention through vaccination is a very active area of research and product evaluation. Over many years, numerous research groups have worked to develop and assess vaccine/adjuvant combinations capable of producing a protective cell-mediated immune response against infection by *Leishmania*. Dog anti-*Leishmania* vaccines should produce a strong T helper 1 response to ward off infection as characterized by production of IFNγ. A protective vaccine should provide robust memory T cells able to mount a recall response at the time of (re)exposure to parasites and reactivate polyfunctional effector mechanisms (1). Protection from CanL depends on the ability of the dog immune system to respond since control requires balanced levels of tissue-specific immunity to not cause immune-mediated tissue damage and subsequent disease.
Several vaccines have been explored as promising vaccine candidate for leishmaniosis. At present no vaccines for human visceral leishmaniosis are available, due to different reasons, two of which are represented by the variability in different species of *Leishmania* that affect people and the high cost associated with development of a product targeted to very poor regions of the world (2). In contrast, four commercial products (Leishmune® (3); Leish-Tec® (4), CaniLeish® (5) and Letifend®) have been licensed on the market for the control of CanL, the first two in Brazil, the others in Europe. Leishmune® was withdrawn from the market under decision of Brazilian Health Authorities. Letifend® was authorized very recently by the European Medicines Agency (EMA) for the European market. Only preliminary studies are available for this vaccine (6). However, as reported on the Agency site the vaccine is composed by a "chimeric" protein (Q protein) including 5 different *Leishmania* antigens and its composition does not include any adjuvant.
In the following table a list of vaccines employed in published field studies is reported; their composition together with their efficacy in terms of protection against disease is reported too.

Antigen	Adjuvant	Dogs (n.)	Follow up (Months)	Country	Protection against disease*	Reference
Fucose-mannose ligand (FML)	QuilA	85	48	Brazil	80%	Borja-Cabrera et al., 2002
Chimeric MML	MPL-SE or	45	24	Italy	0%	Gradoni et

	Adjuprime					**al., 2005**
LiESP	**MDP**	**340**	**24**	**France**	**92%**	**Lemesre et al., 2007**
LiESP	**QA-21**	**90**	**24**	**Italy and Spain**	**68%**	**Oliva et al., 2014**
Gentamicin-attenuated *Leishmania*	**None**	**103**	**24**	**Iran**	**92%**	**Daneshvar et al., 2014**
A2	**Saponin**	**847**	**18**	**Brazil**	**71,4 %**	**Regina-Silva et al., 2016**

* Protection against disease was established with different study designs.

There are no data regarding the efficacy of these vaccines after multiple years of more extensive use of these products by veterinarians in the different countries where they have been marketed. The safety profiles indicate several adverse events mainly in response to saponin adjuvant, characterized by variable grades of severity. Large-scale longer-duration field trials in naturally-infected dogs (phase III trials) are underway to determine the long-term efficacy, tolerance and safety of these vaccines, as also reported in a systematic review (7) on prophylactic measures for CanL. The main problem regarding CanL vaccines is that they do not block the establishment of infection. As vaccinated dogs could still be infectious, a dog without clinical disease healthy but able to spread infection to (immunocompromised) humans and dogs. Both clinically healthy infected dogs and sick dogs have been shown to be infectious to sand flies vectors, but infectiousness is higher from overtly clinical dogs (8). The difficulty of xenodiagnosis in large scale studies did not allow for determination of a definitive answer regarding the role of vaccinated dogs in transmission. A further limitation of vaccination includes possible interference with diagnostic methods, particularly immunofluorescence serology (IFAT). This is of particular importance in countries with regional or national CanL control programs. In conclusion, due to limitations of currently licensed products, vaccination has to be considered as a part of a comprehensive control program for CanL, in which concurrent use of repellents against sand flies on vaccinated dogs.

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BEST CANINE LEISHMANIOSIS PREVENTION STRATEGIES/MEASURES IN DIFFERENT SETTINGS

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There are two predominant means to prevent canine leishmaniosis (CanL). The first means is vector control through insect repellent to avoid infectious bites; the second is chemotherapeutic and/or immunologic control of the infection by using leishmanicide/leishmanistatic drugs, immunomodulators and vaccines, respectively.

As with any vector-borne disease, participation of an arthropod vector in the life cycle of *Leishmania infantum* makes control of leishmaniosis challenging. Phlebotomine sand flies are believed to be the primary route of Infection within endemic areas, so the first line of *L. infantum* infection controlling is the blockade of transmission through the use of insect repellents. Multiple studies have evaluated the use of various active ingredients and compounding, including collars, spot-on and sprays, to prevent *Leishmania* transmission via sand fly. These formulations have a dual anti-feeding effect of both warding off and killing sand flies when they come into contact with a treated dog. These products contain various synthetic pyrethroids that act effectively against sandflies, the main vector of *L. infantum* in southern Europe.

On the other hand, protective and effective vaccines constitute an essential tool of prevention and is the cornerstone for the control of canine leishmaniosis. As anti-*Leishmania* vaccination is not a core vaccination for dogs, the decision to vaccinate should be based upon the following: (i) individual benefit/risk to the dog; (ii) age, breed, life-style or use, habitat, reproductive status; and owner compliance. These factors will determine whether vaccine use is appropriate.

Nevertheless, none of these both control measures should be considered as alone. Together they constitute a well-designed, synchronized, leishmaniosis prevention program for all dogs. Veterinarians should recommend to the owners a tailoring *Leishmania* prevention approaches to specific settings.

This includes dogs living in endemic zones or dogs moving to an endemic zone, in all clinical stages whether they are healthy, infected but clinically healthy, or sick.

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SATELLITE SIMPOSIUM

Research in leishmania vaccines

RIBOSOMAL PROTEINS FOR A HUMAN LEISHMANIASIS PAN VACCINE: BEYOND PROTEIN Q

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Abstract
Conserved antigens from the *Leishmania* parasite, like histones and ribosomal acidic proteins have demonstrated potential to protect towards infection caused by different *Leishmania* species.
In particular Protein Q, the active substance of the canine visceral leishmaniasis vaccine LetiFend®, was designed containing antigenic determinants from three *Leishmania (L.) infantum* ribosomal acidic proteins and histone H2A. Safety and prophylactic efficacy towards canine visceral leishmaniasis infection of LetiFend® has been proven and the vaccine is currently commercialized in Spain and Portugal.
With the objective of deepening into the immunological effect of the ribosome during *Leishmania* infection, a protein extract obtained from *Leishmania* ribosome was purified. It was seen to be able to induce a protective immunological response in BABL/c mice towards infection caused by *Leishmania* visceral, mucosal and cutaneous species and had potential as a sensitive and specific diagnostic tool. The protein extract from the *Leishmania* ribosome is a product difficult to characterize and to be produced consistently, generating the need of studying the specific ribosomal antigens responsible for that activity.
With this aim, recombinant proteins from the *Leishmania* ribosome were purified and the antigenicity and immunogenicity investigated, in the presence of a Th1 adjuvant. From this study, two ribosomal proteins (L3 and L5) were selected for inducing and reducing the lesion size caused by *L. major* and the parasite load. The combination of recombinant proteins L3 and L5 was used to immunize BALB/c mice which were infected with *L. major*, *L. braziliensis* or *L. amazonensis* promastigotes. The combination of both recombinant proteins induces stronger Th1 protective immune responses in BALB/c mice, than the individual proteins and also inhibited the parasite load and the lesion size caused by infection with the cutaneous *Leishmania* species.
Currently, active B cell epitopes from the two molecules are being selected by studying their recognition by sera from naïve dogs immunized with the individual proteins and by sera from infected dogs characterised by the LeishVet classification. From these epitope collection, T cell epitopes will be selected by studying the differential cell response induced in PBMCs of symptomatic and asymptomatic dogs.
From these studies it can be concluded that ribosomal proteins are a promising source to find potential candidates to build a new pan-leishmania vaccine. Preliminary assays have also highlighted their potential as a sensitive and specific diagnostic tool.

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TOLL LIKE RECEPTORS (TLR) IN CANINE LEISHMANIOSIS: ROLE IN DISEASE AND USE ON PREVENTION

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A broad spectrum of clinical manifestations ranging from a chronic subclinical infection to a non-self-limiting illness has been described in canine leishmaniosis (CanL) (1, 18). Therefore, a clinical staging system of CanL based on serological status, clinical signs, laboratory findings, and type of therapy and prognosis for each clinical stage has been proposed (18, 19).This variability in clinical manifestations is determined by differences in the immune response against the infection which are presumably genetically determined. Although different types of adaptive immune responses in dogs with CanL have been investigated in several studies (6), the mechanisms that underlie and determine this variability are still poorly understood. It is currently thought that specific mediators of the innate immune system, such as toll-like receptors (TLRs), play a central role in this polarization. However, there is limited data available concerning the role that TLRs play in canine *Leishmania infantum* infection (6).

The family of toll like receptors (TLRs) is trans-membrane proteins expressed mainly in macrophages, DCs, Natural Killer (NK) cells and lymphocytes (T and B). They are specialized in mediating the innate recognition of pathogens associated molecules patterns (PAMPs), that are presented in a large range of pathogens of clinical and immunological relevance (9) and rarely found in the host cells (12, 15). Recognition of each PAMP appears to be associated to distinct TLRs. Once the response is set, the activation of specific signaling pathways (2) rapidly triggers a variety of phenomena that amplifies parasite immune responses by stimulating the production of pro-inflammatory cytokines, which may play an important role in controlling *Leishmania* infection (16). Some studies confirm the importance of TLRs in the onset of leishmanial pathogenesis, susceptibility, and resistance in mice and human disease models(4, 20).

TLRs and role in disease

There is limited information regarding the role of TLRs in clinical leishmaniosis in dogs (6). Several studies have demonstrated upregulation or high expression of TLR2 in different tissues in dogs with moderate to severe clinical leishmaniosis (3, 5, 8, 14). A lower expression of TLR2 in skin biopsies from dogs with stage I-mild disease was statistically significant compared with stage II-III-moderate to severe disease (3). A study from Brazil found a high parasite load along with increased frequency and expression of TLR2 in the colon of dogs with leishmaniosis (5). In addition, TLR2 upregulation was also observed in the brain, spleen and lymph nodes of sick dogs with naturally-occurring leishmaniosis from Brazil (13). TLR2 upregulation was also noted in skin and liver samples in a canine experimental model of infection (8).

Similar findings were obtained in another study evaluating TLRs in unstimulated blood in sick dogs (14). The relative quantification of TLR2 was significantly higher in sick dogs when compared with seronegative healthy dogs while the relative quantification of TLR4 was similar in both groups (14). In addition, the relative quantification of TLR2 significantly decreased during treatment follow-up at all time points compared with day 0 whereas no changes were observed with TLR4 transcription (14). A significant positive correlation was noted between TLR2 levels and the urinary protein creatinine ratio, total protein, beta and gamma globulins, specific *L. infantum* antibodies and blood parasite load while a negative correlation was observed with albumin, albumin/globulin ratio, hematocrit and hemoglobin (14). TLR4 transcript did not correlate with any parameter (14).

The findings of these studies in conjunction with the current literature available suggest a role for TLR2 in the pathogenesis of CanL. TLR2 appears to be associated with moderate to very severe disease suggesting immune and proinflammatory responses due to the presence of a high *Leishmania* parasite load or cellular damage (endogenous damage-associated molecular patterns (DAMPs) also termed alarmins) as observed for other human diseases (10).

So far, there are few studies that have investigated TLR4 transcripts in CanL. Some authors found that in dogs with naturally-occurring CanL, TLR4 transcript was unchanged in the brain, spleen (13) and unstimulated blood (14) while TLR4 was upregulated in lymph nodes (13) . Another study showed a significant downregulation of TLR4 transcription with disease progression in lymph node and spleen samples and no change in TLR4 transcripts in skin and liver samples in an experimental canine model (8). It is well known that there are different immune responses depending on tissues (compartmentalization) and inflammatory responses in CanL (7, 13). Based on the current literature, it seems that TLR4 transcription is unchanged or decreased in dogs with clinical leishmaniosis when compared with healthy dogs and this might indicate a mechanism of parasite immune system evasion or a less important role for TLR4 in clinical CanL.

Other TLRs have been scarcely investigated in canine *L. infantum* infection (5, 13). Subtle changes or down-regulation

in TLR transcription (TLR2, TLR3, TLR4, TLR9) in several tissues in a canine experimental model (8) and natural infection (5, 13), are indicative of the silent establishment of infection for which *Leishmania* is renown. These observations provide new insights into TLR transcription in several tissues in CanL and highlight possible markers of disease susceptibility.

TLRs use in prevention or immunotherapy

Currently, treatments are not always effective against the disease and development of a long-lasting vaccine would be a cornerstone in the prevention of disease. Therefore, it is important to discover new immunomodulators for prevention and treatment of this important canine zoonotic infectious disease.

The TLR2a agonist Pam3CSK4-TLR2 enhanced the production of the inflammatory cytokines TNF-? and IL-6 in sick, “resistant” and healthy non-infected dogs (11). In addition, a combination of TLR2a + *L. infantum* soluble antigen (LSA) promoted a synergistic pro-inflammatory effect with TNF-? in Ibizian hounds but not in seropositive sick dogs and seronegative healthy dogs (11). These findings

were similar to the ones documented by combinations of TLR4 or TLR7 agonists and vaccine antigens leading to more robust Th1 CD4+ T cell responses in sub-clinically infected dogs (17). Therefore, it is important to highlight that activation of TLR receptors by specific agonists could be a powerful tool in the control and treatment of the CanL, either as adjuvants in future vaccine development or during treatment as immunomodulator to control infection in sick dogs.

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SAFETY MULTICENTER POST AUTHORIZATION PILOT STUDY ON LETIFEND, THE NEWRECOMBINANT VACCINE AGAINST CANINE LEISHMANIOSIS

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BACKGROUND and OBJECTIVES

Canine leishmaniosis (CanL) prevention In dogs can be approached from two well-defined fundamental aspects: control of the vector, through the use of insecticides with repellent action that avoid sand flies biting and, therefore, the transmission of *Leishmania* infection; and control of the parasite, by treating sick dogs and vaccination of healthy animals. Demonstration of the safety of vaccines under quality conditions is a regulatory requirement of the authorities of great importance, since they are directed mainly to healthy and young dogs.

The European Commission has recently approved the marketing of a vaccine against canine leishmaniosis called LetiFend®, composed of Protein Q (PQ), a recombinant protein constructed from the union of five antigenic fragments of four proteins of the parasite *Leishmania infantum* (Carcelén et al, 2009). The vaccine is intended to reduce the risk of developing an active infection, clinical disease or both, following exposure to *L. infantum* (LetiFend®: Summary of Product Characteristics - http://www.ema.europa.eu/ema/).

The main goal of this study was to confirm the safety of LetiFend® vaccine, already authorized by the European Medicines Agency under field conditions in healthy dogs of different ages, breeds, sexes and geographic areas of Spain.

MATERIAL AND METHODS

The present multicenter study was carried out under conditions of "Good Clinical Practice" in five veterinary clinics. The study included a total of 50 dogs located in: Alicante (n = 10), Barcelone (n = 10), Ibiza (n = 9), Madrid (n = 12) and Seville (n = 9). All dogs were healthy, older than 6 months and seronegative with a total of 18 males and 32 females, of different breeds (22 pure breeds and cross-breeds), with very variable weight ranges (between 4 and 63 kg), and ages between 6 months and 11 years. After vaccination with one subcutaneous dose of LetiFend®, enrolled dogs were followed up for 28 days in which a physical examination was carried out along three visits (Day 0, 14 and 28), assessing the physical status (potential clinical signs in different organs / systems, temperature and weight), local tolerance at the site of injection, and haematological and biochemical parameters. In addition, immunological tests were performed for the determination of anti-*L.infantum* antibodies using the indirect immunofluorescence assay test (IFAT) and anti-*Leishmania* ELISA (Ingezim® Leishmania Vet) techniques, and the determination of IgG2 antibodies against PQ by the ELISA.

RESULTS

After vaccination, none of the dogs experienced systemic adverse reactions associated with vaccination; only one of the animals presented a slight edema at the inoculation site that was resolved without treatment within the first 12 hours post-vaccination.

During the 28 days, none of the vaccinated dogs presented clinical signs evident in the general physical examination, at the inoculation site, or clinical-pathological alterations of the hemogram or biochemical profile. Only one dog was excluded from the study because of acute pancreatitis, unrelated to the vaccination administered three weeks earlier.

The presence of anti-*L. infantum* antibodies were not detected in any of the vaccinated dogs by IFAT and/or ELISA techniques after 28 days.

The maximum increase in IgG2 titre against PQ was detected at day 14, and subsequently decreased.

DISCUSSION

The results of the present study show that LetiFend® vaccine is a safe tool for the control of canine leishmaniosis in dogs of different races, weights and ages, since none of the animals showed clinical or clinical-pathological alterations throughout the 28 days of follow-up.

As in previous studies, vaccination with LetiFend® did not interfere with the detection of anti-*L. infantum* antibodies, allowing thus the discrimination between vaccinated animals and naturally infected dogs (Iniesta et al., 2016). In addition, we corroborate that the

administration of a dose of this vaccine generates a significant increase of IgG2 against the PQ at the 14 days post vaccination.

RELEVANT CONCLUSIONS

Safety is a key aspect of vaccination, as it is a preventive measure applied to healthy dogs. This study has shown that LetiFend® is a safe tool in the prevention of leishmaniosis in dogs of different breeds, ages, weight and origin.

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SATELLITE SIMPOSIUM

Tcaks, a publicly available box of chemical compounds: case study of multilateral collaboration to combat leishmaniasis and trypanosomiasis

Welcome and intro to the symposium

Julio Martín. GSK

ABSTRACT:

Using parallel whole-cell phenotypic assays, the GSK HTS diversity set of 1.8 million compounds was screened against the three kinetoplastids most relevant to human disease, i.e. *Leishmania donovani*, *Trypanosoma cruzi* and *Trypanosoma brucei* (which cause visceral leishmaniasis, Chagas disease and sleeping sickness, respectively). Secondary confirmatory and orthogonal intracellular anti-parasiticidal assays were conducted, and the potential for non-specific cytotoxicity determined. Ultimately, three anti-kinetoplastid chemical sets (aka TCAKS for Tres Cantos Anti-Kinetoplastid Sets) of 222 compounds for Chagas-Box, 192 compounds for Leish-Box and 192 compounds for HAT-Box were assembled as new antimicrobial sets available to external collaborators upon request [Peña I *et al.*, Scientific Reports 5, Article number: 8771 (2015); doi:10.1038/srep08771].

In this symposium, we describe the case of the collaborative network which is stemming around TCAKS thanks to multiple researchers and institutions. The expectation is that we foster synergies amongst partners, which otherwise would not have happened. Working together, we envisage to contribute to the research community with new therapeutic targets and chemical tools which may eventually become the source of future drug discovery programs.

Join this workshop to:

- Gain access to a privileged set of compounds to advance your research.
- Become part of a collaborative network for open innovation.
- Learn from researches already participating in this network.

ORAL PRESENTATIONS

Tuesday, 16th May

Session 1. Molecular and Cell biology

11:30 – 13:00

C0141 ROLE OF LEISHMANIA PROTEIN PHOSPHATASE 5 IN STRESS RESPONSE AND PATHOBIOLOGY

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1 Background

Leishmania parasites are responsible for important neglected diseases in humans and animals, ranging from self-healing cutaneous lesions to fatal visceral manifestations. During the infectious cycle, *Leishmania* differentiates from the extracellular flagellated promastigote, found in sandflies, to the intracellular pathogenic amastigote present in mammalian hosts. Parasite differentiation is triggered by changes in environmental cues, mainly pH and temperature. In general, extracellular signals are translated into stage-specific gene expression by a cascade of reversible protein phosphorylation regulated by protein kinases and phosphatases. Though protein kinases have been actively studied as potential anti-parasitic drug targets, our understanding of the biology of protein phosphatases in *Leishmania* is poor despite their implication in critical post-translational modifications and differentiation. Here we report the principal analysis of a novel protein phosphatase 5 (PP5) in *Leishmania* species.

2 Methods

Using in silico analysis we interrogated the *Leishmania major* (*L. major*) genome for PP5. To biochemically characterize PP5 we generated a recombinant protein and measured its catalytic activity using a dose dependent colorimetric assay with the substrate p-nitrophenyl phosphate (pNPP). This assay was repeated after performing site-directed mutagenesis to test the potential negative regulation of PP5 N-terminal tetratricopeptide repeat (TPR) domains. A polyclonal anti-PP5 antibody was raised and PP5 expression levels across different parasite life cycle stages were examined using western blotting. PP5 null mutant parasites were generated using the negative selection system.

3 Results

We determined PP5 to be a feasible potential drug target based on its low similarity with mammalian counterparts. Recombinant PP5 proved it to be a bona fide phosphatase that is enzymatically active and site-directed mutagenesis confirmed auto-inhibitory roles of the N-terminal region. PP5 is developmentally regulated, with increased expression in metacyclic promastigotes, suggesting a possible role in metacyclogenesis. The generation of PP5 null mutant parasites is currently shedding light on the role of PP5 in the pathobiology of the parasite.

4 Conclusions

Collectively these data support further exploration of PP5 to determine its potential future applicability for anti-parasitic intervention.

C0223 INVOLVEMENT OF LEISHMANIA LABCG1 AND LABCG2 TRANSPORTERS IN VIRULENCE, AUTOPHAGY AND OXIDATIVE STRESS

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1 Background

ABC (ATP-binding cassette) transporters in *Leishmania* includes 42 genes distributed in 9 subfamilies (A-I), some of them with relevant biological functions. We are interested in the ABCG subfamily that has been characterized as half-transporters involved in drug resistance, phospholipid translocation and infectivity. Previously, we have described that an inactive mutant for LABCG2 transporter is less infective for macrophages and shows decreased pathogenesis in a mouse model of cutaneous leishmaniasis.

2 Methods

To better understand the biological function of LABCG2 and its nearly identical tandem-repeated transporter LABCG1, we have generated *Leishmania major* null mutant parasites for both genes (ΔLABCG1-2).

3 Results

We have proved that ΔLABCG1-2 parasites present a reduction in phosphatidylserine exposure, infectivity and virulence. In addition, we have shown that ΔLABCG1-2parasites in stationary phase undergo less metacyclogenesis and present differences in the plasma membrane's lipophosphoglycan (LPG) composition. Considering that autophagy is an important process in terms of parasite virulence and cell differentiation, we have shown an autophagy defect in ΔLABCG1-2parasites, detected by monitoring expression of the autophagosome marker RFP-ATG8. This defect correlates with increased levels of reactive oxygen species and higher non-protein thiol content in ΔLABCG1-2 parasites. HPLC analysis revealed that trypanothione and glutathione were the main molecules accumulated in these DLABCG1-2parasites. The decrease in non-protein thiol levels due to preincubation with buthionine sulphoximide restored the autophagy process in ΔLABCG1-2 parasites, indicating a relationship between autophagy and thiol content.

4 Conclusions

LABCG1-2 transporters are involved in virulence and its absence produce a decrease in infectivity in a mouse model of cutaneous leishmaniasis, a defect in metacyclogenesis and changes in LPG composition of *L. major* parasites. In addition, LABCG1-2 transporters could be considered as a GSH and $T[SH]_2$ thiol transporters, as they modulate the levels of these molecules into the parasites influencing the autophagy process.

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C0238 ROLE OF CARBONIC ANHYDRASES IN ACID ACCLIMATIZATION OF LEISHMANIA

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1 Background

One of the longstanding questions in the field of *Leishmania* biology is how the parasite endures and proliferates in acidic phagolysosomes of host macrophages while maintaining a near-neutral intracellular pH. We recently showed that pharmacological inhibition of *L. major* carbonic anhydrase (LmCA) activity resulted in intracellular acidosis and cell death. Interestingly, parasites growing in acidic (pH 5.5) medium were much more susceptible to CA inhibitors than those growing in neutral (pH 7.2) pH [Pal *et al* 2015]. Since *L. major* expresses two CAs, LmCA1 and LmCA2, it was crucial to dissect their role in acid acclimatization of the parasite.

2 Methods

Localisation of LmCA1 and LmCA2 in *L. major* was investigated by immunofluorescence and subcellular fractionation. Gene disruption was achieved by targeted replacement of LmCA1 and/or LmCA2 genes with antibiotic selectable markers. Growth and morphology of parasites were determined by cell counting and scanning electron microscopy, respectively. Cell death and cell cycle were analyzed by MTT assay and FACS, respectively. Intracellular pH of parasites was measured by fluorescent pH sensor, BCECF-AM. Intracellular parasite burden was quantified in *ex vivo* J774A.1 macrophage infection model of *L. major*.

3 Results

Subcellular localization studies confirmed that LmCA1 is localized in the cytosol whereas LmCA2 is a cell surface enzyme. LmCA1 and LmCA2 knockout strains could not be generated in repeated attempts, suggesting their functional indispensability. However, we were able to generate LmCA1$^{+/-}$, LmCA2$^{+/-}$ and LmCA1$^{+/-}$: LmCA2$^{+/-}$ heterozygous strains. Although these strains grew normally in neutral medium, they suffered from intracellular acidosis-induced cell cycle arrest, growth retardation and morphological abnormalities in acidic pH. These phenotypes were reversed by genetic complementation or bicarbonate supplementation. Intracellular parasite burden of heterozygous strains was significantly reduced as compared to the wild type. Pre-treatment of macrophages with lysosome alkalizing agent, chloroquine, increased parasite burden of heterozygous strains to the wild type level.

4 Conclusions

LmCA1 and LmCA2 are crucial for cytosolic buffering of *L. major* and thus play a determining role in parasite proliferation in acidic milieu *in vitro* as well as within phagolysosomes of host macrophages. Above results unravelled a novel pH homeostatic machinery in *Leishmania* and also highlights LmCA1 and LmCA2 as potential anti-leishmanial drug targets.

C0715 ASSESSMENT OF THE MOLECULAR MECHANISM OF ARGININE SENSING IN LEISHMANIA

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1 Background

Visceral leishmaniasis or kala-azar is a deadly disease caused by *Leishmania* donovani. During infection, parasites up-regulate macrophage nitric oxide synthase and arginase activity, both of which use arginine as a substrate. These elevated activities depleted macrophage arginine pools, a situation that invading *Leishmania* cannot tolerate as this is an essential amino acid. *Leishmania donovani* imports exogenous arginine *via* a mono-specific amino acid transporter (AAP3) and utilizes it primarily through the polyamine pathway to provide precursors for trypanothione biosynthesis. We identified that depletion of arginine from promastigote and amastigote growth media induced a rapid up-regulation in AAP3 expression and activity, as well as a few other genes. Further quantitative phosphoproteomic analysis resulted in identification of differentially expressed phosphoproteins such as mitogen activated protein kinase 2 (MPK2) involved in the signaling cascade of arginine deprivation response (ADR) pathway.

2 Methods

Arginine transport in *L. donovani* was determined by using L-[H^3] arginine uptake in a transport assay. To determine changes in the transcript levels of AAP3 and other ADR associated genes upon arginine deprivation northern blotting was undertaken.

3 Results

Our analysis of ADR pathway in *L. donovani* has resulted in the identification of the minimal concentration of arginine required to activate ADR pathway via up-regulation of AAP3. This minimal concentration of arginine is close to the arginine transporter affinity (Km). In addition, our study also has shown that various kinases including protein kinase A (PKA) subunit R', AGC kinase (PKA, PKG and PKC) have been shown previously to be differentially phosphorylated during ADR response have a role in arginine sensing. Lastly, we have used specific inhibitors to block the arginine transport which had a direct effect on the ADR pathway activation and the eventual upregulation of AAP3 expression.

4 Conclusions

We hypothesize that arginine sensing in *L. donovani* is mediated by the arginine mono-specific transporter AAP3 which is also acting as a transceptor. Thus ADR is unique to *Leishmania* and therefore can be a unique drug target.

C0772 PROMOTER AND RIBOZYME DUAL FUNCTION IN THE PR77-HALLMARK OF A LEISHMANIA MAJOR SIDER ELEMENT

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1 Background

Leishmania major genome bears a large amount of Short and Long (SIDERs and DIREs) interspersed degenerated retrotransposons which lack coding capacity and are considered fossil elements. There are around 2000 copies of SIDER per haploid genome. Members of the two large subfamilies of SIDER (SIDER1 and SIDER2) have been implicated in the post-transcriptional regulation of gene expression. Most SIDERs and DIREs maintain conserved at their 5´end a homologous sequence to Pr77 sequence from L1Tc, a coding and autonomous retrotransposon from *T. cruzi*. We have previously reported that L1Tc Pr77 RNA is endowed with autocatalytic HDV-like ribozyme activity and that this function is also present in the homologous Pr77 sequences of transposable elements from *T.vivax*, *T.brucei*, *T.congolense*, *L.mexicana*, *L.donovani*, *L.infantum* and *L. major* SIDER2A sequence. As at DNA level, the L1TcPr77 sequence is an RNA pol II promoter, we wanted to know whether the Pr77 sequence of SIDER2A is able to activate transcription, representing thus a dual promoter/ribozyme system in *L. major* genome.

2 Methods

*Lm*SIDER2A Pr77 sequence was selected on the base of RNAseq data and HDV ribozyme activity and cloned upstream of a luciferase coding gene into pTEX vector. *T.cruzi* was transfected with this construct and control constructs. *Luc* transcripts were detected by Northern-Blot and mRNA 5´-end identified by RT-PCR and sequencing. Luciferase assay system was used to detect Luciferase activity.

3 Results

Analysis of the 81nt of *Lm*SIDER2A$_{28}$ sequence shows a 74% of identity with the L1Tc Pr77 sequence. *Lm*SIDER2A$_{28}$ bears conserved the Downstream Promoter Element (DPE) of L1Tc Pr77 promoter in terms of nucleotide composition (CGTG) and location, nucleotides +25 to +28 relative to the transcription start site. Transfection experiments show that the Pr77 of *Lm*SIDER2A$_{28}$ is able to activate transcription of a reporter gene, generating 3-fold more excess of mRNA level relative to those of the positive control. *Lm*SIDER2A-derived transcripts are not processed by *trans*-splicing as they lack of the 39nt SLRNA at their 5´end, and are not translated.

4 Conclusions

The Pr77-hallmark of SIDER2A$_{28}$ bears a promoter/HDV-ribozyme dual system suggesting that these elements are still active and alive. SIDER elements may play a critical role in the regulation of gene expression, promoting transcription of adjacent genes and downregulating those genes where SIDER2A disrupts their 3´UTRs.

C1238 HEME TRAFFICKING AND METABOLISM IN LEISHMANIA.

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1 Background

One attractive strategy for identifying new leishmanicidal drugs involves exploiting the biochemical differences between *Leishmania* and its human host. The trypanosomatid's auxotrophy for heme constitutes one of these differences and can be considered as an "Achilles heel" for these parasites. Trypanosomatid parasites have lost the heme biosynthesis pathway and, although *Leishmania* has re-acquired the last three enzymes from this pathway from bacteria, they need to scavenge porphyrins from the infected host. *Leishmania* proteins involved in porphyrin trafficking and metabolism are therefore attractive candidates as new drug targets.

2 Methods

Candidate genes were selected using bioinformatic tools and structural analyses were performed using molecular modeling software. Fluorescence microscopy was used to determine the protein's cellular localization. Their levels of expression were modulated by molecular genetic techniques, including the CRISPR-Cas9 technology. Functional assays were performed in homologous and heterologous systems such as bacteria, yeast, *Trypanosoma brucei* and *Xenopus laevis* oocytes. Flow cytometry and HPLC-MS/MS were used to measured porphyrin transport. A mouse model of leishmaniasis was used to analyze their role in *Leishmania* virulence *in vivo*

3 Results

We have identified several *Leishmania* proteins involved in the porphyrin uptake, intracellular trafficking and metabolism. Some of them are essential for the parasite, and even the abrogation of only one allele of these genes prevented the virulence of the parasites in mice. We have also used them to screen inhibitors in a HTS format.

4 Conclusions

Different examples of proteins involved in *Leishmania* heme trafficking and metabolism will be presented.

C1316 A MITOCHONDRIAL FUCOSYLTRANSFERASE IS ESSENTIAL FOR THE VIABILITY OF LEISHMANIA MAJOR

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1 Background

The *L. major* genes *FKP40* and *AFKP80* encode bifunctional proteins with kinase/pyrophosphorylase activities. Both salvage L-fucose to GDP-L-Fuc, but only AFKP80 yielded GDP-D-Ara*p* from D-Arabinopyranose. Correspondingly, Δ*afkp80-* mutants lack D-Ara*p* while Δ*fkp40-* mutants resembled WT. Unexpectedly, we were unable to produce a Δ*fkp40-*/Δ*afkp80-* double mutant. These data raised the possibility that GDP-Fuc (or a related metabolite) is essential. The *Leishmania* genome predicts at least 5 fucosyltransferases, of which LmjF01.0100 is the only one conserved amongst trypanosomatids, showing key motifs characteristic of α-1,2 fucosyltransferases. Enzymatic assays of *L. major* overexpressing C-terminally tagged Lm01 showed transfer of Fuc from GDP-Fuc to a Galβ1,3GlcNAc-β-O-Methyl acceptor. To test essentiality, we used a quantitative 'plasmid segregation' assay, expressing *Lm01* from the multicopy episomal pXNG vector, which additionally expresses GFP, in a chromosomal null Δ*lm01*-background. Single cell sorting for GFP-null lines rarely yielded viable cells, indicating that *Lm01* is essential.
Unlike most eukaryotic glycosyltransferases, Lm01 was predicted to localize to the mitochondrion, which was confirmed experimentally by expression of a C-terminally tagged protein (Lm01-HA). To probe the importance of mitochondrial localization, we used 'plasmid shuffling', asking whether pXNG-*Lm01* could be lost from Δ*lm01*- in the presence of mutated test *Lm01* genes bearing a C-terminal HA tag. A variety of modifications affecting mitochondrial localization could not rescue the Δ*lm01*- mutant; each of these proteins was expressed at high levels but were now found in the cytosol at high levels and showing WT recombinant enzyme activity. Mutation of the predicted FucT catalytic motif resulted in loss of function and enzymatic activity, while maintaining mitochondrial localization. Together these results suggest that both mitochondrial localization and FucT activity are essential for Lm01 functionality. Correspondingly, the rare Δ*lm01*- survivor was quite sick and showed a variety of severe mitochondrial defects. Thus, the Lm01 FUT1 joins the eukaryotic O-GlcNAc transferases as one of the first glycosyltransferases shown to act within the mitochondrion. Current work is now oriented towards identifying the *Leishmania* fucosylated targets therein.

C1375 A MFS-TYPE PLASMA MEMBRANE TRANSPORTER PROTECTS LEISHMANIA PARASITES FROM IRON TOXICITY

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1 Background

Iron is required as a cofactor for various essential cellular processes. However, iron accumulation in the cytosol can generate highly toxic hydroxyl radicals in the presence of oxygen. Therefore, the cytosolic iron concentration must be tightly regulated, by balancing iron uptake with storage or export. One mechanism used by a wide range of organisms to prevent iron toxicity is storage as a complex with ferritin. Other organisms, such as yeast, manage iron accumulation by sequestering it in vacuoles. It is known that iron acquisition in *Leishmania* is mediated by the coordinated action of two plasma membrane proteins, the ferric iron reductase LFR1 and the ferrous iron transporter LIT1. However, it is still unclear how these parasites regulate their cytosolic iron concentration to prevent toxicity. Investigating this issue, we identified and characterized the *Leishmania* Ferrous Exporter 1 (LFE1).

2 Methods

Genome-wide transcriptome analysis (RNA-seq) of iron-deprived *Leishmania amazonensis* revealed LFE1 as a new iron responsive gene. Immunofluorescence of *L. amazonensis* overexpressing LFE1 tagged with triple-Flag or GFP revealed the protein localization. To assess the functional role of LFE1 in *L. amazonensis*, we generated heterozygote and homozygote null mutants using homologous recombination. These mutants were evaluated regarding replication (cell counts), infectivity (macrophage and mouse infections), iron content (ICP-MS) and iron transport (^{55}Fe uptake/export). We also quantified iron content (ICP-MS) in transgenic *Arabidopsis thaliana* plants overexpressing LFE1.

3 Results

LFE1 contains a nodulin-like domain and shows similarity to membrane transporters of the major facilitator superfamily (MFS) in plants. Our data show that LFE1 is targeted to the plasma membrane and mediates iron transport, preventing iron accumulation inside the parasites. LFE1 overexpressed in *Arabidopsis thaliana* also localizes to the plasma membrane and decreases iron content in leaves. LFE1 deficiency increases the sensitivity of *L. amazonensis* to iron toxicity and drastically impairs parasite infectivity.

4 Conclusions

By identifying LFE1 as the first plasma membrane iron exporter from trypanosomatid parasites, our findings add a crucial missing element to the cellular machinery responsible for maintaining iron homeostasis in these important human pathogens.

C1479 NEW FUNCTIONS FOR OLD ENZYMES: REVISITING THE PEROXIREDOXIN REPERTOIRE OF LEISHMANIA INFANTUM

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1 Background

Peroxiredoxins (PRXs) are peroxidases coined to antioxidant protection in *Leishmania* spp. Data collected in our lab do not entirely support this canon. Among the four PRX enzymes of *Leishmania infantum*, the mitochondrial one does not function as antioxidant (published). As for the remaining enzymes (PRX1, 2 and 3), gene knockout studies are missing that unequivocally elucidate their physiologic role in parasites. This work puts forward the first reverse genetic analysis of the PRX1, 2 and 3 repertoire of *L. infantum*, its observations questioning the antioxidant function of at least one member.

2 Methods

Biochemical analyses of PRX1, 2 and 3 were performed with purified recombinant enzymes. Cell localization studies were carried out in transgenic parasites expressing tagged PRX1, 2 or 3. Reverse genetics of each PRX-coding gene was carried out in *L. infantum* promastigotes by homologous recombination. Knockouts were characterized in terms of susceptibility to exogenous H_2O_2.

3 Results

PRX3 is a glycosomal enzyme (the first characterized to date), whose abrogation does not compromise promastigote survival and marginally affects susceptibility to H_2O_2 [IC_{50}(*prx3*$^{-/-}$)=0.85*IC_{50}(control)].
PRX1 and PRX2 are cytosolic enzymes that, despite being almost identical, diverge in their capacity to act as peroxidases *in vitro*: i) PRX1, but not PRX2, is inactivated by H_2O_2; ii) PRX1 is less favorably reduced by tryparedoxin (a critical step for peroxidase activity) than is PRX2. To investigate whether these differences account for unique physiologic functions, *PRX1* and *PRX2* were individually disrupted in promastigotes. PRX quantification in the resulting *prx1*$^{-/-}$ and *prx2*$^{-/-}$ transgenics revealed that PRX1 is 3 times more abundant than PRX2. Despite the higher concentration of PRX1 in Leishmania cytosol, PRX1-depleted promastigotes did not exhibit a deficit in antioxidant response, unlike the *prx2*$^{-/-}$ knockouts which were more sensitive to H_2O_2.

4 Conclusions

Biochemical and functional analyses of PRX1 do not support a direct role for this enzyme in antioxidant protection. Unlike PRX2, PRX1 shares primary structure and biochemical traits with eukaryotic PRXs involved in H_2O_2-sensing and signaling. Work is underway to further elucidate the role of PRX1 in *Leishmania.*

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Session 2. Diagnostic methodology in practice and under development biomarkers in leishmaniasis

C0528 ASSESSING THE EFFECTS OF THE ACQUISITION OF MILTEFOSINE RESISTANCE ON PARASITE VIRULENCE

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1 Background

Oral miltefosine (MIL) is used to treat visceral leishmaniasis (VL) but is increasingly failing. Two isogenic bioluminescent lines of *Leishmania infantum* that originated from a French HIV patient (MHOM/FR/96/LEM3323)were selected for differential susceptibility to MIL. This was achieved by subsequent *in vivo* treatment/relapse rounds in Syrian hamsters followed by transfection to express red shifted luciferase (PpyRE9) which is a proficient tool for downstream *in vivo* bioluminescent imaging. Compared to the susceptible (MIL-S) line, the evolution of tissue parasite burdens in mice revealed a severely hampered infectivity of the resistant (MIL-R) line in the main target organs.

2 Methods

The *in vitro* promastigote proliferation and metacyclogenesis, macrophage infectivity and intracellular parasite expansion of the MIL-R and MIL-S matched lines was compared in relation to the observed *in vivo* infection deficit arising from the acquired MIL resistance. Following the observations made in Balb/c mice, virulence was also compared upon infection of *Phlebotomus perniciosus* sand flies, a key vector of *L. infantum* in the Mediterranean area. Female sand flies were infected by feeding through a chick-skin membrane on heat-inactivated rabbit blood containing 10^6 promastigotes/mL. Flies were dissected at different time intervals post-bloodmeal in order to determine the abundance and location of the *L. infantum* infections with special emphasis to colonization of the stomodeal valve as the prerequisite for successful parasite transmission.

3 Results

Acquisition of MIL resistance, which was linked to a frameshift mutation in the MIL transporter [PLoS One. 2016, 11:e0154101], resulted in a reduced *in vitro* growth rate of promastigotes and a reduced metacyclogenesis based on morphometric analysis. MIL-R parasites were still found capable to infect macrophages *in vitro* but displayed a decreased intracellular growth, indicating reduced fitness as compared to the MIL-S parental line and which is also reflected by the severely compromisedvirulence in mice. Preliminary infection studies in sand flies suggest that the infectivity of the MIL-R strain is also hampered.

4 Conclusions

Evaluation of the *in vivo* fitness of the two *L. infantum* parasite lines with differential drug resistance profile indicate a significant impact of a non-functional MIL transporter on virulence/infectivity in Balb/c mice and in the *P. perniciosus* sand fly vector.

C0823 MICROBIOTA COMPOSITIONS OF LEISHMANIA AND NON-LEISHMANIA SKIN LESIONS, PRELIMINARY FINDINGS

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1 Background

Cutaneous leishmaniasis (CL) is a parasitic disease caused by the protozoan parasite *of the* genus *Leishmania* that cause ulcerative lesions at the site of infection. Secondary bacterial infections are frequently observed in CL patients that impair the healing process of ulcer and require antibiotic treatment. Our study aims at studying the bacterial communities in CL lesions compared to those found in non-*Leishmania* lesions.

2 Methods

A total 298 patients (178 males and 120 females; mean age ±SE: 20.98±1.04) presenting ulcerated skin lesions suspected with CL were included in this study. The dermal tissue scrapings were sampled from the border of one ulcer at least. The tissue scrapings were plotted on sterile filter paper for ITS1-PCR and on slides for Giemsa staining.

3 Results

CL was proved in 153 (51%) cases by ITS1-PCR and microscopy. Among them, 20.9 % (32/153) were infected by *L. major* and 70.6% (108/153) by *L. tropica* as revealed by ITS1-PCR-RFLP. All samples (n=145, 93 males and 52 females) that showed negative ITS1-PCR are designated as non-CL lesions since the causative pathogen was unidentified. The hypervariable V3 and V4 region of the bacterial *16S rRNA* gene from all DNA samples (CL and non CL) was amplified and deep sequenced on Miseq machine.
Among CL patients, 27.5% (42/153) were positive *by 16S rRNA* PCR while 37.2% (54/145) were negatives. Our preliminary results showed highly diverse bacterial community in all lesion samples. The microbial community of CL and non-CL ulcers was statistically different (MRPP test, A=0.0195, P=0.000004). Two OTUs are found significantly more in CL microbiome: *Pseudomonas veronii* and *Delftia* While the most predominating ones in non-CL ulcers were *Skermanella, Delftia, Agrobacterium, Pseudomonas aeruginosa and Escherichia coli. Pseudomonas aeruginosa* and *E. coli* were the most abundant OTUs in all samples (~7% and ~6% of all reads, respectively).

4 Conclusions

The presence of these organisms may play an important role in *Leishmania* infections as well as treatment. However, No significant correlation was observed between the microbial community and *Leishmania* species and number of lesions (R^2<0.3 in all cases).

C0913 PERFORMANCE OF LEISHMANIA RECOMBINANT ANTIGEN-BASED ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) FOR THE SEROLOGICAL DIAGNOSIS OF CANINE VISCERAL LEISHMANIASIS

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1 Background

Visceral leishmaniasis (VL) caused by *Leishmania* (*Leishmania*) *infantum* and transmitted by sandfly *Lutzomyia longipalpis* has the dog *Canis familiaris* as the main peridomestic reservoir. In this context, the identification of infected dogs is considered a crucial step in the control of the transmission of *L.* (*L.*) *infantum* to humans. Currently the Ministry of Health of Brazil recommends the dual path immunochromatographic test, Dual Path Platform (DPP), containing three recombinant k28 proteins of *Leishmania infantum* (TR DPP® Leishmaniose Visceral Canina) as screening and ELISA with total *Leishmania major*-like antigen as confirmatory assay to analyze DPP positive samples. Since culturing the parasite for the preparation of total *Leishmania* antigen is a limiting step, it would be desirable to have assays based on the use of recombinant antigens. We aim to validate the ELISA using different recombinant antigens rK39, rK28, rKR95 and rK18 (Infectious Diseases Research Institute – IDRI, USA) as confirmatory assay for routine use. At present stage, we have evaluated the performance of ELISA using different recombinant antigens.

2 Methods

Serum samples from 57 positive dogs collected from municipalities endemic for canine VL (CVL) of the State of São Paulo and from 71 negative dogs from the city of Jundiaí-SP with no notification of *L. (L.) infantum*-infected dogs, were submitted to DPP and ELISA*L. major*-like. After screening using the direct agglutination (DAT) assay, the samples were tested by ELISA-rK39, ELISA-rK28, ELISA-rKR95, and ELISA-rK18.

3 Results

The sensitivity of ELISA-rK39, ELISA-rK28, ELISA-rKR95 and ELISA-rK18 was respectively 98.3%; 98.3%; 98.2% and 89.6 and the specificity, 100.0%; 98.6%; 100.0% and 98.6%.

4 Conclusions

Except for ELISA-rK18, the performance of rK39, rK28 and rK95 was promising that allow us to proceed with the validation of the tests with 2,321 collected samples from CVL endemic areas in the Northeast, Center West and Southeast of Brazil to be used in the routine diagnosis of *L. (L.) infantum*-infected dogs.

C1083 CAN WE IDENTIFY LEISHMANIA SUPER-SPREADERS TO REDUCE TRANSMISSION TO SAND FLY VECTORS?

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1 Background

In longitunal xenodiagnosis studies of natural infection of dogs with *Leishmania infantum*, a large fraction of transmission events to the sandfly vector *Lutzomyia longipalpis* is due to a small fraction of the infected canine reservoir population, know as super-spreaders. Most available serological tests, and their respective diagnostic cut-off values, are designed to detect infection or disease but also detect infected dogs that don't contributed to transmission; poor test specificity often leads to low dog-owner compliance with control programs. Targeting these super-spreaders to manage visceral leishmaniasis incidence requires a different approach to current blanket control operations that otherwise require extremely high intervention coverage to successfully include the super-spreaders. Increasing the test specificity is also likely improve community compliance. By immunologically testing longituinal sera samples based on current and novel anti-Leishmania antigens, the primary aim of the study is to differentiate super-spreaders in the mixed reservoir population.

2 Methods

To develop a new diagnostic tool to detect infectiousness, five antigen candidates were selected according to previous research. ELISA assays were performed on archived sera collected from a naturally infected cohort population of Brazilian dogs. Their transmission potential was measured by xenodiagnoses during a two years longitudinal study. These dog sera samples are well characterized in terms of exposure, infection, disease and infectiousness; and classified as infected, latent infected and infectious, based on both point xenodiagnosis and infectious history.

3 Results

Preliminary results show that carefully selected threshold based cut-offs allow for a more specific diagnostic test towards reducing canine population transmission potential. For this purpose, some of the new candidates performed better than currently available test antigens (e.g. rK39 and rK28) based on standard cut-offs. The ability of these threshold based antigen tests to detect and remove super-spreaders from the population from time of infectious onset is quantified under different population dynamic scenarios.

4 Conclusions

Choice of antigens and manipulation of antigen thresholds will enable better identification of super-spreaders in a mixed population, aimed to impact *Leishmania* transmission.

C1193 AN OPEN-ACCESS ONLINE WEBSITE FOR DIAGNOSIS AND TYPING OF PATHOGENIC LEISHMANIA SPECIES

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1 Background

Leishmaniases are infectious diseases caused by protozoan parasites from genus *Leishmania* affecting several millions of people in the world. Among tropical infections, it ranks as 2nd and 4th most common cause of death and disease. It constitutes a major public health problem with increasing burden over last decade. Diagnosis of leishmaniasis is complicated by clinical manifestations varying from simple cutaneous to visceral forms. Due to wide spectrum of animal reservoirs and large number of sandfly vectors, accurate diagnosis of *Leishmania* parasites is crucial for treatment and control measures. The diagnosis relies on development of effective methods and suitable biomarkers. Therefore, having knowledge about different methodologies and markers is of importance. Based on an international collaboration with several senior specialists and thanks to huge information coming from our recent exhaustive review paper (in press) and some unpublished data, we constructed an open access database. This website supplies required information for the clinicians and researchers based on type of *Leishmania* species, geographical dispersion, appropriate methods and markers and suitable primers for each goal of diagnosis (detection, identification, discrimination and quantification) and typing.

2 Methods

We: 1) propose the update classification of *Leishmania* species, 2) demonstrate current global distribution map of 21 pathogenic *Leishmania* species, 3) present a complete list of molecular techniques currently in use; 4) assemble a comprehensive list of DNA-based markers including rDNA, kDNA, protein coding genes, RAPD, MLMT, MLST and SNP and classify them according to application in diagnosis and typing, 5) outline a wide map of genes with their distribution on different chromosomes, 6) provide a resource of over 1200 primer pairs with their characteristics and suggest appropriate primers for diagnosis and typing 21 pathogenic *Leishmania*species and 7) highlight the sensitivity of different markers to detect from subsection to subspecies taxonomic levels.

3 Results

Because of big amount of information that have been gathered in this website, it can be considered as a reference database for the parasitologists and molecular biologists as well as the clinicians working in the health centers.

4 Conclusions

There is no test as comprehensive method to diagnosis all pathogenic *Leishmania* species. Chosen method/marker depends on the application. This website supplies reliable source of information for diagnosis.

C1520 DEVELOPMENT, VALIDATION AND CONSTRUCTION OF A SOFTWARE AND ITS UNDERLYING SCORE SET TO PREDICT DEATH BY KALA-AZAR

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1 Background

Clinical presentations of kala-azar, or visceral leishmaniasis, may range from mild to lethal. The early identification of patients with higher risk progressing to a severe state and death is crucial for taking therapeutic and preventive measures, and might reduce kala-azar's morbidity and mortality. Here, a score set composed of four scales along with a software for quick assessment of kala-azar severity at the point of care are described.

2 Methods

Data from 883 patients diagnosed from September 1st, 2005 to August 31th, 2008, was used to derive the score set, and data from 1,031 patients from two subsequent cohorts diagnosed from September 1st, 2008, to August 31th, 2009, were used to validate the model. Stepwise logistic regression was used to derive optimal multivariate prediction models. Model performance was accessed by its discriminatory accuracy (area under receiver operating curve - AUC). After the score set was developed and validated, a computational specialist system (Kala-Cal) was developed to speed up the calculation of the probability of death based on clinical scores. The applet can be applied at http://sbmt.org.br/kalacal/.

3 Results

For children under than two years old, the clinical prediction score showed high discrimination (AUC 0.90, 95%, confidence interval (95%CI) 0.84 - 0.97) to distinguish death from survival. Performance improved after adding laboratory variables (AUC 0.93, 95% CI 0.88 - 0.98). For older children and adults the clinical score showed equivalent discrimination (AUC 0.89 (95% CI 0.84 - 0.93), which also improved after including laboratory data (AUC 0.92, 95% CI 0.88 - 0.96). When applied to the validation cohort the score set also showed a high, although low discrimination (AUC 0.89, 95% CI 0.78 -1.00 for children under two years old and AUC 0.77, 95% CI 0.62 - 0.92 for patients older than two). There was no significant drop in discrimination when the weights of each variable in the model were modified from the 5% to the 95% of the confidence interval of the coefficient of the logistic regression.

4 Conclusions

This score set and Kala-Cal software the opportunity to enhance identification of individuals with greatest probability to present or to progress to severe presentations and death. The associated software may speed up the calculation of probability of death based on the clinical scores and assist physicians with decision making, such as patient referral and on benchmarking, and hopefully may help to reduce mortality by kala-azar.

C1623 CUTANEOUS LEISHMANIASIS IN BRAZIL: UNDENIABLE DIVERSITY OF SPECIES IS STILL POORLY KNOWN

Lourdes Maria Garcez[1], *Marcia Hueb*[2], *Norge Cordies*[3], *Luiz Sanchez*[4], *Luciana Nascimento*[5], *Rogério Santos*[6], *Alzira Oliveira*[7], *Breno Resende*[8], *Daniela Soares*[8], *Walter Santos*[8], *Anadeiva Chagas*[8], *Maria Pinto*[9], *Joelle Rode*[10], *Marina Boni*[10], *Evonnildo Gonçalves*[11]

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1 Background

The knowledge about *Leishmania* species is critical for determining the prognosis and treatment of cutaneous leishmaniasis (CL) patients. This understanding is also important in order to guarantee an adequate treatment efficacy interpretation in clinical trials. Specific species' identification of *Leishmania* using clinical samples is difficult in areas with high biodiversity. We have identified the diversity of *Leishmania* species in clinical samples of CL patients living in three Brazilian states, covered entirely (Acre and Pará), and in part (Mato Grosso) by the Amazon Biome.

2 Methods

Clinical data and skin biopsies were obtained from 78 CL patients with one or more primary lesions, attending health care centres from Pará (18), Acre (30) and Mato Grosso (30). Samples were sent to Instituto Evandro Chagas (Pará). We extracted DNA and sequenced the ITS1 and *Hsp70*-234 PCR products to identify *Leishmania* species.

3 Results

Half of the patients had other manifestations of CL beyond the primary lesion.We identified: *Leishmania (Viannia) braziliensis* (Pará: 61%, Acre: 56%, Mato Grosso: 87%); *Leishmania (Leishmania) amazonensis* (Acre: 3% Mato Grosso: 7%); *Leishmania (Viannia) shawi* (Pará: 11%) and *Leishmania (Viannia) lainsoni* (Acre: 7%). Other results were: *Leishmania (Viannia) braziliensis/Leishmania (Leishmania) infantum* (Mato Grosso: 3%), samples identified up to genus or subgenus (Pará: 28%, Acre: 14%) and negative PCRs (Acre: 20%). Species identification with the markers was more successful on clinical samples from Mato Grosso (93%) when compared to those from Pará (72%) and from Acre (67%).

4 Conclusions

CL in patients from all states was mainly caused by *L. (V.) braziliensis* (7 ITS1 and 5 *Hsp70-234* haplotypes). Other species are less frequent, and *L. (L.) infantum* may cause CL in Mato Grosso. The sequencing of ITS1 and *Hsp70*-234 PCR products did not clarify the entire *Leishmania* species diversity in the samples. Further molecular marker studies are necessary in order to address the lack of discriminatory power to distinguish *Leishmania* species, as well as studies that aim to understand the *L. (V.) braziliensis* polymorphisms.

C1710 AN UPDATE OF THE LEISHMANIASIS DIAGNOSTICS PRIORITIES

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1 Background

Despite substantial advances in leishmaniasis research and control, the disease is still a major public health concern for endemic countries. According to WHO, early diagnosis and effective case management is a cornerstone in the control of leishmaniasis, as it reduces the prevalence of the disease and prevents disabilities and death. FIND, a product development partnership focused on diagnostics for global health, began working on neglected tropical diseases (NTD) in 2006, in order to (i) catalyze development and evaluation of new diagnostic tools; ii) liaise with other organizations, governments and funding agencies to accelerate roll-out of diagnostic solutions; and iii) promote early and accurate diagnosis. HAT became the first NTD to be included in its portfolio, leishmaniasis was added as a core disease in 2010, Chagas disease in 2012 and Buruli ulcer in 2013.

2 Methods

With the aim of updating FIND's leishmaniasis diagnostics strategy, potential diagnostics priorities for leishmaniasis were identified through literature review and expert interviews. In March 2016, forty-seven leishmaniasis experts were invited to rank these priorities.

3 Results

According to the 40 experts who responded to the survey, the top four priorities were: i) Improved RDT for VL diagnosis (with adequate performance across geographical regions and in HIV+ patients); ii) Rapid point-of-care test for dermal leishmaniasis (CL, ML, PKDL); iii) Test of cure/Treatment monitoring; and iv) Strategies to improve access to (early) diagnosis and case management. There was minimal variation in priorities related to the participants' region of origin or field of expertise within leishmaniasis control.

4 Conclusions

This survey identified diagnostic priorities for dermal and visceral leishmaniasis that will guide efforts to develop diagnostic tests that will contribute to improve case management and a reduction in the leishmaniasis burden.

Session 3. Molecular and Cell biology

15:00 – 16:30

C0441 CHARACTERISATION OF AN UNUSUAL LEISHMANIA HSP70 FAMILY MEMBER, HRP4

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1 Background

During the *Leishmania* life cycle, stage differentiations are triggered by several environmental cues, such as pH and temperature. Heat shock proteins (HSPs) are involved in stress sensing and thus are likely to be important to regulate these processes. We identified a novel HSP, LmjF.29.1240 (Heat shock-related protein 4, HRP4), with an unusual protein structure combining a HSP70 and a TPR domain. We hypothesise that this protein could play a role in differentiation.

2 Methods

We used bio-informatics tools to assess HRP4 conservation and evolution in trypanosomatids. We constructed transgenic parasites expressing HRP4 and various domain deletion mutants to study the regulation, localisation and function of HRP4 using multiple approaches such as molecular biology, biochemistry and imaging.

3 Results

We showed that HRP4 is present in all *Leishmania* species and in ancestral trypanosomatids, but absent in the related pathogenic *Trypanosoma brucei* and *T. cruzi*. The study of the endogenous protein regulation revealed that HRP4 levels are high during the growth phase and decrease in stationary phase due to the degradation of HRP4 by two mechanisms: the proteasome pathway in early and an unknown protease in late stationary phase. We investigated whether this degradation could be important to regulate the morphology of parasites. Indeed, in wild type (WT) parasites, the body length/width ratio (L/W) increases as cells progress from logarithmic to stationary phase to become thinner. We compared the L/W ratio of various HRP4 mutants with the WT and found that one particular mutant displayed a slender form already in logarithmic phase. Truncation of part of HRP4 led to early morphological changes, suggesting that HRP4 degradation in stationary phase could be important to regulate these events. As the L/W ratio increases during metacyclogenesis in late stationary phase, we postulate that HRP4 could be implicated in the regulation of this process. HRP4 has a second function, as we showed that it acts also as a heat shock protein. Indeed its level increases in response to high temperature; it interacts with other HSPs, HSP70 and HSP90 and it could have a chaperone function as it binds to ATP-agarose.

4 Conclusions

We identified two potential roles for HRP4, as a chaperone and as a negative regulator of the morphological changes that accompany metacyclogenesis.

C0515 THE ESSENTIAL VALOSIN-CONTAINING PROTEIN P97/VCP/CDC48 PLAYS A CENTRAL ROLE IN LEISHMANIA AMASTIGOTE SURVIVAL

Bruno Guedes Aguiar, *Prasad Kottayil Padmanabhan, Ouafa Zghidi-Abouzid, Carole Dumas, Barbara Papadopoulou*

Research Center in Infectious Diseases, CHU de Quebec Research Center-University Laval and Department of Microbiology, Infectious Disease and Immunology, Faculty of Medicine, University Laval. Canada

1 Background

The valosin-containing protein p97/VCP/Cdc48 is one of the best-characterized type II AAA (ATPases Associated with diverse cellular Activities) ATPases. Most research connects p97/VCP to ubiquitin-dependent processes as it extracts ubiquitylated damaged/misfolded proteins from membranes or different cellular compartments for proteasomal degradation, hence ensuring cellular proteostasis. p97/VCP is associated with an array of biological functions, disease-linked mutations, and has emerged as a novel therapeutic target in cancer cells. Here, for the first time in *Leishmania*, we characterized the *L. infantum Li*p97/VCP ortholog.

2 Methods

*Li*p97/VCP expression was accessed using an anti-*T. brucei* p97/VCP antibody. p97/VCP co-factors were detected by co-IP-MS/MS. Epifluorescence and confocal microscopy revealed its localization. Gene replacement studies were employed to delete p97/VCP alleles. A dominant negative approach was used by overexpressing into the p97/VCP$^{-/+}$ haploid mutant a *p97/VCP* gene harboring point mutations in the Walker B site of ATPase D1 (p97/VCP^{Q1}) or ATPase D2 (p97/VCP^{Q2}) domains or in both sites (p97/VCP^{QQ}). Polyubiquitylated proteins were detected using an FK2 antibody and infection rates were evaluated *in vitro* in THP1 macrophages. DBeQ, a p97/VCP inhibitor, was tested for the first time in trypanosomatids.

3 Results

We showed that *Li*p97/VCP is an abundant protein interacting with most known co-factor/adaptor proteins that determine its functional diversity. *Li*p97/VCP is mostly localized to the cytoplasm and exhibits a differential regulation with higher expression levels in amastigotes. *Li*p97/VCP is essential for parasite growth and null mutant was only obtained when an episomal copy of the *p97/VCP* gene was provided *in trans*. This facilitated null mutant (fdKO) as well as the dominant negative mutants p97/VCP^{Q2} and p97/VCP^{QQ} into p97/VCP$^{-/+}$ strain (sKO^{QQ}) showed a high accumulation of poly-ubiquitylated proteins and were unable to undergo amastigote differentiation. Moreover, both fdKO and sKO^{QQ} exhibited lower infection rates and negligible survival following THP1 infection. Interestingly, DBeQ was shown to effectively inhibit *Leishmania* growth both in culture and inside infected macrophages.

4 Conclusions

Taken together, our results validated p97/VCP as a potential drug target in *Leishmania* and opened new avenues for studying p97/VCP structure, regulation, and function that might be extended to other organisms.

C0630 METABOLOMICS ANALYSIS OF LEISHMANIA PROMASTIGOTES TREATED WITH THE 6-AMINONICOTINAMIDE

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1 Background

The pentose phosphate pathway (PPP) is a major provider of reducing equivalents in leishmania and enzymes of the pathway could serve as good drug targets.6-aminonicotinamide (6AN) is an analogue of nicotinamide and in some species is converted to an analogue of NADP which is able to inhibit the dehydrogenases of the oxidative branch of the PPP. We assayed its effect on *L. mexicana* and *L. infantum* promastigotes, and *L. mexicana* promastigotes and amastigotes. 6AN was only inhibitory of leishmania growth at high concentrations. We went on to investigate how its inhibitory effect was exerted at the level of the metabolome.

2 Methods

M379 *L. mexicana* and PCM5 *L. infantum* promastigotes were treated with 7.8 mM 6AN for 24 h. Small metabolites were prepared in quadruplicate for HILIC-MS analysis according to Creek *et al.(Anal Chem 2011;83:8703–871).*HILIC was carried out on Dionex UltMate 3000 RSLC system (Thermo Fisher, Hemel Hempstead, UK) using a ZIC-pHILIC column (Merck Sequant). For MS analysis Orbitrap machines (Thermo Fisher Scientific) were operated in polarity switching mode. Data were analysed with IDEOMv19 and MetaboAnalyst 3.0.

3 Results

For *L. mexicana,* glutathione, ribose 5P, 6Pgluconate levels and downstream PPP intermediates were similar to controls. For *L. infantum*, it was possible to analyse NAD and NADPH which were found significantly decreased together with the PPP intermediate D-sedoheptulose 7-phosphate. 6AN caused significant depletion in phosphoribosylpyrophosphate (PRPP) and nicotinate (Na). Purine and pyrimidine nucleotides, nucleosides, their derivatives and metabolites related to their synthesis and turnover (ribose and deoxy-ribose) all showed peaks lower in 6AN than in DMSO treated cells. On the contrary, their purine and pyrimidines nucleobase precursors accumulated, indicating that the loss of PRPP was responsible for these changes.

4 Conclusions

In mammals 6AN is converted to 6ANAD/P by NAD^+ glycohydrolase, however in leishmania its toxicity is only seen in millimolar range. Our data indicated that this antimetabolite is capable of depleting cellular PRPP levels by its use in the Preiss-Handler NAD salvage pathway. This in turn diminishes conversion of nucleobases to their nucleoside products. When given in high doses 6AN can kill the parasites, probably due to the depletion of nucleotides required for nucleic acid biosynthesis. It seems that in leishmania a NAD^+ glycohydrolase similar to the mammal one does not work.

C0796 FUNCTIONAL GENOMICS OF LEISHMANIA INFECTION AND IDENTIFICATION OF NOVEL VIRULENT FACTORS

Vyacheslav Yurchenko, *Aygul Ishemgulova Yurchenko, Natalya Kraeva Yurchenko, Anzhelika Butenko Yurchenko, Jan Votýpka, Tereza Leštinová, Jana Hlavacova, Petr Volf*

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1 Background

Comparative genomics and transcriptomics provide promising new tools to identify virulence factors governing pathogenicity. OrthoMCL analysis on a dataset of 27 annotated trypanosomatid genomes delineated 99 orthologue groups (OGs) gained at the basal node of *Leishmania*. The timing of their acquisition suggests they may critical for dixeny, and indeed, this set includes several known virulence factors. However, 87 of 99 OGs are represented by proteins of unknown function, highlighting the need for future gene-focused functional studies. One of such unknown function proteins was encoded by the *L. mexicana* LmxM.30.2090, a gene gained at the Leishmaniinae node.

2 Methods

To ablate the LmxM.30.2090 in *L. mexicana*, both alleles were sequentially replaced with selectable markers for Nourseothricin and Hygromycin. Additional methods used: in vitro macrophage infection, differentiation in culture, infection of sand flies and mice.

3 Results

Comparison of differential gene expression datasets reveals potential virulence factors including LmxM.30.2090 encoding a putative mitochondrial ATP/GTPase. To examine the role of LmxM.30.2090 gene in *Leishmania* virulence, we established LmxM.30.2090$^{-/-}$ *L. mexicana* strains by homologues recombination. Recombinant *Leishmania* show significant deficienciesin their development in insects and not as efficient as their wild type counterparts in vertebrate infection.

4 Conclusions

Functional genomics approaches allowed us to identify several novel putative factors of virulence in *Leishmania* infection. One of them, LmxM.30.2090, is involved in developmental control during insect stage of the life cycle, and, subsequently, influences the outcome of the infection in vertebrates.

C0821 DELETION OF THE A2 VIRULENCE FACTOR THROUGH CRISPR-CAS9 TARGETING RESULTS IN ATTENUATED LEISHMANIA DONOVANI, ROLE OF A2 AS A CHAPERONE PROTEIN

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1 Background

The A2 multicopy gene family has been implicated as a virulence factor for *L. donovani* and may function as a chaperone protein. Protein expression is closely linked to stress response including heat shock or survival in macrophages and experimentally infected mice.

2 Methods

Complete *L. donovani* A2 gene knock-outs were generated for the first time using CRISPR/Cas9. A2-null mutants were characterized for their tolerance to heat-shock, survival in macrophages and survival in visceral organs in mice. To further explore the biochemical activity of A2, *in vitro* studies were performed in investigate the structure of A2 using native gel electrophoresis, gel filtration and negative-staining electron microscopy. A2 chaperone activity was determined by measuring its ability to impair protein unfolding in response to heat.

3 Results

A2-null *L. donovani* mutants displayed a lower tolerance to heat induced stress and were impaired for survival in macrophages compared to WT parasites. No A2-null mutants could be seen in the liver of infected mice at 4 weeks post-infection compared to WT parasites. The transition from its sand-fly vector to a human host is associated with various stresses resulting from host responses including higher temperature associated with fever. We hypothesize that through chaperone-like activity, A2 protects the infecting parasites by preventing the formation of toxic denatured protein aggregates when exposed to elevated temperatures inside the host. *In vitro* studies using native gel electrophoresis, gel filtration and electron microscopy revealed that A2 can assemble into tetramers in a manner similar to small heat shock proteins and these oligomers delayed and reduced the formation of denatured protein aggregates.

4 Conclusions

The A2 virulence factor is required for visceral organ infection and contributes to visceral disease progression by protecting *L. donovani* from heat induced stress through a protein chaperone type activity.

C1002 CHROMOSOMAL SEGREGATION IN THE PROTOZOAN PARASITE LEISHMANIA: THE QUEST FOR CENTROMERES.

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1 Background

Centromeres are the specific DNA sequences on which is established the complex kinetochore machinery responsible for the chromosomal migration during mitosis through interaction with the mitotic spindle microtubules. Our knowledge of the centromeres and kinetochores in divergent eukaryotes remains incomplete. This is particularly true for the protozoan parasite *Leishmania*, which affects millions of people and animals around the world. In the present work, we aimed to define, characterize and localize centromeres in this parasite.

2 Methods

We performed ChIP-seq against a kinetochore member characteristic of the trypanosomatids group (KKTs) in order to identify putative centromeres in *L. major*. The data were then confirmed by Fluorescent In Situ Hybridization (FISH) analysis.

3 Results

First, LmKKT1 is relocated through the cell cycle following a 'kinetochore-like' dynamic. Second, ChIP-seq analysis showed at least one clear peak per chromosome for LmKKT1 in the genome of L. major The LmKKT1 major peaks are narrow and their sizes are comprised between 4 to 12Kb approximately. Fiun ally, we have validated the peaks revealed by ChIP-Seq by immunolocalization combined to Fluorescence in situ Hybridization (FISH).

4 Conclusions

This is the first trypanosomatid genome for which all centromeric sequences are identified for every chromosome. These data are an important step to understand chromosomal segregation in Leishmania, and in particular the link between the constitutive mosaic aneuploidy and the consequent permissive segregation which characterize this divergent eukaryote. To a larger extent, it may also help in understanding the biological processes involved in higher eukaryotes when they have to face aneuploidy.

C1324 LEISHMANIA IFT140 MUTANTS LACK FLAGELLA, ARE RELATIVELY NORMAL AS PROMASTIGOTES, BUT ARE UNABLE TO SURVIVE AS AMASTIGOTES

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1 Background

The *Leishmania* flagellum is dramatically remodeled during development; the promastigote flagellum is long while the amastigote flagellum barely protrudes past the surface. In promastigotes, the flagellum functions in motility and potentially in cell morphogenesis, division, environmental signaling, and maintenance of the flagellar pocket (FP). Amastigotes may share these 'housekeeping' functions, and the flagellum is also proposed as a conduit for transfer of parasite factors into the macrophage. In the long flagellum of trypanosomes, flagellar assembly and cell viability require intraflagellar transport (IFT), but this may differ in the short amastigote flagellum. To test this, we targeted a core retrograde pathway gene *IFT140* in *L. donovani* strain Bob (Goyard *et al Mol. Bioch. Parasitol* 2003). We used a plasmid segregation test of an episomal *IFT140* in a chromosomal *Δift140* knockout (Murta *et al Mol. Micro.* 2009). Unexpectedly, we readily obtained viable knockout promastigotes whichgrew normally, lacked an external flagellum, and were immotile. EM analysis showed no axoneme assembly within the FP. The FP contained many vesicles but was otherwise normal, including maintaining the opening through the collar with relatively normal morphology. Quantitation of several reporters of endo- or exocytosis showed no or small effects (<2-fold), and *Δift140* lacked detectable levels of the major components of the paraflagellar rod. Complementation with episomal *IFT140* restored all mutant phenotypes to WT. When tested in mouse infections in vivo or macrophage infections in vitro, *Δift140* was unable to survive. Correspondingly, plasmid segregation tests carried out in mutants grown as the axenic amastigote stage showed IFT140 to be essential, in contrast to the results obtained as promastigotes.

Thus *Δift140* is the first example of a healthy aflagellate promastigote; its remarkably normal phenotype suggests that unlike trypanosomes, the *Leishmania* promastigote flagellum is not required for cell division, secretion, or FP morphology. In contrast, the ability of *Ld*Bob to differentiate to amastigotes *in vitro* and infect mammalian hosts allowed us to establish a requirement for IFT140 in the infective stage.

C1461 THE LEISHMANIA MRNA-BOUND PROTEOME CHANGES THROUGHOUT THE LIFE CYCLE; CANDIDATE TRANS-REGULATORS OF PARASITE DIFFERENTIATION

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1 Background

Leishmania spp. parasites adapt to drastic changes in environments during their transmission between insect vectors and human hosts. These adaptions require precise gene regulation to coordinate the timely expression of lifecycle stage-specific proteins essential for survival. Gene regulation in *Leishmania* spp. parasites demonstrates near complete reliance on post-transcriptional genetic control, yet insight into *trans*-regulators which control lifecycle progression is limited. In comparison to Trypanosomes, the molecular regulators behind *Leishmania* differentiation remain elusive.

2 Methods

To isolate key trans-regulators implicit in *L.mexicana* parasite differentiation and infectivity, we crosslinked RNA binding proteins (RBPs) to bound mRNAs *in vivo* using an optimised method with reduced stress, then quantitatively identified the mRNA-bound proteome of culture-derived procyclic and metacyclic promastigotes and macrophage- and lesion-derived amastigotes using mass spectroscopy. To verify RBP expression profiles we endogenously tagged genes of differentially-expressed RBPs and performed western blot analyses using constitutive standards. We are currently examining subcellular localisation of endogenously tagged RBPs, genetically characterizing their regulatory function and isolating associating RNAs for relevance to differentiation and infectivity.

3 Results

Results reveal a comprehensive list of developmentally-regulated RBPs with distinct expression profiles. Gene Ontology (GO) term analysis revealed a pronounced enrichment of terms associated with gene regulation and RNA binding. Some non-canonical putative RNA interactors verified in other eukaryotic systems were also identified in our screen. Endogenously tagged RBPs confirmed stage-specific protein expression and showed interesting variance to RNA levels.

4 Conclusions

Results indicate stage-specific control of transcript stability and translation and support a role for identified RBPs in parasite developmental progression and associated virulence.
Our identification and characterization of stage specific RBPs is the first of its kind in *Leishmania* spp. and will provide novel insight into *trans*-regulatory mRNP complexes which drive lifecycle progression to human-infective forms.

C1839 CHARACTERIZATION OF LEISHMANIA DONOVANI EXPERIMENTAL HYBRIDS DERIVED FROM PHLEBOTOMUS ORIENTALIS AND P. ARGENTIPES

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1 Background

It is postulated that genetic exchange in *Leishmania* has implications for heterosis (hybrid vigour), spread of virulent strains, resistance to chemotherapeutics, and exploitation of different hosts and vectors. Previous genetic analyses applied to natural populations imply that genetic exchange is a recurrent feature of some *Leishmania* populations. Here we perform phenotypic and genotypic characterisation, of *Leishmania donovani* hybrids derived from two sand fly species.

2 Methods

Two sand fly species, *Phlebotomus orientalis* and *P. argentipes* were co-infected using two transgenic strains of *Leishmania donovani*. One strain expressing green fluorescent protein (eGFP) and the resistance marker for Neomycin, and the other expressing dsRED and a resistance marker for Hygromycin. Selection in media containing both antibiotics and subsequent confocal microscopy to observe expression of dual fluorescent signals in single cells detected hybrid phenotypes. PCR analysis confirmed integration of both sets of markers into the hybrid parasite genome and Multilocus sequence typing (MLST) applied to evaluate single nucleotide polymorphism (SNP) inheritance patterns. Fluorescence activated cell sorting analysis was undertaken to investigate ploidy along with further phenotypic characterisation via drug susceptibility assays.

3 Results

Hybrids isolated from the two vector species were selected and cloned. Hybrids were resistant to both antibiotics and clones expressing both fluorescent proteins selected. PCR characterisation confirmed genomic integration. MLST identified clear parental and hybrid genotypes with variation within hybrid clones based on SNP inheritance patterns. Sequencing of mitochondrial targets indicate uniparental inheritance from either parental strain. Fluorescence activated cell sorting analysis of DNA content demonstrated that all hybrids and their associated clones were diploid.

4 Conclusions

We generated experimental hybrids of *L. donovani* in two permissive sand fly vectors (*Phlebotomus orientalis* and *P. argentipes*). The results indicate that hybridization occurs more than once in single flies evidenced by multiple hybrid genotypes. The diploid nature of the progeny and apparent uniparental inheritance of mitochondrial markers may be consistent with classical meiosis. We discuss the likely mechanisms of genetic exchange and the subsequent phyloepidemiological implications.

Session 4. Mucocutaneous leishmaniasis, diffuse cutaneous leishmaniasis, post-kala-azar leishmaniasis

C0484 INTRACELLULAR TRAFFICKING OF GFP EXPRESSING LEISHMANIA AETHIOPICA IN TERMINALLY DIFFERENTIATED THP-1 CELLS

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1 Background

Once inside macrophages, *Leishmania* promastigotes differentiate into amastigote forms within membrane bound organelle known as parasitophorous vacuoles (PV). Established amastigotes replicate and spread to neighbouring cells causing a range of diseases known as leishmaniasis. Parasites enter host cells via different receptor depending on their life stage and this is likely to affect intracellular trafficking. Interestingly a novel cell to cell spreading mechanism has been showed indicating a third route of infection. The mechanism through which parasites bind and enter host macrophages has been previously investigated, however very little is known about *Leishmania's* movement through the endocytic pathway and no data is available defining *L. aethiopica* endocytic trafficking. In this research, GFP expressing *L. aethiopica* were used to investigate parasite trafficking inside the host macrophages in all three known infection models.

2 Methods

All infections were carried out using THP-1 monocytes terminally differentiated via PMA treatment as host cells. Metacyclic promastigotes were obtained following peanut agglutination. Axenic amastigotes were isolated following subculturing in JH30 media at 32°C. Heavily infected THP-1 cells were used to study cell-to-cell infection spreading. Trafficking of parasite was studied via detection of co-localization of parasites with early and late endosomal markers (EEA1 and LAMP-1 primary antibodies) from 5 min to 72 h of infection. Samples were visualised using a Nikon ECLIPSE 90i overhead epifluorescent microscope.

3 Results

Following infection with axenic amastigotes parasites co-localization with EEA-1 was detectable from 10 min to 4 h, and co-localization with LAMP-1 at 1 h. Interestingly EEA-1 co-localization following promastigotes and cell-mediated amastigote infection was not detectable at any of the times tested. Moreover, when infected cells were used to start infection, parasites co-localized with LAMP-1 as early as 10 min after co-culture and promastigotes at 4 h after infection.

4 Conclusions

These data showed that *L. aethiopica* trafficking is depend on the parasite stage. This is the first study to compare trafficking of *L. aethiopica* inside host macrophages between promastigotes, axenic and intracellular amastigotes. The data clearly showed differences in internalization which relate to the type of infection and indicate that intracellular parasites might spread to uninfected hosts within the PV of previously infected cells.

C0658 POST-KALA-AZAR DERMAL LEISHMANIASIS WITHOUT PREVIOUS HISTORY OF VISCERAL LEISHMANIASIS: A CASE SERIES ON IDENTIFICATION, CONFIRMATION, AND TREATMENT

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1 Background

Post-kala-azar dermal leishmaniasis (PKDL) is a dermal manifestation which usually occurs after treatment of visceral leishmaniasis (VL), and is being studied as a possible source of transmission. The proper diagnosis and management of PKDL cases are the key to eliminate VL from Southeast Asia. Although PKDL mainly occurs after VL treatment, some particular cases have been reported to occur in patients without a previous history of VL. The identification, confirmation, and treatment of such cases were insufficiently described in previous reports. Here we report three confirmed cases that were successfully treated with miltefosine.

2 Methods

The study physician recorded patients' history and performed the physical examination. Patients proceed for rk 39 strip test based on having a rash like PKDL. Finally, skin samples were collected from the affected areas through skin punch biopsy method to do Polymerase chain reaction (PCR) using taqman primer and probe: 5′-GCGACGTCCGTGGAAAGAA-3′,5′-GGCGGGTACACATTAGCAGAA-3′ and reporter (FAM): 5′-CAACGCGTATTCCC-3′. Patients positive for *Leishmania donovani* (LD) DNA were allocated for 12 weeks oral miltefosine (doses with 2-2.5 mg/kg body weight). Clinical examination and PCR were also done to evaluate the treatment outcome.

3 Results

All the patients stated having a family history of VL and the starting of the rash from chin. The minimum period to be diagnosed as PKDL was ten months and the maximum was 18 months in these three patients. The majority of the patients consulted first with traditional healers, private practitioners before finally being diagnosed with PKDL by the study clinic. They had a macular type of rash which was non-itching and touch sensitive. All of them were rk 39 strip test and PCR positive. During treatment, they experienced anorexia, nausea, vomiting, weakness and diarrhea which was managed by the anti-emetic drug, vitamin supplement, and oral rehydration saline. At 12 months after completion of treatment, their rash disappeared completely, and skin samples tested negative for LD DNA.

4 Conclusions

Family history of VL, rash like PKDL and rk 39 strip test positive can consider as criteria for suspected cases which can standardize through field validation. This report indicates the success of miltefosine in treating PKDL cases without previous VL with minimum and treatable adverse events. This report will be useful to the physicians as well as the policy makers to handle such cases.

C1671 VISCERAL LEISHMANIASIS (VL) BASELINE CLINICAL PRESENTATIONS AND ASSOCIATION WITH INITIAL TREATMENT OUTCOME AMONG EASTERN AFRICAN PATIENTS

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1 Background

Visceral leishmaniasis (VL) is among the most neglected of the tropical diseases, afflicting the poorest of the poor. It is characterized by irregular bouts of fever, weight loss, enlargement of the spleen and liver, and anaemia. It is highly endemic in the Indian subcontinent and in Eastern Africa. An estimated 200,000 to 400,000 new cases of VL occur worldwide each year. Follow up of baseline clinical symptoms is important in monitoring treatment response, and in identifying and reporting of adverse events in trials.

2 Methods

We reviewed data from five open-label randomized clinical trials conducted in the region by Drugs for Neglected Diseases *initiative* (DNDi) between 2004 and 2015. The trials included non-HIV primary VL patients who presented for treatment. The studies were conducted in Ethiopia, Kenya, Sudan and Uganda. Data was analyzed to estimate the proportion of different clinical symptoms at baseline and the association with initial treatment outcome. The analysis also included the distribution of multiple symptoms.

3 Results

A total of 1,426 patients were enrolled in the trials, 1,017 (72%) were male. The median age was 13 years with a range of 4-60 years. Overall, the most common symptoms in addition to fever (100%) were weight loss (62%), inguinal lymphadenopathy (62%) and mucosal pallor (61%); less common symptoms were cervical lymphadenopathy (31%), diarrhea (30%), jaundice (30%), petechial haemorrhages (30%).The symptoms differed by the age, sex and country of residence. The most common symptoms in Kenya were mucosal pallor (75%) and abdominal swelling (79%) while in Sudan common symptoms were weight loss (80%) and inguinal lymphadenopathy (80%). Muscle wasting (63%) was most common symptom in Ethiopia. Multiple symptoms varied by country with high occurrence in Ethiopia (median=7, iqr=4), followed by Sudan (median=5, iqr=2) then Kenya (median=3, iqr=2). Abdominal swelling, epistaxis, jaundice, cervical lymphadenopathy and petechial haemorrhages were among the common VL symptoms that were significantly associated with initial treatment failure (p-value <0.05). Fever was the only symptom that had disappeared at the end of treatment (Day 28-30) among those with treatment success outcome.

4 Conclusions

Fever, abdominal swelling, weight loss, inguinal lymphadenopathy and mucosal pallor were the most common symptoms. Symptoms varied among the countries and fever clearance remains a better indicator of treatment success.

C1676 REASONS FOR SCREEN FAILURES IN CLINICAL TRIALS CARRIED OUT IN THE LEISHMANIASIS EAST AFRICA PLATFORM TRIAL SITES.

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1 Background

Screen failures are common in clinical trials and impact recruitment rate and study duration. In clinical trials it is important to understand the reasons for patient screen out, and seek to include into the trials populations most representative of the intended research beneficiaries. The information is also useful for planning study recruitment, whilst keeping in mind safety considerations when defining eligibility criteria.

2 Methods

We reviewed the reasons for screen outs from 6 clinical trials conducted by the Leishmaniasis East African Platform (LEAP) clinical research sites from 2003 to 2016. Five phase II and one Phase III study conducted in Kenya, Uganda, Ethiopia and Sudan were reviewed. Four studies included patients who were 4 to 60 years old, one study 7 to 60 years and the other 4-12 years. All the trials reviewed enrolled patients who were parasitologically confirmed to have visceral leishmaniasis (VL), were HIV negative and excluded patients with severe VL, among other eligibility criteria.

3 Results

Overall 5,831 patients were screened and 1,929 (33%) enrolled in the 6 VL trials. Out of the 5,831 screened, 2,969 were in phase II trials with an enrolment of 752 (25%) subjects while 2,862 patients were screened in the phase III trial with an enrolment of 1,177 (41%) giving a 4:1 and 2.4:1 screen to enrolment ratio respectively. The main reasons for screen outs were VL parasite negative (55%), abnormal laboratory values (12%), and refusal to consent (2%). 5% were due to other eligibility criteria such as age, recent VL treatment, concomitant conditions, pregnancy or lactating, malnutrition and undocumented reasons.

4 Conclusions

The screen to enrolment ratio was better in the phase III trial, as expected due to the usually less stringent criteria compared to phase II studies. A significant reason for screen outs was negative parasitology and laboratory abnormalities. To improve on patient enrolment in the studies, there is a need for efficient screening, increased community mobilization and attention to laboratory abnormalities. The rate of refusal to consent to participate in clinical trials was low. Further study of the specific laboratory abnormalities leading to screen outs is required.

C1891 POST-KALA-AZAR DERMAL LEISHMANIASIS (PKDL) TREATED WITH AMBULATORY SHORT COURSE AMBISOME

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1 Background

Post-Kala-Azar Dermal Leishmaniasis (PKDL) is a skin disease caused by *Leishmania donovani*, which usually appears 6 months to several years after apparently successful treatment of visceral leishmaniasis (VL). In the Indian subcontinent, PKDL occurs in 5-10% of VL patients. It rarely poses any other than cosmetic problems; however, untreated it constitutes a reservoir of infection. A safe and effective short course treatment for PKDL is a prerequisite for the elimination of VL, which currently does not exist. MSF-supported facilities have used short course liposomal amphotericin B (AmBisome®) regimens for clinically diagnosed PKDL in India and Bangladesh between 2010 and 2015.

2 Methods

A 30mg/kg AmBisome regimen was given on ambulatory basis over 3 weeks in 6 divided doses of 5mg/kg. Medical photographs were taken of each patient at baseline, and at 1, 3, 6 and 12 months after treatment. Main outcomes were the clinical assessment of lesions at 12 months compared to baseline, and safety during treatment, with an emphasis on hypokalaemia. Following safety concerns in Bangladesh, a further clinical study was initiated to assess the effectiveness and safety of a lower 15mg/kg total dose, administered in 5 doses of 3mg/kg over 3 weeks.

3 Results

A total of 271 patients, 110 in Bangladesh and 161 in India, were treated with the 30mg/kg regimen. In India, of 89 patients who completed 12 months' follow-up, 75 (84.3%) cases showed substantial or complete cure, with excellent tolerance and safety. In Bangladesh, 88 patients completed 12 months' follow up, and 70 (79.5%) showed substantial or complete cure; however 6.5% developed severe hypokalaemia.
In Bangladesh, of 272 patients that were treated with the 15 mg/kg regimen and completed 12 months follow up, 245 (90.1%) showed a significant improvement of lesions, with the majority of lesions completely resolved in 213 (78.3%) patients. In 27 (9.9%) patients lesions did not improve and new lesions had appeared in 13 (4.8%) patients. The treatment regimen proved very safe with no severe adverse effects.
Moderate (2.5-3.0 mmol/L) hypokalaemia occurred in 7 (2.5%) patients.

4 Conclusions

Short-course ambulatory AmBisome regimens may be considered as an effective and safe option for treatment of PKDL patients in the Indian subcontinent. In Bangladesh, a 15 mg/kg dose regimen proved effective and safe in the treatment of PKDL.

C1666 SAFETY AND EFFICACY OF A COMBINATION OF PAROMOMYCIN AND MILTEFOSINE FOR TWO VERSUS THREE COURSES IN PATIENTS WITH POST KALA-AZAR DERMAL LEISHMANIASIS (PKDL) - AN EXPLORATORY PILOT STUDY

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1 Background

Post Kala-Azar Dermal Leishmaniasis (PKDL) is considered as a reservoir of visceral leishmaniasis (VL). It plays an important role in disease transmission. As there is no treatment that will be 100% safe and efficacious moreover, the parasite also shows a gradual increase in tolerance and resistance. Since the combination of Paromomycin and Miltefosine has been found to be safe, highly effective and cheap, this observational pilot study was undertaken to find out whether this combination is effective in PKDL. This study will help to the program maker to make a short term treatment regimen for PKDL with minimal side effect.

This study evaluates the efficacy and safety of a novel combination, i.e. paromomycin plus miltefosine in the treatment of PKDL, which has not been tried previously.

2 Methods

This is a pilot exploratory study. Thirty patients were enrolled in two groups. Group A (n=15) received two courses of paromomycin 11mg/kg/day IM plus miltefosine 2.5 mg/kg/day orally (10 days) at a gap of 15 days between each course, whereas group B (n=15) received three courses of paromomycin plus miltefosine combination. The efficacy of these therapies were ascertained by complete resolution of signs and symptoms of PKDL and grade 0 parasitic score in the skin lesions at one year follow up.

3 Results

Lesion of the group A patients did not disappear completely at the end of therapy so all the patients were shifted to receive three courses of the same combination. All the thirty patients completed the study. Initial and Final parasitological cure was found to be 100% and 83.33% (95% confidence interval 69.5-96.5%) respectively. A total of 63 AEs were reported in 24 patients. All the AEs were CTC grade one. Pain at the site of injection (28.6%) was most commonly occurring AE and increase in hepatic enzymes level, eosinophilia, vomiting, gastritis etc. were among others.

4 Conclusions

Efficacy of this novel combination therapy appears to be optimal with excellent safety and tolerability profile. Further studies with a higher number of samples are recommended.

Session 5. Drug development: conventional and alternative. Clinical and experimental therapy in cutaneous and visceral leishmaniasis

C0640 MODELING MILTEFOSINE EXPOSURE AFTER ALLOMETRIC DOSING IN PEDIATRIC VISCERAL LEISHMANIASIS PATIENTS IN KENYA AND UGANDA

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1 Background

Conventional dosing of the oral drug miltefosine at 2.5 mg/kg/day has demonstrated lower efficacy in pediatric visceral leishmaniasis (VL) patients compared to adults, most probably due to significantly reduced drug exposure in children. To assess whether exposure in children could be increased, an open-label clinical trial was conducted in Kenya and Uganda to evaluate the pharmacokinetics (PK) of an allometric dose, in which pediatric patients received a relatively higher mg/kg dose.

2 Methods

Miltefosine plasma concentrations were measured using sparse sampling in 30 VL patients aged 4-12 receiving 2.7-3.9 mg/kg/day for 28 days. Samples were analyzed by liquid chromatography tandem mass spectrometry. A population PK model was developed using non-linear mixed effects modelling. Observed miltefosine exposure after allometric dosing was compared to PK results after conventional 2.5 mg/kg/day dosing in East-African pediatric patients, and compared to simulations with a population PK model based on this linear dosing trial.

3 Results

Miltefosine PK was best described by a two-compartmental model with a decreased relative bioavailability of -62% the first six treatment days, as is observed for conventional dosing. Plasma concentrations in the first week of treatment accumulated faster after allometric compared to linear dosing (C_{day7} 5.88 vs 2.67 µg/mL). However, stagnation in miltefosine accumulation in week 3 of treatment was observed in ~40% of patients. Total exposure increased (median $AUC_{0-\infty}$ 12%), but not dose-proportionally (median dose increased 28%), indicating unexpected non-linearity in PK. More importantly, variability in total exposure was decreased for allometric vs conventional dosing (CV% 16.3 vs 35.5%), resulting in a two-fold decreased proportion of patients who fail to attain the 17.9 µg/mL threshold previously associated with probability of cure (15 vs 29%).

4 Conclusions

This is the first population PK model describing miltefosine exposure in children after allometric dosing. Miltefosine exposure was increased, but not dose-proportionally. Exposure was notably higher in the first treatment week, despite decreased bioavailability. Due to decreased variability more patients reached the threshold previously associated with clinical cure. Both these factors might have positively contributed to the increased efficacy of this allometric miltefosine regimen and emphasize its value in the treatment of pediatric patients.

C0976 THE ASSOCIATION OF MILTEFOSINE AND PENTOXIFYLLINE TO TREAT MUCOSAL LEISHMANIASIS: A PILOT CLINICAL TRIAL IN BRAZIL

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1 Background

Mucocutaneous leishmaniasis (ML) is endemic in the central region of Brazil and other countries worldwide. The standard treatment with meglumine antimoniate has high rates of severe adverse effects and the rate of drug resistance is increasing.

2 Methods

This study consists in a randomized clinical trial to access efficacy of the association of oral miltefosine and oral pentoxifylline compared to intravenous meglumine antimoniate and oral pentoxifylline. Patients diagnosed with ML seen at the *Hospital Universitário de Brasília*, Brazil were randomized into two groups. Active control group: received 20mgSb+5/kg/day meglumine antimoniate intravenous for 28 days and pentoxifylline 20mg/kg/day up to 400mg 3 times a day for 28 days. Experimental group: received the experimental combined oral treatment: miltefosine 2,5mg/kg/day up to 50mg twice a day and pentoxifylline 20mg/kg/day up to 400mg three times a day for 28 days. Patients were monitored once a week for side effects and cure was established 90 days after treatment, based on clinical and laboratorial criteria. Patients were followed up to 1 year after treatment.

3 Results

A total of 8 patients with ages ranging from 19 to 79 years were included, 4 in each group. Five patients were male. Five patients had comorbidity such as hypertension, insulin resistance, treated cerebral astrocytoma and asthma; 3 patients in control group and 2 in experimental group. Three patients from the control group needed to interrupt treatment and change to liposomal B amphotericin due to side effects. The other patient in this group completed treatment and was cured after 90 days. Three patients in the experimental group were cured 90 days after treatment. One patient in this group needed to interrupt treatment due to liver enzyme persistent elevation, and treatment was completed with to liposomal B amphotericin.

4 Conclusions

Treatment of ML with the oral combination of miltefosine and pentoxifyline was well tolerated and had a high rate of clinical and laboratorial cure when compared to the combination of intravenous meglumine antimoniate and oral pentoxifylline. It may represent a good alternative to the increasing resistance and high toxicity of antimoniate treatments. The possibility of oral therapy may also increase patient adherence to treatment. Larger studies are needed and are being conducted by this team.

C1245 THERMOTHERAPY ALONE OR COMBINED WITH A SHORT-COURSE SYSTEMIC TREATMENT AS AN ALTERNATIVE THERAPY IN PERUVIAN CUTANEOUS LEISHMANIASIS: A CASE SERIES

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1 Background

Current guidelines for treatment of cutaneous Leishmaniasis (CL) have included thermotherapy as accepted first line option for treatment of CL both caused by old world or new world strains. Although a systematic review has recently demonstrated no difference in treatment outcomes between systemic pentavalent antimonial (S-Sb5+)and thermotherapy, experience and evidence of thermotherapy for treatment of CL in Peru is still limited as well as long-term data supporting its safety.

2 Methods

Due to S-Sb5+ sometimes cannot be administrated due to medical contraindications, here we report treatment outcomes of 43 patients treated both with thermotherapy as well as thermotherapy combined with a short course of miltefosine (21 days) or S-Sb5+ (10 days). Thermotherapy was applied in a single session using the Thermomed device. S-Sb5+ and Miltefosine were administrated using conventional dose but for a short period. All patients were followed-up for at least six months or until treatment failure was confirmed.

3 Results

Patients were mainly males (60%) with a median age of 39 years-old. They acquired the infection both in jungle and highland regions and the predominant strain was L. peruviana (58%). Individuals harbored two or less lesions (80%) with a median duration of 2 months (0.75-48). Lesions were mainly ulcerative (93%) with a median diameter of 31 mm (8-105). Eighty two percent were treatment naive and they received our treatments due to a medical contraindication for standard S-Sb5+ course (age, prolongued QTc, pregnancy, multiple prior S-Sb5+) or personal reasons that prevented the stay for 20 days in Lima. Cure was achieved in 14/19 subjects treatment with the combined therapy (73%) whereas 18/24 cured after Thermotherapy (75%). Cure rates were sustained even according infecting strain: L. braziliensis (71%), L. peruviana (64%) and L. guyanensis (100%).

4 Conclusions

Although our patients represent a very heterogeneous group and our sample size is reduced we identified that both thermotherapy alone or combined with a short course of systemic therapy has probably similar efficacy to the one widely reported for standard S-Sb5+. Cure rates were acceptable in infections caused by L. braziliensis and L. peruviana and highly efficacious in infections by L. guyanensis.

C1265 ORAL PENTOXIFYLLINE ASSOCIATED WITH PENTAVALENT ANTIMONY: A RANDOMIZED TRIAL FOR CUTANEOUS LEISHMANIASIS.

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1 Background

Cutaneous leishmaniasis (CL) by *L. braziliensis* is associated with decreasing cure rates in Brazil. Standard treatment with pentavalent antimony (Sb^v) cures only 50 to 60% of the cases. The immunopathogenesis of CL ulcer is associated with high interferon gamma (IFN-γ) and tumor necrosis factor (TNF) production. Pentoxifylline, a TNF inhibitor, have been successfully used in association with Sb^v in mucosal and cutaneous leishmaniasis.

2 Methods

A randomized, double blind and placebo controlled trial to evaluate the efficacy and safety of oral pentoxifylline plus Sb^v versus placebo plus Sb^v in patients with CL caused by *L. braziliensis* in Bahia, Brazil. A total of 164 patients were randomized in two groups to receive the combination or the monotherapy.

3 Results

Cure rate six months after treatment was 45% in the pentoxifylline group and 43% in the control group. There was also no difference between the groups regarding the healingtime (99.7 ± 66.2 days and 98.1 ± 72.7 days respectively).Adverse events were more common in the pentoxifylline group (37.8%), versus 23% in the placebo group.

4 Conclusions

Sb^v combined therapy with pentoxifylline is not more effective than Sb^v monotherapy in the treatment of CL caused by *L. braziliensis*.

C1476 IDENTIFICATION OF SNPS ASSOCIATED WITH THE THERAPEUTIC RESPONSE IN PATIENTS WITH CUTANEOUS LEISHMANIASIS.

Mariana del Socorro Rosales Chilama[1], *Julieth Murillo*[2], *Alvaro Martinez*[2], *Adriana Navas*[2], *Deninson Alejandro Vargas*[2], *Maria Claudia Barrera*[2], *Andres Castillo*[3], *María Adelaida Gómez*[4]

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1 Background

Treatment failure to first line pentavalent antimonials is as high as 40% in patients with cutaneous leishmaniasis (CL) in Colombia, risking the only available control measure. The absence of therapeutic alternatives and of predictive and prognostic tools of the outcome of treatment contribute to maintain the high rates of treatment failure. The aim of this study was to identify SNPs in immune response genes, membrane transporters and metabolic enzymes as potential biomarkers of treatment response in CL patients.

2 Methods

Candidate genes for SNP profiling were selected based on differential expression between patients with divergent therapeutic responses and of experimental evidence of functional impact on the antileishmanial effect of antimonials. SNPs were prioritized based on their relationship with other pathologies, allelic frequencies, in silico functional effects, among others. Genotyping was conducted by bidirectional sequencing of PCR amplification products on a cohort of 56 CL patients (33 responders and 23 non-responders) with a reported minimum adherence to treatment of 80%.

3 Results

Twenty-nine SNPsin *ccl2, cxcl5, cxcl10, tlr7, abcc1, abcb1, abcb6 and mt2a* genes were selected for genotyping. Among all evaluated gene variants, an association between two polymorphisms in *ccl2* and therapeutic response was established (OR: 2.55, 95% CI: 1.17-5.55 and OR: 3.08, 95% CI: 1.40-6.74). The genotype distributions met the Hardy-Weinberg equilibrium ($p > 0.05$). Combinatorial analyzes showed potential synergistic contribution between SNPs in *ccl2* (OR: 14.3, 95% CI: 1.71-119) and the therapeutic response.

4 Conclusions

This study provided the basis for identification of SNPs as biomarkers of therapeutic response in CL and for the design of subsequent validation studies.

C1552 PHASE II RANDOMIZED CLINICAL TRIAL OF THE EFFICACY AND SAFETY OF AMBISOME® IN COMBINATION WITH SODIUM STIBOGLUCONATE OR MILTEFOSINE VERSUS MILTEFOSINE MONOTHERAPY FOR AFRICAN VISCERAL LEISHMANIASIS.

MONIQUE WASUNNA[1], *Simon Njenga*[2], *Manica Balasegaram*[3], *Neal Alexander*[4], *Raymond Omollo*[5], *Tansy Edwards*[4], *Thomas Dorlo*[6], *Brima Musa*[7], *Mohammed Hassan Sharaf Ali*[7], *Mohamed Yassein*[7], *George Kirigi*[2], *Rashid Juma*[2], *Anke E. Kip*[6], *Gerard Schoone*[8], *Asrat Hailu*[9], *Joseph Olobo*[10], *Sally Ellis*[11], *Robert Kimutai*[12], *Susan Wells*[11], *Eltahir Awad Gasim Khalil*[7], *Nathalie Strub–Wourgaft*[11], *Fabiana Alves*[11], *Ahmed Musa*[7]

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1 Background

Visceral leishmaniasis, or kala-azar, is a parasitic disease which is fatal without treatment. A 17-day treatment of sodium stibogluconate (SSG) with paromomycin (PM) is the recommended treatment in eastern Africa, but requires painful injections, causes side effects, and patients need to stay in the hospital during treatment. Combination regimens including AmBisome® and miltefosine are safe and effective in India, but there are no published data from trials of combination therapies including these drugs from Africa.

2 Methods

A phase II open-label, non-comparative randomized trial was conducted in Sudan and Kenya to evaluate the efficacy and safety of three treatment regimens: 10 mg/kg single dose AmBisome® plus 10 days of SSG (20 mg/kg/day), 10 mg/kg single dose AmBisome® plus 10 days of miltefosine (2.5mg/kg/day) and miltefosine alone (2.5 mg/kg/day for 28 days). The primary endpoint was initial parasitological cure at Day 28, and secondary endpoints included definitive cure at Day 210, and pharmacokinetic (miltefosine) and pharmacodynamic assessments.

3 Results

In sequential analyses with 49-51 patients per arm, initial cure was 85% (95% CI: 73-92) in all arms. At D210, definitive cure was 87% (95% CI: 77-97) for AmBisome® + SSG, 77% (95% CI 64-90) for AmBisome® + miltefosine and 72% (95% CI 0.60-0.85) for miltefosine alone, with lower efficacy in younger, less heavy patients. Miltefosine PK data indicates under-exposure in children compared to adults.

4 Conclusions

No major safety concerns were identified, but point estimates of definitive cure were less than 90% for each regimen so none will be evaluated in Phase III trials in their current form. The results also suggest miltefosine was under-dosed in children and so allometric dosing, which takes into account the differences in drug uptake and metabolism seen in children compared to adults, needs to be evaluated.

C1584 SAFETY OF AVAILABLE TREATMENTS FOR VISCERAL LEISHMANIASIS IN BRAZIL: RESULTS FROM THE LV BRASIL TRIAL

Joelle Rode[1], *Dorcas Lamounier Costa*[2], *Carlos Henrique Nery Costa*[2], *Roque Pacheco de Almeida*[3], *Enaldo Viera de Melo*[3], *Sílvio Fernando Guimarães de Carvalho*[4], *Ana Rabello*[5], *Andréa Lucchesi de Carvalho*[6], *Anastácio de Queiroz Sousa*[7], *Robério Dias Leite*[7], *Simone Soares Lima*[2], *Thais Alves Amaral*[8], *Fabiana Piovesan Alves*[9], *Gustavo Adolfo Sierra Romero*[10]

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1 Background

In order to provide evidence for the rational use of visceral leishmaniasis (VL) treatments, a multicenter, randomized, open label, controlled trial was conducted to assess the safety and efficacy of the treatments recommended for VL in Brazil.

2 Methods

Eligible patients were randomized to standard treatment with meglumine antimoniate (MA), 20 mg Sb^{+5}/kg/day for 20 days, amphotericin B deoxycholate, 1 mg/kg/day for 14 days, liposomal amphotericin B (LAMB), 3 mg/kg/day for 7 days and a combination of liposomal amphotericin B, 10 mg/kg single dose, plus meglumine antimoniate, 20 mg Sb^{+5}/kg/day for 10 days. Safety was monitored during the whole duration of patients´ participation in the trial through clinical examination, registry of complaints, laboratory tests and ECG according to scheduled evaluation time-points. Adverse events (AE) were evaluated according to the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, version 1.0, December 2004, and AEs were classified as related or not-related to the trial medications. Higher toxicity of amphotericin B deoxycholate prompted an unplanned interim safety analysis leading to this arm being dropped. This report is based on data from the remaining three arms. ClinicalTrials.gov identification number NCT01310738.

3 Results

Among the 332 patients, a total of 1,744 AEs were recorded, of which 124 were serious adverse events (SAE). 568 (32.6%) AEs were medication-related, among them 71 SAEs. The most frequent medication-related SAEs were respectively: increase of pancreatic and/or liver enzymes (28.5%), cardiac toxicity (19%), anaemia and haematological parameter alterations (14.3%) in the MA arm; anaemia (40.9%) and other haematological parameter alterations (13.6%) in the LAMB arm; and anaemia (39.3%), increase of pancreatic enzymes (17.8%) and cardiac toxicity (14.2%) in the combination arm. Overall, the most frequent medication-related AEs were alteration of biochemistry parameters (23.6%), gastrointestinal disorders (20.4%), systemic disorders (19.5%) and haematological parameter alterations (13.9%).

4 Conclusions

LAMB intervention showed a significantly better safety profile, suggesting it would be a more suitable first line treatment for VL in Brazil. Descriptive analysis of safety results will contribute to a better VL case management and guidance for treatment recommendation adoption by health care providers.

C1625 PILOT STUDY OF SAFETY/EFFICACY OF TOPICAL LIPOSOMAL AMPHOTERICIN B FOR THE TREATMENT OF CUTANEOUS LEISHMANIASIS CAUSED BY L. MAJOR

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1 Background

Cutaneous leishmaniasis (CL) is endemic in more than 70 countries and is the most common form of leishmaniasis. Ninety percent of CL is reported from seven countries including Iran. Treatment of CL is a dilemma and solely depends on using antimoniate derivatives, which needs multiple injections as such the compliance is low and is not always effective. Amphotericin B (AmB) is insoluble in aqueous media and the traditional formulation accompanies severe toxic adverse effects, including the acute infusion related toxicities and the chronic nephrotoxicity introduces limitation in the clinical use of traditional micelle AmB. In order to reduce the adverse effects and increase tolerance to AmB; three different lipid-based formulations of AmB (Amphotec™, Abelcet™ and AmBisome™) were developed and used to treat visceral leishmaniasis. Topical treatment formulations have been developed against CL but yet none has reached to the market. In the current study Nano-liposomes containing 0.4% Amphotericin B (Nano-Amph B) was developed and used to treat CL patients.

2 Methods

Nano-Amph B was produced with support of DNDiunder GMP condition andwas tested *in vitro* and *in vivo* against *L. tropica*. Safety of the preparation was checked *in vivo* in animal model. Due to the political sanctions, Glucantime was not easily available in the country for a while, so Nano-Amph B was used to treat CL patients. The potential candidate patients were interviewed, physically examined, their lesions were checked by a dermatologist. Skin samples were collected from the patients for direct smear, culture and PCR. The patients received topical Nano-Amph B twice a day for 28 days

3 Results

Safety Nano-Amph B was tested in healthy human volunteers to be safe. Then 22 CL patients with 46 lesions caused by *L. major* were treated with Nano-Lip Amphotericin 0.4%, twice a day for 4 weeks. There was no side effect in any of the lesions or the patients. The follow up results showed that one patient did not use the medicine, 2 patients did not show up for any follow up, one patient with 4 lesions was visited only on day 42; one of his lesions was completely healed and 3 lesions showed partial cure, the patient was not seen after day 42. Eighteen patients with 36 lesions showed complete cure at day 42. The cure rate was 78%.

4 Conclusions

Nano-liposomal form of Amphotericin B 0.4% showed to be safe and the efficacy rate in a pilot study was high (78%), it worth to use this formulation in Phase 3 trial

C1588 COHORT OBSERVATIONAL STUDY TO ESTIMATE THE PROPORTION OF POST KALA-AZAR DERMAL LEISHMANIASIS (PKDL) WITH AT LEAST TWO YEARS FOLLOW UP AFTER TREATMENT WITH THREE NEW TREATMENT REGIMENS IN VL ENDEMIC DISTRICTS OF BIHAR

Vishal Goyal[1], *Vidya Nand Rabi Das*[2], *Krishna Pandey*[2], *Fabiana Alves*[3], *Suman Rijal*[4], *Pradeep Das*[2], *Jorge Alvar*[3]

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[3]*Drugs for Neglected Diseases initiative (DNDi) Geneva, Switzerland*

1 Background

DNDi conducted a field pilot implementation study between 2012 and 2015 to assess the feasibility of using three new treatment regimens to treat visceral leishmaniasis (VL) at public health facilities in Bihar: single dose AmBisome (SDA) and the combinations miltefosine with paromomycin (M+P) and AmBisome with miltefosine (A+M). As a result, the national program revised its treatment policy, recommending SDA as the 1st option and M+P as the 2nd option. The present study was conceptualized at the request of the Indian National Program to provide evidence to policy makers of the occurrence of Post Kala-Azar Dermal Leishmaniasis (PKDL) in this large cohort of VL patients treated with three new treatment modalities.

2 Methods

All VL patients (n=1761) who had previously been treated in the pilot implementation study in two districts of Bihar (Patna and Saran/Vaishali) with any of the new treatment regimens, SDA, M+P for 10 days, or A+M for 8 days, are invited to participate in the study. Ethical clearance was obtained from RMRI Ethical Committee.

After obtaining informed consent, subjects are assessed for history and clinical manifestation of PKDL at a follow-up visit at least 24 months after VL treatment. All clinically suspected PKDL cases (skin lesions with past history of VL treatment) are screened by rK39 rapid diagnostic test, and probable PKDL patients (skin lesions and rK39 positive) are referred to RMRI for parasitological confirmation by microscopy and qPCR in skin slit tissue.

3 Results

Up to 30 Oct 2016, 556 VL treated patients were screened for PKDL. 16 suspected cases were identified, of which 13 were probable PKDL cases (5 in SDA, 4 in A+M and 4 in M+P). 2 of these had been identified before the 24-months follow-up visit and had been already treated for PKDL, and parasites were not demonstrated in 5 cases. Another 6 cases had parasitological confirmation of PKDL (2 in SDA, 1 in A+M and 3 in M+P). Results of a larger sample will be presented at the conference. The proportion and timing of patients developing PKDL will be described for each new VL treatment regimen.

4 Conclusions

Preliminary results show that PKDL was observed in 1.4% of patients at least 24 months after treatment of VL. Measuring the burden of PKDL, the rates according to the drug used in the VL episode, mapping cases and getting them treated is important for the control of VL, especially given the VL elimination program target of 2017 in the Indian sub-continent.

Wednesday, 17th of May
Greco room D1-D2
9:30 – 11:00

Session 6. Genetics, taxonomy and omics. Tools and reverse genetics

C0161 THE DYNAMICS OF LATERAL GENE TRANSFER IN GENUS LEISHMANIA

Elisabet Vikeved, *Anders Backlund, Cecilia Alsmark*

Uppsala University, Sweden

1 Background

The need for new effective drugs to treat leishmaniasis, a neglected tropical disease, estimated to threaten 350 million people in 88 countries worldwide according to WHO estimates, is urgent. The genome of *Leishmania major*, one causative agent of leishmaniasis, includes a number of genes acquired by lateral gene transfer (LGT) from a prokaryote donor, and these LGTs have been suggested as potential drug targets. However the current understanding of the incorporation and fate of lateral acquired genes into eukaryote genomes is limited.

2 Methods

We have used bioinformatics tools to analyse orthologous sequences of LGTs previously confirmed in *L. major* in the sequenced genomes of *L. braziliensis L. infantum, L. mexicana*, *L. tarentolae*, and in the close relatives *Trypanosoma brucei* and *Trypanosoma cruzi.* To further evaluate the dynamics of LGTs within genus *Leishmania*, we have used a PCR-based approach to investigate ten additional strains of *Leishmania* parasites with unpublished genomes, for the presence of a subset of twenty LGTs that are unique to genus *Leishmania*. Three universal trypanosomatid LGTs were included for comparison. For these genes we have also evaluated features of amino acid and nucleotide composition, as well as phylogeny.

3 Results

We found that nearly all of the universal trypanosomatid LGTs are distributed in the entire genus *Leishmania* and they are conserved in the host genomes,whereas the LGTs unique to genus *Leishmania* display a relatively high gene loss and evolves fast. Only eleven of these twenty LGTs are uniformly distributed in genus *Leishmania*, whereas the remainder of the LGTs have undergone differential pseudogenization, degradation or loss in one or more species.

4 Conclusions

Hence, lateral gene transfer in genus *Leishmania* is a continuously dynamic process, foreign beneficial genes can be conserved along genus diversification contributing to specie differentiation and specification, but they can also degrade and eventually cease to exist. The prokaryote origin of LGTs in eukaryotic parasites motivates their potential as drug targets, however attention needs to be paid to the dynamics of LGTs while selecting such gens as drug targets.

C0192 MULTIPLEXED SPLICED-LEADER SEQUENCING: A HIGH-THROUGHPUT, SELECTIVE METHOD FOR RNA-SEQ OF LEISHMANIA

Bart Cuypers[1], *Malgorzata A. Domagalska*[1], *Géraldine De Muylder*[1], *Pieter Meysman*[2], *Manu Vanaerschot*[4], *Hideo Imamura*[1], *Thomas-Wolf Verdonckt*[1], *Peter J. Myler*[4], *Gowthaman Ramasamy*[4], *Kris Laukens*[2], *Jean-Claude Dujardin*[1]

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1 Background

Leishmania causes a wide array of diseases in its mammalian host, where it resides within mononuclear phagocytes. Transcriptome studies of these intracellular parasites are important for the development of therapeutic agents. However, host cell RNA is much more abundantly present than parasite RNA, imposing parasite isolation prior to sequencing. Interestingly, the parasite's mRNA transcripts differs from the host's by starting with a fixed 39 nucleotide sequence or spliced-leader (SL). We exploited this feature and developed a protocol (SL-seq) to specifically amplify and sequence SL-containing parasite RNA out of a pool of host cell RNA and assessed its validity, specificity and sensitivity.

2 Methods

Briefly, SL-Seq first converts *Leishmania* mRNA to cDNAusing a SL-specific primer. Amplification is carried out with overhang-extension PCR, adding additional motives for compatibility with Illumina sequencers. Unique indexes can be added to each sample, allowing multiplexing hundreds of samples on the same sequencer. We benchmarked our method by comparing results from SL-seq with those obtained with the Illumina Stranded mRNA kit (ILL-seq). Both methods were used in parallel to sequence mRNA from: 1) logarithmic (LOG) and stationary (STAT) phase *Leishmania* promastigotes, and 2) THP-1 monocytes infected with clinical *Leishmania* strains. Finally, to assess SL-seq sensitivity, we diluted parasite RNA with human RNA in a 1:100 and 1:000 ratio (*Leishmania*:human) and performed SL-seq.

3 Results

Expression changes observed between LOG and STAT promastigotes were highly correlated between SL- and ILL-seq (p<2^-16, R^2=0.78). Both methods detected 97% of the annotated genes. We successfully sequenced *Leishmania* transcriptomes from infected THP-1 cells, without prior parasite isolation. With ILL-seq only 1.6% of the data was *Leishmania* mRNA, while this was 65.0% using the SL-seq, indicating SL-seq resulted in a 42 fold enrichment of parasite mRNA. Diluting *Leishmania* mRNA with human mRNA increased aspecific amplification: 57.69% and 12.44% of the 1:100 and 1:1000 dilutions data respectively was *Leishmania* mRNA, still significantly outperforming undiluted ILL-seq.

4 Conclusions

We developed a novel method for RNA-seq in *Leishmania* called SL-Seq. This method shows high selectivity and sensitivity, and allows sequencing the parasite transcriptome directly from the infected host cell. This method might also be useful for other SL-containing organisms like trypanosomes and nematodes.

C0276 CRISPR-CAS9-MEDIATED GENOME EDITING IN LEISHMANIA

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1 Background

Given its simplicity and high efficiency, CRISPR-Cas9 has been used for genome engineering in a wide variety of organisms. In this study, we have adapted CRISPR-Cas9 genome editing technology in *Leishmania*.

2 Methods

The *L. donovani* ribosomal RNA promoter, the Hepatitis Delta Virus and Hammerhead ribozymes were used to develop various *Leishmania* gRNA and Cas9 expression vectors.

3 Results

It was revealed that *L. donovani* mainly used microhomology (3-20 nt) mediated end joining (MMEJ) to repair Cas9 nuclease created double strand DNA break (DSB). MMEJ resulted in deletion mutations ranging from 10 to more than 3000 bp. The nonhomologous end-joining (NHEJ) pathway appears to be absent in *L. donovani.* The activity of different gRNAs could vary significantly with double gene alleles inactivation frequency ranging between 0.05 to 5 % four weeks post gRNA vector transfection. Gene editing is 30 times more efficient with the ribosomal RNA promoter than with the U6 promoter to drive gRNA expression.The use of oligonucleotide and double strand DNA donors (antibiotic selection marker or GFP) with only 25 nt (bp) microhomology sequences flanking the DSB, and the dual (or multiple) gRNAs expression vector greatly improved the precision and efficiency of CRISPR mediated genome editing. They were used to generate various gene disruption mutants, single amino acid substitution mutant, GFP fusion with an endogenous gene and the precise whole gene deletion mutants. Co-selection for CRISPR-Cas9 activity by co-targeting the miltefosine transporter followed by miltefosine selection significantly enriched CRISPR edited mutants, which was used to delete all 11 copies of A2 virulence gene family, an unattainable task with the traditional gene targeting method. By simultaneously targeting sites from two different chromosomes, we generated all 4 types of targeted chromosome translocations regardless of the polycistronic transcription direction in the parents' chromosomes. A single gRNA and Cas9 co-expression CRISPR vector was also developed and used to delete genes efficiently in *L. donovani*, *L. major* and *L. mexicana*. By directly observing death of CRISPR targeted *Leishmania* cells, this CRISPR system can be effectively used to determine the essentiality of *Leishmania* genes, and we demonstrated that RAD51, a DNA recombinase involved in homology directed repair, is essential for *L. donovani*.

4 Conclusions

CRISPR-Cas9 is a powerful and versatile genome editing tool for *Leishmania*.

C0395 SELF-MATING GENERATES GENOTYPIC AND PHENOTYPIC VARIANTS IN LEISHMANIA

Ehud Inbar[1], *Jahangheer Shaik*[1], *Jeffrey Brendan*[2], *Deborah Dobson*[3], *Stephen Beverley*[3], *David Sacks*[1]

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1 Background

Leishmaniasis is caused by a large, biologically diverse genus of protozoan parasites, transmitted by a wide variety of sand fly species and associated with a range of clinical outcomes in many different mammalian hosts. In recent years, evidence for hybrid genotypes in natural populations has been accumulating, suggesting a potential role for sex in generating this diversity. We have formally demonstrated genetic exchange in *Leishmania* by recovering full genomic experimental hybrids from sand flies that were co-infected with different strains and species of *Leishmania.* However, since opportunities for outcrossing in the sand fly vector are likely to be extremely rare, we suggest that self-mating is the more relevant sexual reproductive strategy

2 Methods

To investigate the frequency and consequences of self-mating, we used two clonal lines of *L. major* Friedlin marked with different antibiotic resistance genes to co-infect a natural vector, *Phlebotomus duboscqi*, and recovered and cloned 23 double drug resistant hybrids. We also generated clonal populations from each parental line passed individually through flies. All lines were submitted to whole genome DNA- and RNA-seq and their virulence phenotypes assessed by subcutaneous injection into BALB/c mice

3 Results

Despite the virtual genetic identity between the parents, the crosses yielded progeny that were genotypically divergent from the parents and from each other. Loss of heterozygosity was observed in the progeny at a frequency consistent with Mendelian inheritance and/or homologous recombination. Importantly, we observed significantly more changes in ploidy and somy levels in the sexual products compared to the clonal populations, and these changes were directly correlated with the transcriptomic profiles in the progeny. Employing a virulence test in BALB/c mice, many of the hybrids exhibited faster lesion development than either parent, a phenotype that was only obtained by self-mating. We are currently exploring possible associations between the hyper-virulent phenotype and the genomic variability that was generated by self-mating

4 Conclusions

We have experimentally demonstrated the role of self-mating in producing significant genomic and transcriptomic variations in the parasite, as well as altering its virulence capacity. Since self-mating as opposed to outcrossing can potentially occur during normal cyclical development, we suggest that self-mating has an important role in contributing to the remarkable diversity of the genus

C1637 GENOMIC ANALYSIS OF INTRA-SPECIFIC HYBRIDS AND ANCESTOR-LIKE DONORS AMONG NATURAL ETHIOPIAN ISOLATES OFLEISHMANIA DONOVANI, AND THE EXPERIMENTAL PRODUCTION OF ABUNDANT, VIABLE L. DONOVANI HYBRIDS IN TWO SAND FLY SPECIES

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1 Background

Visceral leishmaniasis is endemic in Ethiopia where it has also been responsible for fatal epidemics. It is postulated that genetic exchange in *Leishmania* (Kinetoplastida: Trypanosomatidae) has implications for heterosis (hybrid vigour), spread of virulent strains, resistance to chemotherapeutics, and exploitation of different hosts and vectors.

2 Methods

Here we perform whole genome sequencing of 11 Ethiopian *Leishmania donovani* isolates from humans consisting of seven putative parent-like and four putative hybrid like genotypes.
L. donovani strains from the same region of Ethiopia were transformed to carry green (eGFP) and red (dsRFP) fluorescence and drug resistance markers. Co-infection of sand flies with red and green transgenic strains produced abundant proven ('yellow') hybrid clones, which were viable and thrived *in vitro*.

3 Results

128,000 SNPs were identified over 30MB with a total of 50,000 parent-distinguishing SNPs, where all samples of one parent are homozygous for one allele and all samples of the other parent homozygous for one different allele. Results indicate that five isolates, including one isolate previously thought to be parent- like, are genetic hybrids. Hybrids possessed heterozygous regions from both parents as well as heterozygous blocks, which were analysed for SNP distribution, recombination hotspots, and estimates of recombination frequency. There was substantial genetic distance between most putative hybrids, which could suggest independent recombination events or historical divergence. Most isolates possessed a somy between 2 and 4 at the level of individual chromosomes. However large differences in somy were observed between two phylogenetically similar hybrid isolates, which may be indicative of a genome duplication or genome reduction following non-classical meiotic hybridisation.

4 Conclusions

The likely parental origins, frequecy, mechanisms and epidemiological implications of hybridisation are discussed.

C1655 ASSEMBLY AND COMPARISON OF THE L. NAIFFI AND L. GUYANENSIS GENOMES ISOLATED FROM DOGS PROVIDES INSIGHTS INTO THE EVOLUTION OF THE VIANNIA SUBGENUS

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1 Background

In many cases, leishmaniasis is a zoonosis - 12 of the 21 human infecting *Leishmania* spp.are also capable of infecting domestic dogs(*Canis familiaris*)and causing canine leishmaniasis (CanL). CanL is caused by *L. infantum* (syn. *L. chagasi*) in the Old World and mainly *L. donovani (infantum)* and *L. (Viannia) braziliensis* in the New World. In Colombia, dogs are *reservoirs of L. braziliensis and L. panamensis/L. guyanensis as well as L. infantum. Leishmania* DNA was sampled from two dogs with the cutaneous form of CanL in Colombia in 1985/86 and whole genome sequenced. Genome assembly of the short-read data and subsequent phylogenomic analysis identified the species as *L. guyanensis* and *L. naiffi.*

2 Methods

Read data for *L. guyanensis* MCAN/CO/1985/CL085 and *L. naiffi* MCAN/CO/1986/CL223 were assembled to chromosome level from Illumina short reads using a mixture of *de novo* assembly and reference based alignment with *L. braziliensis* M2904 as a reference genome. Annotation was performed using the Companion webserver.

3 Results

The genomes were identified as *L. guyanensis* CL085 and *L. naiffi,* using a phylogenomic approach. Over 8,000 genes were annotated on each genome, in common with other *Leishmania* spp. assembled to date. *L. naiffi* had not previously been reported to cause disease in dogs or humans in Colombia, making this the first report of this species in Colombia. Comparisons of these genomes with other *Viannia* genomes, revealed the presence of a minichromosome in an unassembled *L. shawi* sample and a 45 kb amplification common to many *Viannia* genomes including *L. naiffi* and *L. guyanensis*. High copy number of TATE transposons, a *Vianni*a specific feature, was found in *L. guyanensis* CL085 and high copy number of leishmanolysin, a putative virulence factor, was found in *L. naiffi* CL223. Few genes specific to *L. naiffi* and *L. guyanensis* or restricted to the *Viannia* subgenus were discovered. Both species exhibited mosaic aneuploidy and had expanded gene arrays.

4 Conclusions

Our results demonstrate that genome sequencing and assembly of short-read data has an important role to play in disease surveillance and the characterisation of *Leishmania* spp. and subgenera. This study reports for the first time, to our knowledge, both the presence of *L. naiffi* in Colombia and *L. naiffi* as an etiologic agent of cutaneous CanL. It also adds to our understanding of the *Viannia* subgenus, by contributing the annotated genomes of *L. guyanensis* and *L. naiffi*, which will aid future work in this area.

C1896 GENOME INSTABILITY AND GENETIC DIVERSITY - HOW TO ADAPT IN THE ABSENCE OF SEX IN THE PROTOZOAN PATHOGEN LEISHMANIA DONOVANI

Pablo Prieto Barja[1], *Pascale Pescher*[2], *Giovanni Bussotti*[3], *Frank Dumetz*[4], *Hideo Imamura*[4], *Darek Kedra*[5], *V Chaumeau*[6], *Heinz Himmelbauer*[7], *M Pages*[8], *Yvon Sterkers*[8], *Jean Claude Dujardin*[9], *Cedric Notredame*[10], *Gerald Spaeth*[2]

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1 Background

The process through which L. Donovani adapts to environmental is poorly understood. During its life cycle itundergoes a major developmental transitions. Itcan also adapt to a variety of unpredictable fluctuations inside its human host during infection progression. As a result parasites selected for higher fitness, impact the disease and can give rise to drug resistance.Aneuploidy has emerged as an important driver in evolutionary adaptation of fungal pathogens, but beyond its effect on gene count, it can also impact it's response through the selection of beneficial alleles. Yet, the role of aneuploidy on allelic selection is as well poorly understood.

2 Methods

We applied HTseq analysis on the protozoan parasite Leishmania donovani. We have revisited genotype and haplotype variations derived from genomewide aneuploidies events ocurring in two datasets. One obtained from a large-scale study of 204 field isolates previously published, together with new passages of a hybrid species LD1S. In the former longitudinal analysis for which both DNA and RNA were ultimately obtained and correlated using Htseq together with phenotypic measurements such as infectivity and cell count. Inspection and confirmation of chromosome wide aneuploidies were confirmed by FISH.

3 Results

We show that beneficial haplotype combinations in a given parasite population arise independently in a polyclonal fashion. We observed stable co-existence of subpopulations that share common driver aneuploidies but maintain significant differences in karyotypes and haplotypes thus providing the genetic substrate for new populations to be rapidly founded in response to environmental change. Second, we reveal aneuploidy as a mechanism to generate genetic diversity on an evolutionary scale through the accumulation of polymorphisms in super-numerous chromosomes.

4 Conclusions

From our work emerges a novel strategythatuses genomic instability as a substitute for gene regulation. This process relies on the dynamics of aneuploidy to modulate transcriptome and the ability to change its genetic diversity and to explore combinations of karyotypes and haplotypes in order to increase its fitness. This mechanism has important implications inpathogenesis as it may explain the phenotypic variability observed infield isolates with respect to drug susceptibility, virulence, and tissue tropism. Karyotype and haplotype selection likely represents a new bet hedging strategy with broad relevance to other aneuploidy prone microbial pathogens.

C0295 INCORPORATING DRUG RESISTANCE STUDIES IN THE QUEST FOR NOVEL ANTILEISHMANIAL AGENTS

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1 Background

Chemotherapy of visceral leishmaniasis (VL) is threatened by growing drug resistance (DR) and treatment failure. Resistance has rendered antimonials (SSG) obsolete in the Indian Sub-Continent (ISC) and miltefosine's (MIL) efficacy is decreasing. New chemotherapeutic options are needed and novel compounds are being introduced like the 'Leishbox'[a]. We recently recommended embedding DR-studies in the drug development pipeline[b], by (i) validating novel compounds' activity on recent clinical isolates with DR and (ii) generating experimental DR to promising compounds at an early stage of development, to further optimize them and monitor clinical trials. In the present study, we explored the implementation of this recommendation.

2 Methods

In this academic/industrial collaboration, two approaches were used. First, the Leishbox compounds were screened against one SSG-sensitive and one SSG-resistant strain of *L. donovani* recentlyisolated from ISC patients, using an intracellular assay of *L. donovani*-infected THP1-derived macrophages. Then, results were compared with those originally observed with *LdBob*, an East African strain. Secondly, stepwise resistance selection was performed on an SSG-sensitive clinical isolate of *L. donovani*, using increasing concentrations of an advanced Leishbox compound and MIL as positive control.

3 Results

Almost 25% of the compounds active against *LdBob* showed inactivity against the clinical isolates. Differences in activity were also observed depending on the SSG susceptibility. Currently, after 7 rounds of drug dose increments, the promastigotes have achieved significant resistance to MIL while the IC_{50} has only increased 4 folds with the Leishbox compound.

4 Conclusions

In conclusion, we demonstrate the potential value of including clinical isolates (including resistant strains) in HTS progression cascade.. Resistance selection will help understand the compounds' mode of action, hereby assisting drug combinations or novel drug design.

This research is funded by the European Union's H2020 Programme under the MSCA GA nº 642609.

References: [a] Peña et al., 2015, Sci. Rep. 5: 8771; [b] Hefnawy et al., 2016, Trends in Parasitology, in press

Miradero Room

Session 7. Drug development: conventional and alternative. Clinical and experimental therapy in cutaneous and visceral leishmaniasis

C0200 DETC-BASED BACTERIAL CELLULOSE BIO-CURATIVES FOR TOPICAL TREATMENT OF CUTANEOUS LEISHMANIASIS CAUSED BY L. BRAZILIENSIS

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1 Background

The treatment of leishmaniasis still relies on drugs with potentially serious adverse effects. Dysregulation of the Superoxide Dismutase 1 (SOD1) axis has been identified as key problem in CL and SOD1 plasma levels can predict therapeutic failure in CL caused by *L. braziliensis*. Herein, we tested a topical formulation of biocellulose (BC) membranes containing Diethyldithiocarbamate (DETC), a SOD1 inhibitor, as an additional treatment to CL.

2 Methods

Macrophages were infected with *L. braziliensis* and exposed to BC-DETC to cytotoxicity and leishmanicidal effect evaluation. Additionally, *L. braziliensis*-infected BALB/c mice were topically treated with BC, BC-DETC or BC-DETC plus pentavalent antimonial (Sb^v) and the parasite load and lesion development were assessed. Human macrophages were differentiated from healthy donors blood and the leishmanicidal property of BC-DETC was verified.

3 Results

Leishmania-infected macrophages treatment resulted in parasite killing, without pronounced toxic effects to host cells. This outcome was associated with lower SOD1 activity and higher production of superoxide and cytokine mediators. Topical application of BC-DETC *in vivo* significantly decreased lesion size, parasite load and the inflammatory response at the infection site, as well as the production of both IFN-γ and TNF. Combination of topical BC-DETC plus intraperitoneal Sb^v also significantly reduced disease development and parasite load. The leishmanicidal effect of BC-DETC was extended to human macrophages infected with *L. braziliensis*, highlighting the feasibility of BC-DETC as a topical formulation for chemotherapy of cutaneous leishmaniasis caused by *L. braziliensis*.

4 Conclusions

BC-DETC topical bio-curative is an effective and translatable addition to the existing chemotherapies currently available for CL. A proof of concept clinical trial is under way in an endemic area to CL caused by *L. braziliensis* to evaluate the efficacy of BC-DETC.

Source of funding: CNPq, FIOCRUZ, FAPESP and CAPES.

C0305 URSOLIC ACID DISPLAYS LEISHMANICIDAL EFFECT AND MODULATES IMMUNE RESPONSE IN EXPERIMENTAL VISCERAL LEISHMANIASIS

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1 Background

In the New World, visceral leishmaniasis (VL) is caused by *Leishmania infantum* with an incidence of 1.9 cases per 100,000 inhabitants and a 90% mortality rate if not properly treated. Although glucantime and amphotericin B are the standard drugs used in treatment, they induce serious side effects in the patients, justifying the search for new leishmanicidal drugs. In this regard, ursolic acid (UA) seems to be an interesting molecule, because different works showed its action against *Leishmania* species mainly in *in vitro* studies. Thus, this work aimed at analyze the leishmanicidal effect of UA in experimental VL and its impact on immune system of infected animals.

2 Methods

UA was isolated from the leaves of *Baccharis uncinella* (Asteraceae) by chromatographic methods and identified by NMR and MS. *Mesocricetus auratus* were intraperitoneally infected with promastigotes of *L. infantum*, after 60 days animals were treated with 1.0 or 2.0 mg/kg of UA as well as the standard drug amphotericin B (5.0 mg/kg) daily, for 15 days. Fifteen days after the last injection, splenic and liver parasitisms were quantified, and the expression of IFN-γ, IL-4 and IL-10 mRNA were analyzed in spleen of experimental animals. In addition iNOS production was evaluated in spleen and liver by immunohistochemistry. Toxicity of UA was evaluated in hamsters, by analyzing creatinine, urea, AST and ALT; as well as changes in kidney, spleen, liver, heart and lung

3 Results

Animals treated with UA had fewer parasites in the spleen and liver in comparison with untreated animals. This low parasitism correlates with the increased accumulation of IFN-γ mRNA observed in the spleen, and with the production of iNOS in spleen and liver. UA was not toxic for gold hamster, since seric levels of creatinine, urea, AST and ALT were similar among groups, and changes in histology of internal organs were not observed. By contrast, groups treated with amphotericin B increased the levels of creatinine, and histological changes in kidney were observed.

4 Conclusions

These findings indicate that UA has a good activity in eliminating tissue amastigotes and in inducing with a pro-inflammatory immuneresponse. Therefore, UA is an interesting natural compound for *in vivo* treatment of VL that should be considered for the development of prototype drugs against this parasitic disease. **Funding:** São Paulo Research Foundation (2013/16297-2 and 2016/00468-0), LIM-HCFMUSP.

C1274 IN VITRO ACTIVITY OF 1H-PHENALEN-1-ONE DERIVATIVES AGAINST LEISHMANIA SPP. AND ITS MECHANISMS OF CELL DEATH.

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1 Background

The current available leishmanicidal treatments include pentavalent antimonials, amphotericin B, miltefosine, paramomicine and pentamidine. These drugs are normally highly toxic to the patient even at low doses. Nevertheless, the appearance of resistant strains to these active compounds is raising as a main problem in the current therapeutic measures against these parasites. With no immediate prospect of vaccines, there is a great need to develop new leishmanicidal agents with an acceptable efficacy and safety profile. 1H-Phenalen-1-one (PH) containning compounds have been isolated from plants and fungi, where they play a crucial role in the defense mechanism of plants. Natural as well as substituted PHs exhibit a diverse range of biological activities against fungi (Fusarium oxysporum, Mycosphaerella fijiensis), protozoan parasites (Leishmania donovani, L. amazonensis, L. infantum, Plasmodium falciparum) or human cancer cells. New substituted PHs have been tested in this study.

2 Methods

The sensitivity and activity of the PH was tested in vitro against the promastigote and the intramacrophage amastigote stage of L. amazonensis using a colorimetric assay based on Alamar Blue® reagent. For the evaluation of drug-induced cytotoxicity effects was used a commercial kit based on the measurement of lactate dehydrogenase (LDH) activity released to the media. Assays to measure the mitochondrial membrane potential using JC-1, or the phosphatidylserine (PS) externalization using annexine v, were performed in order to determine the type of induced cell death after incubation with P. Also we evaluate the membrane permeability and the ATP level after treatment.

3 Results

These compounds showed a significant dose-dependent inhibition effect on the proliferation of the parasites, with IC50 values up to 1 µM for the promastigote stage of both strains, and 0.6 µM for the intramacrophage stage. The majority of the PHs showed low cytotoxic effect and high Selectivity Index. Moreover, PH derivatives induced externalization of PS as well as a decrease in the mitochondrial membrane potential in both strains of Leishmania. The ATP level of the parasites were not altered after the treatment with some PHs, in addition the cytoplasmic membrane did not experience strong alterations.

4 Conclusions

In conclusion, substituted PHs induce an apoptosis-*like* process on *Leishmania* at very low doses, avoiding an unnecessary immune response, and considering PHs as a good compounds for further studies

C0864 IN VITRO AND IN VIVO ANTILEISHMANIAL ACTIVITY OF ALYEYUBA® AND POTENTIAL UTILITY IN THE TREATMENT OF UNCOMPLICATED CUTANEOUS LEISHMANIASIS

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1 Background

Phytotherapeutics are extracted from medicinal plants and their active ingredients may relieve symptoms and even cure diseases, though they occasionally present adverse effects. Because 80% of the world population use traditional medicine and plant derived materials, the World Health Organization has prioritized the validation of traditional medicine products. Alyeyuba® is a mixture of *Caesalpinia spinose* gum, *Plantago major* leaf extract and *Calendula officinalis* flower extract which is used in the management of ulcers for its astringent, antiseptic, anti-inflammatory and healing properties.

2 Methods

The antileishmanial activity and cytotoxicity of Alyeyuba® was determined by *in vitro* and *in vivo* studies. A preliminary evaluation of the therapeutic potential of Alyeyuba® (lotion and cream) as antileishmanial treatment in humans was done in five patients with uncomplicated cutaneous leishmaniasis (CL).

3 Results

Alyeyuba® showed moderate *in vitro* activity against amastigotes of *L. panamensis* and no cytotoxicity in U-937 macrophages. Five of 6 hamsters (83.3%) infected by *L. braziliensis* treated with Alyeyuba® cream and 6/6 hamsters (100%) treated with Alyeyuba® lotion twice a day during 28 days cured at the end of the study. Indeed, the five patients with uncomplicated CL caused by *L. panamensis* treated with a combination of cream (twice a day) and lotion (three times a day) during 3 weeks showed complete cure after 45 days of treatment ended without adverse reactions. All patients remain cured after have finished the treatment.

4 Conclusions

Alyeyuba® may represent an option for treating uncomplicated CL and therefore will be an opportunity to evaluate the efficacy and safety of this phytotherapeutic by randomized controlled trials to have an alternative for the treatment of CL.

C1139 EVALUATION OF HISTOPATHOLOGICAL CHANGES AND THERAPEUTIC EFFICACY IN MICE INFECTED WITH LEISHMANIA INFANTUM TREATED WITH A MIXED FORMULATION OF CONVENTIONAL AND PEGYLATED LIPOSOMES CONTAINING MEGLUMINE ANTIMONIATE (GLUCANTIME®)

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1 Background

Pentavalent antimonial has been the first choice treatment for visceral leishmaniasis in Brazil. However, it has several side effects that leads to low adherence to treatment. In this context, the use of liposome-encapsulated meglumine antimoniate (MA) arise as an important strategy for chemotherapy enhancement.

2 Methods

The aim of this study was evaluate the histopathological changes and therapeutic efficacy after treatment with a mixed formulation of a conventional and pegylated liposome containing MA in BALB/c mice. The mice were infected intravenously with $1x10^7$ promastigotes of *L. infantum* and a single dose treatment regimen was performed. The experimental groups (n = 12) were divided as follows: 1) Control [PBS]; 2) Empty Liposomes [Empty Lipo]; 3) Free MA, 20 mg/kg [MA]; 4) Liposomal formulation of MA (20 mg/kg) consisting of mixed conventional and pegylated liposomes [Lipo MA]. The treatment regimens were administered 14 days post-infection (d.p.i) and the euthanasia was performed 28 d.p.i.

3 Results

Histopathological analyses of the hepatic tissue demonstrated that animals treated with MA and Lipo MA, showed a significant decrease in the quantitative evaluation of inflammatory cells compared to control group. Analysis of the inflammatory process showed that animal of group PBS exhibited moderate inflammatory granulomatous a typical pattern, with exudate cells that are organized in the form of aggregates circumscribed epithelioid cells and lymphocytes. Animals treated groups with Empty Lipo, MA and Lipo MA showed discreate inflammatory process with focal accumulation of mononuclear cells. The therapeutic efficacy was evaluated by qPCR in spleen and we observed a positivity of 41.7%, 50.0%, 25.0%, and 0.0% in the PBS, Empty Lipo, MA and Lipo MA groups, respectively. In the liver, the percentages were 83.3%, 100.0%, 83.3%, and 41.4%, respectively to the PBS, Empty Lipo, MA and Lipo MA groups. Thus, Lipo MA group had a significant decrease of parasite burden in the spleen and liver compared to the other groups.

4 Conclusions

Given the absence of a liposomal formulation with meglumine antimoniate (Glucantime®) in the market, our results demonstrated that the Lipo MA is a promising antileishmanial formulation which decreases the inflammatory response in the liver (target organ) accompanied by a significant reduction of the parasite burden in the spleen and liver of treated mice.

C1309 IMMUNOTHERAPY BY USE OF PCDNA ENCODING IP-10 IN BALB/C MICE INFECTED WITH L. MAJOR

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1 Background

Interferon gamma-induced protein 10 (IP-10) also known as C-X-C motif chemokine 10 (CXCL10) is one of the most important cytokine in the immune system in favor of Th1 response. Today there are interesting efforts to employ IP-10 to combat against leishmaniasis as an immunotherapy tool. Because of the IP-10 potency in inducing interferon gamma (IFN-γ), it seems this chemokine can overcome to *leishmania* through nitric oxide (NO) production resulting from IFN-γ activation as a powerful tool for parasite elimination. So we decided to explore the plasmid encoding IP-10 (pcDNA-IP10) in the treatment of susceptible mouse model after infection with *L. major*.

2 Methods

The potency of therapeutic effects of pcDNA-IP10, were evaluated in BALB/c mice infected with *L. major.* For comparison, a DNA plasmid without any manipulation (wild type) as a control group was investigated in parallel. Finally the efficacy of intervention was examined at 3 weeks after treatment and have been compared to before treatment. For obtaining a valid result and valuation, different parameters were tested, including parasite burden (PB), NO and cytokine assay including IFN-γ, interleukin 4 (IL-4), and interleukin 6 (IL-6) as well as arginase activity assay.

3 Results

After analysis the result, it was found that the number of parasites (PB) was decreased after the intervention as compared to before treatment. The level of NO also is increased as a result this intervention. The arginase activity also shown significant decreasing in treated mice. About cytokine assessment, significant increase of of IFN-γ and reduction of IL-4 and IL-6 was observed ($p < 0.05$)

4 Conclusions

Although not expected much, it can be concluded from these results that naked DNA as DNA plasmid containing IP-10 is a powerful tool against *leishmania* infection, especially in terms of parasite load decreasing lymph nodes of infected BALB/c mice and its effect on NO production. In an overall view, we can conclude that pcNDA encoding IP-10 may represent a promising therapeutic approaches to improve cutaneous leishmaniasis and presumably with a few modifications the better outcome is possible.

C1687 ORAL ADMINISTRATION OF 18-METHOXYCORONARIDINE (18-MC) SHOWED TO BE EFFICIENT AGAINST EXPERIMENTAL CUTANEOUS LEISHMANIASIS DUE TO LEISHMANIA (L.) AMAZONENSIS IN THE MONKEY CHLOROCEBUS AETHIOPS (PRIMATES: CEBIDAE)

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1 Background

Leishmaniasis still remains a health problem affecting more than 1.5 million people yearly around the world. Cutaneous forms have worldwide incidence of 0.7 to 1.2 million cases per year and in Brazil is possible to observe all forms of the disease: localized (LCL), mucocutaneous (MCL), borderline disseminated (BDCL), and anergic diffuse (ADCL). 18-MethoxyCoronaridine (MC) is an indole alkaloid investigated due to its anti-addictive properties and leishmanicidal effect also. *Leishmania (L.) amazonensis* is the most important causative agent of ADCL in Brazil, and this species has been used for our group since the first *in vitro* studies. Non-human primate models are excellent source of information in drug development program due to their phylogenetic proximity with human beings.

2 Methods

16 young specimensof *Chlorocebus aethiops* monkeys (9 male and 7 female) were intradermal inoculated with 10^6 cultured stationary-phase promastigotes of *L. (L.) amazonensis* (IFLA/BR/2015/Bragança, Pará State, Brazil), fresh isolated from the phlebotomine vector, *Lutzomyia flaviscutellata*, in five different points on the dorsal surface of the tail. Animals were then randomized and distributed into four groups for oral treatment started one month P.I.: Control – untreated; 30mg/kg of 18-MC for 28 days; 90mg/Kg of 18-MC for 10 days and 90mg/kg of 18-MC for 28 days.

3 Results

Results suggest a strong and robust dose-dependent effect of 18-MC in the lesion area reduction, especially after 30 and 90 treatments for 28 days, inhibiting 67 and 98% the lesion area at the end of the experiment (5 months P.I.). Moreover, when compared to controls lesions the treated animals exhibited better re-epithelization and higher number of cured lesions per animals with no or less satellite lesions.

4 Conclusions

Taking together, these results reinforce 18-MC as an important candidate for leishmaniasis treatment, especially for cutaneous forms.

C1694 TRANSLATIONAL PHARMACOKINETIC-PHARMACODYNAMIC MODELING FRAMEWORK TO ASSESS THE PREDICTIVE PERFORMANCE OF DIFFERENT PRECLINICAL VISCERAL LEISHMANIASIS ANIMAL MODELS

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1 Background

Efficacy of novel compounds against visceral leishmaniasis (VL) is typically assessed by reduction of splenic or hepatic intra-macrophageal *Leishmania* parasite burden in hamster or mouse infection models. Selection of adequate clinical dosing regimens based on these experiments is difficult, since the predictive value of these models remains unassessed. The aim of this study was to develop a translational framework to incorporate and model available preclinical pharmacokinetic (PK) and pharmacodynamic (PD) data of oral miltefosine, derive appropriate PKPD targets, and assess their predictive performance by comparing PK target attainment in human VL patients.

2 Methods

First, a population PK model for miltefosine was developed on PK data from healthy hamsters and mice. Animals were treated with various single and multiple (5 day) oral dose regimens between 5 and 40 mg/kg/day. Subsequently concentration-time curves and secondary PK parameters for both free and total concentrations were simulated for *Leishmania* infected animals in various early curative studies. PD endpoint in these studies was Leishman-Donovan Unit (LDU) in liver/spleen at various days post-treatment, depending on animal species. Exposure-response curves were fitted using 4-parameter log-logistic models. PK analyses and simulations were performed in NONMEM 7.3 and PKPD using the 'drc'-package in R.

3 Results

Developed miltefosine compartmental PK models adequately described the mouse and hamster PK data respectively, with various non-linearities characterized. Exposure-response curves for various PK parameters ($AUC_{0\text{-}14d}$, Time>IC_{50}, etc.) for both total and free miltefosine were successfully fitted. Various PK targets corresponding with 50%, 95% and 99% reduction of the parasite burden were calculated for each PKPD relationship. The probability of PK target attainment in human patients, accounting for species differences in protein binding, were compared to clinical outcome. In hamster, free $AUC_{0\text{-}14d}$associated with 99% LDU reduction in hamster/mouse corresponded best with the observed fraction of human patients reaching this target in relation to clinical outcome.

4 Conclusions

For the first time PKPD relationships were quantified in *Leishmania*-infected hamsters and mice. The translational framework may be a valuable tool to establish the best preclinical model and targets for drug discovery for leishmaniasis and help in the design of future first-in-human clinical trials.

Greco Room D1-D2

Session 8. Host-parasite cellular and molecular interactions

C0296 VESICLE SECRETION FROM THE LEISHMANIA FLAGELLUM AS A POTENTIAL MECHANISM OF VIRULENCE FACTOR DELIVERY

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1 Background

Upon injection into the mammalian host by the sandfly promastigote-form parasites are phagocytosed by macrophages, where they differentiate into amastigotes. Although many virulence factors are known to modulate macrophage signalling pathways to favour infection, the delivery mechanisms are largely unknown. During differentiation to amastigotes the promastigote flagellum shortens dramatically and the fate of the excess flagellar membrane is unknown. Here we investigate the possibility that during *Leishmania* differentiation, shedding of the flagellar membrane is a source of extracellular vesicles (EVs) which provide a virulence factor delivery mechanism.

2 Methods

Ultracentrifugation was used to isolate EVs from a differentiating parasite culture (DIF) or a control promastigote parasite culture (PRO). Isolated EVs were analysed by label-free protein mass spectrometry to identify proteins enriched in the DIF sample. To study the biological effects of EVs, human macrophages were exposed to isolated EVs or live promastigotes and cytokine secretion was quantified by ELISA. CRISPR-Cas9 gene editing was used to produce an *LPG1* null mutant to assess the contribution of lipophosphoglycan (LPG) to the observed effects.

3 Results

Mass spectrometric analysis showed that known virulence factors and EV-associated proteins as well as many uncharacterised proteins were enriched in the DIF sample. The proteomic datasets contained proteins with a range of known localisations, including flagellar-localised proteins. Work is ongoing to confirm whether these proteins are EV-associated. Human macrophages exposed to EVs or live promastigotes showed reduced secretion of CXCL1, CXCL10, CXCL11 and IFNγ. This suppressive effect was less pronounced in response to *LPG1* null cells and EVs and was fully restored when an ectopic copy of LPG1 was re-introduced into the mutant.

4 Conclusions

Known protein virulence factors and LPG are present in *Leishmania* EV fractions as well as known flagellar proteins.Isolated EVs and live promastigotes induce changes in cytokine secretion by human macrophages, including reductions in chemokines CXCL1, CXCL10 and CXCL11 and pro-inflammatory IFNγ. LPG contributes to the effects observed on cytokine production, and EVs may be an important delivery mechanism for LPG.

C0510 THE D2 SUBUNIT OF MACROPHAGE VACUOLAR ATPASES CONTROLS THE BIOGENESIS OF LARGE LEISHMANIA AMAZONENSIS PARASITOPHOROUS VACUOLES THROUGH REGULATION OF CHOLESTEROL EFFLUX.

Carina Carraro Pessoa[1], *Luiza de Campos Reis*[1], *Erica Valadares de Castro Levatti*[1], *Vanessa Gonçalves Pereira*[1], *Joyce Umbelino da Silva Yamamoto*[1], *Eduardo Milton Ramos Sanchez*[1], *Vânia D Almeida*[1], *Anita Hilda Straus*[1], *Hiro Goto*[1], *Renato Arruda Mortara*[1], *Fernando Real*[2]

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[2]*Cochin Institute France*

1 Background

Vacuolar ATPases (v-ATPases) are proton pumps present in the membrane of intracellular organelles including *Leishmania* parasitophorous vacuoles (PVs). v-ATPases are composed of subunits arranged in two domains (V1 and V0), mainly regarded as regulators of organelle acidification. Some subunits present different isoforms depending on cell lineage, controlling additional v-ATPase functions. The d subunit (domain V0) presents an isoform d2 that regulates membrane fusion in osteoclast formation and is up-regulated in macrophages infected with *Leishmania amazonensis*. Considering that this *Leishmania* species develops within large and fusogenic PVs which importance in the parasite intracellular life cycle is not clear, we investigate the participation of this v-ATPase subunit in the biogenesis of *L. amazonensis* PVs.

2 Methods

RAW 264.7 macrophages were silenced for d2 (d2-KD) and infected by *L. amazonensis.* Expression of isoform d2 was assessed by quantitative PCR and the morphological and biochemical development of PVs were assessed by multidimensional microscopy, mass spectrometry of lipids and enzymology.

3 Results

The d2 isoform (ATP6V0d2) is downregulated in macrophages activated with interferon-gamma and lipopolysaccharide. *L. amazonensis* infection reverts this d2 downregulation, suggesting that induction of d2 expression by the parasite counteracts the participation of v-ATPases in macrophage response against *Leishmania*. Although silencing d2 do not interfere in amastigote multiplication, *L. amazonensis* failed to develop large PVs in d2-KD macrophages, demonstrating the requirement of d2 for the biogenesis of large *L. amazonensis* PVs. d2-KD macrophages presented a three-fold depletion in cholesterol levels and an exogenous replenishment of cholesterol (via ox-LDL) restored PV enlargement despite d2 silencing, demonstrating that d2 controls the biogenesis of *L. amazonensis* PVs by regulating cholesterol efflux.

4 Conclusions

L. amazonensis modulates the function of v-ATPases (by induction of ATP6V0d2 expression) favoring the biogenesis of large PVs. Isoform d2 participates in building *L. amazonensis* PVs via cholesterol metabolism, by mechanisms currently under investigation. This study highlights molecular and biochemical mechanisms of *Leishmania* PV biogenesis that could help design improved drugs, especially liposomal drug delivery systems, to be employed in the treatment of leishmaniasis.

C0700 THE ROLE OF ABCG1 IN THE STEROL COMPOSITION AND IN THE SUSCEPTIBILITY TO AZOLES IN LEISHMANIA AMAZONENSIS

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[3]*Université Laval Canada*

1 Background

Trypanosomatids have their own machinery for sterol synthesis, productin the ergosterol. However, a high percentage of exogenous cholesterol is found in *Leishmania* spp. Transport and intracellular distribution of cholesterol and other sterols in their free form in *Leishmania* spp. is not clear. The ABCG1 transporter is implied with translocation of cholesterol and phospholipids in mammalian cells. The genome of *Leishmania* spp. has 42 genes belonging to ABC family. ABCG1 is in the genome of *Leishmania* spp., but its function has not been described.This project aims to study the role of ABCG1 in the maintenance of the sterol homeostasis in *Leishmania amazonensis,* correlating these findings to sensitivity to inhibitors of ergosterol biosynthesis.

2 Methods

To generate overexpressing parasites, ABCG1 gene was cloned in the pGEMT vector using *E. coli* DH5α competent bacteria, subcloned in PSP72αNEOα vector and transfected in *L. amazonensis*. Gene sequencing of pGEMT+ABCG1 and qPCR of gene expression were performed. Posteriorly, we evaluated the resistance and susceptibility to inhibitors of the esterol biosynthesis pathway (ketoconazole, terbinafine, simvastatine) and the reference drugs (miltefosine, amphotericin B, SbIII). From these results we evaluated the composition of the sterols by Thin Layer Chromatography (*TLC)*, incubating the promastigotes with different concentrations of ketoconazole and terbinafine.

3 Results

Gene sequencing and qPCR of gene expression were performed, confirming the process. No significant change in sterol profile of ABCG1 overexpressing parasites was observed in homeostasis conditions. Nevertheless, when the transfected parasites were exposed to ketoconazole, we observed a dose-dependent increase in cholesterol content in relation to the mock transfected parasites. This increase in cholesterol content was paralleled to an increase in the resistance to ketoconazole, with a raise of the IC_{50} from 4.5 uM in control parasites to 23.7 uM in ABCG1 overexpressing parasites.

4 Conclusions

These results are suggestive of the involvement of ABCG1 in transport of exogenous cholesterol in *Leishmania amazonensis.*

C0825 IDENTIFICATION OF MICRORNA-21 AS A BIOMARKER IN LIVE ATTENUATED LEISHMANIA VACCINE INDUCED PROTECTIVE IMMUNITY

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US FDA USA

1 Background

No vaccine exists against visceral leishmaniasis.Towards developing an effective vaccine, we have reported extensively on the immunogenicity of live attenuated LdCentrin$^{-/-}$ mutants in animal models. . The correlates of protection in human studies reported in literature so far have limited value in predicting the safety and immunogenicity of anti-leishmanial vaccines since such markers do not predict early immune response. Hence, there is a need is a need to develop biomarkers that can predict the immunogenicity of LdCen$^{-/-}$ at an earlier stage of vaccination.

2 Methods

Differential expression of genes including microRNAs (miRs) in human macrophages after infection with LdWT or LdCen$^{-/-}$ was determined by human microarray hybridization and RNA sequencing. miR-21 inhibition was done either by anti-sense oligos or LNA based inhibitors. Expression of miR-21, IL-12 was measured by RT-PCR. Antigen specific CD4^{+} T cells were enriched using tetramers against CD4 epitope (2W) and analyzed by flow cytometry.

3 Results

Unbiased gene expression profiling of US blood donor's PBMCs infected with *Leishmania* parasites (LdWT or LdCen$^{-/-}$) *ex-vivo* resulted in the identification of several miRs that regulate important cytokine genes. Importantly, we found a strong induction of miR-21, known to control the expression of IL-12 mRNA, in LdWT infection compared to LdCen$^{-/-}$ infection. IL-12 produced by DCs is critical for priming a host protective Th1 cell response during *Leishmania* infection. Validation of human PBMC data in a mouse model revealed an enhanced expression of miR-21 in virulent LdWT infected dendritic cells (DCs) compared to LdCen$^{-/-}$. This correlated with low levels of IL-12 mRNA in WT compared to LdCen-/- infected DCs. Silencing of miR-21 resulted in an augmented induction of IL12 in LdWT infected DCs both *in vitro* and *in vivo*, illustrating the role of miR-21 in LdWT infection mediated suppression of IL12. In contrast, LdCen$^{-/-}$ infected DCs showed a strong induction of IL12, and miR-21 silencing resulted in a further increase in IL12 levels. Additionally,blocking of miR-21 enabled strong proliferation of CD4^{+} T cells in mice infected with LdWT parasites. Thus lower level of miR-21 in a vaccinee may indicate the safety and immunogenicity consistent with a protective response of the vaccine.

4 Conclusions

Our data suggests that miR-21 could be used as a biomarker of protection in anti-leishmanial vaccines.

C0829 CELL CONTACT-DEPENDENT AND SOLUBLE FACTORS OF INFECTED MONOCYTES ARE REQUIRED TO ACTIVATE HUMAN NATURAL KILLER CELLS BY LEISHMANIA PARASITES

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1 Background

Activated natural killer (NK) cells release interferon (IFN)γ, which is crucial for the control of intracellular *Leishmania*. In contrast to mouse leishmaniasis the human NK cell response to *Leishmania* is poorly characterized. Here, we investigated whether and how human NK cells get activated by promastigotes.

2 Methods

Human PBMCs, purified NK cells, dendritic cells (DC) and monocytes were co-cultured with *Leishmania (L.) spp*. promastigotes or exposed to supernatants of these co-cultures for 20 h. Cytokines were neutralized by antibodies. Expression of surface markers was measured by flow cytometry. Cytokine release was determined by ELISA or intracellular cytokine staining, NK cytotoxicity by ^{51}Cr-release assay.

3 Results

Coculture of PBMCs with *L. spp*. resulted in increased expression of the activation marker CD69 on NK cells. The extent of CD69 expression was influenced by the parasite species and the ratio of added *Leishmania* but not by the organotropism of the respective species. Up-regulation of CD69 on NK cells required monocytes in direct contact with *Leishmania*. Addition of anti-interleukin (IL)-18 antibodies inhibited this effect. Interestingly, no IL-18 was detected in supernatants of NK cell/monocyte/parasite co-cultures. Confocal microscopy with non-permeabilized cells suggested that *Leishmania*-infected monocytes trans-present IL-18 to NK cells. As supernatants of monocyte/*Leishmania* cultures were also able to induce CD69 on NK cells, a soluble heat-labile factor other than IL-18 might be additionally involved in the activation of NK cells. NK cell-activating cytokines such as IL-1β, IL-12, IL-15 and IFN-αβ could be excluded. Increase of CD69 on human NK cells was not accompanied by NK cell IFN-γ production or enhanced cytotoxicity, which, however, could be triggered by adding IL-12. Remarkably, exogenous IL-12 alone was not sufficient to trigger release of IFNγ and to up-regulate CD69 in human NK cells. The human DC subpopulation mDC1 was shown to release IL-12 in response to *Leishmania* promastigotes. Unlike to CD69, direct contact between parasites and NK cells led to a reduced expression of CD56 mRNA and protein.

4 Conclusions

Leishmania stimulate NK cells via trans-presentation of IL-18 by monocytes for the expression of CD69, whereas additional exogenous IL-12 is required to induce NK cell IFNg release. The downregulation of CD56 on NK cells by *Leishmania* could affect adhesion or signaling events favoring parasite survival.

C0830 GENETIC BACKGROUND SHIFTS MIRNA PROFILE OF LEISHMANIA AMAZONENSIS INFECTED MACROPHAGES AND ADJUSTS NO/POLYAMINES PRODUCTION

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1 Background

The microRNAs (miRNAs) can modulate immune response by post-transcriptional regulation of genes involved in inflammatory mechanisms. Nitric Oxide (NO) versus polyamines production during *Leishmania* infection plays a pivotal role in the survival of parasite. *Leishmania amazonensis* can alternatively modulate the expression of host inducible Arginase 1 (ARG 1), supplying the polyamines pathway for parasite survival or Nitric Oxide Sintase 2 (NOS 2) producing NO to kill the parasite. L-arginine is substrate of both ARG 1 and NOS2 and its absence is an attenuating factor of infectivity of macrophages.

2 Methods

Bone Marrow-Derived macrophage from BALB/c (BALB/c-MO) or C57BL/6 (B6-MO) mice were infected with *L. amazonensis* (*La*-WT) or arginase null-*L. amazonensis* (*La*-arg$^-$), MOI 5:1, at 34° C, 5%CO_2. The miRNAs were analyzed using miScript miRNA PCR arrays (Qiagen). The mRNA quantification was performed by qPCR.

3 Results

The *La*-WT infection of BALB/c-MO modulated 27% of the 84 miRNAs analyzed when compared to uninfected-macrophages, 78% were upregulated. The *La*-WT infection of B6-MO modulated 32% of miRNA analyzed, but only 50% were upregulated.The infection with *La*-arg$^-$ increased the percentage of miRNAs modulated to 43% in BALB/c-MO, but only 22% were upregulated. In B6-MO, *La-arg-* infection modulated about 25% of miRNAs tested, 60% upregulated.*Nos2* mRNA amount was higher in 4-24h-B6-MO *La*-WT infection compared to non-infected control, but was reduced in *La*-arg$^-$ infection. However, *Nos2* levels were lower in BALB/c-MO *La*-WT infection than in the *La-arg-* infection. Host ARG 1 and cationic transporter-2B mRNAs were increased in BALB/c-MO infected with WT or *La-arg*$^-$, but not altered in B6-MO. *In silico* search indicated that miR-291-3p, miR-294-3p and miR-721could regulate *Nos2* mRNA.Moreover, miR-294-3p and miR-721 were increased in *La*-WT 4-24h-infected BALB/c-MO, but presented a lower increased in *La*-arg$^-$ infection. Conversely, the levels of miR-291-3p, miR-294-3p and miR-721 were higher in 4-24h-*La*-arg$^-$ infected B6-MO, but lower in the WT-infection. The blocking of these miRNAs binding regions to *Nos2* mRNA caused an increase in NOS2 expression and NO production in BALB/c-MO infected with *La*-WT and reduced infectivity.

4 Conclusions

We concluded that *L. amazonensis* infection could alter the miRNAs profile of mouse macrophages to subvert the host immune responses as well as the production of NO. Financial Support – FAPESP and CNPq.

C1014 SNARES IMPORTANCE IN LEISHMANIA COMMUNAL AND INDIVIDUAL PV FORMATION

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1 Background

Leishmania is the parasite responsable for leishmaniasis, a disease endemic in roughly 98 countries around the world. This disease can be visceral, cutaneous or mucocutaneous depending on the *Leishmania* species involved. To survive into host macrophages, *Leishmania* creates parasitophorous vacuoles (PV). These PVs can be either individual, small and tight around the parasite for species such as *L. major* or communal and spacious for species such as *L. amazonensis*. **We propose that Soluble N-éthylmaleimide-sensitive-factor Attachment protein REceptors (SNAREs) play a central role in the formation of large communal PVs by providing membrane from various organelles**.

2 Methods

We proceeded to in vitro infection of bone marrow macrophages (BMM) from WT and SNAREs KO macrophages fallowed by confocal microscopy to establish SNAREs recruitment to the PV and the parasite survival.

3 Results

We first looked at the different membrane compartments directly involved in the phagosome maturation process. The SNAREs SNAP23 (plasmic membrane), VAMP3 (early endosome) and the lysosomal associated membrane protein 1 (LAMP1) were found to be recruited to the communal vacuoles while only VAMP 8 (late endosome) was accumulated to individual vacuoles. These recruitment patterns suggest that the *Leishmania* communal species undergo and survive a complete phagosome maturation process while conserving membrane and augmenting the PV size to dilute microbicidal molecules produced by macrophages, while the individual species blocks the maturation process to the late endosome stage. We also found that the trans-Golgi (Vti1a) and the endoplasmic reticulum (Stx18) were recruited to communal vacuoles suggesting that *L. amazonensis* can hijack other membrane sources. We also used VAMP3 KO macrophages to study the role of VAMP3 in communal PV formation. Within these cells we noted an increased growth of *L. amazonensis* and a larger vacuole size while the individual specie was left unaffected. We also noted higher level of recruitment for LC3, an autophagy marker, to the communal PV. VAMP3 KO cells have a deregulation of autophagy leading to an augmentation of LC3 that could possibly be leading to the hijacking of the autophagy system and to the augmentation of the PV size.

4 Conclusions

SNAREs play an important and active role to Leishmania PV's formation. Further studies to understand the autophagy machinery and VAMP 3 involvement into the PV's formation are currently underway in vivo and in vitro.

C1454 IL-4-PRODUCING B CELLS REGULATE T HELPER CELL DICHOTOMY DURING CUTANEOUS LEISHMANIASIS IN BALB/C MICE

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1 Background

Immunity to the protozoan parasite *Leishmania major* is dependent on the successful generation of a Th1 response whilst IL-4Rα-driven Th2 immunity renders susceptibility to this parasite in BALB/c mice. B cells have also been implicated indisease progression but it is unclear if IL-4Rα-responsive B cells play a significant role.

2 Methods

To investigate this further, a novel BALB/c mouse lacking IL-4Rα expression on B cells (mb1^{cre}IL-4Rα$^{-/lox}$) was infected with *L. major* LV39 parasites and using a range of immunological texhniques, ELISA, FACs, RT-PCR, adoptive transfer studies and mixed bone-marrow chimeras, host immune parameters were analysed at 8 weeks post-infection.

3 Results

Following infection with *L. major*, mb1^{cre}IL-4Rα$^{-/lox}$ BALB/c mice efficiently controlled cutaneous disease as shown by significantly reduced footpad swelling and parasite burdens as well as increased dendritic cell-derived IL-12p40 production. This in turn led to enhanced IFN-γ production and significantly reduced secretion of IL-4, IL-13 and IL-10 by CD4^{+} Th cells as well as augmented type 1 antibody responses and upregulated iNOS production in mb1^{cre}IL-4Rα$^{-/lox}$ mice, altogether leading to a protective type-1 immune response. Surprisingly, IL-4 production by B cells was found to be induced as early as day 1 after infection leading to susceptibility in cutaneous leishmaniasis whilst in contrast, B cells unable to express or respond to IL-4 prevented *L. major*-induced cutaneous leishmaniasis in B cell-deficient μMT animals. Mechanistically, we confirmed in mixed-bone marrow chimeras that B cell-derived IL-4 was important in regulating and assisting a detrimental Th2 response during *L. major* infection in BALB/c mice.

4 Conclusions

Together, this data demonstrates that early IL-4Rα-responsive B cells producing IL-4 influence early T helper dichotomy towards detrimental T helper 2 responses, which leads to *L. major*-induced cutaneous leishmaniasis in BALB/c mice.

Miradero Room

Session 9. Drug development: conventional and alternative. Clinical and experimental therapy in cutaneous and visceral leishmaniasis

C0166 MIRNA MEDIATED NEGATIVE REGULATION OF MACROPHAGE SURFACE RECEPTOR LEADING LEISHMANIA PARASITIC INHIBITION: A THERAPEUTIC APPROACH AGAINST VISCERAL LEISHMANIASIS INFECTION

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1 Background

Visceral leishmaniasis (VL), a tropical neglected disease caused by *Leishmania donovani*, results in significant mortality in Indian subcontinent. The extracellular promastigotes form of *Leishmania* parasite is transmitted by the bite of sand-fly vector to the mammalian host. Within human body, the major site of residence for *Leishmania* parasite is macrophage. Initially, promastigotes ligates itself to macrophage surface receptors and triggers phagocytosis. The cellular component which helps in the parasite encounter to the macrophage receptors is immunoglobulins, third complement protein (C3), and neutrophils. In this study, we have proposed that miRNA mediated negative regulation of macrophage surface receptor may lead to parasitic inhibition.

2 Methods

Macrophage cell receptors, which facilitates the promastigotes internalization were identified which include third complement receptor (CR3), first complement receptor (CR1), mannose receptor (MR), Fc gamma receptor (FCγR) and Fibronectin receptors (FnRs). Bioinformatics analysis was performed to search for the potent miRNA against 3'-UTR sequence of targeted mRNA that encodes aforementioned receptor using miRDB database. Respective target score for each miRNA was also calculated to find the potential miRNA inhibitor against the identified targets.

3 Results

MiRDB outputs were able to show that hsa-miR-8485 and hsa-miR-338-5p has a putative binding site at the 3'-UTR region of CR3. Suggesting that, these miRNAs have the capability to hinder the attachment of promastigotes to the macrophage, which is essential for the internalization of promastigotes. Similarly, hsa-miR-6511a-5p mediates the same, but targets CR1 of macrophage. Seed region present in the hsa-miR-23a-3p and hsa-miR-23b-3p has the putative binding site at the 3'-UTR region of mannose receptor coding mRNA, suppress the expression of mannose receptor, and lower the parasite internalization. Similarly, hsa-miR-4261 and hsa-miR-4307 play an important role by inhibiting the binding of immunoglobulin attached promastigotes to the FCγR2B receptor. Furthermore, we speculated that hsa-miR-4447 may suppress the early stage parasite attachment to macrophages. Until and unless the parasite enters into the macrophage via phagocytic pathway they can't survive more.

4 Conclusions

This study concludes that microRNAs mimic can be used as a therapeutic agent in combination therapy to control the visceral leishmaniasis infection in early infection stages.

C0475 IDENTIFICATION OF RESISTANCE MARKERS FOR DRUG LEADS

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1 Background

The European Union-funded project New Medicines for Trypanosomatidic Infections (NMTrypI) aims at identifying new lead compounds for the treatment of Leishmaniasis, HAT and Chagas' Disease. New leads against Leishmaniasis are urgently needed in the face of the disease spread threat caused by globalisation, climate change and drug resistances. The evaluation of new drug leads requires an assessment of possible resistance mechanisms and cross resistance with drugs already in use.

2 Methods

To identify recessive markers of resistance we selected for spontaneous resistance in *Leishmania* promastigotes by repeated passage of the parasites under drug pressure at IC50 to IC75. Parasites with an acquired resistance against the applied leads were then subjected to genomic DNA isolation and deep sequencing. To identify dominant genes involved in lead resistance, we applied the Cos-Seq strategy (1). Leishmania parasites were transfected with a cosmid DNA library derived from a donor strain and grown under drug pressure to select for protective gene loci. Selected cosmids were isolated and analyzed by NGS.

3 Results

The analysis of the Miltefosine(MIL)-resistant parasite populations revealed SNPs inside the gene for the MIL trasnporter, leading to single amino-acid exchanges close to positions described in the literature (2,3). Selection with MIL derivatives also affected the transporter, introducing amino acid exchanges and chain termination mutations. All resistance phenotypes could be reversed by episomal expression of a non-mutated transporter transgene, confirming the causative nature of the transporter mutations for resistance. The Cos-Seq approach was applied for both intracellular amastigotes and axenic promastigotes. Data analysis for the experiments with the promastigotes is still pending, but the NGS analysis for intracellular selection showed a limited number of selected gene loci under the various drug pressures and limited selectivity even for known resistance gene loci, likely due to the limited number of cell divisions during *in vitro* infections.

4 Conclusions

The comparison of the results from the experiments with intracellular amastigotes and axenic promastigotes will show wether the same gene loci are selected in the different parasite stages and wether selection is more stringent in the axenic culture. Analysis of the selectd gene loci will provide patterns of resistance marker loci to be compared between drug leads, enabling an assessment of cross resistance.

C1064 THE POTENT ITK/BTK INHIBITOR PROMOTES PROTECTIVE IMMUNITY AGAINST L. DONOVANI AND IS EFFECTIVE IN TREATMENT OF VISCERAL LEISHMANIASIS (VL)

Sanjay Varikuti, Steve Oghumu, Noushin Saljoughian, Cesar Terrazas, ***Abhay Satoskar***

Ohio State University USA

1 Background

B/I-Inh is an irreversible inhibitor of Bruton tyrosine kinase (BTK, important for B-cell receptor activation) and interleukin-2-inducible kinase (ITK, important for TCR stimulation and promoting Th2 differentiation). B/I-Inh has multiple immunomodulatory effects and is approved for clinical use. We have found that B/I-Inh treatment alters the balance between Th1/Th2 cells toward Th1 subset, which is critical for immunity against many intracellular pathogens including *Leishmania*

2 Methods

Here, we evaluated the efficacy of orally administered B/I-Inh in the treatment of visceral leishmaniasis caused by *L. donovani* and compared it to the standard antileishmanial drug sodium stibogluconate. In addition, we analyzed immune responses by assessing cytokine production by the spleen cells as well as characterizing immune cell populations in the target organs.

3 Results

B/I-Inh treatment significantly reduced parasitic loads in the livers and spleens of *L. donovani* infected mice and reduced pathology. This decrease in parasite loads of B/I-Inh-treated mice was comparable to mice treated with sodium stibogluconate (SSG). Analysis of cytokine responses in the infected spleens revealed that *L donovani* antigen-stimulated spleen cells from B/I-Inh-treated mice produced significantly higher levels of disease protective cytokines IL-4 and IL-13 compared to spleen cells from vehicle treated mice. On the other hand, levels of disease exacerbating cytokines IL-6 and IL-10 were significantly lower in culture supernatants from B/I-Inh treated mice compared to controls. Interestingly, spleen cells from B/I-Inh-treated mice also produced significantly less IFN-γ than WT spleen cells. B/I-Inh-treated mice also showed a significant decrease in the number of macrophages and monocytes in their livers and spleens.

4 Conclusions

Our study shows that B/I-Inh promotes protective immunity by modulating innate and acquired immune responses during *L. donovani* infection and is therapeutically as effective as SSG in controlling the infection. Thus, B/I-Inh could be a novel host-targeted drug that warrants further investigation for treatment of VL caused by *L. donovani*.

C1233 NANOSYSTEMS FORMED BY AMPHIPHILIC ANTIMONY COMPLEXES AS CARRIERS OF THE LEISHMANICIDAL DRUG MILTEFOSINE

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1 Background

Pentavalent antimonials and miltefosine (HePC) are reference medicines for the treatment of leishmaniasis. However, their use is limited by their toxicities and the onset of resistance. Considering the need to improve the treatment of leishmaniasis, the World Health Organization (WHO) encourages the reformulation of the available drugs, as well as the development of new association protocols. The present work evaluated as a new therapeutic strategy of cutaneous and visceral leishmaniasis (CL and VL), the co-administration of Sb(V) and HePC by oral route in the form of nanosystems made from amphiphilic Sb(V) complexes which incorporated HePC.

2 Methods

SbL8 and SbL10 were synthesizedfrom $KSb(OH)_6$ and N-alkyl-N-methyl glucamide surfactants (L8 and L10, hydrocarbon chain of 8 and 10 carbons, respectively). After dispersion of SbL8 and SbL10 in saline, HePC was added. The SbL8/HePC and SbL10/HePC formulations were characterized forHePC incorporation using a fluorescent analog and the stability of SbL10 nanosystems in media of different pHs. The therapeutic efficacy of SbL8/HePC and SbL10/HePC by oral route was evaluated in BALB/c mice infected with *Leishmania amazonensis* and *Leishmania infantum*through qPCR.Comparison was madeto SbL10 (oral),meglumine antimoniate (IP)or HePC (oral).

3 Results

The spectral changesof the HePC fluorescent analogue in the presence of SbL8 and SbL10 supportthe spontaneous incorporation of HePC in the nanosystems.SbL10 nanosystems showed disappearance of hydrophobic environment in acidic medium, suggesting a change of the nanosystem structure and probable release of incorporated HePC in the acidic environment of the stomach. In the murine model of VL, treatment with the mixed formulation SbL8/HePC for 10 days promoted significant reduction of the parasite load in the spleen, in relation to control. In the murine model of CL, treatment with SbL8/HePC, SbL10/HePC or HePC for 30 days induced lesion closure in all animals, however,only the mixed formulations resulted in significant reduction of the parasite load in relation to the control group. It is noteworthy that the SbL8/HePC and SbL10/HePC formulations promoted, in both LV and LC models, significantly greater parasite suppression compared the antimonial drugs and HePC, given separately.

4 Conclusions

This work establishes for the first time the potential of the mixed nanosystem SbL10/HePC as an oral formulation for the treatment of LV and LC.

C1261 NANOSYSTEMS DESIGNED FROM AMPHIPHILIC COMPLEXES OF ANTIMONY(V) INCORPORATING AMPHOTERICIN B FOR THE ORAL TREATMENT OF VISCERAL LEISHMANIASIS

***Arshad Islam**, Arshad Islam, Juliane Souza Lanza, Flaviana Ribeiro Fernandes, Delia Chaves Moreira dos Santos, Gisele Abreu, Lucas Ferreira, Maria Norma Melo, Cynthia Demicheli, Frédéric Frézard*

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1 Background

In an attempt to promote the oral delivery of antimonials, in this work we investigate the incorporation and state of aggregation of Amphotericin B (AmB) in the nanosystem SbL10, obtained from amphililic complex of pentavalent antimony and N-decanoyl-N-methylglucamide surfactants (L10), and also their in vitro and in vivo antileishmanial activity.

2 Methods

The incorporation rate and aggregation state of AmB into the SbL10 dispersion was determined using an HPLC-based technique and circular dichroism (CD) and UV-vis, respepctiviely . The SbL10 nanosystem and its AmB-incorporated formulation SbL10-AmB, and free AmB were evaluated in vitro in *Leishmania infantum* promastigotes and compared to Anforicin B (reference drug). The efficacies of SbL10 and SbL10-AmB were also evaluated in BALB/c infected with *L. infantum*, via oral administration.

3 Results

HPKC analysis showed 74±1% incorporation of AmB in SBL10. Characterization of SbL10-AmB by CD and UV-Vis absorption spectroscopies indicated that AmB is present predomininatly under the monomeric form, which is the least toxic form to the host. SbL10-AmB showed in vitro acitivity against L. infantum promastigotes at a slighlty higher level than the commercial drug (Anforicin B®. BALB/c mice infected with L. infantum and treated orally with SbL10-AmB mixed formulation (170 mg Sb/kg, 14 mg AmB/kg per day) showed significant reduction of parasite loads in the spleen and liver, when compared to the untreated control, to a similar level as animals treated parenterally with Anforicin B® (>1 mg/kg).

4 Conclusions

This study established for the first time the potential of SbL10-AmB mixed formulations for the oral treatment of visceral leishmaniasis, indicating its potential as antileishmanial drug candidates for further developments and applications.

C1379 NANOFORMULATIONS OF TRIFLURALIN ANALOGUES

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1 Background

The eradication of leishmaniasis is difficult to achieve by conventional therapies. The key to convert an active molecule into an efficient drug is an appropriate formulation, able to target the drug to the sites of infection at desirable concentrations, without producing uncontrolled toxic effects. This task can be fulfilled by Nanodrug delivery systems (nanoDDS), including liposomes and solid lipid nanoparticles (SLNP). A class of drugs that have attracted our attention are dinitroanilines, due to their mechanism of action, enabling to display antimitotic activity on the parasite without affecting the host. Analogues of one dinitroaniline (Trifluralin-TFL) were produced and characterized in our laboratory. In this work we will report the behaviour of some analogues (TFL-A) after incorporation in liposomes and SLNP, and analyse the possible interest of these formulations as antileishmanial agents.

2 Methods

TFL-A were prepared by hemi-synthesis methodologies in which TFL amine group was modified with different substituents. The in vitro hemolytic activity, cytotoxicity and anti-leishmanial activity were evaluated in red blood cells, THP1 cell line and L. infantum infected macrophages, respectively. TFL-A were incorporate in liposomes by the dehydration-rehydration method and in SLNP by the emulsion-solvent evaporation (dichloromethane) method. The in vivo therapeutic activity of free and liposomal TFL-A was evaluated in a murine model of L. infantum visceral leishmaniasis

3 Results

The most promising TFL-A, identified according to in vitro lower cytotoxicity and higher anti-leishmanial activity were incorporated into NanoDDS. The incorporation parameters of TFL-A in both NanoDDS are similar, evidencing high incorporation efficiency and loading capacity. The resulting nanoformulations display similar sizes, zeta potential and no haemolytic activity and reduced cytotoxicity compared to the free forms. These nanoformulations consistently evidenced in vivo significant reduction of parasitic loads higher than the respective free drugs and Glucantime®. Both nanoformulations have different preference for the target organs: liposomal for the liver and SLNP for spleen. Both Nanoformulations can be stabilized in lyophilized form and scaled-up, without changing their properties.

4 Conclusions

Analogues of Dinitroanilines associated to lipidic NanoDDS, such as Liposomes and SLNP, seem to be promising candidates for leishmaniasis treatment.

C1380 AMPLIFYING THE ANTILEISHMANIAL POTENTIAL OF ARTEMISININ IN NANO AND NANO LIPOSOMAL FORM

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1 Background

In the absence of effective vector control measures and vaccines, chemotherapy remains the main armament in control of visceral leishmaniasis (VL), a neglected disease of poverty. There is a pressing need for alternate rescue therapy due to escalating drug refractoriness, coupled with adverse effects, emergence of HIV co-infection, and resurfacing in the form of PKDL after apparent cure. Since *Leishmania* parasites evade host defensive machinery, a promising therapeutic option that would concurrently rejuvenate an impaired cell-mediated immune response is needed. Artemisinin is a sesquiterpene lactone with potent antileishmanial activity. However, its lipophilicity limits its accessibility to parasitized macrophages harbouring *Leishmania* amastigotes. To meliorate its bioavailability, we incorporated artemisinin in nanoparticles, and in nanoliposomes to further enhance its stability with targeted delivery to macrophages.

2 Methods

Artemisinin-loaded poly lactic co-glycolic acid nanoparticles (ALPLGA) were prepared by solvent displacement and nanoliposomal artemisinin (NLA) by thin film hydration and the formulations optimized using Box-Behnkehn design and characterized by Dynamic Light Scattering. Cytotoxicity on murine macrophages *ex vivo* as well as *in vivo* in BALB/c mice was tested. Antileishmanial efficacy was evaluated *ex vivo* on macrophage-infested *Leishmania* amastigotes. The protection *in vivo* was determined in infected BALB/c mice coupled with the immune correlates of protection.

3 Results

ALPLGA and NLA had spherical surface with particle size of 220 and 83 nm, respectively. The drug loading was 29.2 and 33.2 % in nano and nanoliposomal form, respectively. ALPLGA and NLA were free from concomitant signs of toxicity and significantly denigrated the intracellular *Leishmania* infection. Following treatment in murine model of VL, artemisinin formulations demonstrated superior efficacy over artemisinin with 85.5 (ALPLGA) versus 82.4 % (NLA) protection in the liver, and 82.1 (ALPLGA) versus 77.6 % (NLA) in the spleen. The protection coincided with modulation of cell-mediated immunity towards Th1 type as evidenced by positive DTH, augmented lymphoproliferation, induction of Th1 signature cytokines and IgG2a antibody isotypes.

4 Conclusions

Ours is the first report on the use of nano and nanoliposomal drug delivery systems of artemsinin with synergistic Th1 immunopotentiation, as a promising alternative intervention against VL. Our published findings in these lines will be discussed.

C1413 ENHANCED ANTI-LEISHMANIAL ACTIVITY OF CARBON NANOTUBE BASED MILTEFOSINE FORMULATION

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1 Background

The strategic use of nanoparticle (NP) applications in therapies has emerged as a viable option to more effectively deliver toxic drugs and combat drug resistance since the same therapeutic effect can be achieved by NPs with lower and less frequent doses. Of note, single-walled carbon nanotubes (SWNTs) are selectively localized to and internalized by macrophages and exhibit high innate antimicrobial activity via cell membrane damage from direct physical contact and indirect activation of inflammatory cytokine pathways. As a result, if delivered in combination with an anti-leishmanial drug, there exists the potential for a synergistic therapeutic effect of enhanced, targeted uptake and subsequent superior parasite killing. Given the recent emergence of miltefosine (HePC) resistance in India, the exploration of NPs formulations of HePC, especially SWNTs, is an urgent need.

2 Methods

Miltefosine-attached carbon nanotubes (HePC-SWNTs) were prepared by mixing SWNTs with HePC to facilitate surface adsorption. Following synthesis, the morphology, size, and surface properties were characterized to ensure attachment and to determine stability, drug loading, and a dose-release profile. Cytotoxicity was assessed in vitro using the RAW 264.7 macrophage cell line. Anti-leishmanial activity was assessed in vitro using a RAW 264.7 macrophage model of L. viannia infection.

3 Results

HePC-SWNTs were readily internalized by L. viannia infected macrophages and exhibited amplified anti-leishmanial activity, likely due to selective infected macrophage localization and the slow release profile of HePC from the SWNTs (>12 hours). Ultimately the parasitic load was reduced by 27% more than miltefosine alone during in vitro testing. Cytotoxicity in healthy cells was noted in the highest tested concentration (60 mg/mL particles); however, the findings indicate the potential applicability of low-doses (10 mg/mL particles) for enhanced treatment.

4 Conclusions

HePC-SWNTs can be successfully employed in vitro as a novel miltefosine formulation. As such, HePC-SWNTs represent a more specific and less toxic formulation for miltefosine, potentially reducing drug-associated side effects and delaying the emergence of population-level miltefosine resistance due to the lower encapsulated dose and targeted delivery to leishmania-infected macrophages. The in vitro effects must now be assessed in vivo. Overall, this study establishes a foundation for further exploration of nanoparticle applications involving miltefosine.

C0456 DDD853651, A PRE-CLINICAL CANDIDATE FOR VISCERAL LEISHMANIASIS, ACTS THROUGH INHIBITION OF CYCLIN-DEPENDENT KINASE 12

Susan Wyllie
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1 Background

Visceral leishmaniasis causes significant mortality and morbidity in many parts of the world. Current drugs are inadequate and there is an urgent need for the development of new, effective oral treatments for this disease. In the current study, we report the discovery of an exciting new anti-leishmanial compound (DDD853651), which inhibits a novel molecular target within these parasites.

2 Methods

Phenotypic screening, followed by medicinal chemistry, led to the development of a novel anti-leishmanial chemical series based around a pyrazolopyrimidine scaffold. Further optimisation identified the lead compound DDD853651.To determine the mode of action and specific molecular target(s) of this compound series, a number of complementary methodologies in the fields of genomics and chemical proteomics were employed. Specific details will be presented.

3 Results

DDD853651 has potent efficacy in a mouse model of visceral leishmaniasis and has suitable physicochemical, pharmacokinetic and toxicological properties for further development. Compounds from this series have a profound effect on the parasite cell-cycle inducing arrest at both G1/S and G2/M. Comprehensive mode of action studies utilising orthogonal approaches such as genomics and chemical proteomics indicate that compounds from this series act principally by inhibiting cyclin-dependent kinase 12 (CRK12) of Leishmania parasites.

4 Conclusions

DDD853651 shows real potential to become a much needed oral treatment for visceral leishmaniasis and has recently progressed into advanced pre-clinical development, with the aim of entering into human clinical trials. Our comprehensive mechanism of action studies confirm that the principal target of DDD853651 is CRK12. This cyclin dependent kinase, whose endogenous function remains to be determined, represents a novel, druggable target within Leishmania. The discovery of CRK12 as a viable anti-leishmanial drug target opens up new possibilities for drug discovery.

Greco Room D1-D2
15:00-16:30

Session 10. Host –parasite cellular and molecular interactions

C0114 MALNUTRITION AND SUSCEPTIBILITY TO VISCERAL LEISHMANIASIS

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1 Background

Patients with visceral leishmaniasis (VL), a potentially fatal infectious disease caused by *Leishmania* parasites, frequently suffer from severe malnutrition. Malnutrition is commonly associated with increased infectious disease susceptibility and severity. Whereas malnutrition might enhance the incidence of disease as well as its severity, active infection can in turn exacerbate malnutrition.

2 Methods

Here, we performed a prospective study with apparently healthy individuals with normal BMI and low BMI in Ethiopia. We recruited apparently healthy individuals from Gondar, Ethiopia; with normal BMI (>18.5), moderately malnourished (16.5-18.4 BMI) and severely malnourished (<16.5 BMI). Our aim was to compare in malnourished individuals and individuals with normal BMI, several immunological parameters that have been shown to be impaired in patients with nonhealing VL: hematological profile, cytokine profiles in the plasma, $CD4^+$ and $CD8^+$ T cell ratio and activation status, as well as neutrophil effector functions.

3 Results

Our results show a similar hematological profile in all three groups, as well as a similar ratio of CD4+ and CD8+ T cells. We found significant correlations between low BMI and increased levels of T helper (Th) 1 and Th2 cytokines (Interferon (IFN)-γ, (interleukin (IL)-2, IL-12), (IL-4, IL-5, IL-13), as well as IL-10, IL-33 and tumor necrosis factor-α, but not IL-8 or C-reactive protein. The activities of arginase, an enzyme associated with immunosuppression, were similar in plasma, peripheral blood mononuclear cells (PBMC) and neutrophils from all groups and no differences in the expression levels of CD3ζ, a marker of T cell activation, were observed in CD4+ and CD8+ T cells. Furthermore, whereas the capacity of neutrophils from the malnourished groups to phagocytose particles was not impaired, their capacity to produce ROS was impaired. Finally, we evaluated the frequency of a subpopulation of low-density neutrophils and show that they are significantly increased in the malnourished individuals. These differences were more pronounced in the severely malnourished group.

4 Conclusions

Our results show that even in the absence of apparent infections, healthy malnourished individuals display dysfunctional immune responses that might contribute to increased susceptibility and severity of VL.

C0706 L. MEXICANA AMASTIGOTES SURVIVE AND REPLICATE WITHIN NEUTROPHILS

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1 Background

The protozoan *Leishmania (L.) mexicana* parasites cause chronic non-healing cutaneous lesions in humans and mice with poor parasite control. Promastigote forms of the parasites are injected by sand flies and transform into intracellular amastigote stage forms in the vertebrate hosts. We have previously shown that within hours of infection, *L. mexicana* promastigotes induce the early local recruitment of neutrophils that sequester the parasites, shaping the microenvironment and eventually favoring the development of a chronic lesion. We show here that neutrophils are also present in chronic lesions induced by *L. mexicana* infection, and therefore investigated interactions between neutrophils and the intracellular amastigote form of the parasite.

2 Methods

The interaction between neutrophils and fluorescently labelled parasites was assessed by flow cytometry and imaging flow cytometry both *in vitro* and *ex vivo*. Furthermore, we generated a strain of *L. mexicana* parasites expressing a photoconvertible fluorescent protein in order to measure parasite replication.

3 Results

We first show that *L. mexicana* amastigotes are 2-3 times less internalized by neutrophils than promastigotes *in vitro*, however opsonization with immune serum led to comparable internalization. Furthermore, the majority of infected neutrophils were not apoptotic. Several weeks post *L. mexicana* infection, neutrophils represent 6-10% of the local cells in chronic lesions. Strikingly,these neutrophils are heavily infected with over 70% of them harboring two or more intact amastigotes, as determined by the use of fluorescently labelled parasites and imaging flow cytometry. Using *L. mexicana* parasites expressing a photoconvertible fluorescent protein, we could further show that amastigotes not only survive within neutrophils but also can replicate within these cells.

4 Conclusions

Taken together, our data suggest that neutrophils play a negative role upon *L. mexicana* infection, contributing to parasite persistence within chronic lesions, therefore serving as an alternative host for amastigote replication.

C0917 KUPFFER CELLS AND BLOOD DERIVED MACROPHAGES RESPOND DIFFERENTLY TO L. INFANTUM PARASITES

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1 Background

Canine leishmaniasis (CanL) is a zoonotic disease caused by *Leishmania infantum*. Macrophages (MØ) are the natural host cells for this parasite and Kupffer cells (KC), the liver resident MØ also constitute a target for the parasite. However, communication between infected KCs and hepatocytes remains unclear. In this study, the interrelation of *L. infantum* parasites with the two MØ lineages, blood's and liver's was analyzed. Activation of infected KCs in hepatic environment was addressed in order to investigate the liver immune response.

2 Methods

Liver of healthy stray dogs captured by official services were excised by veterinary clinicians under anesthesia prior to euthanasia. After liver perfusion, hepatocytes and KCs were cultured in William's or RPMI medium as appropriate. Blood monocytes were also isolated and cultured in RPMI until reach MØ differentiation. KCs and MØ were infected with *L. infantum* axenic amastigotes or virulent promastigotes for 24 h. Infected KCs were co-cultured with hepatocytes. Gene expression of innate receptors TLR2, TLR4, TLR9, NOD1 and NOD2 and of cytokines IL-12, TNF-α, IL-10, IL-4 and TGF-β were quantified by real-time PCR.

3 Results

L. infantum rapidly infected KCs, inducing an anergic state with no activation of innate immune receptors or cytokine generation. On the other hand, MØ reacted exuberantly in the first 3 h of infection, increasing gene expression of innate immune receptors and of cytokines. However, after 5 h of infection silencing of inflammatory mechanisms were imposed. Curiously, when in contact with hepatocytes infected KCs exhibited significant increases of NOD1, NOD2, TLR2, TNF-α, TGF-β and IL-4 mRNA.

4 Conclusions

The fast and intense reaction of blood MØ to *L. infantum* parasites contrast with KCs tolerance,indicating that these two cell lineages have different activation potential. KCs seem to reflect the immune tolerance characteristic of the liver, which associated with the later silencing of blood MØ can account for parasite survival. However, hepatocytes seem to have a synergistic role in activating infected KCs and possible in orchestrating liver immune response. Thus, hepatocytes can be key cells in determining the infection outcome.
Funding: Portuguese Foundation for Science and Technology (FCT), projects PTDC/CVT/118566/2010, UID/CVT/00276/2013 and GHTM- UID/Multi/04413/2013. A. Rodrigues holds a FCT PhD scholarship SFRH/BD/73386/2010.

C1069 A MULTI-DIMENSIONAL ANALYSIS OF MICRORNA-MEDIATED REGULATION IN LEISHMANIA-INFECTED HUMAN DENDRITIC CELLS

Nicholas Geraci, ***Mary Ann McDowell***

Dept. of Biological Sciences/University of Notre Dame United States

1 Background

The innate immune response is an emergent property resulting from the integration of a multitude of signals and pathways. As the gatekeepers, the functional response of dendritic cells (DC) is crucial for innate immunity and the subsequent generation of adaptive immunity. Immune response regulation occurs at multiple levels including DNA modification, transcriptional and translational mechanisms. We utilized an integrative systems-level approach to identify novel regulatory circuits that direct immune responses in human DC infected with *Leishmania* parasites. *Leishmania* parasites cause a spectrum of clinical manifestations, ranging from self-healing to fatal disease. The clinical outcome is primarily attributed to differences in the *Leishmania* species initiating the infections, with *L. donovani* eliciting fatal visceral disease and *L. major* associated with self-resolving cutaneous disease and protective immunity.

2 Methods

Here we performed a multi-dimensional analysis of miRNA, mRNA, and protein expression with matched samples of human monocyte derived dendritic cells (MDDC) infected with either *L. major* or *L. donovani*. Active mature miRNAs, in physical association with RNA induced silencing complex proteins (RISC), were immuno-precipitated and sequenced; mRNA expression was characterized by microarray analysis; and total cellular proteins were quantified by Western Blot.

3 Results

Results revealed a differential expression of >100 miRNAs between MDDCs across time and between those infected with either *L. major* or *L. donovani*, with a general down regulation of miRs in *L. major*-infected human DC. Correlative expression analysis, guided by *in silico* miRNA:mRNA targeting predictions, revealed that *L. donovani* infected DC upregulated miR expression, targeting MAP kinase, STAT and TGF-β signaling pathways.

4 Conclusions

Together these data revealed distinct features of *Leishmania* species that may regulate disease outcome and provide new directions for the investigation of molecular mechanisms of *Leishmania* infection in human host cells.

C1230 HLA-DR AS A GENETIC DETERMINANT OF SUSCEPTIBILITY TOWARDS VISCERAL LEISHMANIASIS

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1 Background

HLA class II is a highly polymorphic cell surface glycoprotein, crucial for regulating host immune responses by their ability to present the foreign antigens to CD4+ T lymphocytes. Genome wide association study (GWAS) had highlighted HLA-DRB1 as major genetic risk factor for disease. This study aims to evaluate the immunological and genetic basis of HLA-DR being a risk factor for VL. We hypothesize that polymorphism in HLA-DR could be the result of regulatory upstream elements.

2 Methods

The study was performed on PBMCs of VL cases (pre-and post-treatment) and healthy controls for gene expression by Real time PCR. The study was further extended to look for HLA-DR on different immune cells at protein levels by flow cytometry on different immune cells in whole blood and splenic aspirate. The whole blood was also used for the stimulation experiment with recombinant human IFN-g followed by flow cytometry. The genomic DNA from
We found that transcriptional regulation of HLA-DR is hampered during VL. This perturbation in the HLA-DR and cellular responsiveness during *Leishmania* infection depends upon the host genetics. Thus, altering the antigen processing and presentation abilities depending upon the genotype of individual could be helpful in designing prophylactic options for VL.
VL cases and healthy controls were used for SNP genotyping.

3 Results

We found significantly decreased expression of HLA-DR on monocytes but no such changes in B-cells where HLA-DR was elevated in follow-ups, while non-professional APCs i.e. T-cells, had significantly higher expressions of HLA-DR in active cases. We found that the HLA-DR expression by cell types was similar in splenic aspirate cells. The results from recombinant human IFN-g stimulation studies indicated for elevated expression of HLA-DR under the influence of recombinant human IFN-g, on different immune cells in healthy controls as compared to cases. The analysis of allelic architecture of HLA-DR in VL cases and healthy controls defined the association of risk haplotype with the susceptibility towards disease, therefore, suggesting the role of allelic variations during the antigen presentation process as the key factor for determining the disease outcome.

4 Conclusions

We found perturbation in the HLA-DR regulation during *Leishmania* infection depends upon the host genetics. Thus, altering the antigen processing and presentation abilities depending upon the genotype of individual could be helpful in designing prophylactic options for VL.

C1591 WHOLE GENOME TRANSCRIPTION PROFILING OF THE LEISHMANIA TROPICA INFECTED LESIONS FROM CUTANEOUS LEISHMANIASIS PATIENTS

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1 Background

Leishmania tropica is the main causative agent of anthroponotic cutaneous leishmaniasis (CL) in Iran.Due to difficulties in obtaining human skin biopsy, most studies of CL have been limited to the analysis of peripheral blood. Although these systemic responses are important, recent studies of lesion biopsies from CL patients have revealed unexpected new data about this disease, which would not be reachable from studies on peripheral blood. Therefore, defining the host inflammatory response in the lesions is crucial for the development of new treatment modalities.To our knowledge, this study is the first report to build a comprehensive picture of transcriptome changes in acute human skin lesions during infection by *L. tropica*.

2 Methods

Skin biopsies were obtained from *L. tropica* infected patients with acute lesion prior to therapy using a 4-mm punch. Normal skin samples were taken from volunteers who were living in a non-endemic area without a history of leishmaniasis. All patients provided written informed consent to participate. The CL diagnosis was based on both clinical examination and PCR.Total RNA isolated from frozen tissues was used for library preparation after rRNA depletion. RNA-seq was run on an Illumina HiSeq 2500 platform. Transcript quantification and differential expression analysis were done using Bioconductor package edgeR.

3 Results

Comparing the transcription profile of the *L. tropica* skin lesions with normal skin identified 5396 differentially regulated genes. Forty-seven of the top 60 up-regulated genes encode immunoglobulin fragments.Among these top 60 genes, also the most strongly 'induced' members included genes associated with inflammation and immune response activation (CHIT1,PLA2G2D, MMP9 and GBP5), inflammatory cell recruitment (CXCL9), cytotoxicity (KLRC3) and antimicrobial genes (Lys).Gene set enrichment analysis revealed that cytotoxity, inflammasome, B and T cell activation, cell surface receptors and signaling pathway are associated with acute lesions.

4 Conclusions

We observed an unexpectedly prominent role for B cells and immunoglobulins in lesions, suggesting that B cells may be strong contributors to parasite persistence. Defining the host inflammatory pathways within *L. tropica* lesions will be critical for development of new therapeutic interventions.

C0116 VISCERAL LEISHMANIASIS PATIENTS DISPLAY ALTERED COMPOSITION AND MATURITY OF NEUTROPHILS AS WELL AS IMPAIRED NEUTROPHIL EFFECTOR FUNCTIONS

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1 Background

Immunologically, active visceral leishmaniasis (VL) is characterised by profound immunosuppression, severe systemic inflammatory responses and an impaired capacity to control parasite replication. Neutrophils are highly versatile cells, which play a crucial role in the induction as well as the resolution of inflammation, the control of pathogen replication and the regulation of immune responses. Neutrophil functions have been investigated in human cutaneous leishmaniasis; however, their role in human visceral leishmaniasis is poorly understood.

2 Methods

we evaluated the activation status and effector functions of neutrophils in patients with active VL and after successful anti-leishmanial treatment. Neutrophils were isolated from blood sample by double layer density gradient technique. Neutrophil effector functions were assessed by measuring reactive oxygen species (ROS) production and phagocytic capacity by flow cytometry. Neutrophil activation status was assessed by measuring phenotypic markers on and in neutrophils by flow cytometry and by measuring degranulation products by ELISA in the plasma. The level of inflammation was assessed by measuring inflammatory markers by ELISA in the plasma

3 Results

Our results show that neutrophils are highly activated and have degranulated; high levels of arginase, myeloperoxidase and elastase, all contained in neutrophils' granules, were found in the plasma of VL patients. In addition, we show that a large proportion of these cells are immature. We also analysed effector functions of neutrophils that are essential for pathogen clearance and show that neutrophils have an impaired capacity to release neutrophil extracellular traps, produce reactive oxygen species and phagocytose bacterial particles, but not *Leishmania* parasites.

4 Conclusions

Our results suggest that impaired effector functions, increased activation and immaturity of neutrophils play a key role in the pathogenesis of VL

C0285 DOES INFLAMMATORY MONOCYTE DIFFERENTIATION GOVERN LEISHMANIA TISSUE TROPISM?

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1 Background

Leishmania (*L*) species produce a spectrum of clinical outcomes in their mammalian hosts. Following transmission to the skin by the bite of an infected sand fly, *L. major* (*L.m*) produces localized cutaneous lesions while *L. infantum* (*L.i*) disseminates to the liver and spleen, producing little or no pathology in the skin. The parasite- and host-related factors that control there respective tissue tropisms and pathologies remain poorly defined.

2 Methods

To compare the dermal immune response to *L.m* and *L.i,* 2 million metacyclics promastigotes were injected intradermally in the ear of C57Bl/6 mice. At different times post-infection the phenotypes of infected and recruited cells were analyzed by flow cytometry. Bone marrow inflammatory monocytes were cultured with metacyclic promastigotes and monocyte differentiation and intracellular parasite survival and growth were studied.

3 Results

Intradermal injection of *L.i* metacyclics promastigotes in C57Bl/6 mice results in the dissemination of the parasite to spleen and liver as early as 1 day post-infection (p.i). Cutting off the site of inoculation 16h p.i did not change the level of infection in the viscera suggesting that the dissemination is independent of establishment of infection in the dermal site. At 18h p.i, we observed that both parasites species were mainly detected within neutrophils, where they remained viable. At day 3 p.i, they transitioned into dermal macrophages during *L.i* infection, while monocytes-derived DCs and conventional DCs harboured *L.m*. *In vitro* infection demonstrated that *L.i* parasites induced bone marrow inflammatory monocytes to differentiate into macrophages whereas *L.m* favoured their differentiation into Tip-DCs. The *in vitro* bone marrow and *in vivo* dermal inflammatory monocytes seemed to impair *L.i* fitness but were permissive to *L.m* multiplication.

4 Conclusions

Our results demonstrate that *L.i* and *L.m* induce different programs of differentiation in Ly6C^{hi} monocytes. Further studies are in progress to confirm that *Leishmania* skin tropism is governed by the differential maturation of inflammatory monocytes early during the infection.

C0874 THE ROLE OF NRF2 IN LEISHMANIA INFECTION

Marta Reverté Royo[1], *Remzi Onur Eren*[1], *Dmitry Kopelyanskiy*[1], *Matteo Rossi*[1], *Patrik Castiglioni*[1], *Florence Prevel*[1], *Chantal Desponds*[1], *F. Matthew Kuhlmann*[2], *Stephen M. Beverley*[2], *Nicolas Fasel*[1]

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1 Background

Leishmania parasites are exposed to the anti-microbial reactive oxygen and nitrogen species, ROS and RNS respectively, generated by the host immune system to restrict parasite growth. However, *Leishmania* parasites have developed an elaborated antioxidant defence system to scavenge these reactive species. Nrf2 is a key transcriptional factor mediator of antioxidant genes, protecting the cell from oxidative stress. In response to oxidative stimuli, as observed with *Leishmania* infection, Nrf2 is released from its negative regulator Keap1 and translocates to the nucleus, where promotes the expression of genes such as Hmox1. In this study we focus on *L. guyanensis* (*Lgy*). *Lgy* exacerbates disease severity owing to the presence of the endosymbiotic double stranded RNA virus (LRV1). LRV1 is recognised by the Toll-like receptor-3 (TLR-3), which is a dsRNA sensor in the innate cell. LRV1 activates the key master regulator of cell survival, Akt, which is a positive regulator of Nrf2. Akt, in turn, downregulates Gsk3β, preventing Nrf2 degradation. In this study, we investigated which TLR pathway is implicated in Nrf2 activation.

2 Methods

Bone marrow macrophages (BMM) were isolated from tibias and femurs of C57BL/6 (WT), Nrf2 deficient mice (Nrf2 -/-), TLR-3 deficient mice (TLR-3 -/-), Trif deficient mice (Trif -/-) and MyD88 deficient mice (MyD88 -/-). BMMs were stimulated using Medium, PolyI:C, t*ert*-Butylhydroquinone (tBHQ) or incubated with Akt and Gsk3β inhibitors, infected with *Lgy LRV+* or *Lgy LRV-* and tested for Nrf2 activation. WT, Nrf2 -/- and Hmox1-Lysm Cre mice were infected with LRV+ or LRV- *Lgy*. Footpad swelling was measured every week for 8 weeks.

3 Results

We could conclude that Nrf2 expression did not depend on LRV1 or on TLR3 signalling; Akt and Gsk3β inhibitors validated these results. Nrf2 activation relied on MyD88 signalling in *Lgy* infection. *In vivo* experiments showed that Nrf2 promoted footpad swelling in *Lgy* infection as Nrf2 -/- infected mice showed decreased footpad swelling. Mice deficient in the Nrf2 target gene (Hmox1) did not present a phenotype in *Lgy* infection, as footpad swelling was the same as in WT infected mice.

4 Conclusions

Our data identified a critical role for Nrf2 in *Lgy* infection. Nrf2 could partly be responsible for the antioxidant defence system to scavenge reactive species that *Leishmania* parasites have developed and thus increase inflammation in *Lgy* infection.

Miradero Room

Session 11. Diagnostic methodology in practice and under development Biomarkers in leishmaniasis

C0536 DISSECTING THE IN VITRO DRUG SUSCEPTIBILITY ASSESSMENT OF CLINICAL ISOLATES: WHICH CELL TYPE BEST TO USE?

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LMPH - University of Antwerp Wilrijk - Antwerp Belgium

1 Background

Monitoring drug susceptibility of clinical *Leishmania* isolates still relies on *in vitro* drug susceptibility assays. Although such assays are extensively used in laboratories all over the world, no acceptable level of standardization is yet available, resulting in the use of different parasite stages (promastigotes versus amastigotes), host cells (primary cells versus cell lines), drug exposure times, detection methods and endpoint criteria. The need for assay standardization to decrease inter-laboratory variation and to facilitate comparison of results has already been expressed at previous WorldLeish meetings. Since our research already advocated the use of intracellular amastigotes for evaluation of drug susceptibility, identification of the most suitable host cell was a next relevant item.

2 Methods

Particularly primary cell types (peritoneal exudate cells, bone marrow-derived and blood monocyte-derived macrophages) and a few cell lines (*e.g.* J774 and RAW) were compared. The cells were screened for their susceptibility to infection with promastigotes of four different *L. infantum* strains (1 lab strain and 3 clinical isolates) and their permissiveness for intracellular amastigote proliferation after a multiplicity of infection of 15/1. To link intracellular survival to the activation status of the host cell, the production of ROS (superoxide and nitric oxide) and the cytokine responses were evaluated. In addition, the impact of cell stimulation and cell adherence time was explored, next to identifying the minimal treatment duration time. The different cell systems were assessed with the objective to propose a cheap and straightforward assay procedure.

3 Results

Despite differences in susceptibility to *L. infantum* infection *in vivo*, primary macrophages derived from Swiss, Balb/c and C57BL/6 mice only showed minimal variations in terms of susceptibility to infection and activation status of the host following different cell stimulation procedures and cell adherence times. Minor dissimilarities were noted between the different cell lines/types with regard to intracellular amastigote proliferation.

4 Conclusions

Based on the extensive *in vitro* comparison of different primary cell types and cell lines, a practical protocol to measure drug susceptibility of visceral *Leishmania* isolates will be presented in support of current attempts to improve harmonization of standard operating procedures worldwide.

C1079 FIELD SAMPLING AT ENDEMIC VILLAGES AND MOLECULAR DIAGNOSIS AT DISTANT RESEARCH CENTERS: A COUPLED AND ARTICULATED STRATEGY FOR MOLECULAR DIAGNOSIS AND STRAIN IDENTIFICATION OF CAUSATIVE AGENTS IN SUSPECTED CUTANEOUS LEISHMANIASIS LESIONS IN RURAL CITIES FROM PERU AND BOLIVIA

Braulio Mark Valencia Arroyo[1], *Lineth Garcia*[2], *Ernesto Rojas*[2], *Milena Alba*[1], *Nair Montaño*[2], *Cecilia Angulo*[2], *Aleida Verduguez*[2], *Ana Margarita Montalvo*[3], *Jorge Fraga*[3], *Jorge Arevalo*[1], *Gert Van der Auwera*[4], *Alejandro Llanos-Cuentas*[1], *Andrea K Boggild*[5], *Vanessa Adaui*[1]

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1 Background

Cutaneous leishmaniasis (CL) is a public health problem in South America from where almost one-third of the global burden is reported, in particular from Brazil, Colombia, Peru and Bolivia. It is well known that direct examination has a modest sensitivity ranging from 40-75%, and it's efficiency can be significantly affected by conditions such as chronicity of disease and experience of laboratory technicians. Although many "point of care" approaches are currently been evaluated for diagnosis of CL, none of them have demonstrated to be comparable to conventional PCR at experienced laboratory facilities

2 Methods

We have developed an articulated strategy founded on local health promoters and sample shipping through public transportation directed to distant expert research centers for molecular diagnosis and strain identification in two endemic villages from Peru and Bolivia. One hundred and twenty two subjects with clinically suspected CL were evaluated by direct examination, Leishmanin skin test and kDNA PCR. Samples for PCR were obtained by scraping or brushing from CL lesions

3 Results

Sensitivity and specificity of smear were 59.5% (95% CI 47.8–70.2%) and 89.3% (95% CI 76.1–96%) respectively. In the case of Leishmanin skin test, the sensitivity and specificity were 82.3% (95% CI 71.7–89.6%) and 80.8% (95% CI 66.2–90.4%). Sensitivity and specificity of kDNA PCR were 92.4% (95% CI 83.6–96.8%) and 93.6% (95% CI 81.4–98.3%) for samples obtained by scraping and 97.5% (95% CI 90.3–99.6%) and 85.1% (95% CI 71–93.3%) for samples obtained by brushing. Species identification was randomly performed in 46 parasite-probe samples by hsp70 PCR in which 33 samples were undoubtedly characterized as L. (V.) braziliensis (11 samples), L. (V.) guyanensis (21 lesions) and L. (V.) lainsoni (2 lesions) in Peru and Bolivia. Blinded species identification performed in Cuba as a part of a ring trial confirmed the vast majority of this results as well as identification of strain in 10 lesions without quantifiable DNA. Results in which discordant results were identified at ring trial were examined by DNA sequencing at Belgium and Canada. At the end of the ring trial 95% of cumulative strain identification (44/46) was possible.

4 Conclusions

Our intervention allows successful molecular diagnosis and species identification using field sampling at endemic villages and molecular diagnosis at distant research centers.

C0181 THERAPEUTIC ROLE OF CHOLESTEROL IN EXPERIMENTAL VISCERAL LEISHMANIASIS

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1 Background

In patients suffering from Kala azar there is a significant decrease in cholesterol level. Cholesterol forms an adduct with a number of membrane proteins to maintain their conformation. The causative agent Leishmania donovani (LD) replicates within the macrophages (MΦs) depletes membrane cholesterol. Therapeutic role of cholesterol has been studied in tuberculosis. We asked the similar question and dissected the mechanism.

2 Methods

MΦs were harvested from mice. The T-cell stimulating ability of MΦs was studied using class II restricted peptide specific T-cell hybridomas and resulting IL-2 production was used as functional read out. Conformation of cell surface associated MHC II protein was studied using conformation specific mAb (11.5–2). Liposomal cholesterol was prepared with cholesterol and phosphatidyl-choline. For molecular dynamics (MD) studies exhaustive (1 microsecond) all-atom of the MHC-II 3D model was carried out in presence and absence of cholesterol. Cholesterol was docked onto the CRAC motif region of the MHC-II 3D model and complex structure embedded in POPC bilayer was used for MD simulation. The lateral mobility of a membrane protein was carried out using GFP-tagged PLCd1 as a probe and was analyzed from the FRAP experiment.

3 Results

The infected MΦs (I-MΦs) display increased lateral mobility of membrane proteins and functional defects in terms of class-II restricted T-cell activation. As compared to the normal counterpart, there is faster dissociation of the cognate peptide from the peptide-MHC complex by the I-MΦs. Using conformation specific mAb we show that there is an alteration in the conformation of the MHC-II protein; the conclusion is substantiated by MD simulation studies. MD analysis showed that presence of bound cholesterol at the transmembrane domain of MHC-II protein provides suitable structural conformation and stability of the extra cellular domain required for proper antigen presentation. Since liposomal cholesterol delivery in I-MΦs corrects above defects and restores T-cell stimulating ability we studied the therapeutic role of cholesterol. We found that injection of liposomal-cholesterol in infected hamsters resulted in organ parasite clearance.

4 Conclusions

Since liposomal cholesterol corrects infection induced cellular defects and decreases organ parasite in infected hamsters thus liposomal cholesterol may be used as an adjunct to treatment of visceral leishmaniasis.

C1393 POINT OF CARE DNA ASSAYS FOR DIAGNOSIS OF CUTANEOUS LEISHMANIASIS IN TUNISIA AND IN THE OLD WORLD

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1 Background

Leishmaniases constitute major public health problems in many countries, and are particularly endemic in the Afro-Mediterranean region. In Tunisia and other countries of the region, complex epidemiological situation with proven implication of at least 3 *Leishmania* (L.) species: *L. major*, *L. infantum*/*L. donovani* and *L. tropica* which are responsible for Cutaneous leishmaniasis. Visceral leishmaniasis is caused by *L. infantum* or *L. donovani* according to the country. The aim of this study is to develop rapid, simple, sensitive and specific DNA assays for POC *L.* species detection and identification. Such tools could provide early alert on changes in clinico-eco-epidemiological patterns. For this purpose, 2 technologies were considered: Multiplex PCR (Mx-PCR) and a rapid and ultra sensitive colorimetric detection of DNA hybrids on DNA miniarrays

2 Methods

RAPD analysis and *in silico* comparative genomic analyses of *L.* genomes were used to identify 7 targets. Different primer pairs were designed using "primer3" and assessed for criteria of taxa-specific amplification. Retained primer pairs were used to develop Mx-PCR assays. Their specificity was tested experimentally. A total of 201 *L.* strains or samples were used in this study including 80 DNAs extracted from typed *L.* strains belonging to 8*L.* species of the Old World, 82isolates from Tunisian CL patients and 39 remains of biological samples taken for diagnosis of suspected CL. DNA miniarrays were developed which allowed a drastic increase in sensitivity of detection and a rapid results delivery time as compared to classical detection on agarose gels

3 Results

6 primer pairs were selected for the development of 2 Mx-PCR assays that generated consistent and well differentiating amplification profiles for species specific identification of the 3 *L.* species/complexes encountered in the Afro-Mediterranean region. Mx-PCR assays were assessed on DNA extracted from CL samples offering up to 88% sensitivity of detection. DNA arrays developed allowed a gain of 100 to 1000 fold in sensitivity of detection in controlled reconstruction experiments where down to 2 parasites could be detected, therefore demonstrating a good potential for diagnosis

4 Conclusions

Our Mx-PCR coupled to DNA miniarrays have a very good potential to constitute POC CL diagnosis tools. We are currently establishing protocols for clinical evaluation of this diagnosis method in Tunisia.
Financial support: IAEA, AUF and the Ministry of Higher Education and Research in Tunisia

C1533 SLA-CELL PROLIFERATION TEST USEFUL ON MANAGEMENT OF VISCERAL LEISHMANIASIS PATIENTS UNDER IMMUNOSUPRESSOR TREATMENTS

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1 Background

Treatment with immunosupressors (IS) drugs (esteroids, methotrexate, anti-TNF) is a predisposing factor for VL, but optimal treatment regimen is not well stablished. SLA-cell proliferation test has been confirmed as marker of specific cellular immunity for VL. Largest leishmaniasis outbreak in Europe was declared since June 2009 in Fuenlabrada (Madrid, Spain). In this setting, our aim was to validate the usefulness of this cellular test for the management of VL on IS patients

2 Methods

From January 2013 IS patients, excluding trasplanted, with previous or active VL, were recruited to 1st November 2016 (201 weeks). Leishmania-specific PCR, serology (ELISA or IFAT) or SLA-cell proliferation test were used besides habitual test. Relapse was defined as symptomatic VL after the end of the treatment

3 Results

18 VL cases in IS patients were identified: 13 on steroids, 9 on methotrexate and 5 on antiTNF therapies, alone or in combination.
3 patients presented mucosal leishmaniasis, no relapses (mean70 weeks) in the follow up. SLA-cell proliferation test were negative.
15 VL were asymptomatic and clinically cured when they were recruited. SLA-cell proliferation test was positive in 14 cases and negative in one. All of them were PCR negative.
The patient with negative SLA-cell proliferation test needed to continue on steroid treatment, received monthly prophylaxis with LAB and was monitorized for Leishmania tests. Steroid doses were tappered and after 25 weeks SLA-cell proliferation test became positive. Prophylaxis with LAB was stopped and after 116 weeks the patient had no relapse of VL.
14 patients with positive SLA-cell proliferation test was allowed to reintroduce IS drugs and were monitorized for Leishmania tests. In all 14 patients, blood PCR was negative along the follow up.
In 11 of them, SLA-cell proliferation test remained positive, 9 are still under IS therapies and none of them presented relapses (mean 235 weeks).
In last 3 patients, SLA-cell proliferation test became negative during the follow up, 2 presented clinical relapse and the third one was apparently asymptomatic but PCR was positive in bone marrow

4 Conclusions

In this small sample of IS patients with VL (not mucosal), positive SLA-cell proliferation test, as a marker of specific cellular immunity, was useful to monitor these patients after treatment: it avoids secondary prophylaxis when positive, and predicted relapse when negative. Steroids and methotrexate treated patients seems more prone to relapse than those under antiTNF therapies

C0153 LEISHMANIA HASP AND SHERP GENES ARE REQUIRED FOR IN VIVO DIFFERENTIATION, PARASITE TRANSMISSION AND VIRULENCE ATTENUATION IN THE HOST

***Johannes Doehl*[1]**, *Jovana Sádlová[2], Hamide Aslan[3], Katerina Pružinová[2], Sonia Metangmo[3], Jan Votýpka[2], Shaden Kamhawi[3], Deborah Smith[1]*

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[3]Vector Molecular Biology Section, Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health USA

1 Background

Differentiation of *Leishmania* promastigotes within their sand fly vector, termed metacyclogenesis, is essential for parasites to regain mammalian host infectivity. It is accompanied by changes in the local parasite environment, including secretion of complex glycoconjugates within the promastigote secretory gel and colonization and degradation of the sand fly stomodeal valve. Deletion of the stage-regulated HASP and SHERP genes on chromosome 23 of *Leishmania major* is known to stall metacyclogenesis in the sand fly, but not *in vitro*.

2 Methods

We generated and characterized a series of new *L. major* HASP and/or SHERP replacement lines using standard *in vitro* methods. Fully tested lines were inoculated into the footpads of BALB/c mice to measure parasite infectivity and used in blood-feeding sand flies to test the contribution of HASP and SHERP proteins to metacyclogenesis and parasite transmission *in vivo*. Light and confocal microscopy, RT-PCR, immunoblotting, limiting dilution assay, in vitro osmotaxis and growth assays were employed for detailed analyses in these experiments.

3 Results

Metacyclogenesis was stalled in HASP/SHERP mutants in vivo and, although still capable of osmotaxis, these mutants failed to secrete promastigote secretory gel, correlating with a lack of parasite accumulation in the thoracic midgut and failure to colonise the stomodeal valve, preventing parasite transmission. Sand fly midgut homogenates were shown to modulate parasite behaviour in vitro, suggesting a role for molecular interactions between parasite and vector in *Leishmania* development within the sand fly. Further, we showed for the first time stage-regulated expression of *L. major* HASPA2 (promastigote-specific) and HASPA1 protein (amastigote-specific). Despite the lack of HASPA2 expression in amastigotes, its gene replacement into the null locus background delays onset of pathology in BALB/c mice. This HASPA2-dependent effect is reversed by HASPA1 gene addition, suggesting that the HASPAs may have a role in host immunomodulation.

4 Conclusions

Our work confirms the importance of HASP and SHERP genes in metacyclogenesis in vivo and demonstrates their importance in successful sand fly midgut colonization, transmission and host infectivity. We also show important experimental differences between parasite behaviour in vitro and in vivo, which we suggest is attributed to yet-to-be identified midgut molecules. This work emphasises the importance of validating data generated in in vitro culture in the sand fly.

Greco Room C1-C2
15:30-17:00

Session 12. Epidemiology

C0410 MOLECULAR EPIDEMIOLOGY OF HUMAN CUTANEOUS LEISHMANIASIS IN JERICHO AND ITS VICINITY IN PALESTINE FROM 1994 TO 2015

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1 Background

Cutaneous leishmaniases (CL) are vector-borne parasitic diseases endemic in many countries of the Middle East including Palestine.

2 Methods

Between 1994 and 2015, 2160 clinically suspected human cases of CL from the Jericho District were examined. Stained skin tissue smears and aspirates were checked by microscopy and cultured for promastigotes, respectively. For leishmanial species identification, amplification products from a PCR-ITS1 followed by RFLP analysis using Hae III. Data were analyzed using Epi Info free-software.

3 Results

The overall infection rate was 41.4% (895/2160), 56.3% (504/895) of the cases were male, 43.7% (391/895) female, 60.5% (514/849) children under age 14, 41.3% (259/627) of the cases were caused by Leishmaniamajor and 57.3% (359/627) by Leishmaniatropica. The case numbers peaked in 1995, 2001, 2004, and 2012. Statistically-significant clusters of cases caused by L. major were restricted to the Jericho District; those caused by L. tropica were from the districts of Jericho, Bethlehem, Nablus and Tubas. CL is seasonal and trails the sand fly season. Distribution of cases was parabolic with fewest in July. The monthly total number of cases of CL and just those caused by L. major correlated significantly with temperature, rainfall, relative humidity, evaporation, wind speed and sunshine ($P<0.05$, $r2=0.7-0.9$ and $P<0.05$, $r2=0.5-0.8$, respectively). Cases caused by L. tropica, significantly, had a single lesion compared to cases caused by L. major ($P=0.0001$), which, significantly, had multiple lesions ($P=0.0001$).

4 Conclusions

This and previous studies showed that CL is present in all Palestinian districts. The surveillance of CL has increased public awareness and molecular biological methodology for leishmanial species identification is an essential addition to classical diagnosis. The overall results are discussed, correlated to climatic and environmental changes and large-scale human activities.

C0568 INCIDENCE AND RISK FACTOR FOR POST-KALA-AZAR DERMAL LEISHMANIASIS AND VISCERAL LEISHMANIASIS RELAPSE: EVIDENCE FROM A PROSPECTIVE COHORT STUDY IN BANGLADESH

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1 Background

Post-Kala-azar Dermal Leishmaniasis (PKDL) and visceral Leishmaniasis relapse (VLR) are potential threat for the success of the visceral leishmaniasis (VL) elimination program of the Indian sub-continent. Single intravenous infusion of liposomal amphotericin B (AmBisome) (SDA) in a dose of 10mg/kg body weight is currently the first choice for treatment of VL in Bangladesh, India and Nepal. Incidence of and risk factor for VLR and PKDL after treatment with SDA is unknown. In this study, we investigated incidence of PKDL and VLR their risk factor among cured VL patients after treatment with SDA.

2 Methods

Study participants include 290 cured (six months after start of treatment) VL patients, treated with SDA under a controlled study. Study participants had been followed and clinically evaluated every six months for a period of four years. Study physician referred suspected cases with VLR / PKDL to the Surja Kanta Kala-azar Research Centre for confirmation of diagnosis and treatment. VLR had been confirmed by demonstration of LD bodies in splenic aspirate and PKDL by demonstration of LD bodies and / isolation of LD DNA by qPCR in skin specimens of skin lesions.

3 Results

Three percent and 13% of cured VL patients developed respectively VLR and PKDL within 4-years observation. The annual incidence of VLR and PKDL was respectively 1% and 4%. However, 90% of VLR and 94% of PKDL occurred respectively within 180 days and 730 days after cure from VL. VLR was more frequently observed in younger when treated for VL (mean ± SE, 14±3.5 yrs vs. 19±0.83 yrs, p=0.24). However, cases with and without subsequent PKDL were comparable regarding age during treatment for VL (mean ± SE, 20±2.23 yrs vs. 19±0.83 yrs, p=0.53). There was no predilection to sex for development of PKDL and VLR. We did not find any association of spleen size and blood hemoglobin level before treatment and 30 days after treatment with development of VLR or PKDL.

4 Conclusions

This is the largest cohort study with active follow up of cured VL patients for development of VLR and PKDL after treatment with SDA. After cure from VL the incidence of VLR was insignificant but incidence of PKDL is a concern. These study findings will be useful for VL elimination program of the region for defining case management strategy during maintenance and consolidation phases of the program.

C0782 AN EMERGING CLUSTER OF CUTANEOUS LEISHMANIASIS IN NORTH-EASTERN ITALY: IS A NOVEL STRAIN CIRCULATING IN THIS AREA?

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1 Background

Human leishmaniasis is on increase in the Mediterranean Europe. Nevertheless, the exact prevalence of cutaneous leishmaniasis (CL) is largely unknown as underdiagnosis and underreporting are common. In this study, we evaluated epidemiological, clinical and molecular features of CL cases occurring in the Bologna province, north-eastern Italy.

2 Methods

We performed a retrospective study on CL cases diagnosed in the Bologna province (Emilia-Romagna region, north-eastern Italy) between January 2013 and October 2016.Species identification was carried out by *hsp70* amplification and sequencing, while strain characterization was performed by multilocus microsatellite typing (MLMT) targeting 15 highly polymorphic markers.MLMT genotypes were compared with a collection of Italian *L. infantum* strains.

3 Results

During 2013-2016, 36 cases of CL were identified in the Bologna province with an average incidence of 1.00/100,000, with a 4- up to 12-fold increase as compared to previous years. Twenty one out of 36 (58%) CL cases presented as single, typical lesions. Molecular identification of *Leishmania* species by *hsp*70-based PCR showed signatures of both *L.infantum* and *L.donovani*. MLMT provided preliminary evidence of genetic diversity between strains from CL from the Bologna's province and those commonly circulating in Italy. Further molecular evaluation of strain characteristics is ongoing.

4 Conclusions

We report an increased number of cases of CL in a focal area of north-eastern Italy in 2013-2016. Our preliminary results suggest that an unusual Leishmania strain circulates among humans in the Bologna province, north-eastern Italy. This study highlights the importance of CL surveillance in endemic areas and emphasize the need of molecular laboratory surveillance for CL.

C1011 LEISHMANIASIS IN SWEDEN 1993-2016

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1 Background

In Sweden, leishmaniasis is an imported disease, and its epidemiology and incidence was not known until now. Immigration to Sweden reached a peak in 2014-2015, with over 150 000 people, who now constitute 1,5% of the Swedish population (10 million). Many come from areas endemic for leishmaniasis. We conducted a retrospective, nationwide, epidemiological study from 1993 through 2016.

2 Methods

We conducted a search for all probable cases of leishmaniasis reported to the Swedish Patient registry, which collects data on all admitted patients in Swedish healthcare since 1993 and out-patient visits since 2001. A search for laboratory-verified cases were conducted in databases of the parasitology laboratory at the Swedish Public Health Agency from 1993 through 2016. Laboratory verification included microscopy, culture, serological and molecular testing. Clinical and demographic data, referring clinic, travel history and sample type were collected.

3 Results

In total, 299 probable cases and 182 verified cases were identified. However, data from out-patient clinics has only been registered since 2001. Annual incidence ranged from 0,023 to 0,35 per 100 000 with a rapid increase in the last four years. Of 182 laboratory-verified cases, 100 were diagnosed from 2013 through 2016, and in this group almost half of the patients were children under 18 years. Patients were not concentrated in the large cities, but presented in different healthcare settings in all regions of Sweden. Cutaneous leishmaniasis was the most common clinical manifestation (>95% of cases) and the majority of infections were acquired in Asia, specifically Syria and Afghanistan. *Leishmania tropica* was responsible for the majority of cases (42%). A combination of laboratory methods increased the sensitivity of diagnosis among verified cases.

Conclusions: Our study presents the first estimation of incidence of imported leishmaniasis in Sweden, and highlights the importance of having a high index of suspicion for leishmaniasis for certain groups of immigrants and travellers presenting to Swedish healthcare professionals. Swedish residents who have spent time in Leishmania-endemic areas, could be at risk of developing disease some time during their lives. Increased awareness and knowledge is needed for correct diagnosis and management of this rare disease in Sweden.

4 Conclusions

There has been a fourfold increase in laboratory-verified cases of leishmaniasis in Sweden the past two years. Most of these cases are Syrian immigrants infected with L. tropica. Our study highlights the importance of having a high index of suspicion for leishmaniasis for certain groups of immigrants and travellers presenting to Swedish healthcare professionals.

C1452 EMERGING FOCI OF VISCERAL LEISHMANIASIS IN ARMENIA – MOLECULAR EPIDEMIOLOGY AND PILOT RISK ASSESSMENT BY ECOLOGICAL NICHE MODELING

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1 Background

Visceral Leishmaniasis (VL) is re-emerging in Armenia. From 1926 till 1969 919 cases were reported in 16 districts, dogs were identified as reservoir. After a control program in 1954-1969 VL disappeared until 1999 when again a single case emerged. Until 2016 >81 cases were recorded in different parts of Armenia. Entomological studies in active foci identified *Ph. balcanicus* and *Ph. kandelaki* as main vectors. Objectives of this study were (i) to determine for the first time the genetic diversity and population structure of *L. infantum* in Armenia and to compare the genotypes with those from endemic regions worldwide to draw conclusions about the mode of spread and to enable epidemiological monitoring of VL in Armenia; (ii) to predict the spread of VL and the vectors in time and space based on ecological niche modeling to provide control strategies.

2 Methods

20 human samples from different parts of Armenia previously identified by ITS-1 RFLP as *L. infantum* were studied by Multilocus Microsatellite Typing (MLMT). These data were combined for comparison with the set of all previously typed *L. infantum* strains from the main endemic regions in the World (19 countries, 520 strains). Ecological niche modeling was performed based on registered VL cases and identified sandflies collected in 2009-2015. Both datasets were used for calculation of distribution models for different time periods.

3 Results

Within the 20 Armenian *L. infantum* strains 19 different genotypes were identified, which clustered into two main populations that were not related to the geographic origin of the cases. The combined analysis revealed the closest relationship to MON-1 subpopulations from Greece, Turkey, Cyprus, Israel, Uzbekistan. The ecological niche models identified regions at the borders to the neighboring countries Georgia, Turkey, Iran, Azerbaijan as most suitable for the vectors and with the highest risk for VL.

4 Conclusions

This study identified for the first time genotypes and their diversity of *L. infantum* circulating in Armenia assigning them to specific geographic MON-1 populations. Based on these results further genotyping studies should be performed with human and animal reservoir samples also from the neighboring countries to understand re-emergence, spread and epidemiology of the disease. An important role for developing control strategies in the whole region of Southern Caucasus will play further modeling based on a comprehensive collection of epidemiological and entomological data in this region.

C1490 SPREAD OF ZOONOTIC VISCERAL LEISHMANIASIS FROM NORTHERN TO CENTRAL TUNISIA

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1 Background

During the last twenty years, zoonotic visceral leishmaniasis (ZVL) was spreading from the north characterized by humid, sub-humid, and semi-arid bio-climates to the arid areas located in Central Tunisia. In order to understand the eco-epidemiological factors involved in the distribution of ZVL, a sero-epidemiological and an entomological investigation were performed in two governorates situated in two distinct geographical areas: Bizerte in the north and Kairouan in the center.

2 Methods

All human cases of ZVL diagnosed in each governorate between 2004 and 2013 were included in the study. ZVL cases were identified from the Regional University Hospital that serves the whole population of each governorate. A total of 299 sera collected from clinically healthy owned dogs used for guarding houses in the governorate of Bizerte and tested for anti-*L. infantum* antibodies by indirect immunofluorescence antibody test (IFAT). Sand flies were collected inside animal shelters located in the peri-domestic areas by using CDC light traps. Collected sand flies were identified to the species level. The dominance of each species was measured by its relative abundance. Sand flies were examined for the presence of *Leishmania* DNA by using a nested PCR.

3 Results

Among 108 sera collected from clinically healthy owned dogs used for guarding houses in the governorate of Bizerte and tested for anti-*L. infantum* antibodies by indirect immunofluorescence antibody test (IFAT), 8.3% were positive. From of total of 191 serum samples collected from clinically healthy owned dogs used for guarding houses in the governorate of Kairouan and tested for anti-*L. infantum* antibodies by IFAT, 26.7% were seropositive. The cumulative incidences of ZVL in humans in the governorates of Kairouan and Bizerte were 39.17/100,000 and 0.2/100,000 inhabitants, respectively. *Phlebotomus perniciosus*, *P. perfiliewi* and *P. longicuspis* are the most abundant sand fly species in both governorates. While sand fly species of the subgenus *Larroussius* are abundant in the two governorates, the infection rates of sand flies with *Leishmania* DNA in the governorate of Bizerte and Kairouan were 3.8% and 9.4%, respectively.

4 Conclusions

A high prevalence of *L. infantum* in dogs is a key factor for triggering transmission to humans. Thus, it is an important parameter in the control of ZVL transmission.
These results suggest a higher force of infection in emerging foci such as the governorate of Kairouan leading to an important incidence of ZCL in humans.

C0892 CANINE POPULATION DYNAMICS DETERMINE LEISHMANIA INFANTUM TRANSMISSION RATES AND INTERVENTION EFFORT.

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1 Background

In zoonotic visceral leishmaniasis (VL), high turnover rates of canine reservoir populations, through both natural loss and targeted culling, involve the loss of both infectious and non-infectious individuals (such as uninfected or never-infectious dogs). The associated rapid replacement of lost dogs with young/immunologically naïve individuals or infected immigrants has the potential to promote transmission and long-term persistence of infection in the population. As such, reservoir population dynamics determine the effort required to maintain interventions targeted at reservoir populations, including impregnated dog collars or vaccinations.

2 Methods

Here, we formally describe the dynamics of a range of canine populations and use individual based stochastic models to mathematically explore the practical implications of population turnover rates for VL transmission, and to assess intervention strategies.

3 Results

Constructed models were validated by demonstrating similar results to empirically derived infection estimates. Sensitivity analyses identified key parameters that drive transmission under different demographic and epidemiological conditions. The implications of these results to the implementation of control interventions are discussed.

4 Conclusions

Reservoir population dynamics, and the forces that determine these rates, have significant implications for transmission, and consequently on the effort required to drive down infection incidence. The importance of demography and intervention interval is well characterised in the case of human vaccine controlled infections. These data represent the first attempt to consider similar dynamics in dog populations, where turnover not only influences transmission but also has the potential to dilute any community-level effects of individual-based interventions if not well understood.

C1547 ASYMTOMATIC LEISHMANIA INFECTION IN FUENLABRADA HIV COHORT. LEISHMANIASIS OUTBREAK IN FUENLABRADA, SPAIN

*Alicia Castro[1], Eugenia Carrillo[2], **Juan San Martín López[1]**, Laura Botana[2], Laura Molina[1], Laura Fernández[2], Belén Matía[1], Carmen Chicharro[1], Javier Nieto[1], Sheila Ortega[1], Ana Ibarra-Meneses[1], Luis Horrillo[1], Carmen Sánchez[2], Javier Moreno[2], Jose Manuel Ruiz Giardin[1]*

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1 Background

The largest leishmaniasis outbreak in Europe was declared since June 2009 in Fuenlabrada (Madrid, Spain). Our aim was to estimate the prevalence of asymptomatic infection in the HIV cohort of Fuenlabrada, in this high exposure setting, and analyze the risk factors for the development of visceral leishmaniasis (VL) in these patients

2 Methods

Fuenlabrada's HIV cohort was checked for exposure to *Leishmania*. A *Leishmania* infected case (LIC) was defined as a positive result in, at least, one of following blood tests: *Leishmania*-specific PCR, serology (ELISA or IFAT) or SLA-cell proliferation test(marker of cellular immunity). LIC could be symptomatic (cutaneous or visceral) or asymptomatic (never symptoms of active leishmaniasis). LIC vs not infected, and VL vs asymptomatic LIC, were analyzed with statistical tests looking for risk factors.

3 Results

583 patients of the Fuenlabrada's HIV cohort were followed since 1st June 2004 to 31th December 2015. 135 representative patients were selected for exposure test to *Leishmania*, including 12 symptomatic patients (10 visceral and 2 cutaneous). 19 (15.4%) of the 123 asymptomatic patients fulfilled the definition for *Leishmania* infection, none of them became symptomatic during the study period. Total prevalence of the cohort was estimated 17.5%.
Multivariate analysis was performed in *Leishmania* infected cases (LIC) vs not infected and find association to the "distance to Bosquesur park", considered the focus of the outbreak, 1082 meters media distance to the park in infected vs 1719 meters not infected (ANOVA p=0.02). Multivariate analysis performed between patients who developed VL vs asymptomatic LIC showed association to illness development only for patients with "negative SLA-cell proliferation test with positive PCR or serology" (p<0,001, OR not calculable) and "less than 100 CD4 during outbreak period" (p=0,001, OR not calculable)

4 Conclusions

In the TARGA era, 15.4% of our HIV cohort has been infected by *Leishmania* but remain asymptomatic. Negative SLA-cell proliferation test with positive PCR or serology, reflects the absence of specific immune response to Leishmania, and is therefore an independent risk factor for visceral leishmaniasis development

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Thursday, 18th of May
Greco Room D1-D2
9:30-11:00

Session 13. Host-parasite cellular and molecular interactions

C0214 LEISHMANIA DONOVANI DELAYS PHAGOSOME-LYSOSOME FUSION BY SABOTAGING THE HOST AKT-AS160-RAB14 PATHWAY

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1 Background

Intramacrophage pathogens upon invasion, faces the challenge to survive within the acidic environment of phagolysosomes (PL) which are meant for pathogen destruction. To successfully reside and multiply within these, the *Leishmania* parasites need to delay the phagosome maturation process to transform from the acid labile promastigotes to the acid tolerant amastigote forms. As small GTPases are known to play important roles in vesicular fusion events, here we demonstrate the critical role of AKT-mediated-activation of small GTPase Rab14, which blocks the phagosome maturation process giving the parasite the optimum time for differentiation thereby aiding its survival.

2 Methods

To elucidate the roles of key molecules playing instrumental roles in phagosome maturation events extensive use of confocal microscopy has been undertaken. Along with this, biochemical studies and molecular biological techniques have been put to use to substantiate and validate the data obtained from microscopical observation. All experiments were done in triplicates in RAW264.7 cells and validated in BMDM isolated from the Balb/c mice.

3 Results

Our work reliably elucidates that the PL fusion events are delayed in live *Leishmania donovani* infected macrophages (mφ) as opposed to the fixed parasite infected mφ where early fusion of the PL is observed. Further work demonstrated that this PL fusion delay is dependent on AKT-mediated phosphorylation and inactivation of AS160 which is a GTPase-Activating protein (GAP). Upon phosphorylation by AKT, AS160 can no longer inactivate Rab14 which continues to persist on the parasitophorous vacuole and inhibit phagosome maturation. Prolonging Rab14 persistence on phagosomal membrane by using constituitively active Rab14 or siRNA targeting of AS160 in fixed parasite infected cells showed delayed PL fusion kinetics. On the other hand, chemical inhibition of AKT or use of dominant negative constructs of AKT which would diminish presence of Rab14 on phagosome membrane or using dominant negative constructs of Rab14 in live parasite infected cells showed early and increased kinetics of PL fusion further supporting our hypothesis that PL fusion events in *Leishmania* infected mφ are dependent on AKT mediated AS160-Rab14 regulation.

4 Conclusions

To our knowledge this is the first report on manipulation of host AKT-AS160-Rab14 by *Leishmania* to delay phagosome maturation which provides sufficient time to the parasite to differentiate into amastigotes.

C0422 DEVELOPMENT OF A NEW SYSTEMS-WIDE SCREEN TO IDENTIFY HOST SUBSTRATES OF THE LEISHMANIA EXCRETED CASEIN KINASE ISOFORM 1.2

Despina Smirlis[1], *Florent Dingli*[2], *Damarys Loew*[2], *Gerald F. Späth*[1], *Najma Rachidi*[1]

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1 Background

Leishmania secreted proteins, particularly signaling proteins, are important modulators of macrophage functions for establishing a permissive environment for intracellular parasite survival. A known *Leishmania* excreted signaling molecule is Casein Kinase 1.2 (LmCK1.2), and our current hypothesis is that LmCK1.2 is released through exosomes into the host cell to phosphorylate host proteins allowing the parasite to subvert macrophage functions. To investigate the role of LmCK1.2 on macrophage reprogramming, it is essential to identify its host substrates.The major difficulties for determining *bona fide* kinase substrates are the laborious methodology, the small yield, and the background kinase activities. To address these major challenges, we developed a new method to quickly identify LmCK1.2 substrates from host macrophages.

2 Methods

We used Stable-Isotope Labelling by Amino acids in Cell culture (SILAC), phosphatase treatment, *in vitro* kinase assays, TiO2 phospho-peptide enrichment and mass spectrometry analysis to compare the phosphorylation status of heat inactivated THP-1 protein extract after *in vitro* kinase assay with recombinant LmCK1.2 or LmCK1.2-K40A (inactive).

3 Results

We showed that (1) heat inactivation of the THP-1 protein extract completely abolished the background endogenous kinase activities, (2) treatment of the extracts with phosphatase unmasked occupied phosphosites, and (3) use of SILAC simplified quantification. These steps allowed over 2-fold enrichment of more than 800 phospho-peptides, among which several showed known CK1 phosphorylation motifs ($S/TX_{(2-3)}S/T$). We identified over 450 potential LmCK1.2 substrates, implicated in activities critical for the modulation of macrophage functions, such as lipid binding, transcription activation activity and receptor signaling. In addition, we identified known substrates of mammalian CK1, such as vinculin, stathmin or ankyrin.

4 Conclusions

We have developed a new high throughput method to identify kinase substrates that could be applicable to most kinases. Using this method, we identified, for the first time, putative host substrates of the parasitic CK1.2, suggesting the potential of this kinase to interfere with many host pathways. These findings will enlighten our knowledge on trans-signalling events induced by *Leishmania* in the macrophage, highly relevant for the subversion of macrophage functions.

C0474 LEISHMANIA AMAZONENSIS AMASTIGOTES COORDINATE A REGULATORY DICHOTOMY AT THE TRANSCRIPTOME LEVEL TO INHIBIT NF-KB AND INFLAMMASOME ACTIVATION IN PRIMARY MACROPHAGES

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1 Background

Leishmania amazonensis (*L. am.*) – a causal agent of cutaneous Leishmaniasis - has the capacity to subvert macrophage metabolic pathways and immune functions. However, the impact of virulent *L. am.* amastigotes on the NLRP3 inflammasome activation in primary macrophages has not been investigated.

2 Methods

Primary macrophages were differentiated from bone marrow precursors of BALB/c mice in presence of CSF-1. Virulent *L. am.* amastigotes were isolated from lesions of nude mice and used to infect macrophages (MOI of 4 to 1). NLRP3 inflammasome activation was evaluated up to 30 days post infection in presence or absence of LPS / ATP through IL-1b and IL-18 secretion by ELISA, ASC speck formation and caspase-1 cleavage by Western blotting. NF-kB activation was assessed monitoring RelA nuclear translocation by fluorescence microscopy. Transcriptional analyses were performed by RT-qPCR.

3 Results

We describe for the first time that infection of BMDMs with *L. am.* amastigotes does not trigger inflammasome activation even after 30 days of infection. Infection caused coordinated transcriptional reprogramming of host macrophage functions, including down-modulation of inflammasome activating pathways (TLRs, signaling kinases) and up-modulation of inflammasome inactivating pathways, such as deubiquitinating enzymes. Modulations of key regulators of inflammatory signaling were associated with lower translocation of RelA into the nucleus of infected cells and decreased transcription of genes whose products define different inflammasomes (NLRP3, NLRC4, AIM2, RIG1), IL-1b and IL-18. Of note, these subversions were maintained after LPS / ATP stimulation, resulting in a strong decrease in IL-1b and IL-18 secretion in infected macrophages. Surprisingly, inhibition did not concern cytokines whose secretion does not depend upon inflammasome activation, such as TNF.

4 Conclusions

Our study describes a new subversion mechanism deployed by amastigotes that target transcription of key regulators of the NF-kB and inflammasome activation pathways to dampen the macrophage pro-inflammatory response and favor parasite survival.

C0502 UNDERSTANDING THE EXOSOMAL RELEASE OF THE LEISHMANIA CASEIN KINASE 1 ISOFORM 2 BY REVEALING ITS BINDING PARTNERS

Daniel Martel[1], *Damarys Loew*[2], *Florent Dingli*[2], *Gerald Späth F*[1], *Najma Rachidi*[1]

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1 Background

L. donovani is the causative agent of visceral Leishmaniasis, a major public health problem worldwide. We are interested in understanding how *Leishmania* interacts with macrophages, and particularly the role of secreted parasite proteins on intracellular *Leishmania* survival. Casein Kinase 1 isoform 2 (CK1.2), a protein kinase involved in signalling, has been identified in *Leishmania* exosomes by proteomics analyses and is important for intracellular parasite survival. However little is known about the functions it carries outside of the parasite. We postulate that CK1.2 is secreted in the macrophage to phosphorylate host proteins in order to modulate the host cell biology and evade the innate immune response. To understand how the secretion of CK1.2 into the host cell via exosomes is regulated, we identified CK1.2 binding partners in the parasite.

2 Methods

We immunoprecipitated V5-tagged CK1.2 from transgenic promastigotes and axenic amastigotes, and the eluted samples were analysed by mass spectrometry. We used bioinformatics and immunofluorescence analyses to study the interaction and localisation of the selected CK1.2 partners as well as their functions.

3 Results

We identified 46 CK1.2 binding partners in promastigotes and 102 in amastigotes. In promastigotes, CK1.2 partners were mostly involved in vesicular processes, folding and transport, whereas in axenic amastigotes they were involved in metabolic pathways, translation and RNA processing. We identified interacting partners orthologous to those of mammalian CK1 such as HDAC, but also novel partners including many hypothetical protein. In order to gain a first insight into their putative function, we used I-TASSER (Iterative Threading ASSEmbly Refinement) software for *de novo* structure prediction and protein tagging, which provided useful elements toward the understanding of their functions. For further characterisation and to study their interaction with CK1.2 in the parasite, we selected several proteins implicated in vesicular sorting and are currently performing immunofluorescence experiments and functional studies allowing us to better understand the mechanisms underlying CK1.2 secretion.

4 Conclusions

We identified different CK1.2 binding partners in promastigotes and in amastigotes, suggesting that CK1.2 regulates different pathways in each parasite life stage. Proteins implicated in endosomal processes and vesicular transport are currently studied to determine their implication in CK1.2 exosomal loading.

C0761 TRANSCRIPTIONAL PROFILING BY RNA-SEQ REVEALS DIFFERENTIAL GENE EXPRESSION IN ARGININE PATHWAY IN LEISHMANIA AMAZONENSIS WILD TYPE AND LEISHMANIA AMAZONENSIS ARGINASE KNOCKOUT

Juliana Ide Aoki[1], *Sandra Marcia Muxel*[1], *Ricardo Andrade Zampieri*[1], *Audun Helge Nerland*[2], *Lucile M. Floeter-Winter*[1]

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1 Background

Leishmania is a protozoan parasite that alternates its life cycle between the sand fly vector and the mammalian host. This alternation involves environmental changes and submits the parasite to dynamic modifications in morphology, metabolism, cellular signaling and regulation of gene expression to allow a rapid adaptation to new conditions. Our research group have been studying the role of arginase in *L. amazonensis* during the parasite life cycle and in the establishment and maintenance of the infection in mammalian macrophages. Arginase is an immune-regulatory enzyme that can reduce the production of nitric oxide by activated macrophages, directing the availability of L-arginine to polyamines pathway, what result in parasite replication.

2 Methods

Next generation sequencing have been proving high-throughput transcriptome data. In this work, we performed the transcriptome profiling using Illumina HiSeq 125 bp paired-end to identify differentially expressed genes from promastigotes (pro) and axenic amastigotes (ama) of *L. amazonensis* wild type (LaWT) and arginase knockout (Laarg$^-$). The sequence reads were aligned with *L. mexicana* genome. All these data provided knowledge about the mechanism of gene regulation by arginase activity during replication and differentiation of the parasite life cycle, which can elucidate the metabolism to maintain the infection and/or to subvert macrophage defenses.

3 Results

Total 8,700 transcripts and 443 novel transcripts were identified and revealed 85% of genes were constitutively expressed. However, among the 1,268 genes differentially expressed, 7, 43, 14 and 2% were exclusively expressed in pro LaWT, ama LaWT, pro Laarg$^-$ and ama Laarg$^-$, respectively. A large proportion of transcripts that were up or down-regulated were associated with metabolic processes. In this work, we focused in the biosynthesis of amino acids, arginine and proline metabolism validating the differentially expressed profile of argininosuccinate synthase, acetylornithine deacetylase, spermidine synthase, arginase, glutamate dehydrogenase, proline oxidase and pyrroline carboxylate dehydrogenase, among the samples.

4 Conclusions

The gene expression patterns yielded signatures of multiples developmental stages of *L. amazonensis* and the existence of a coordinated response in the absence of the enzyme arginase, providing additional insights into how *Leishmania* is able to modulate its biological functions to survive into the macrophage. Supported by: FAPESP and CNPq (Brazil), SiU (Norway).

C0763 PROTEOMIC ANALYSIS OF LEISHMANIA-INFECTED MACROPHAGES REVEALS MODULATION OF PROTEINS INVOLVED IN IRON METABOLISM AND INFLAMMATION

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1 Background

CBA macrophages (MØ) control infection by *L. major* and are permissive to *L. amazonensis*, which suggests that MØ play an important role in the outcome of *Leishmania* infection.

2 Methods

In order to evaluate the global MØ response to *Leishmania* infection, proteomic studies were carried out using CBA bone marrow derived macrophages (BMMØ). Protein expression was identified 6—24 hours after infection with *L. major* or *L. amazonensis* using LC-MS/MS with a MudPIT approach. We then validated differently expressed proteins and pathways by FACS, ELISA or western-blot at different time points varying from 6 to 48 hours after infection.

3 Results

Results from 6 independent experiments were analyzed, and 382 proteins were found to be differently expressed, comparing *L. amazonensis* and *L. major* infection. Using IPA software, proteins related to lipid metabolism and small molecule biochemistry were grouped into a network and showed higher expression in *L. amazonensis*-infected cells. Proteins related to cell signaling and cellular assembly, organization and movement formed another network showing higher expression levels in *L. major*-infected cells. These results clearly demonstrate that *L. amazonensis* and *L. major* modulate MØ functions differently. *L. major* activates cell-signaling networks, while *L. amazonensis* induces the expression of proteins involved in cell metabolism. Two of the molecules with higher expression in *L. amazonensis*-infected BMMØ were transferrin receptor (CD71) and heme oxygenase 1 (HO-1). After validation, we showed a higher expression of CD71 in *L. amazonensis*-infected macrophages. We also found a higher binding and uptake of holotransferrin after *L. amazonensis* infection. A higher expression of HO-1 and ferritin, in addition to a lower production of superoxide (O2-) and nitric oxide (NO) were also observed in *L. amazonensis*-infected cells. Reduced expression of the anti-inflammatory and anti-oxidant HO-1 found in *L. major*-infected MØ can be related to a higher production of iNOS and subsequently higher NO production found in these infected cells.

4 Conclusions

These findings suggest that the differences observed may be correlated to the role played by iron metabolism in favoring L. amazonensis differentiation and survival inside macrophages by contrast in controlling L. major infection. Taken together, the data indicate that parasites play the important role, affecting cell activation related to infection outcome.

C0667 TARGETING HOST AND PARASITE HISTONE MODIFYING ENZYMES FOR ANTI-LEISHMANIAL DRUG DISCOVERY

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1 Background

The incidence of human leishmaniases shows an important increase over the last decades due to failing preventive and therapeutic measures, and the emergence of drug resistant parasites in developing countries. Surprisingly, even though it is very well established that *Leishmania* reprograms its host cell to subvert the immune response and to meet the nutritional and metabolic needs for intracellular parasite survival and proliferation, there is only little effort to exploit these crucial effects of the parasite on the host cell for anti-parasitic drug discovery. The impact of intracellular Leishmania infection on the host cell transcriptome and conservation of all classes of histone modifying enzymes (HME) in *Leishmania* suggest epigenetic regulation both at host and parasite level as interesting targets for chemotherapy.

2 Methods

We screened 480 epigenetic inhibitors against extracellular *L. amazonensis* and *L. donovani* promastigotes using a dye reduction assay, and intracellular *L. amazonensis* amastigotes using a high content phenotypic assay. Additionally, we studied the expression of histone marks on free parasites and infected primary mouse macrophages using antibodies specific for methylated and acetylated residues by immunofluorescence microscopy and Western blotting.

3 Results

We identified 25 hits that kill extra- or intracellular parasites at 10 µM or 1 µM targeting all major classes of HMEs, suggesting a potential essential role of epigenetic regulation in *Leishmania*. Interestingly, 10 hits exclusively killed intracellular parasites, revealing a host cell-dependent mechanism of action likely through modulation of macrophage epigenetic regulation. Current studies are focusing on hits potentially targeting histone methylation enzymes. By applying antibodies against epigenetic marks of histone H3 we showed (i) a nuclear localization for H3K4me3, H3K9me3, and H3K27me3 marks in *Leishmania* promastigotes and intracellular amastigotes, and (ii) quantitative differences of these marks between non-infected and infected macrophages.

4 Conclusions

Our data reveal a surprising complexity of epigenetic host/parasite interactions that opens interesting new venues for anti-leishmanial intervention. Targeting the host cell rather than parasite biology represents a new strategy in anti-leishmanial drug discovery that likely will be more refractory to the emergence of drug resistant parasites.

C0197 INHIBITOR OF SERINE PEPTIDASE 2 (ISP2) IS REQUIRED FOR LEISHMANIA MAJOR PERSISTENCE IN THE SKIN THROUGH CONTROL OF MONOCYTES AND MONOCYTE-DERIVED SUBSETS

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1 Background

In the mouse model of *Leishmania major* infection, protective immunity is associated with inflammatory responses. It is well-characterised that neutrophils are the first responders in *Leishmania* infection and that the neutrophils can influence the subsequent waves of cellular infiltration, particularly the monocytes. In this study, we evaluated the dynamics of cellular influx, and responses associated with cellular activation, at the lesion site during long-term, low dose intradermal infection of the ear. We used this model to also address whether these responses were altered during infection with genetically-modified *L. major* deficient in Inhibitor of Serine Peptidase 2 (ISP2), a virulence factor that inhibits the activity of host serine peptidases, which are key regulators of the inflammatory response.

2 Methods

We performed flow cytometry to analyse the innate cell populations within the infected ears of C57BL/6 mice over a period of 5 weeks. Through the use of quantitative non-invasive bioluminescence imaging of myeloperoxidase (MPO) activity and tracking of bioluminescent *L. major*, we show that *Leishmania* ISP2 is required to modulate phagocyte activation and is important for parasite persistence at the infection site but not in the draining lymph node.

3 Results

During *L. major* infection, significant infiltrates of neutrophils and monocytes occurred at the infection site, at 1-4 days and 2-4 weeks, while numbers of dermal macrophages and dendritic cells were only slightly elevated in the first days. However, during infection with *L. major* deficient in ISP2, there was a higher proportion and number of monocytes, monocyte-derived macrophages and dendritic cells (moDCs) during a ´second wave´ of infiltration. We show here that ISP2 plays a key role in the control of monocyte and monocyte-derived cell population influx, while it does not affect the recruitment of neutrophils. *In vivo* imaging of MPO activity over an 8-week infection also suggested that ISP2 controls local inflammation. The presence of ISP2 was linked to the regulation of iNOS expression in monocytes and macrophages, moDC activation measured by co-stimulatory molecule expression, and the levels of IFN-γ *in situ*, having an impact on lesion development and the control of infection.

4 Conclusions

Taken together, our findings indicate that early survival and persistence of *L. major* in the dermis is associated with the downregulation of inflammatory monocytes and monocyte-derived macrophages and moDCs via ISP2.

Miradero Room

Session 14. Vector biology and leishmania-sand fly interactions

C0127 INCREASED TRANSMISSIBILITY OF LEISHMANIA DONOVANI FROM MAMMALIAN HOST TO VECTOR SAND FLIES AFTER MULTIPLE EXPOSURES TO SAND FLY BITES

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1 Background

Visceral strains of *Leishmania* sp. can be detected in blood and in the skin of the mammalian host suffering from visceral leishmaniasis (VL), however, the role of these tissues as a source of infection back to vector sand flies remains unclear. Using the experimental hamster model of VL, we have addressed the efficiency of transmission of *L. donovani* to sand flies, the role of the skin and blood, and most interestingly, the effect of multiples exposures to sand fly bites on the transmissibility.

2 Methods

L. donovani infected male hamsters exhibiting at least 20% weight loss, consistent with progressive VL, were exposed to xenodiagnoses with *Lutzomyia longipalpis* on the same sites on the belly on two consecutive days. Two days after feeding, the blood fed flies were dissected, and the midguts cultured to determine the percentage of infected flies. After the second feeding, the hamsters were euthanized, and their blood and excised belly skin were used for artificial feeding of sand flies.

3 Results

The transmission from the hamsters to the flies after the first feeding varied between 0 to 57% of positive flies for parasites (median: 20%). Importantly, following the second feeding on the same site, we observed a dramatic increase in the percentage of flies infected (7.7% - 100%, median: 54.6%; $p<0.0001$, $n=20$). During artificial feeding, the flies could acquire infection from the hamster blood (56.2%), as well as from the excised hamster skin (18.6%). However, there was a strong correlation between natural feeding and artificial feeding on the blood ($R=0.696$, $p=0.0174$), but not on the skin. In addition, we observed an important increase in blood parasitemia after sand fly exposures as determined by serial dilution ($p=0.0136$) and PCR ($p=0.0216$), that correlated with an increased infection in flies comparing artificial feeding on blood obtained pre- and post-sand fly exposure ($p=0.0049$).

4 Conclusions

There is a strong increase in *L. donovani* transmissibility after multiple exposures to sand fly bites due to a remarkable systemic effect that exposure to uninfected fly bites has on the number of infected cells or parasites released into the peripheral blood.

C0154 SKIN RESIDENT PARASITE BURDEN AND DISTRIBUTION DETERMINE HOST INFECTIOUSNESS IN VISCERAL LEISHMANIASIS

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1 Background

The infectiousness of patients with visceral leishmaniasis for the sand fly vector of disease is widely thought to be linked to blood parasitemia. Recent work, however, challenges this hypothesis by suggesting skin parasite burden plays a vital role in host infectiousness.

2 Methods

We developed a new murine model using C57BL/6 RAG-/- mice that supports skin infection with *Leishmania donovani* to investigate spatial parasite distribution and burden at macro- (qPCR) and micro- (confocal microscopy) scale in the mouse skin. Biological data was fed into a mathematical model developed in this study to investigate *in silico* the predicted impact of skin parasite distribution and burden to outward transmission to a sand fly vector.

3 Results

Skin parasite burden in long-term infected C57BL/6 RAG-/- mice strongly correlated with outward transmission success to sand flies fed on the shaven animals flank. Further, *L. donovani* distribution in infected mouse skin was markedly skewed. Mathematical models accounting for this heterogeneity demonstrate that whilst a patchy distribution reduces the expected number of flies acquiring parasites, it increases the infection-load for sand flies feeding on a patch and hence the potential for onward transmission. A model including micro and macro-patchiness provides the best fit for experimental sand fly feeding data.

4 Conclusions

Our data indicate that parasite burden and distribution in the skin collectively provide the strongest predictors of host infectiousness. These are critical factors to consider when assessing treatment efficacy, transmission competence and the impact of the ongoing VL elimination campaign in South Asia.

C0160 SAND FLY FAUNA (DIPTERA: PSYCHODIDADE: PHLEBOTOMINAE) AND PUTATIVE VECTORS OF AMERICAN CUTANEOUS LEISHMANIASIS FROM NORTHERN BRAZIL BORDERING FRENCH GUIANA

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1 Background

In Brazilian Amazon borders, American Cutaneous Leishmaniasis (ACL) is endemic and regarded as an important problem of public health. The present study aimed to provide knowledge on the Phlebotominae fauna and putative ACL vectors in northern Brazil bordering French Guiana.

2 Methods

Were selected three ecologically distinct forested areas, approximately 6km far from urban area of Oiapoque municipality (Amapá State, Northern Brazil), as captures sites for sand flies, as follow: Vila Vitória (area under recent deforestation), Clevelândia (ancient colonization area), BR156-Km6 (primary forested area). Captures were done in 2015-2016, during months of rainy (April-May) and dry (October-November) seasons. During these periods, each area was sampled in four consecutive days using: eight CDC light traps disposed at 1,5m ground level and two at 20m in tree canopy; two Shannon collections and one daily aspiration on three bases. Species identification was based on morphological characters and females were dissected under sterile conditions for search of natural infection by flagellates; after microscopically analysis, infected guts were inoculated into tubes containing NNN media. DNA extracted from successfully isolated parasites or even from remaining content from glass slides of positive guts was used for *Leishmania* typing by PCR-RFLP technique.

3 Results

A total of 9.175 specimens were captured, belonging to 48 species. Amongst them, it must be drawn attention to the following sand flies considering literature-based evidences to be potentially associated with transmission of ACL in that studied region: *Nyssomyia umbratilis* (most frequent), *Psychodopygus squamiventris maripaensis, P. ayrozai, Bichromomyia flaviscutellata, P. davisi* and *P. paraensis*. Flagellate infections were found in 23 occasions, mainly from CDC ground, in 17 *N. umbratilis* (8 from Vila Vitória, 8 from BR156-Km6, and 1 from Clevelândia) and two *Migonemyia migonei*, one *Viannamyia furcata*, one *Sciopemyia fluviatilis,* one *Evandromyia infraspinosa* and one *S. sordellii* from Vila Vitória. Parasites from 11 *N. umbratilis* and one *E. infraspinosa* were characterized as *Leishmania* (*Viannia*) *guyanensis.* Negative PCR-RFLP for other sand flies suggested culture contamination, few *Leishmania*-DNA or infections by other trypanosomatids.

4 Conclusions

Continuous entomological surveillance is needed to ensure possible participation of other phlebotomine sand fly species in the Guianan ACL transmission.

C0255 ROLE OF THE PERITROPHIC MATRIX IN VECTOR COMPETENCE OF SERGENTOMYIA SCHWETZI TO LEISHMANIA PARASITES.

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1 Background

The peritrophic matrix (PM) is an acellular chitin-containing envelope which in most insects encloses ingested blood meal, protects the midgut epithelium against abrasion and creates a physical barrier that can prevent escape of parasites from the endoperitrophic space. The rate of formation and disintegration of the PM may contribute to the vector competence of sand flies. Here we test the hypothesis that kinetics of the PM formation is connected with refractoriness of *Sergentomyia schwetzi* to *Leishmania* infections.

2 Methods

Key developmental parameters of *L. donovani* in *S. schwetzi* were compared with development in two susceptible (permissive) sand fly species, *Phlebotomus orientalis* and *P. argentipes,* and in *P. papatasi* which is refractory to *L. donovani.* Morphology, ultrastructure and timing of formation and degradation of the PM was studied using light microscopy, histology and electron microscopy. In addition, the effect of the PM persistence on *Leishmania* development in *S. schwetzi* was tested using co-feeding of *L. donovani* and *L. major* together with exogenous chitinase obtained from the culture of the fungus *Beauveria bassiana*.

3 Results

The four sand fly species studied did not differ substantially in morphology and ultrastructure of the PM, however, the kinetics of the PM was highly species-specific. Both susceptible vectors showed slower formation of the PM than refractory species. In both refractory species infections were lost with defecation, but this failure was caused by different mechanisms: while parasites reached the abdominal midgut in *P. papatasi*, they remained enclosed within the PM in *S. schwetzi* wherethe time period between degradation of the PM and defecation of bloodmeal remnants was extremely short. Addition of exogenous chitinase to the infective dose containing *L. donovani* or *L. major* promastigotes caused earlier disintegration of the PM and significant enhancement of infection rates in *S. schwetzi*: *L. donovani* and *L. major* survived defecation and developed late stage infections in up to 60% of females.

4 Conclusions

The crucial aspect of the refractoriness of *S. schwetzi* to leishmania parasites is the relative timing of subsequent digestion steps: persistence of the PM till defecation of blood remnants does not allow parasites to colonize the midgut and to develop mature sand fly infection. Therefore, in some sand fly species, PM represents the crucial barrier for establishment of *Leishmania* infections.

C0434 QUANTIFICATION OF INFECTION RESERVOIRS IN HUMAN VISCERAL LEISHMANIASIS BY XENODIAGNOSIS: A PROOF OF CONCEPT STUDY

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1 Background

Visceral leishmaniasis (VL) is the most fatal form of leishmaniasis caused by protozoan parasite *Leishmania donovani* complex and transmitted between people by the bites of blood sucking vector sand fly *Phlebotomous argentipes*. An important factor influencing the transmission dynamics of disease is the unknown contribution of human host with different parasitemia to infected sand fly population. The aim of present study is to investigate the infectiousness profile of VL patients by xenodiagnosis during repeat exposure of colony reared sand flies.

2 Methods

Direct xenodiagnosis was performed on 20 active VL patients using 10 male and 30 female pathogen free colonies of *P. argentipes* sand flies. After 60 hrs of post blood meal, fed sand flies were dissected and infections of sand flies were evaluated by microscopy and PCR analysis. Repeat xenodiagnosis was made at same body place after 24hrs to see the effect of biting response on parasite transmission.

3 Results

We found varying sand fly feeding response (3-94%) with VL patients, and 30% of the fed *P.argentipes* females flies found infected with promastigote parasites under microscopy. No significant effects on transmission were seen in repeated xenodiagnosis. PCR were positive in 8 of 18 (44%) and 9 of 18 (50%) fed flies of first and second xenodiagnosis respectively, and only 14% of sand flies were found positive by both microscopy and PCR.

4 Conclusions

The study shows first time on human that VL patients harbored parasites in sufficient numbers to promote infections in vector sand flies.

C0477 PREDICTING THE DISTRIBUTIONS OF BRAZILIAN VECTORS OF LEISHMANIA FOR DIFFERENT CLIMATE CHANGE SCENARIOS

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1 Background

This ongoing project, funded by the Brazilian government (Conselho Nacional de Desenvolvimento Científico e Tecnológico, 446274/2015-1) aims to map the current and future potential distributions of the major sand fly vectors of *Leishmania* species causing human cutaneous and visceral leishmaniasis in Brazil.

2 Methods

Occurrence records of *Lutzomyia flaviscutellata*, *L. intermedia*, *L. neivai*, *L. whitmani*, *L. migonei*, *L. umbratilis*, *L. wellcomei*, *L. complexa*, *L. longipalpis* and *L. cruzi* are obtained from the literature, museum collections and Brazilian health departments. Ensemble models for each vector's current distributions are generated with a selection of ecological niche modelling algorithms. Intergovernmental Panel on Climate Change (IPCC) climate change scenarios are then employed to predict the future distributions of currently suitable bioclimates. Bioclimatic variables are of remote-sensed and ground-based origins and come from open access databases. The approach relies on using a Geographical Information Systems framework, and is based on methods published for *L. flaviscutellata*, South American (SA) vector of *Leishmania amazonensis* (Carvalho et al. PLoS One 2015;10:e0143282).

3 Results

Each vector's distribution has been mapped to administrative level 3 ('município') and capture site, when available. The current bioclimates associated with *L. flaviscutellata*, *L. intermedia* and *L. neivai* have been identified. Based on the IPCC greenhouse gas emission scenarios "stabilization" (RCP 4.5) or "high increase" (RCP 8.5), southward expansion of the bioclimatic ranges of *L. flaviscutellata* and *L. neivai* are expected, whereas subtle improvements in climatic conditions in northeast Brazil are anticipated for *L. intermedia*. This may reduce the co-occurrence of *L. intermedia* and *L. neivai*. Vectors of cutaneous leishmaniasis show a variety of local range expansion and/or contraction patterns in Brazil and elsewhere in SA.

4 Conclusions

Climate change may not lead to all sand fly vectors spreading into more southerly regions or to higher elevations, because their ecological niches are not determined solely by climate. Consequently, it should not be assumed that transmission of human leishmaniasis will automatically spread southwards in response to climate change. Future aims include modelling the incidence of infection and disease in geographical regions predicted to support sand fly vectors in the future.

C1427 LUTZOMYIA LONGIPALPIS TGF-BETA HAS A ROLE IN LEISHMANIA INFANTUM CHAGASI SURVIVAL IN THE VECTOR

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1 Background

Leishmaniasis is transmitted to the vertebrate host through the bite of sand flies. At the end of blood digestion the parasites adhere to the sand fly midgut epithelium avoiding parasite elimination with digested blood remnants. This intimate relationship with the parasite leads to an immune response by the insect in the attempt to control infection. Previous studies from our group have shown that caspar silencing decreases parasite load inside *Lutzomyia longipalpis* and that different bacterial challenges modulate the expression of a defensin. In relation to TGF-β, studies with the malaria vector *Anopheles* showed that the modulation of a TGF-β expression could influence the production of NO and that this modulation affected the *Plasmodium* infection cycle.

2 Methods

We are presently characterizing immune responses in *L. longipalpis* by investigating the expression of specific genes and by gene silencing approaches.

3 Results

We found that an *L. longipalpis* Transforming Growth Factor–β from the activin-inhibin family (LlTGF-β) was overexpressed 72h post infection with *L. i. chagasi*, when parasites are starting to attach to the midgut epithelium. LlTGF-β seems to be modulated by the Toll pathway, since the silencing of cactus caused an increase in LlTGF-β expression. In the absence of infection, the silencing of LlTGF-β increased the expression of the immune effector molecules cecropin and defensin, but not INOS. We also investigated the effect of LlTGF-β silencing on immune responses and *L. longipalpis* infection with *L. i. chagasi*. With the depletion of LlTGF-β there is a drastic reduction of infection starting at 48h and also an increase of AMPs and INOS expression indicating that LlTGF-β probably is a negative regulator of immune responses and has a possible role in controlling *L. i. chagasi* infection inside the sand fly. We have also used the TGF-β receptor inhibitor SB-431542 in similar studies and found that depletion using gene silencing or receptor inhibition led to similar results, namely increased immune responses and decreased *Leishmania* infection.

4 Conclusions

Thereby we can assume that in *L. longipalpis* the *Leishmania* contact with the gut or the increase of NOS might be causing an activation of LlTGF-β and a negative regulation of immune effectors.

C1653 CAN PHLEBOTOMUS PERNICIOSUS PLAY A ROLE OF PERMISSIVE VECTOR FOR LEISHMANIA TROPICA? EXPERIMENTAL EVIDENCE MAY SUGGEST THIS HYPOTHESIS.

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1 Background

Climate changes and human travels may have an impact on the geographic spread of the leishmaniases. The competence of indigenous sand fly species to transmit non-indigenous *Leishmania* parasites, however, is a prerequisite for disease establishment and spreading. *Phlebotomus perniciosus*, a permissive vector in laboratory conditions, is the proven vector of *L. infantum* in the western Mediterranean, a region recently interested by intense human migration. Imported cutaneous leishmaniasis (CL) due to *L. tropica* is increasingly documented in southwestern Europe, but the role of *P. perniciosus* as a potential vector of this parasite was never evaluated experimentally.

2 Methods

The *L. tropica* strain MHOM/IT/2016/ISS3183, recently isolated from a CL case imported from Afghanistan, was used to infect laboratory-reared *P. perniciosus*. Sand flies were artificially fed with different promastigote doses in rabbit blood. Furthermore, chronic footpad lesions developed in hamsters after syringe infection with culture metacyclics, were offered to *P. perniciosus* bites to mimic a natural infectious dose of amastigotes. Infection in sand flies was evaluated individually by dissection and gut microscopy, and by quantitative (q) RT-PCR.

3 Results

In experiments using artificial promastigote feeding, 850 engorged females were examined, of which 78% showed microscopically viable parasites. In *in vivo* amastigote-initiated infection experiments, 30 to 67% of 400 females which fed on footpad lesions at different clinical stages, showed viable parasites at the dissection. Promastigote load and developmental stages in gut were highly variable. Heavy late-stage infections with colonization of the stomodeal valve and presence of morphologically-identified metacyclics were observed from 5-7 days post bloodmeal until 13-16 days of observation, though in a variable proportion of infected flies (14-70%). qPCR confirmed the variable parasite load, in a range of 2 – 10^5 parasites/gut at various days post infection.

4 Conclusions

Although experiments of *L. tropica* transmission to naïve hamsters by *in vivo*-infected *P. perniciosus* are still in course, we demonstrated that an Asian strain of *L. tropica* can develop infectious stages in *P. perniciosus* artificially fed with promastigotes or after biting on chronic hamster lesions. This may support the hypothesis that, in particular epidemiological situations, *P. perniciosus* might play the role of occasional *L. tropica* vector.

C1892 THE PARAMETERS OF EXPERIMENTAL LEISHMANIA INFECTION IN THE SAND FLY VECTOR DETERMINE TRANSMISSION OUTCOME IN THE MAMMALIAN HOST: IMPLICATIONS FOR THE EPIDEMIOLOGY OF HUMAN DISEASE

Nathan Peters[1], *Lisa Stamper*[1], *Rachel Patrick*[1], *Michael Fay*[2], *Phillip Lawyer*[1], *Dia-Eldin Elnaiem*[1], *Nagila Secundino*[1], *Alain Debrabant*[3], *David Sacks*[1]

[1]*LPD/NIAID USA*
[2]*NIH USA*
[3]*CBER/FDA USA*

1 Background

In nature, sand flies likely become infected with varying doses of parasites depending upon their feeding behavior and the concentration of parasites in the lesion or blood upon which they feed. Reservoir hosts, including humans for the anthroponotic forms of Leishmaniasis, with active infections containing large numbers of parasites may be more likely to give rise to heavily infected flies that may transmit greater numbers of parasites resulting in more severe infections. The influence of the initial infectious dose in the sand fly vector on subsequent transmission efficacy and disease severity has been difficult to study because it is impossible to control for the influence of the number of parasites an individual sand fly deposits into the skin of the mamalian host.

2 Methods

To identify both the influence of the infectious dose of *Leishmania* in the sand fly vector and the parameters of infection within infected sand flies on subsequent transmission and disease severity in the mammalian host, we sampled groups of flies infected with varying doses of parasites and compared infection intensity and degree of metacyclogenesis with the frequency of transmission and disease severity.

3 Results

The percentage of parasites within the midgut that were metacyclic promastigotes had the highest correlation with the frequency of transmission. Sand fly infections initiated with high doses of parasites resulted in higher percentages of metacyclic promastigotes, higher transmission frequency, and increased disease severity.

4 Conclusions

These observations have implications for transmission of *Leishmania* by the sand fly vector in both the laboratory and in nature, including how the number of organisms acquired by the sand fly from an infection reservoir may influence the clinical outcome of infection following transmission by bite. I particular, they suggest that the reservoir for new severe infections may be correspondingly severe existing infections.

Greco Room D1-D2
11:30-13:00

Session 15. Early drug discovery. Animal models and genetic susceptibility. Drug resistance mechanism

C0379 EMERGENCE OF MILTEFOSINE RESISTANT CASES OF VISCERAL LEISHMANIASIS IN INDIA

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1 Background

Miltefosine unresponsive and relapse cases of visceral leishmaniasis (VL) are being increasing reported. However, so far there is no report of laboratory confirmed miltefosine resistance in old world visceral leishmaniasis. Here, we report laboratory confirmed miltefosine resistant VL cases from India.

2 Methods

Two patients of visceral leishmaniasis were referred to us with suspected VL. First patient was a native of Bihar, but residing in a kala-azar non-endemic area. He was treated with broad spectrum antibiotics and antipyretics but without any response. Second patient was from Jharkhand state of eastern India adjoining to Bihar. He was refractory to anti-leishmanial treatment including miltefosine. On investigations blood buffy coat from both the patients grew *Leishmania donovani*. The isolates were characterized for their drug susceptibility profile, nucleotide sequencing and SNP analysis of LdMT and LdROS gene expression. Both patients were successfully treated with amphotericin B.

3 Results

The*in-vitro* drug susceptibility assays on both isolates showed good IC_{50} value to amphotericin B (0?1 ± 0?0004 µg/ml and 0?07 ± 0?0019 µg/ml respectively). One strain was refractory to SbIII also with IC_{50} of >200 µM while the second isolate was sensitive to SbIII with IC_{50} of36?70 ± 3?2 µM. However, the IC_{50} against miltefosine was more than 10-fold higher (>100 µM) as compared to the standard strain DD8 (6?8 ± 0?1181 µM), confirming resistance of the parasite against miltefosine. Further, the gene analysis demonstrated single nucleotide morphisms (SNP) (Tyr354Phe and Phe1078Tyr) in the LdMT gene could represent a molecular marker of miltefosine resistance in *L. donovani*.

4 Conclusions

This is the first documentation of laboratory confirmed miltefosine resistant visceral leishmaniasis from India, needing urgent steps to stop spread of these strains.

C0397 THE UP-REGULATION OF IRON SUPEROXIDE DISMUTASE IN LEISHMANIA DONOVANI ASSOCIATED WITH MILTEFOSINE RESISTANCE AND THEIR INHIBITION RESTORED THE DRUG EFFICACY IN-VITRO.

Radheshyam Maurya Maurya[1], *Jalaja Veronica*[2], *Shyam Sundar*[3]

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[2]*Department of Animal Biology, University of Hyderabad, Hyderabad India*
[3]*Department of Medicine, Banaras Hindu University, Varanasi India*

1 Background

The emergence of a drug-resistant parasite is the primary challenge to control visceral leishmaniasis (VL) in the endemic area. Miltefosine, which is used for treating VL, has recently reported ineffective due to the emergence of resistant L. donovani strains. Hence, there is an urgent need to explore the Miltefoesine resistant mechanism in the parasite.

2 Methods

L. donovani (DD8; Sensitive and BHU875; Resistant) promastigotes were cultured in vitro in M199 medium containing 15% FBS and 1% penicillin-streptomycin at 26oC. MTT assay was performed confirmed the IC50 of sensitive and resistant parasites. The cell cycle was performed using PI dye exclusion test and ROS estimation using H2DCFDA dye to confirm the resistant parasites in-vitro. The comparative proteome profile of DD8 and BHU875 parasites were resolved by using 2D gel electrophoresis and spots were analyzed by MALD-TOF which revealed Iron superoxide dismutase (FeSODA) protein is up-regulated. Then we performed the enzyme assay with parasite crude lysate to determine the FeSODA activity. Further, we cloned and expressed FeSODA protein and raised the antibodies in mice. Next, we cloned the antisense orientation of FesodA gene in pEGFP-C1 plasmid and transfected the resistant and sensitive parasites by the electroporation method.

3 Results

In-vitro experiments with drug showed resistant parasite (BHU875) revealed 4-fold higher cytotoxicity, normal morphology, coherent cell cycle, and suppression of reactive oxygen species (ROS) after miltefosine treatment. Comparative proteome analysis of resistant and sensitive parasites revealed up-regulation of FeSODA in BHU875 strain as compared to its sensitive strain. In agreement with the proteome profile, the crude lysate protein of BHU875 showed higher FeSODA enzymatic activity as well as compared to its sensitive strain. Confirming its role in miltefosine resistance, the episomal inhibition of gene coding for FesodA in BHU875 parasites restored drug sensitivity and also affected the intracellular survival of the parasites in the infected macrophage.

4 Conclusions

In conclusion, our study provides a direct link between miltefosine resistance and FeSODA activity in the parasites. Hence, our study could help in the rational redesigning for the FeSODA targeted drugs which can successfully combat the miltefosine-resistant L. donovani parasites in the endemic area.

C0459 PRE-ADAPTION TO ANTIMONIALS IS GENERAL IN LEISHMANIA DONOVANI FROM THE INDIAN SUBCONTINENT

Franck Dumetz[1], *Bart Cuypers*[1], *Hideo Imamura*[1], *Malgorzata Domagalska*[1], *Erika D'Haenens*[1], *Ilse Maes*[1], *Suman Rijal*[2], *Khana Basudha*[2], *Shyam Sundar*[3], *Syamal Roy*[4], *Jean-Claude Dujardin*[1], *Géraldine De Muylder*[1]

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[4]*Department of Infectious Diseases and Immunology, Council of Scientific and Industrial Research, Indian Institute of Chemical Biology India*

1 Background

Antimonials (SSG) have been used for many decades in the Indian Sub-Continent (ISC) to treat patients with visceral leishmaniasis (VL). We previously sequenced the whole genome of 204 *L. donovani* isolates and found out that all isolates originating from the lowland hyper-endemic region (93% of the sample), possessed an exceptional, intra-chromosomal amplification of the gene encoding MRPA (involved in efflux of trivalent antimony, Sb^{III}); this amplicon was absent in 'Yeti' strains originating from Nepalese highlands. This unique feature could represent a pre-adaptation of lowland strains to antimonials. To verify this, we experimentally selected Sb^{III}-Resistance in lowland and Yeti strains and characterized the time-to- and the type-of-resistance.

2 Methods

Promastigotes of 3 cloned strains, BPK282 and BPK275 (lowlands) and BPK026 (Yeti) were selected *in vitro* using progressively increasing doses of Sb^{III}. Genomic and metabolomic changes were followed by high-throughput sequencing and mass-spectrometry, respectively. We also performed a 'flash-selection', by directly exposing parasites to the single highest drug concentration.

3 Results

Baseline Sb^{III} sensitivity of BPK282 and BPK275 was comparable and 10 times lower than BPK026. Time-to-resistance was shorter for the 2 lowland strains (4 cycles) than for BPK026 (7 cycles). All quadruplicates of BPK275 and BPK282 reached the last cycle but only 1 replicate of BPK026 was recovered at the final concentration. Intracellular amastigotes of the selected Sb^{III}-R strains were also resistant to SSG. At genomic level there were no local CNVs, no indels and very few SNPs (only in BPK026); in contrast, chromosomal copy number increased (noteworthy chr23 carrying MRPA), even more in BPK026. From a metabolomic point of view, lowland and Yeti isolates were very different. During SbIII-R selection, the number of changed metabolites was much higher for BPK026: among them, arginine was unchanged during selection of lowland isolates, but increased and reached an equivalent level in Yeti strains. Pre-adaptation of lowland strains was further confirmed by flash-selection: in two weeks, both strains were growing in highest Sb^{III} concentration, while Yeti was killed.

4 Conclusions

Lowland strains are pre-adapted to Sb^{III}, among others because of multiple copies of MRPA present on chr23 and Yeti strains need much more time and molecular changes to become resistant. Results will be discussed in the context of treatment outcome in patients.

C0460 MOLECULAR MARKERS FOR MILTEFOSINE RESISTANCE IDENTIFIED IN LEISHMANIA INFANTUM CLINICAL ISOLATES: POTENTIAL MILTEFOSINE PROGNOSTIC MARKERS FOR VISCERAL LEISHMANIASIS TREATMENT

Juliana Brambilla Trindade Carnielli[1], *Kathryn Crouch*[2], *Nicholas Dickens*[2], *Jeziel Dener Damasceno*[3], *Silvio Fernandes Guimaraes Carvalho*[4], *Carlos Henrique Nery Costa*[1], *Vladimir Costa Silva*[1], *Elaine Brown*[5], *Reynaldo Dietze*[1], *Jeremy Charles Mottram*[5]

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1 Background

Visceral leishmaniasis (VL) treatment relies on a few chemotherapeutic drugs including pentavalent antimony, amphotericin B and miltefosine. Miltefosine has been highly effective against VL in India. However, the clinical trial conduced in Brazil showed that about 50% of VL patient relapsed after the miltefosine treatment (2.5 mg/Kg for 28 or 42 days). Miltefosine resistance mechanisms are being elucidated in laboratory *Leishmania* spp. strains but they are less clear in clinical isolates.

2 Methods

In this study, we used comparative genomics to identify molecular differences between *L. infantum* (= *L. chagasi*) isolates from VL patients in Brazil with different miltefosine treatment outcomes. Whole genome sequencing was carried out by Illumina technology with isolates from cured (n=14) and relapsed (n=12) patients.

3 Results

Genomics approach highlighted 93 orthologs groups that exhibited a significant difference ($p<0.01$) in gene dosage between the two groups, including a deletion of a locus on chromosome 31 (containing four genes; Miltefosine Sensitivity Locus, MSL) which was strongly associated ($p<0.001$; reciprocal relative risk=9.4) with treatment failure. It was inferred that this deletion process occurs by homologous recombination using repetitive sequence flanking the MSL and apparently is not induced by miltefosine pressure. Nevertheless, re-expression of individual MSL genes in a miltefosine resistant promastigote line did not restore the *in vitro* miltefosine susceptibility phenotype. Moreover, MSL screening in 107 more *L. infantum* isolates from different regions of Brazil (ES, MG, BA, MA, and PI) revealed that MSL frequency in *L. infantum* varies geographically in Brazil: southeast region (Espírito Santo and Minas Gerais) have a very low number of isolates that are likely to respond to miltefosine treatment (3.7% of isolates with MSL), whereas in MA and PI, in the northeast part of Brazil, 67.7% of isolates have the MSL.

4 Conclusions

These data indicate that miltefosine-resistance mechanisms in *Leishmania* clinical isolates are beginning to be elucidated and suggest that miltefosine could successfully be used for treatment of VL in Brazil after stratification for the presence of the MSL.

C0527 IMPACT OF MILTEFOSINE RESISTANCE AND HOST IMMUNE SUPPRESSION ON PARASITE FITNESS

***Eberhardt Eline**, Hendrickx Sarah, Maes Louis, Caljon Guy*

University of Antwerp Wilrijk - Antwerp Belgium

1 Background

The increase in treatment failures and occurrence of drug resistance is a growing problem in the treatment of visceral leishmaniasis (VL). To study the impact of the acquisition of miltefosine (MIL) resistance on the parasite infection dynamics, bioluminescent *L. infantum* reporter lines were generated of a MIL-susceptible strain (MIL-S, MHOM/FR/96/LEM3323) and a syngeneic experimental MIL-resistant (MIL-R) strain that is hampered in MIL uptake due to a frameshift mutation in the transporter gene. The *in vivo* fitness and course of infection were compared in single and in co-infections in immunocompetent and immunocompromised hosts using bioluminescent imaging.

2 Methods

BALB/c mice were infected intravenously with $1x10^8$ metacyclic promastigotes of the luminescent MIL-S or MIL-R lines, while only one of the 2 strains was luminescent in co-infections. To assess the effect of immunosuppression, half of the (single) infected animals received 150 mg/kg cyclophosphamide weekly. *In vivo* imaging was performed at 1 day post-infection (dpi) and at 1, 2, 3, 4, 6, 8, 10 and 12 weeks post-infection (wpi) upon which they were sacrificed to determine parasite burdens by RT-qPCR and microscopy.

3 Results

The MIL-S parental strain produced a distinct liver and spleen peak respectively at 3 wpi and 12 wpi. In contrast, MIL-R only showed a limited bioluminescent signal in the liver disappearing at about 3-4 wpi, despite fully comparable initial levels to the MIL-S line at 1dpi. In the co-infected mice, the MIL-S infection course was unaltered while the MIL-R line disappeared more rapidly, actually suggesting that MIL-S outcompetes MIL-R. Immunosuppression did not rescue the observed fitness loss of MIL-R, but resulted in higher overall organ burdens for both strains.

4 Conclusions

Both in single and co-infections, the MIL-R strain fails to multiply and establish visceral infections *in vivo*, while the MIL-S strain induces a clear and typical VL infection course. This impaired fitness is associated to the resistant phenotype of the parasite and cannot be rescued by immunosuppression.

C0628 MILTEFOSINE UNRESPONSIVENESS IN CLINICAL ISOLATES OF LEISHMANIA DONOVANI: APPARENT MECHANISMS AND CLINICAL IMPLICATIONS

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1 Background

Miltefosine (MIL) is an oral antileishmanial drug used for visceral leishmaniasis (VL) treatment in the Indian subcontinent. Recent reports indicate a significant decline in its efficacy and a high rate of relapse in VL as well as post kala-azar dermal leishmaniasis (PKDL). We investigated the parasitic factors apparently involved in miltefosine unresponsiveness in clinical isolates of *Leishmania donovani.*

2 Methods

L. donovani isolated from pretreatment cases (LdPreTx, n=6), from cases of VL and PKDL patients that relapsed after MIL treatment (LdRelapse, n=5) and parasites made experimentally resistant to MIL (LdM30) were included in this study. MIL uptake was estimated using liquid chromatography coupled mass spectrometry. Reactive oxygen species (ROS) and intracellular thiol content were measured fluorometrically. Q-PCR was used to assess the differential expression of genes associated with MIL resistance.

3 Results

LdRelapse parasites exhibited higher IC_{50}, increased infectivity to macrophages and significantly lower accumulation ($P<0.001$) of miltefosine when compared with LdPreTx parasites. Miltefosine induced ROS levels were significantly lower ($p<0.05$) in macrophages infected with LdRelapse while intracellular thiol content were significantly higher in LdRelapse compared to LdPreTx, indicating better tolerance for oxidative stress in LdRelapse isolates. Reduction in drug and ROS accumulation and increase in thiol levels were the highest in LdM30 parasite. Genes associated with oxidative stress, metabolic processes, transporters and cell motility showed modulated expression in LdRelapse and LdM30 parasites in comparison with LdPreTx parasites.

4 Conclusions

The present study highlights the parasitic factors and pathways responsible for miltefosine unresponsiveness in VL and PKDL.

C1006 MAGNETIC HYPERTHERMIA AS A NOVEL APPROACH FOR TREATMENT OF CUTANEOUS LEISHMANIASIS

Sarah Berry, Clare Hoskins, Neil Telling, ***Helen Price***

Keele University, United Kingdom

1 Background

The use of magnetic nanoparticles to produce heat (magnetic hyperthermia) has gained considerable interest in development of novel cancer therapies due to the increased sensitivity of cancerous cells to heat shock. In the current study, we are taking the first steps to establish whether magnetic hyperthermia could be used as an inducible, controlled and localised form of thermotherapy for cutaneous leishmaniasis.

2 Methods

Stable ferrofluids were produced by coating a range of iron oxide nanoparticles with citric acid. Uptake of nanoparticles by the human monocytic cell line THP-1 and *L. mexicana* axenic amastigotes was analysed by ferrosine assays, microscopy and flow cytometry. Magnetic hyperthermia was induced by the application of an alternating magnetic field and the resulting effects on cell viability and morphology were assessed using resazurin-based assays, luminescence, flow cytometry and electron microscopy.

3 Results

We have confirmed there are significant differences in heat sensitivity between cultured *L. mexicana* amastigotes and THP-1 cells. A panel of magnetic nanoparticles are readily taken up by differentiated THP-1 cells and a controlled increase of temperature of up to 25°C can be achieved by application of an alternating magnetic field. Treatment with nanoparticles alone has a limited effect on viability of macrophages and amastigotes, whereas the subsequent induction of magnetic hyperthermia is highly effective in killing parasites in vitro.

4 Conclusions

Magnetic hyperthermia may be an exciting new avenue for development of novel treatments for cutaneous leishmaniasis. Studies are ongoing to characterise the uptake, localisation and effects of magnetic nanaoparticles in THP-1 and *L. mexicana* amastigotes. We are also performing critical experiments to determine the effects of magnetic hyperthermia on *L. mexicana* infected macrophages.

C1728 GLYOXALASE PATHWAYS INVOLVED IN DETOXIFICATION OF METHYLGLYOXAL COULD BE A POTENTIAL TARGET FOR DRUG DEVELOPMENT AGAINST LEISHMANIA DONOVANI

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1 Background

Visceral leishmaniasis is a tropical disease that has posed a major problem in many tropical and temperate countries including Indian subcontinent. Occurrence of disease at a slow but steady rate indicates the persistence of parasite as well as a potential threat of an outbreak sooner or later if not properly taken care. The current treatment options are not cost-effective and exhibit one or other side effects. Moreover, these drugs require the help from the immune system of the host. Therefore, there is a need to focus on the development of new drugs which is independent of the host's immune responses. Similar to their mammalian counterpart, *Leishmania(L.) donovani* neutralizes methylglyoxal (MG), which is a by-product in glycolysis pathway. However, it differs in one or more way(s) from the humans. It is known that methylglyoxal (MG) is predominantly eliminated through glyoxalase (GLO) pathways. Treatment with antimony also targets the glyoxalase pathway (via trypanothione reducatse) but failed to show accumulation of MG in resistant parasites. Our approach herewith is to encourage the accumulation of MG to contain the growth of parasite.

2 Methods

We cultured the DD8 strain of *L. donovani* parasite under various physiological conditions. The levels of methylglyoxal were measured along with expression of enzymes involved in glyoxalase pathway i.e. glyoxalase I (GLOI) and glyoxalase II (GLOII). *In silico* approaches were also adopted to identify the transporters of methylglyoxal.

3 Results

Our findings indicate an increase in the intracellular levels of MG as well as increase in the levels of enzymes (GLOI and GLOII) involved in its detoxification. Under several culture conditions, we observed accumulation of MG. Our findings are also suggestive of the fact that *Leishmania* avoids the accumulation by MG by promoting its excretion across the cell membrane. Our *in silico* approaches identify certain transporters, which may be used as therapeutic targets.

4 Conclusions

Targeting glyoxalase pathway along with the MG transporters can be a potential therapeutic approach. Our study also provides an explanation for antimony resistant parasite.

C1814 CYSTEINE SYNTHASE A KEY PROTEIN MODULATING AMPHOTERICIN B RESISTANCE AND SURVIVAL OF LEISHMANIA DONOVANI UNDER OXIDATIVE STRESS

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1 Background

Cysteine biosynthesis pathway of *Leishmania* may serve as a potential drug target because it is different from human host and regulates downstream components of redox metabolism of the parasites; essential for their survival, pathogenicity and drug resistance. However, despite the apparent dependency of redox metabolism of cysteine biosynthesis pathway, the role of *L. donovani* cysteine synthase (LdCS) in drug resistance and redox homeostasis has unexplored.

2 Methods

In this study, for LdCS-GFP over-expressor, C-terminal-GFP construct was produced and transfected by electroporation in the *L. donovani* promastigotes. Further, *in vitro* drug sensitivity, MTT, enzyme activity (CS, peroxidase, SOD, GST & APx) assays were performed between wild type (S1) and LdCS over-expressor (S1-OE) strains. We also used *ex vivo* model to assess the rate of infection in mouse peritoneal macrophages between S1 and S1-OE strains.

3 Results

Our results showed that over-expression of LdCS in Amphotericin B sensitive strain (S1-OE) modulates resistance towards oxidative stress and drug pressure. We observed that antioxidant enzyme activities were up-regulated in S1-OE parasites and these parasites alleviate intracellular reactive oxygen species (ROS) efficiently by maintaining the reduced thiol pool. But in contrast toS1-OE parasites; Amp B sensitive strain (S1) showed higher levels of ROS which was positively correlated with the protein carbonylation levels and negatively correlated with cell viability. However, further investigations showed that LdCS over-expression also augments the ROS-primed induction of LdCS-GFP as well as endogenous LdCS and thiol pathway proteins (LdTryS, LdTryR and LdcTXN) in *L. donovani* parasites; which probably aids in stress tolerance and drug resistance suggested its role in parasite survival. Thus, we tested its role in infectivity using ex-vivo model which showed that LdCS over-expression increases the virulence of *L. donovani* parasites, suggesting the potential role of LdCS in survival under oxidative burst conditions inside macrophages.

4 Conclusions

Our results reveal a novel crosstalk between LdCS and thiol metabolic pathway proteins play a crucial role in drug resistance and redox homeostasis of *Leishmania*. In addition, the expression of LdCS was up-regulated in Amp B resistant isolates and during stationary stages of growth, accompanied by higher thiol content & supports parasites survival under ROS.

Miradero Room

Session 16. Clinical and experimental immunopathology and pathogenesis

C0140 MACROPHAGE ACTIVATION MARKER NEOPTERIN AS A PHARMACODYNAMIC BIOMARKER IN VISCERAL LEISHMANIASIS

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[4]*Drugs for Neglected Diseases initiative, Geneva Switzerland*

1 Background

Visceral leishmaniasis (VL) is caused by the *Leishmania* parasite, which replicates within host macrophages, thereby increasing overall macrophage biomass which decreases again with waning parasitic infection. The aim of this study was to evaluate neopterin - a macrophage activation marker - as possible pharmacodynamic (PD) marker to monitor VL treatment response, for which recrudescence of parasites is a long-term event which is difficult to predict.

2 Methods

Samples were collected from VL patients in Sudan and Kenya receiving an 1) 11-day combination therapy of a liposomal amphotericin B (L-AMB) infusion on day 1 followed by 10 days of oral miltefosine (L-AMB+MIL, 45 patients) or 2) 28 days of oral miltefosine (MIL, 46 patients). Neopterin was quantified with an ELISA kit in 498 plasma samples collected on miltefosine treatment day 1, 7, 11 (L-AMB+MIL), 14 and 28 (MIL) and 1 and 6 months after treatment. Values are reported as median (inter-quartile range). The Welch t-test on log-transformed data was used in comparisons.

3 Results

Neopterin levels were elevated in all VL patients at baseline 98.8 (63.9-135) nmol/L compared to normal (<10 nmol/L), regressing during treatment to 33.6 (21.3-52.0) nmol/L for the L-AMB+MIL and 21.9 (16.3-40.0) nmol/L for the MIL treatment arm. 19 patients relapsed and received rescue treatment within 6 months after treatment. In combination therapy, neopterin concentrations one day after L-AmB infusion were significantly higher for cured than for relapsing patients at 137 (98.5-197) nmol/L and 84.4 (68.9-106) nmol/L, respectively ($p<0.01$). Neopterin levels remained stable for monotherapy patients during the first treatment week, while concentrations in combination therapy patients almost halved to 55.1 (37.2-83.2) nmol/L. Comparing the neopterin concentration of the last day of treatment to one month after treatment, patients that relapsed showed a significantly ($p<0.001$) higher fold-increase of 2.2 (1.5-2.8) compared to 0.78 (0.53-1.4) for patients that cured.

4 Conclusions

Neopterin levels were elevated in VL patients and decreased during treatment in both treatment arms, though the neopterin dynamics differed between the two arms. The, possibly prognostic, initial surge in neopterin levels in cured combination therapy patients could imply an instant immunomodulatory effect of L-AmB on the Th1 response. Another possible marker in predicting treatment failure in VL is the relative neopterin concentration increase within one month after treatment.

C0679 CHANGES IN RNA EXPRESSION PATTERN IN NEUTROPHILS OF PATIENTS WITH INDIAN VISCERAL LEISHMANIASIS

Shweta Srivastva*[1]*, Smriti Sharma[1], Richard Davis[2], Siddharth Sankar Singh[1], Susanne Nylen[3], Mary Wilson[2], Shyam Sundar[1]

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1 Background

Visceral leishmaniasis (VL) is a disease caused by *Leishmania donovani* in India. Neutrophils act as first line of defense against invading microorganisms and their interaction with other immune cells and environmental signals during disease can influence overall immune response. We observed HLA DR expressing low density "neutrophil like cells" which stains CD66b in PBMC and whole blood samples in active VL subjects by Flowcytometry previously. In continuation to our previous study we also tried to look at changes in mRNA expression in active VL pre and post treatment (paired) groups and endemic healthy controls (EHC) to understand the contribution of these cells during active disease.

2 Methods

Patient Selection: All patients presented with VL symptoms at KAMRC, Muzaffarpur, India were confirmed to be VL positive by detection of amastigotes in splenic aspirates and/or by positive rk39 test and EHCs were enrolled from local area.
Gene Expression: Human neutrophils were isolated using Militenyi CD66abce micro bead kit to get highly pure neutrophils (> 97%). cDNA was prepared using High capacity cDNA archive kit. Real time PCR was performed using ABI-Prism 7500 using specific Sybr Green primers for mRNA of interest.

3 Results

Differences were observed in expression of cytokine and chemokine mRNAs in the same subjects (paired) in pre and post treatment (n=8). Some, but not all differences mirrored profiles in EHCs (n=5).
IFN γ,IL10, were significantly up regulated during Active VL while cytokine IL1β and β defensins and chemokines CXCL8,CCR4,CCR5,CXCR1,CXCL9 were found to be down regulated. We looked at some important markers like MPO, PD1, Arginase 1, and HLA DR which were found to be up regulated during active VL. These markers were studied to address functional competency of neutrophils during active VL at mRNA levels.

4 Conclusions

Neutrophils from subjects with active disease differ from the healthy subjects and from the same subjects after drug induced cure showing neutrophils during active disease are capable of extensive changes in gene expression and can influence development of overall immune response.

C0724 MEGLUMINE ANTIMONIATE AND MILTEFOSIN TREATMENT PROTOCOLS TEND TO RESTORE CYTOKINE NORMAL LEVELS IN CANINE LEISHMANIOSIS

***Marcos Ferreira Santos*[1]**, *Graça Alexandre Pires[1], Cátia Marques[1], Ana Duarte[1], Joana Gomes[1], Lídia Gomes[1], Alexandra Basso[1], Ana Reisinho[1], José Meireles[1], Maria Pereira[2], Armanda Rodrigues[2], David Santos-Gomes[2], Gabriela Santos-Gomes[2], Isabel Pereira da Fonseca[1]*

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1 Background

Canine leishmaniosis (CanL) caused by *Leishmania infantum* is an endemic zoonotic disease of worldwide concern. Sick dogs are usually treated with a combination of leishmaniostatic and leishmanicidal drugs, which improves clinical condition. Thus, further research aiming at better understanding the dog's cellular immune response when subjected to antileishmanial drugs is needed. Therefore, this study intends to evaluate the effect on cell mediate immune response of two treatment protocols commonly applied to CanL.

2 Methods

Symptomatic dogs, naturally infected with *L. infantum* were selected and randomly treated with allopurinol (10 mg/kg, BID, 6 months) in combination with either meglumine antimoniate (100 mg/kg, SC, SID, 28 days; MG+A) or miltefosine (2 mg/kg PO, SID, 28 days; MT+A). Peripheral blood, lymph node and bone marrow samples collected before treatment (Tp0) and one (Tp1), two (Tp2) and three (Tp3) months after treatment were used to assess the gene expression of IL-2, IL-4, IL-5, IL-10, IL-12, TNF-a, TGF-b and IFN-g by qPCR. In parallel, samples obtained from non-infected dogs were also evaluated.

3 Results

When compared with non-infected dogs, peripheral blood of sick dogs (Tp0) evidenced gene expression reduction of all cytokines with the exception of IFN-g which increased. However, in addition to IFN-g bone marrow also showed enhanced IL-12 and TNF-a and the lymph node exhibited an increase accumulation of IL-10 mRNA. Taken together, these results indicate that changes in cytokine gene expression caused by *Leishmania* parasites seem to be tissue dependent. Although chemotherapy tends to restore normal cytokine gene expression three months after MG+A treatment a reduction below normal levels of IL-4, IL-5, IL-10 and TGF-b in blood and of IL-4 and TGF-b in the bone marrow were observed. These results point through a drug induced reduction of anti-inflammatory cytokines.

4 Conclusions

CanL causes a lymphocyte deactivation, leading to lower production of pro- and anti-inflammatory immune mediators that seems to be more accentuated in the peripheral blood. Both treatments induce the normalization of cellular immune response associated with the clinical improvement of the dog. Even so, some protocol adjustments should be considered, precluding the baseline gene expression of Th2 and regulatory cytokines.

Funding: Portuguese Foundation for Science and Technology (FCT) (PTDC/CVT/118566/2010) and UID/CVT/00276/2013. M. Santos holds a PhD scholarship SFRH/BD/101467/2014.

C0838 BLOOD MONOCYTES ARE SKEWED TOWARDS M2 LIKE PHENOTYPE, REFLECTED BY ACQUISITION OF TYPICAL M2 MARKERS AND INHIBITION OF PRO-INFLAMMATORY PHENOTYPE IN VISCERAL LEISHMANIASIS PATIENTS

***Neetu Singh*[1]*, Rajiv kumar*[1]*, Shashi Bhushan*[1]*, Christian Engwerda*[2]*, Shyam Sundar*[1]

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1 Background

Monocytes and macrophages show pleiotropic activity by virtue of their phenotypic and functional heterogeneity. In *in-vitro* cell culture systems they are known to display M1 (classically activated) or M2 (alternatively activated) phenotypes, depending upon activation signals. The M1 phenotype is inflammatory, while the M2 phenotype is associated with suppression of cell mediated immunity and can result in susceptibility to intracellular parasites. *Leishmania* is known to deactivate different macrophage signalling pathways to ensure it's survival, but it remains unclear whether monocytes and macrophages are deactivated or differentiate to an alternatively activated phenotype in human VL (Visceral Leishmaniasis). This study aims to characterise phenotypes and functions of monocytes in VL patients by measuring M1- and M2-like monocytes and testing whether they are associated with disease pathology.

2 Methods

We assessed various M1 and M2 markers at phenotypic, genotypic and functional levels using real time PCR, ELISA and Flow cytometric based functional assays.

3 Results

Monocytes in VL patients before drug treatment lacked a classically activated phenotype, whereby they had a significant decrease in expression of IL1-β, IL-6, MPO, ROS production, NADPH subunit expression and phagocytosis. Monocytes in VL patients are associated with a skewing towards a M2-like phenotype with elevated expression of TGM2, PKM, PPARG, Vitamin D signaling receptor, SOCS1 and other typical M2 markers at the mRNA level.
These findings suggest that encounters between *Leishmania* and monocytes in VL promote a monocyte phenotype which is non inflammatory with reduced phagocytic capacity, ROS production and is skewed towards a M2-like phenotype, an activation state that might sustain chronic infection. Strategies to manipulate the polarization of these cells may provide therapeutic advantage with appropriate pharmacological interventions.

4 Conclusions

These findings suggest that encounters between *Leishmania* and monocytes in VL promote a monocyte phenotype which is non inflammatory with reduced phagocytic capacity, ROS production and is skewed towards a M2-like phenotype, an activation state that might sustain chronic infection. Strategies to manipulate the polarization of these cells may provide therapeutic advantage with appropriate pharmacological interventions.

C0165 LEISHMANIA DONOVANI EXPLOITS SIALIC ACIDS, HOST SIGLECS AND SIALIDASE TO TURN DOWN MACROPHAGE ACTIVATION FOR SUCCESSFUL INFECTION

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[3]*National Institute of Pharmaceutical Education and Research, Kolkata India*

1 Background

Linkage-specific sialic acids (Sias) were reported on virulent *L.donovani*. Sias binding immunoglobulin type lectins (siglecs) are present in hematopoetic cells. Here we aim to investigate the utilization of Sias by the parasite for siglec-mediated binding for establishment of successful infection.

2 Methods

Binding of parasites with siglecs and phagocytosis was determined by flow cytometry and confocal microscopy. Reactive oxygen species (ROS) and nitric oxide (NO) were detected by H_2DCFDA staining and Griess reaction and cytokines by ELISA and real time PCR. The status of host signalling molecules was analysis by western and immunoblotting. The level sialic acid-regulating enzymes (sialyltransferase/sialidases), TLR2/4 in infected macrophages were determined by PCR, Western blotting and enzyme activities. A prooxidant molecule was isolated from an edible plant and checked its activity through several approaches.

3 Results

We have observed enhanced binding of high Sias-containing virulent parasite with siglec-1 and siglec-5 compared to low Sias-containing avirulent strain. Siglec-1 mediated interaction induced enhanced phagocytosis while Sias-siglec-5 binding suppressed ROS, NO and Th1 biased cytokines in infected macrophages. The parasite induced host phosphatase and suppressed MAPK, PI3K pathway signalling molecules which ultimately reduced translocation of NF-κβ to the nucleus through Sias-siglec-5 interaction. The specificity of Sias-Siglec5 interaction was further confirmed by either removing Sias using sialidase from parasites or knocking down siglec from macrophages by siRNA. Additionally, modulations of sialidase and sialyltransferase were observed in infected macrophages which were linked to reduced TLR 2/4 level and immune suppression. Furthermore, newly-identified molecule enhanced ROS in infected cells by binding to the catalytic domain of anti-oxidant enzymes of parasite. It also exhibited immunomodulatory activity *in vitro/in vivo*.

4 Conclusions

Our study first time conclusively demonstrated the role of Sias-siglec interactions in phagocytosis, modulation of innate immune response and altered downstream signaling pathways contributing impairment of immune effector functions of macrophages. *Leishmania* modulated sialidase and sialyltransferase to malfunction TLR-signalling for successful infection. Additionally, our novel herbal prooxidant molecule could be developed as an inexpensive and nontoxic therapeutics either alone or in combination.

C1327 INFLAMMATION AND PARASITE METASTIZATION IN THE PATHOGENESIS OF DISSEMINATED CUTANEOUS LEISHMANIASIS

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1 Background

Disseminated leishmaniasis (DL) is characterized by the presence of more than 10 papular, acneiformes and ulcerated lesions spanning for two different parts of the body. The primary lesion is a classical cutaneous leishmaniasis (CL) ulcer and suddenly a large number of lesions occur associated with fever, chills and malaise. DL is clinically, immunologically and pathologically distinct of diffuse cutaneous leishmaniasis and it's relevance is due to the 20 fold increasing in the number of cases in the last 30 years, the presence of great number of lesions, occurrence of mucosal disease in up to 40% of the patients, and high rate of failure to antimony (Sb^5) therapy. The possibility that DL is associated with impairment in the immune response is attractive, but immunopathologic analysis in tissue from CL and DL did not show difference. In vitro lymphocyte from DL patients produce significantly less IFN-γ and TNF than cells from CL. However this is due to the migration of leishmania reactive cells to the multiple DL lesions.

2 Methods

Participants of this study will include patients in disseminated leishmaniasis (N=20), patients with cutaneous leishmaniasis (N=20) and healthy subjects as controls (N=10). Evaluation of the respiratory burst will be performed by FACS analysis using the *Dihidrorodamina* (DHR). Determination of reactive oxygen species (ROS) and nitric oxide was determined with specific probes. Leishmania infection in macrophages was performed using the ratio of *L. braziliensis* macrophage 5:1. A number of amastigotes was determined by microscopy analysis. The determination of cytokines was done with by ELISA in the supernatants of cells uninfected or infected with *L. braziliensis* at different times 2 hours and 48 after infection. Molecular biology techniques were used to determine genotypic difference in *L. braziliensis* isolates.

3 Results

Isolates of *L. braziliensis* from DL patients were genotipically different from isolates of CL. After infection of macrophage DL isolates induced a higher respiratory burst and more ($P<0.01$) cytokine production (TNF, CXCL9 and CXCL10) than CL isolates. Moreover there was no impairment in DL macrophage in leishmania killing.

4 Conclusions

Isolates of *L. braziliensis* are distinct from those of CL, enhances inflammatory response mediated by macrophages, factors that are associated with metastization of parasites.

C0530 NOVEL THERAPEUTIC ADJUVANT FOR PROTECTION AGAINST LEISHMANIA DONOVANI INFECTION THROUGH ACTIVATION OF CD40 & TLR4 LEADING TO RENEWAL OF GRANULOCYTE MONOCYTE PROGENITORS IN BONE MARROW: A NON-SELF 'CARBOHYDRATE-COMPLEX'.

Aritri Dutta[1], Priyankar Maji[2], Debasri Mukherjee mukherjee[3], Suvadip Mallick[1], Somaditya Dey[1], Dibyendu Kanti Majumdar[4], Jhuma Ganguly[2], Syed Sirajul Islam[2], Bhaskar Saha[3], ***Chiranjib Pal[1]***

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1 Background

Decades of effort in Leishmania prophylactic/therapeutic adjuvant development resulted in efficacy trials of a few first generation Leishmania vaccines but currently there is no prophylactic/therapeutic adjuvant available for human leishmaniasis. It seems that the use of an adjuvant and/or delivery system is necessary for almost any modern vaccine particularly vaccines against leishmaniasis which has been estimated to cause the ninth largest disease burden among individual infectious diseases.

2 Methods

A novel 'carbohydrate-complex' has been isolated from Indian edible mushroom *Pleurotus florida*, further investigated for its novel structure and tried for immunotherapeutic adjuvant against *L. donovani* infection, *in vitro* and *in vivo*. Cellular involvement of CD40-TLR4 has also been investigated and its efficacy in renewal of repopulating hematopoietic stem cells in bone marrow.

3 Results

The 'carbohydrate-complex' inhibited the pathogenic morph of *L. donovani* (amastigotes) *in vitro* (IC50: 50 µg/ml) and *in vivo* (IC50: 25 µg/Kg body weight), induced the nitric oxide production significantly. It efficiently reinstated the expression of CD40 in infected-macrophages which is correlated with the release of IL-12, required for the disease demolition. The 'carbohydrate-complex' also induced the expression of TLR4 in *L. donovani*-infected macrophages. Interestingly, the 'carbohydrate-complex' failed to inhibit the intracellular amastigotes in presence of anti-CD40 and anti-TLR4 (antagonist) whilst the TLR4 agonist and CD40L in adition to this 'carbohydrate-complex' augmented the inhibition of intracellular amastigotes. Thus the involvement of CD40 and TLR4 has been further confirmed by the up-regulation of relevant signaling cascades in treated macrophages. It induced the pro-inflammatory mediators MCP-1, IL-12, IL-6, TNF-α, IFN-γ in spleen and bone marrow and Th1 directing chemokines CXCL10 and CCL5 in spleen which in combination increased the Lin-Sca1-Kit+FcgRII/III^{hi}CD34+ granulocyte-monocyte progenitors (GMP) leading to development of phagocytic & pro-inflammatory CD11b+CD115+Ly6C$^{high/middle}$ monocytes.

4 Conclusions

We may claim that, to our knowledge, this is the first conclusive report of its kind to establish a novelwater soluble 'carbohydrate-complex' isolated from mushroom as an antileishmanial molecule which may be exploited as CD40-TLR4 directed therapeutic adjuvant against *L. donovani* infection.

Greco Room D1-D2
15:00-16:30

Session 17. Clinical and experimental immunopathology and pathogenesis

C0174 HIF-1ALPHA IS CRITICAL FOR INDUCING MDSC-LIKE FUNCTIONS AND M2 MACROPHAGE POLARIZATION DURING CHRONIC VISCERAL LEISHMANIASIS

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INRS – Institut Armand-Frappier Canada

1 Background

Leishmania donovani impairs the immune response by triggering a hypoxic micro-environment that facilitates the establishment of a chronic infection. Under this condition, HIF-1α, the master regulator of the response to low oxygen tension, is stabilized in all cell populations. The effect of hypoxia on the immune response to *Leishmania* has not yet been investigated. Particularly, the role of HIF-1α in macrophage and monocyte function during chronic visceral leishmaniasis remains unknown.

2 Methods

Here, we investigate the role of HIF-1α in inflammatory monocyte and dendritic cell functions, and in the modulation of macrophage polarization during chronic *L. donovani* infection. To this end, we generated CD11c-specific HIF-1α knock-out mice that were then infected with *L. donovani*.

3 Results

Our data demonstrate that that *L. donovani* promotes the output from the bone marrow of monocytes with a regulatory phenotype. We also show that, when myeloid cells reach the spleen, they acquire MDSC-like functions in a HIF-1α-dependent manner. HIF-1α is also involved in driving the polarization towards M2-like macrophages in response to extracellular lactate. Ablation of HIF-1α in $CD11c^+$ cells resulted in decreased intracellular lactate concentrations, lower expression of M2 macrophage markers, greater expansion and effector capacity of monocytes, and enhanced $IFNY^+$ production by $CD4^+$ T cells.

4 Conclusions

Moreover, mice with a targeted depletion of HIF-1α in $CD11c^+$ cells had a significantly lower splenic parasite burden, suggesting that induction of HIF-1αin $CD11c^+$ cells may represent a key factor in the establishment of persistent *Leishmania* infections.

C0665 BLIMP-1 MEDIATED REGULATION OF B-CELLS FUNCTIONS IN VISCERAL LEISHMANIASIS

Bhawana Singh, *Om Prakash Singh, Neetu Singh, Siddharth Sankar Singh, Shyam Sundar*

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1 Background

B-cells have a spectrum of functions ranging from antibody production to antigen presentation and have additional vital roles in immune mechanisms. There has been rudimentary knowledge for the role of B-cells in intracellular infections with contradictory findings. We have explored the role of B-cell dysfunctions in visceral leishmaniasis (VL) pathogenesis in terms of the phenotypic and functional properties of B-cells during the course of VL as compared to healthy controls.

2 Methods

We had collected the splenic aspirates and blood samples from VL cases at different time points of treatment, splenic aspirates were used for microscopic examination and scored wereserum samples from VL cases were used for direct agglutination assay(DAT) .

Whole blood samples were used for analysing the B-cell profile during the course of treatment by flow cytometry. We also looked for the transcriptional levels of B-cell effector molecules by real-time PCR and at protein levels by flow cytometry.

3 Results

VL cases had decreased B-cell percentages during the entire course of disease and attained exhaustive phenotype with atypical memory cell makers indicative of B-cell dysfunctions in VL. Additionally, B-cells had compromised abilities of antigen processing and presentation under the influence of Blimp-1. Blimp-1 with TNF-receptor members- BCMA and TACI, increased plasma cell survival and antibody production; therefore, increasing VL-specific antibody titres that directly correlated with the exhausted phenotype and also with disease severity and these cells were acquiring regulatory properties. Anti-leishmanial treatment gradually restored the functional features of B-cells.

4 Conclusions

Therefore, this study for the first time indicated for impaired B-cell functions and pathological consequences in human VL paving the way for immunotherapeutic targeting this cell population for disease intervention.

C0988 TNF-MEDIATED SUPPRESSION OF ARGINASE 1 IN MACROPHAGES IMPROVES CONTROL OF LEISHMANIA MAJOR DURING THE ACUTE PHASE OF INFECTION, BUT DELETION OF ARGINASE 1 DOES NOT PREVENT LONG-TERM PARASITE PERSISTENCE

Christian Bogdan, *Katrin Paduch, Andrea Debus, Stephanie Obermeyer, Ulrike Schleicher*

Microbiology Institute, University Hospital Erlangen Germany

1 Background

Tumor necrosis factor (TNF) is essential for the inducible NO synthase (iNOS)-mediated control of *Leishmania (L.) major* parasites by macrophages *in vitro* and during acute infections *in vivo*, but its mode of action is poorly understood. After clinical resolution of acute *L. major* infections, small quantities of parasites will persist lifelong despite sustained levels of iNOS protein and activity. Here, we investigated whether an altered expression of arginase (Arg) 1, an enzyme that competes with iNOS for their common substrate L-arginine, is a component of TNF-mediated protection and whether Arg1 contributes to *Leishmania* persistence.

2 Methods

Bone marrow-derived macrophages (BMM) were costimulated with IL-4 (inducer of Arg1) and TNF. C57BL/6 wildtype (WT), C57BL/6 TNF-/-, C57BL/6 Arg1fl/fl Tie2Cre-/- or Tie2Cre+/- mice were infected s.c. with *L. major* promastigotes. Arg1 and iNOS expression were analysed by RT-qPCR, Western blot, flow cytometry and confocal fluorescence microscopy (CLSFM) of tissue sections. CLSFM was also used to identify cell types producing NO or harboring *Leishmania*. To delineate the mechanism of action of TNF, anti-histone 3-lysine 27(acetylated) chromatin immunoprecipitations (ChIP), STAT6 nuclear translocation assays and STAT6 ChIP-analyses were performed. Parasite burdens were determined by limiting dilution.

3 Results

TNF suppressed IL-4-induced Arg1 mRNA and protein in BMM. ChIP analyses revealed that TNF reduced the chromatin accessibility of the Arg1 promotor and enhancer, notably for the transcription factor STAT6 that is essential for Arg1 expression, whereas STAT6 phosphorylation and nuclear translocation remained unaffected. *L. major*-infected TNF-/- mice showed a hyperexpression of Arg1 in infected skin lesions and draining lymph nodes, which was paralleled by a reduced *in situ* production of NO and an increased parasite load during the acute phase of infection. In C57BL/6 WT mice, in contrast, Arg1 expression was largely limited to the skin infection site. Deletion of Arg1 neither altered the course of acute infection nor prevented long-term parasite persistence in clinically cured mice.

4 Conclusions

At least part of the protective effect of TNF results from an epigenetic shut-down of the Arg1 promotor/enhancer, which improves the availability of arginine for iNOS and thereby facilitates the production of NO. Although persistent *Leishmania* are under the control of iNOS, deletion of arginase 1 did not lead to parasite elimination.

C1077 MIR155 DEFICIENT MICE MOUNT AN EFFICIENT TH1 RESPONSE AND RESOLVE CUTANEOUS L. MAJOR INFECTION

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Ohio State University/Pathology Ohio USA

1 Background

The outcome of *Leishmania major* infection mainly correlates with the ratio of Th1/Th2 responses where a Th1 response is associated with protection and a Th2 response leads to disease susceptibility. Micro RNA 155 (miR155) plays an important role in regulating Th1 immune responses and associated IFN-γ production. In this study we examined the role of miR155 in the control of *L. major* infection using miR155 knock out (miR155KO) mice.

2 Methods

Age and sex matched WT and miR155KO C57BL/6 mice were infected with 2 x 10^6 *L. major* (LV39) by foot pad inoculation and disease progression was monitored by measuring lesion growth and assessing parasite loads temporally. Groups of infected WT and miR155KO mice were euthanized at weeks 4, 8 and 14 post-infection and immune responses in the draining lymph nodes were analyzed by performing T cell proliferation and measuring cytokine production by *Leishmania* antigen activated lymph node cells.

3 Results

Following foot pad infection with *L. major*, miR155KO C57BL/6 mice rapidly developed large lesions compared to wild type (WT) C57BL/6 controls. However, as the infection progressed, miR155KO mice resolved their lesions faster than WT mice. Rapid resolution of infection in miR155KO mice was associated with significantly higher T cell proliferative responses to *Leishmania* antigen as well as consistently increased levels of IFN-γ and IL-12 in the culture supernatants. In addition, dendritic cells isolated from the *L. major* infected mir155KO mice produced significantly more Nitric Oxide (NO) and displayed increased expression of co-stimulatory molecules upon IFN-γ stimulation *ex vivo.*

4 Conclusions

Taken together, our results indicate that although miR155 plays a role in mediating early immunity against *L. major* infection, it is not required for the induction of a Th1 response, IFN-γ production and disease resolution following *L. major* infection.

C1256 TARGETING INHIBITORY RECEPTORS LAG-3 AND TIM-3 TO ENHANCE ANTI-PARASITIC CD4+ T CELL RESPONSES DURING VISCERAL LEISHMANIASIS

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1 Background

Visceral leishmaniasis (VL) caused by protozoan parasite *Leishmania donovani* remains a major public health problem in tropical countries. CD4+ T cells exhaustion is a common phenomenon during chronic leishmania infection which causes reduced IFN-γ secretions, critical for controlling the parasite replication. This can be mediated by abnormal expression of immunosuppressive receptors LAG-3 and TIM-3 on the surface of CD4+ T cells. The present study aims to investigate the role of Lag-3 and Tim-3 in patients with VL.

2 Methods

Peripheral blood mononuclear cells (PBMCs) were collected from VL patients before and after drug treatment as well as from endemic healthy control. $CD4^+$ T cells were enriched using magnetic beads. Ex-vivo mRNA expression of TIM-3 and LAG-3 was measured in whole PBMCs as well as enriched $CD4^+$ T cells by Real-Time PCR. PBMCs were also subjected to fluorescent staining for flow cytometry to measure the surface expression of these molecules. To know the functional relevance of LAG-3 and TIM-3, whole blood cells of VL patients were stimulated for 24 hours with soluble leishmania antigen in presence of anti LAG-3 or anti TIM-3 antagonistic antibodies or isotype controls and IFN-γ secretion was measured in culture supernatant.

3 Results

We observed enhanced mRNA expression of TIM-3 and LAG-3 in whole PBMCs as well as $CD4^+$ T cells of VL patients in pre treatment stage compared to post treatment and endemic controls. Similarly, $CD4^+$ T cells showed enhanced surface expression of TIM-3 and LAG-3 as revealed by flow cytometry analysis. We observed an enhanced IFN-γ secretion in whole blood culture after LAG-3 blockade compared to isotype controls but there was no any effect of TIM-3 blockade on IFN-γ secretion.

4 Conclusions

These results identify LAG-3 as an important immunotherapeutic target to enhance anti-parasitic $CD4^+$ T cell response and treat VL patients.

C1296 MYELOID CELL COUNTER-REGULATORY MECHANISMS MEDIATE IMMUNE SUPPRESSION AND DISEASE PROGRESSION IN EXPERIMENTAL VISCERAL LEISHMANIASIS

Elvia Yaneth Osorio, Omar Saldarriaga, Fanping Kong, Bruno Travi, Heidi Spratt, ***Peter Melby***

University of Texas Medical Branch, USA

1 Background

Progressive visceral leishmaniasis (VL) is driven by the failure of cellular immune responses to control the intracellular parasite. We studied the Leishmania donovani infected Syrian hamster (*Mesocricetus auratus*) as a model because it mimics the progressive nature of active human VL.

2 Methods

Outbred Syrian hamsters were infected with 10e6 metacyclic L. donovani promastigotes. Spleen tissue and splenic macrophages were harvested and differential gene expression determined by RNA sequencing coupled with *de novo* transcriptome assembly. Macrophage function was determined in in vitro infected bone marrow-derived macrophages and in ex vivo cultures of splenic macrophages from infected animals.

3 Results

Differentially expressed transcripts from the spleen and splenic macrophages identified a highly inflammatory spleen environment during VL. There was abundant expression of type I and type II interferon response genes. However, high IFN-g expression was ineffective in directing exclusive M1 macrophage polarization, suppressing M2-associated gene expression, and restraining parasite replication and disease. Transcriptional profiling showed that splenic macrophages had markers of myeloid-derived suppressor cells that included profound upregulation of enzymes that deplete arginine, tryptophan, and cysteine (arginase 1 [Arg1], indoleamine 2,3 dioxygenase [Ido1], and cysteine dioxygenase [Cdo1], respectively). Splenic macrophages from hamsters with VL suppressed T cell responses in a co-culture assay. Paradoxically, IFN-g enhanced parasite growth in ex vivo cultured splenic macrophages from hamsters with VL, especially later in the course of infection. IFN-g also promoted expression of the amino acid-depleting enzymes. This suggests that macrophages in the inflammatory environment of the spleen are conditioned to respond to macrophage activation signals with a counter-regulatory program that is ineffective and even disease-promoting. This was regulated through activation of STAT3 and expression of IL-10. Delivery of a STAT3 inhibitor significantly reduced parasite load and expression of the amino acid-depleting enzymes in infected animals.

4 Conclusions

STAT3 appears to be a central regulator of the immunosuppressive phenotype of splenic macrophages in VL. The STAT3 pathway offers a rational target for adjunctive host-directed therapy to interrupt the immunosuppressive function of myeloid cells in VL.

C1350 REGULATORY IGDHI B CELLS SUPPRESS T CELL FUNCTION VIA IL-10 AND PD-L1 DURING PROGRESSIVE VISCERAL LEISHMANIASIS

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1 Background

During visceral leishmaniasis(VL), T helper 1 (Th1)-based inflammation is induced to control intracellular parasites. Inflammation-based pathology has been shown to be dampened by interleukin 10 and eventual Programmed Death1 (PD1)-mediated T cell exhaustion. Cell type(s) responsible for the initiation of T cell-produced IL-10 during VL are unknown.

2 Methods

Whole blood and in some instances splenic samples from fifty U.S. and Brazilian dogs were sampled in a cross-sectional fashion to evaluate CD19+ B cell populations across progressive VL. Information on each dog's age, sex, geographic location, and *Leishmania* status (determined via PCR, serology, and clinical signs) was obtained. Cells from whole blood were processed for Flow Assisted Cell Sorting and analyzed via FlowJo for their regulatory phenotype and ability to alter the function of other B cells and CD4+ T cells. Statistical analysis was performed via Graph Pad prism, with statistical significance established at $p > 0.05$.

3 Results

$CD19^{+}$, $CD5^{-}$, $CD1d^{-}$, IgD^{hi} regulatory B cells from healthy controls produced IL-10 in absence of infection or stimulation in contrast to $IgD^{lo/neg}$ B cells. IgD^{hi} B cells may have a *de novo* vs. induced regulatory program. IgD^{hi} B cells increased three-fold in population size as VL progressed. B cells from VL dogs were necessary and sufficient to suppress T helper 1 (Th1) cell effector function ex vivo. IgD^{hi} B cells induced T cell and IgD^{lo} B cell IL-10 production. Blockage of B cell-specific PD-L1 restored Th1 responses. IgD^{hi} regulatory B cells represent a novel regulatory B cell which may precipitate T cell exhaustion during VL.

4 Conclusions

These novel regulatory B cells represent an important component of immune regulation during chronic *L. infantum* infection and greatly expand our understanding of non-experimentally induced regulatory B cells.

C1660 ROLE OF PD1/PDL1 IN THE INDUCTION OF REGULATORY T CELLS DURING LEISHMANIA AMAZONENSIS INFECTION

Herbert L. De Matos Guedes[1], Eric D. Carlsen[2], Yuejin Liang[2], Alessandra M. F. Martins[1], Iryna V. Pinchuk[2], Lynn Soong None None[2], ***Lynn Soong[2]***

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1 Background

Leishmania amazonensis is one of the etiological agents of diffuse cutaneous leishmaniasis in South America. In murine models of this infection, dysregulated expansion of effector T cells is related to pathogenesis, while the induction of regulatory T cells (Treg) promotes lesion resolution. Several important co-stimulatory ligand/receptor pairs, including PD1/PDL1, ICOS/ICOSL, OX40/OX40L, and GITR/GITR have been identified for Treg induction. However, the roles of these molecules in *L. amazonensis*-induced chronic cutaneous leishmaniasis are unclear.

2 Methods

In this study, we used *L. amazonensis*-infected C57BL/6 mice, lesion-derived CD4+ cells, as well bone marrow-derived dendritic cells to examine the expression levels of PD1/PDL1, ICOS/ICOSL, OX40/OX40L, and GITR/GITR by using multicolor flow cytometry, qRT-PCR, and Western blot assays.

3 Results

We found that infected footpad tissues exhibited an increase in expression of PD1 and PDL1 (10- and 5-fold, respectively), while expression of other Treg-inducing molecule pairs was unchanged during infection. We also revealed an increase in the percentage of $CD11c^{+}PDL1^{+}$ dendritic cells (DC) and $PD1^{+}CD4^{+}$ T cells in draining lymph nodes of infected mice. To evaluate parasite-induced PDL1 expression on DC, we infected bone marrow-derived DC with promastigotes and amastigotes *in vitro*. *L. amazonensis* infection selectively increased PDL1 expression, but decreased PDL2 expression, on the surface of DC, implying a role of PDL1 in DC dysfunction. Additional analyses suggested that infection-mediated PDL1 expression was dependent on mTor and partially dependent on STAT3, PI3K, MAPK, and MyD88. Infected DCs were more competent in inducing $CD25^{+}FoxP3^{+}$ Treg *in vitro* than were the controls; this Treg-promoting effect was dependent on PDL1 expression but not on TGF-β production.

4 Conclusions

Together, these data support a role for PD1/PDL1-mediated signaling for modulating local inflammatory responses during *L. amazonensis* infection. This study provides new insights on immune regulation of cutaneous leishmaniasis.

C1852 REGULATION OF IMMUNITY TO VISCERAL LEISHMANIA IN A NON-HUMAN PRIMATE MODEL OF INFECTION

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[4]CHU de Québec Research Center QUEBEC CANADA

1 Background

Mouse models have been extensively employed in studies aiming to understand the immune dynamics during visceral leishmaniasis (VL). However, they fail to accurately mimic human disease and, as such, alternative models are required. Non-human primates (NHP) provide relevant models to study immune responses during infections that target deep tissues, such as VL. Furthermore they provide a window to the early events after infection, which are often silent in human patients. The role of CD8 T cells and CD4 T follicular helper cells remain some of the least studied aspects of the VL immunology. In particular, how Tfh cells impact on the humoral response against the parasite and whether CD8 T cell exhaustion is associated with loss of control of parasite growth.

2 Methods

We infected rhesus macaques with *Leishmania infantum* to gain insight into the CD4, CD8 and B cell responses occurring during VL. We employed multicolour flow cytometry and tissue imaging to follow the dynamics of these immune populations during the course of infection and compare the events ocuring in the blood, lymph nodes and spleen. The parasite burden and cytokine mRNA levels in the different organs was assessed by quantitative PCR and antibody levels in the serum by ELISA.

3 Results

We found that, during VL, the differentiation of splenic T follicullar helper cells is impaired, which impacts the humoral response against the parasite, resulting in a disruption of the normal architecture of germinal centers, decrased levels of parasite-specific antibodies despite the persistent hypergammaglobulinemia. Following the CD8 T cell response we observed a progressive loss of effetor function that was concomitant with increased expression of CD8 T cell exhaustion markers and a ramping parasite burden.

4 Conclusions

Altogether, our work highlights the usefulness of NHP models to understand the immune dynamics occurring during human VL.

Miradero Room
15:00-16:00

Session 18. Epidemiology

C0412 SEROPREVALENCE STUDY OF CANINE LEISHMANIASIS IN NORTHERN AND PYRENEAN AREAS OF SPAIN. IDENTIFICATION OF RISK FACTORS.

Rita Velez[1], *Cristina Ballart*[1], *Ester Domenech*[2], *Alba Abras*[1], *Anna Fernández-Arévalo*[3], *Silvia Tebar*[3], *Carme Muñoz*[4], *Montserrat Portús*[3], *Jordi Cairó*[2], *Montserrat Gállego*[1]

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1 Background

Canine leishmaniasis caused by *Leishmania infantum* is an important zoonosis widespread in more than 70 countries. Although considered an endemic country, many regions in Spain, mainly in northern and Pyrenean areas, still lack an updated description of CanL prevalence and vector characterization. These epidemiologic studies are of utmost importance for understanding the disease extension and trends, and should be the basis for any prevention or control programmes.

2 Methods

Between April 2012 and March 2016, cross-sectional CanL surveys were conducted in different locations of Girona province and Pyrenean areas in north-eastern Spain, regions for which no previous data on the disease exist. Thirty-nine sampling points, mainly located in rural and periurban areas, and with altitudes ranging from 50 to 1300 m a.s.l. were selected. In these locations, dog densities ranged from 3 to 34 per site, totalling 601 sampled animals. The majority of dogs were hunting animals, but breeding, racing and shelter dogs were also included. Blood samples were collected by cephalic or jugular venepuncture for serology testing through a previously validated ELISA against *L. infantum*. Information on dogs' age, sex, breed, use, habitat and presence of CanL clinical signs were also collected. CanL prevalence was calculated and a mixed logistic regression model was used to assess the relationship between dog seropositivity and individual and location variables.

3 Results

One hundred and fifty dogs were seropositive, which represent 25% of the population sampled. Seropositivity ranged from 0% to 41.7%, with a higher prevalence observed at altitudes ranging between 200 and 600 m a.s.l. Preliminary results of the statistical analysis of individual and location related variables on dog seropositivity suggest that dog habitat, age and use may have an influence on the risk of dogs becoming seropositive.

4 Conclusions

The results presented here show that *L. infantum* infection in dogs is present in the area of study. The high number of seropositive dogs detected in some locations, reinforces the need to implement an effective programme of CanL detection and treatment, as well as a reinforcement of preventative measures. Epidemiologic surveys should be regularly performed in order to obtain comparative data and understand CanL trends in this region.

Research funded by Ministerio de Ciencia e Innovación, Spain (CG12010-22368-CO2-01) and European Union's H2020 Programme under the MSCA GA nº642609.

C1051 SEROLOGICAL EVIDENCE OF CANINE VISCERAL LEISHMANIASIS IN IÑAPARI - PERU, FREE COUNTRY OF THE DISEASE.

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[2]*Fundação Oswaldo Cruz Brazil*
[3]*Escola Nacional de Saúde Pública Sergio Arouca, Rio de Janeiro Brazil*

1 Background

Visceral Leishmaniasis (VL) is lethal when it is not detected and treated promtly. In Latin America (LA) it is caused by *Leishmania (L.) chagasi* being endemic in 11 countries such as Bolivia, Colombia and Brazil, neighbouring countries of Peru; in recent years LV has spread to new geographical areas of LA, by migration of the population associated with socioeconomic phenomena and the presence of Interoceanic corridors with endemic countries for LV, increasing the risk of the entry of dogs infected by *L. (L.) chagasi.* In Peru no cases of VL have been reported. The aim of the study was to highlight the epidemiological risk factors associated with the occurrence of VL in free areas with high risk of transmission.

2 Methods

An exploratory cross-sectional descriptive study was conducted in two stages: I) Serological search using the Inmunocromatografic Dual Path Platform and Indirect Immunofluorescence tests, to detect specific antibodies against *L. (L) chagasi* in canine population of: a) Iñapari, urban location b) Villa Primavera, rural location, c) Belgica, native rural location. Cross-reactivity for Ehrlichia, Borrelia, Anaplasma and Dirofilaria was evaluated with the CANIV 4 Rapid Test. II) Entomological survey with CDC traps and taxonomic identification with Young & Duncan's keys. Data and clinical signs of dogs were recorded.

3 Results

We sampled 134 dogs, detecting antibodies against the rK28 protein of *L. (L.) chagasi* in 7/134 (5.22%) of the population studied; 2/28 (7.14%) for Belgica, where close coexistence between native Peruvians and Brazilians of the Acre River was observed; 4/87 (4.6%) for Iñapari and 1/19 (5.26%) in Villa Primavera. No cross-reactivity was detected with other etiological agents. Clinical manifestation presented with loss of body weight, dermatitis, alopecia and onychogryphosis in 12% of dogs. Phlebotomines were captured in the three locations, six species of Lutzomyias were identified, highlighting *Lu. nevesi*, close *Lu. evansi*, vector of VL.

4 Conclusions

This is the first study of VL performed in Peru, in which we detected 5.22% of specific antibodies for VL in dogs from Iñapari, district border with Brazil and Bolivia; this finding is significant because up to now Peru is considered to be a free country of VL. It is recommended to implement epidemiological surveillance studies in search of the vector and the etiologic agent of VL in high risk living borders in Peru, in order to control the spread into new geographic areas of LA.

C1153 EVALUATION OF THE EFFICACY OF COLLARS IMPREGNATED WITH DELTAMETHRIN IN THE CONTROL AND PREVENTION OF CANINE VISCERAL LEISHMANIASIS IN ENDEMIC AREA

Bruna Martins Macedo Leite*[1]*, Manuela da Silva Solcà[1], Lívia Brito Coelho[1], Liliane Celestino Sales Santos[2], Adriana Oliveira de Almeida[3], Sandra Maria de Souza Passos[3], Lucas Edel Donato[4], Leila Denise Alves Ferreira Amorim[5], Patrícia Sampaio Tavares Veras[1], Deborah Bittencourt Mothé Fraga[1]

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[4]Grupo Técnico das Leishmanioses - Ministério da Saúde, Brazil
[5]Instituto de Matemática, Departamento de Estatística, Universidade Federal da Bahia. Salvador ,Brazil

1 Background

Visceral leishmaniasis is a zoonosis of great importance in public health. In Brazil, Leishmania infantum is transmitted by Lutzomyia longipalpis and dog is considered the main domestic reservoir. The VL control measures have limitations that compromise their efficacy and point to the need of new strategies. The aim of this study was to evaluate the efficacy of the use of deltamethrin-impregnated collars in dogs to control and prevent canine visceral leishmaniasis (CVL).

2 Methods

A interventional study was implemented in an endemic area in Brazil. In the baseline, CVL diagnosis was performed using DPP and EIE®CVL. Negative dogs were included in the study, while positive animals were euthanized. The study area was divided into: i) intervention area, where seronegative dogs used the collar with deltamethrin and ii) control area, where seronegative dogs did not use the collar. In addition to the baseline, two other serological evaluations were performed, one every 6 months, when dogs were reevaluated by serological tests. Dogs that seroconverted in both areas were euthanized. Dogs that remained as seronegative between evaluations, in the intervention area received a new colar, and in the control area did not receive, but remained in the study. Seroprevalence and incidence rates were calculated in both areas and were compared using EPIINFO. Survival analysis using Cox model was performed in STATA.

3 Results

In the first serological evaluation, seroprevalence was similar in both areas, 26% in intervention area and 32% in control area. In the second evaluation, there was a significant reduction in seroprevalence (8%) in both areas. In the third evaluation, the seroprevalence reduced in intervention area to 6% and increased to 11% in control area. This increase was stastically significant and risk ratio (RR=0.55) indicated protection against CVL among dogs in intervention area. In the survival analysis, the Wilcoxon test showed longer survival time among dogs in intervention area ($\chi 2=7.2$; $p=0.0074$). Although, considering CVL incidence, there was no significant change between the areas. In the Cox model, the CVL protection was 51% in the intervention area, however, there was no association between seroconversion of dogs and the use of deltamethrin-impregnated collars ($p=0.1$).

4 Conclusions

The evaluation of the efficacy of the use of the collar as a control measure did not present conclusive results and its implementation represents an operational challenge for the health authorities.

C1458 EXPLORATORY STUDY OF CANINE LEISHMANIOSIS IN A FOCUS OF THE ANDEAN STATE TRUJILLO, VENEZUELA

Maria Sanchez Rodriguez[1], Orquidea Rodriguez Garcia[2], Melcenia Moreno Nuñez[2], Vestalia Rodriguez[2], Martin Sanchez Silva[2]
[1]Instituto Witremundo Torrealba, Nurr- ula,Venezuela
[2]Instituto de Biomedicina Jacinto Convit, UCV, Venezuela

1 Background

Visceral Leishmaniasis is a public health problem that in the last years has been of great importance in several endemic areas of Venezuela, being school age children the most susceptible. The canine is the reservoir of disease, which in addition to representing the source of infection for humans also suffers it ; The Andean region in Venezuela is known to be a Cuteneous leishmaniasis endemic area and few reports has been associated in humans with the visceral disease. In the present work, an exploratory study of seroprevalence for canine visceral leishmaniasis was carried out in the Simón Bolívar neighborhood of the Sabana de Mendoza parish of Sucre municipality, Trujillo state, a population selected for presenting a recent history of human visceral leishmaniasis.

2 Methods

A total population of 520 canines (65% male and 35% female) was evaluated, each of them underwent physical examination and blood sampling with informed consent of their respective owners. Epidemiological data were collected through a survey and direct observation of the study area. The climatological and sociodemographic data was georeferenced using QGIS software. The infection status of the canine population was determined using the ELISA-rk39 immunoassay and compared with the commercial rk39 Dipstick.

3 Results

There were no significant differences in variables such as age and sex regarding canine infection status. It was also demonstrated the high sensitivity of the ELISA vs the rapid test. The majority of infected dogs were classified as asymptomatic (61%) and of the symptomatic 20% showed multiple signs and symptoms including hepatosplenomegaly. A large proportion of houses with infected dogs are located towards the edge of the forest area and a garbage dump with sewage favoring the vector-reservoir contact.

4 Conclusions

The results obtained suggest a high prevalence in a well delimited area and a potential risk of acquiring the disease by both canines and humans, the fact that asymptomatic infected dogs represent the highest proportions imply an increased risk for which makes it necessary to implement measures of epidemiological control in the area. Financed by CDCH UCV PSU09-7878 MPPCTI, PEII G-20012000976 MS.

C1086 EVALUATING THE EFFICACY OF THE MASS USE OF DELTAMETHRIN-IMPREGNATED DOG COLLARS FOR PREVENTING CANINE VISCERAL LEISHMANIASIS IN A BRAZILIAN MUNICIPALITY.

Sara Silva, Laiza Gomes, Aline Cerqueira, Paloma Carvalho, Luiz Felipe Borges, Renato Capanema, Camila Oliveira, Helena Teotonio, Elen Silveira, Maria Helena Morais, Pedro Pereira,Danielle Soares
Federal University of Minas Gerais. Belo Horizonte, Brazil

1 Background

Visceral leishmaniasis (VL) is an important zoonosis in Public Health, especially in developing countries. The adopted measures for its prevention and control are controversial and the real impact caused by them is questionable. The aim of this study was to evaluate the efficacy of using deltamethrin-impregnated dog collars in the prevention and control of canine VL (CVL) in a municipality of Minas Gerais, Brazil.

2 Methods

An epidemiological intervention study was conducted by comparing morbidity measures (prevalence and person-time incidence) in two distinct areas: the first one the Experimental Area (AE), where the proposed measure was implemented, and the second one the Control Area (CA). In both areas blood samples were collected from dogs. In the EA the animals also received collars impregnated with deltamethrin 4%. For diagnosing the animals, two serological tests were used in series: a rapid test (TR-DPP) as triage, and an enzyme-linked immunosorbent assay (ELISA) as confirmatory, as preconized by Brazil's Ministry of Health. Six months after the first visit, a second blood sample was taken from the animals, in order to determine the incidence of LVC, the relative risk and the efficacy of the intervention.

3 Results

In the first intervention, 1020 dogs from the EA, and 589 from the CA were examined. The prevalence calculated for the EA was 4,41% (CI95% 3,27-5,91), and for the CA was 7,13% (CI95% 5,25-9,59). The odds of infection observed, 1,6636 (CI95% 1,08-2,57), indicated that dogs from the CA had naturally more chances of infecting with VL than dogs from the EA. In the second intervention, 454 dogs from the EA, and 292 from the CA were examined. The person-time incidence observed in the EA was 3,51% (CI95% 1,30-7,48), and in the CA was 18,11% (CI95% 12,15-25,33). Due to the difference found in the odds of infection, a correction in the number of the new cases from CA was made by maintaining 66% (n = 17) of them, and the new person-time incidence value found was 11,81% (CI95% 7,03-18,23). The difference between the calculated incidences was statistically significant (p=0,009). The relative risk value found was 0,2972 (CI95% 0,1204-0,7339; p=0,009), which demonstrated that the proposed measure was a protection factor, and the efficacy of the mass use of collars was estimated in 70,27%.

4 Conclusions

These results give scientific based evidence that the mass use of insecticide-impregnated dog collars are an alternative to be considered in the combat against CVL.

C1266 SEROLOGICAL AND ENTOMOLOGICAL SURVEY ON CANINE LEISHMANIASIS IN LAMPEDUSA ISLAND, ITALY.

Valentina Foglia Manzillo[1], *Manuela Gizzarelli*[1], *Fabrizio Vitale*[2], *Salvatore Daniele Sotera*[3], *Alessandra Torina*[2], *Gaetano Oliva*[1]

[1]*Department of Veterinary Medicine and Animal Production Naples Italy*
[2]*Istituto Zooprofilattico Sperimentale della Sicilia – CreNaL, Palermo Italy*
[3]*DVM Italy*

1 Background

Lampedusa is a small island (20,2 km^2) of the Italian Pelagie archipelago, the southernmost of Italy and the nearest to North Africa (113 km). Lampedusa has a population of 6000 inhabitants, less than 1000 dogs have been estimated. The unique study on Canine Leishmaniasis (CanL) in this island was performed on 87 dogs about fifteen years ago. In this trial a seroprevalence of 39,1% was assessed and the presence of phlebotomine sand flies was reported (*P. pappatasi:* 88,8%).During last decade Lampedusa has been interested by a deep social change caused by the massive arrival of migrants from north Africa. In this new scenario, it was interesting to add more data on CanL.

2 Methods

Two hundred and forty-two dogs were enrolled from November 2015 to February 2016. Each dog was submitted to clinical examination and blood sample. *L. infantum* infection was assessed by using rapid test and confirmed by IFAT (OIE protocol). The cut-off dilution was set at the 1:160 titer; lower titers were considered a marker for *Leishmania* exposure. Dogs showing clinical signs were submitted to RT- PCR (blood/lymph node). *Leishmania* vectors were monitored from five resting sites for sand flies. Sticky traps and CDC miniature light traps were used.

3 Results

Seventy/242 dogs (28,9%) resulted negative. The remaining 172 (71,1%) showed positivity at least to one of the test. In detail 5/172 (2,9%) showed a positive rapid test alone; 148/172 resulted positive to IFAT. These dogs showed antibodies equal or higher to cut-off (47,9%) or lower (52%). Total seroprevalence was of 61,1%. Twenty-three positive dogs (13,4%) showed clinical signs; 19 resulted positive to RT-PCR. Among 223 sand flies specimens collected 3 species were identified (*P. perniciosus*, *P.papatasi*, *S. minuta*). Based on male identification (34,9%), *P. perniciosus* was the most prevalent species (75%); female identification is ongoing.

4 Conclusions

Our findings suggest a severe increasing of CanL seroprevalence when compared with the previous study. The high proportion of seropositive dogs demonstrates that *L. infantum* abundantly circulates in the island and constitutes a risk for people, particularly for hosted migrants affected by immune depressive diseases. The high presence of *P. perniciosus* increases the risk of zoonotic transmission. The high level of infection among the canine reservoir host. Restrictive sanitary measures should be considered by using large scale application of sand flies repellents.

Greco Room C1-C2
15:30-17:00

Session 19. Operational research

C0461 EXPERIENCES FROM IMPLEMENTATION OF FOCUSSED PHARMACOVIGILANCE FOR VISCERAL LEISHMANIASIS TREATMENT IN BANGLADESH

Md. Sakhawat Hossain[1], *Varun Sharma*[2], *Amresh Kumar*[2], *AFM Akhtar Hossain*[3], *Md. Mahshin*[4], *Abhijit Sharma*[2], *Md. Akter Hossain*[4], *Rashidul Haque*[1], *Abul Khair Mohammad Shamsuzzaman*[3], *Vivek Ahuja*[2], *Dinesh Mondal*[1]

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1 Background

Adverse effects of anti-leishmanial drugs can significantly affect the quality of life and adherence to therapy for visceral leishmaniasis (VL) and Post-kala-azar Dermal Leishmaniasis (PKDL). An effective model of pharmacovigilance (PV) may ensure maximum therapeutic benefit for patients by early detection and prevention of ADRs. We investigated feasibility of focussed PV for VL (VLPV) through National VL Elimination Program of Bangladesh.

2 Methods

This activity involves stimulated spontaneous reporting of suspected ADR with drugs used for VL and PKDL by health care professionals (HCP) to a central authority. This program does not dictate the use of any particular drug and management is in accordance with VL elimination program. All ADR reports are forwarded to the Directorate General of Drug Administration (DGDA). Operational plan developed for focussed VLPV in Bangladesh describes the purpose, procedures, roles and responsibilities of HCP and responsible person at the central level. Focal VLPV teams at UHC level with one medical officer, one nurse, and one statistician have been trained in PV concepts and their roles and responsibilities in reporting ADR encountered during patient care.
ADR information is collected on a standard reporting form prepared for this purpose and is sent to contact person at Center for Disease Control (CDC), Ministry of Health. The contact person at CDC sends acknowledgment and collects missing information.

3 Results

During Dec 2014 - Jan 2016, 703 VLPV reports were received for 907 treated patients from 23 treatment centres. Out of these 703 reports, 137 (20%) had at least 1 ADR and remaining contained no ADR. Out of 137 reports, 92(67%) were patients of VL, 40 (29%) of PKDL, 4 (3%) of VL relapse and 1 (1%) were others. Out of 137 reports, Liposomal Amphotericin B (LAMB), Miltefosine (MF) and Combination were administered to 110 (80%), 26 (19%) and 1 (1%) respectively. Most frequent ADR was fever reported in 65% (89/137) of patients treated with LAMB followed by vomiting in 20% (27/137) by LAMB (14/137) and MF (13/137). Chills and rigor were mostly caused by LAMB with a frequency of 16%. Four cases with fatal outcome were reported. Two of these were with LAMB and assessed as related to treatment by the causality assessment committee.

4 Conclusions

Focussed VL Pharmacovigilance in public health system of Bangladesh is effective and should be continued.

C0618 BARRIERS OF VISCERAL LEISHMANIASIS REPORTING AND SURVEILLANCE IN NEPAL: COMPARISON OF GOVERNMENTAL VL-PROGRAM DISTRICTS WITH NON-PROGRAM DISTRICTS

DaJull Lim[1], *Megha Raj Banjara Banjara*[2], *Anand Ballabh Joshi*[3], *Chitra Kumar Gurung*[3], *Piero Olliaro*[4], *Greg Greg Matlashewski*[5], *Axel Kroeger*[4]

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[2]*Tribhuvan University Kathmandu Nepal*
[3]*Public Health and Infectious Disease Research Center, Kathmandu, Nepal Nepal*
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[5]*Department of Microbiology and Immunology, McGill University, Canada Canada*

1 Background

Nepal finds itself on the verge of reaching the maintenance phase of the Visceral Leishmaniasis (VL) elimination program. There is considerable reduction of reported cases but there are new challenges due to spread of VL cases in new areas of the country including previously non-endemic areas without a program of vector control or patient management. This study points out shortcomings in surveillance and timely reporting that need strengthening to sustain elimination and prevent future endemics.

2 Methods

In a cross sectional study design mixed methods were applied to collect data from two study populations in VL-program and non-program districts during the months of March to May 2016. Four periods of delay were recorded using structured interviews with 40VL patients. In-depth interviews and semi-structured interviews were also conducted with 13 health managers to identify shortcomings of the surveillance and the reporting system. Quantitative data was entered into Excel and SPSS 21 for analysis and qualitative data was analyzed through qualitative content analysis using MAXQDA.

3 Results

Total delay from onset of symptoms to successful reporting increased to 115 days to previous years. Comparison of non-program districts to program districts showed 24 days of difference. Patient delay was 2 times higher in non-program districts (31days) compared to program districts (14 days). The diagnostic delay showed no significant differences between program (73 days) or non-program districts (80 days). Delay to successful therapy (4 days) and report to central authority (17 days) showed no significant differences amongst two areas of comparison or to previous years. Main barriers of reporting were: 1) geographical access, poverty and low awareness 2) exclusion of private sector in referral, treatment and reporting 3) complexity and backwardness of reporting system 4) verticality of existing programs lack of responsible coordinating position.

4 Conclusions

In order to address the prolonged patient delay, VL program should address efficiently to new pattern of endemicity. The private sector needs to be involved into the program in order to address access issues and reduce delay times. A clear referral structure of patients and validation structure of reports need to be implemented to strengthen and simplify surveillance to ensure timely and reliable reporting.

C0689 A CROSS SECTIONAL STUDY OF VISCERAL LEISHMANIASIS RELAPSE (VLR) CASES IN SURJA KANTA KALA-AZAR RESEARCH CENTRE (SKKRC), MYMENSINGH, BANGLADESH

Shomik Maruf[1], *Ariful Basher*[1], *Progganandа Nath*[1], *Md. Golam Hasnain*[2], *A.F.M. Akhtar Hossain*[3], *A.F.M. Akhtar Hossain*[3], *Sanya Tahmina*[3], *Md. Nasir Uddin Ahmed*[4], *Jorge Alavar*[5], *Dinesh Mondal*[6]

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1 Background

Surja Kanta Kala-azar Research Centre (SKKRC), Mymensingh, Bangladesh is the only specialized centre in the country for treatment of complicated cases with Visceral Leishmaniasis (VL) and Post Kala-azar Dermal Leishmaniasis (PKDL) and also for cases with Visceral Leishmaniasis Relapse (VLR). As there is no clinical, demographic, geographic information along with geographic distribution available for VLR in Bangladesh, we aimed to describe this knowledge gap in this study.

2 Methods

This is a cross sectional study. We used 4-year hospital registry of the SKKRC, for the VLR cases between January, 2012 and December, 2015. We included in the analysis only the records of VLR cases defined by past history of VL, fever > 2 weeks, splenomegaly and having parasitological confirmation, either by Giemsa stained microscopy of spleen or bone marrow aspirate, or by blood Real Time – Polymerase Chain Reaction (RT-PCR).

3 Results

Of total 310 hospitalization records of VLR, 151 cases had parasitological confirmation. Cases came from 38 sub-districts as highest number of patients (20.5%) came from Trishal sub-district, followed by Gafargaon (13.2%) and Bhaluka (9.9%). Ninety two percent had single relapse and eight percent had multiple relapses. Mean age for the patients was 31.22 years and most of the cases were male 111/151 (73.5%). Cases with single and multiple VLR did not differ regarding and sex and age. The median duration for VL relapse was 2 years. Six of 151 patients had other comorbidities; 2 had extrapulmonary tuberculosis and rest had diabetes mellitus and other chronic diseases. During admission 60% of VLR cases were malnourished and 89 % patients were anemic (Hb<11g/dl). The average size of spleen during admission was 5.5 cm and hepatomegaly was found in 2.7% of cases. Forty three percent, 26%, 22% were treated respectively with Miltefosine, Sodium Stibogluconate and AmBisome for primary VL. Most of the VLR cases received treatment with multi dose AmBisome followed by combination therapy and Amphotericin B Deoxycholate. 70% cases with comorbidities were treated with multi-dose AmBisome.

4 Conclusions

SKKRC is successfully managing VLR cases from all over Bangladesh. Most of the VLR are coming from Trishal and other sub-districts of Mymensingh district. Malnutrition and anemia after cure from primary VL could be potential risk factor for VLR. Prevalence of malnutrition and anemia was evident in most of the cases demanding further research.

C0866 VISCERAL LEISHMANIASIS INCIDENCE REPORTED BY THE ELIMINATION PROGRAM IN BIHAR, INDIA IS MUCH CLOSER TO TRUE INCIDENCE THAN PREVIOUSLY ESTIMATED

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1 Background

The incidence of visceral leishmaniasis (VL) in India is among the highest in the world and the state of Bihar is estimated to account for four-fifth of the cases in the country. It has previously been estimated that India under-reports VL cases by a factor of 4-8 times. Given the imperative to eliminate the disease, it is important to identify as many cases as possible to enable disease control strategies to be accurately and comprehensively targeted. Two consecutive situation assessments, designed and led by CARE India, aimed, inter alia, at estimating the true incidence of the disease.

2 Methods

Using an elaborate index case tracing method, we attempted to locate all the new VL cases during the reference period of **July**'2013 to December'2014 in 33 VL-endemic districts of Bihar. Neighbourhood families and key informants were interviewed and all private labs, pharmacists and selected physicians in affected blocks were contacted for information about VL patients known to them. Secondary cases identified in this manner were then tracked down and interviewed to see if they met the case definition. The identified cases were stratified by their residential blocks (sub-districts) for estimation of true block-level incidence. Blocks were then classified as above or below the elimination level of 1 case/10,000 population.

3 Results

In total, 7,897 VL cases meeting the case definition were traced from across the state. We found that about 9% (721/7,897) cases were not captured by the government reporting system. Using the mid-year population as the denominator, the annual incidence proportion was estimated to be 0.71/10,000 population. In all 334 blocks in the state had one of more cases of VL, of which 86 blocks had incidence above the elimination level. About 23% of blocks having high endemicity and 16% of blocks with low endemicity were observed to have been misclassified by program reports. These findings were similar to the results of a similar assessment carried out in 2013 in eight districts of Bihar by the same team.

4 Conclusions

The magnitude of under-reporting of VL-cases was found to be substantially lesser than previously estimated, and very close to officially reported levels. This could be because the VL patients are attracted to public facilities due to the free availability of expensive diagnostics and medicines, wage loss compensation and the lack of equally attractive private sector alternatives.

C1247 SETTING THE POST-ELIMINATION AGENDA FOR KALA-AZAR IN INDIA (SPEAK INDIA): WORKING TOWARDS SUSTAINED ELIMINATION OF VISCERAL LEISHMANIASIS IN THE INDIAN SUBCONTINENT

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1 Background

The governments of India, Bangladesh and Nepal have set 2017 as the target year for visceral leishmaniasis (VL) elimination as a public health problem (defined as <1 case/10,000 people at the sub-district/block level) from the Indian subcontinent. To date, Bangladesh and Nepal are on track to meet this aims, however blocks in India (notably those north of the Ganges in Bihar state) are not, and others (particularly in southern Bihar state) are currently experiencing a resurgence in disease incidence. It is unclear whether these differing levels of success are due to differences in surveillance, disparities in health systems and community awareness, or variations in the epidemiology of VL. Numerous aspects of VL biology and epidemiology will need to be addressed for elimination to be achieved and sustained.

2 Methods

To identify the additional knowledge required to ensure the VL elimination target is met and sustained, the Setting the Post-Elimination Agenda for Kala-Azar in India (SPEAK India) consortium has been established. SPEAK India will bring together scientific, practical and logistics experts to analyse new and existing data, develop protocols, actions and methodologies, and formulate the research questions that need to be addressed for sustainable VL elimination to be achieved.

3 Results

Through an evaluation of India's current control programme for kala-azar and VL response strategy, and discussions between scientific and operational experts, three strategic areas requiring further research attention have been identified:

1. Surveillance
2. Health systems
3. Transmission

Considering these areas, three overarching research questions have been identified to guide future VL elimination research:

1. In a close to elimination or post-elimination surveillance programme kala-azar (KA) cases will be uncommon, so what should the surveillance be looking for?
2. What are the knowledge gaps in the role of sandflies in transmission, and how can these be exploited to optimise vector control?
3. How should health systems be strengthened to prevent the resurgence of KA?

4 Conclusions

Through constructive discussion around the transmission dynamics of VL, SPEAK India has and will continue to, facilitate the identification of research priorities and necessary health system adaptations for VL elimination to be achieved throughout the Indian subcontinent.

C1557 PHLEBOTOMUS ARGENTIPES (DIPTERA: PSYCHODIDAE) CONTROL IN BANGLADESH: A CLUSTER RANDOMIZED CONTROLLED TRIAL

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1 Background

A number of studies on visceral leishmaniasis (VL) vector control tools have been conducted during the past decade with sometimes confirmative and sometimes contradictory results. The present study investigated in a large sample different options which have not yet been fully explored including: (1) indoor residual spraying (IRS) with alpha cypermethrin 5WP; (2) long lasting insecticide impregnated bed-net (LLIN); (3) impregnation of local bed-nets with slow release insecticide KOTAB 123 (KOTAB); (4) insecticide spraying in possible breeding places using chlorpyrifos 20EC (OUT) and five different combinations.

2 Methods

The study is a controlled cluster randomized trial where 3089 houses from 11 villages were divided into 10 sections, each section having six clusters and each cluster having about 50 houses. Vector density was measured before and up to 22 months after intervention using CDC light traps.

3 Results

A total of 17,434sand flies were collected during baseline and 9 follow-up surveys. At baseline, the average *P. argentipes* density per household was 10.6 (SD=11.5) in the control arm and 7.3 (SD=8.46) to 11.5 (SD=20.2) in intervention arms. The intervention results presented as the range of percent reductions of sand flies and rate ratios in 9 measurements over 22 months. Among single type interventions, the effect of IRS with 2 rounds of spraying (applied by the research team) ranged from 13% to 75% reduction of *P. argentipes* density compared to control arm (rate-ratio [RR] ranged from 0.25 to 0.87). LLINs caused a vector reduction of 9% to 78% (RR, 0.22 to 0.91). KOTAB reduced vectors by 4% to 73% (RR, 0.27 to 0.96). The combination of LLIN and OUT led to a vector reduction of 26% to 86% (RR, 0.14 to 0.74). The reduction for IRS and OUT together was 8% to 88% (RR, 0.12 to 0.92). IRS and LLIN combined resulted in a vector reduction of 13% to 85% (RR, 0.15 to 0.77). IRS plus KOTAB reduced vector densities by 16% to 86% (RR, 0.14 to 0.84). Some intermediate measurements for KOTAB alone and for IRS plus LLIN; and IRS plus KOTAB were not statistically significant. The bioassays on sprayed surfaces or netting materials showed favourable results (>80% mortality) for 22 months (IRS tested for 12 months). In the KOTAB a gradual decline was observed after 6 months.

4 Conclusions

LLIN and outdoor spraying was the best combination to reduce VL vector densities for 22 months or longer. Operationally this is much easier to apply than IRS. A cost analysis of the preferred tools will follow.

C1693 INSECTICIDE TREATED DURABLE WALL LINING FOR CONTROLLING VISCERAL LEISHMANIASIS IN INDIAN SUBCONTINENT: A 24-MONTH FOLLOW-UP STUDY

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1 Background

Vector control is one of the important strategies of the visceral leishmaniasis (VL) elimination program in Bangladesh, India and Nepal. Recently we found and reported that Durable Wall Lining (DWL) was the most effective sand fly control tool compared to impregnated bed net with insecticide and household wall wash with lime. Here we investigated the longevity of the efficacy of DWL for sand fly control to support the VL elimination program in the Indian sub-continent.

2 Methods

This multi-country cluster randomized controlled trial had 12 clusters in each site: six for intervention and six for control and each cluster included 45-50 households. The durable wall lining is a thin sheet of woven shade cloth impregnated with insecticides (ZeroVector® Durable Lining, Vestergaard, Frandsen, Switzerland). Trained village volunteers installed DWL in the allocated households under supervision of trained field research supervisors. Five households from each cluster were randomly selected for entomological activities for sand fly density (CDC light trap method) and mortality measurement (WHO con method). Household interviews were conducted for collecting information on household socioeconomic status, knowledge about VL and acceptance of the DWL intervention at the community. A generalized estimating equation (GEE) modelling with Poisson distribution approach was used to identify the intervention effect on female *P. argentipes* density.

3 Results

Female *Phlebotomus argentipes* density was similar in intervention and control clusters at baseline, but was lower in intervention cluster over the entire 24-month follow-up period compared with control clusters. Statistical significant intervention effects on female *P. argentipes* sand fly reduction measured by adjusted incidence rate ratio (95% CI) at 1, 3, 9, 12 and 24 months were respectively: 0.28 (0.23 - 0.34), 0.27 (0.21 - 0.34), 0.38 (0.31 - 0.47), 0.62 (0.51 - 0.75) and 0.75 (0.63 - 0.89). Average sand fly mortality (95%CI) at 1, 3, 9, 12 and 24 months was respectively 84% (81% - 87%), 84% (79% - 88%), 84% (79% - 89%), 74% (71% - 78%) and 75% (70% - 80%). Community acceptance of DWL intervention was 93.7% (95%CI, 92.0% - 95.2%).

4 Conclusions

Sustained efficacy of DWL was achieved throughout the 24-month follow-up with unyielding acceptance by the community. The DWL should be considered as a long-lasting effective tool for sand fly control for the VL elimination program of the Indian sub-continent.

C1709 VILLAGE POPULATION-AT-RISK BASED ACTIVE CASE DETECTION IN INDIA – A FINAL FRONTIER FOR ELIMINATION

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1 Background

India aims to achieve Kala-Azar (KA) elimination as a public health problem by the end of 2017. But however in 2016, 123 blocks remain above the target of 1/10000 cases. AThe average time to treatment from onset of illness remains over 2 months and substantial numbers of undiagnosed cases of Post Kala-Azar Dermal Leishmaniasis (PKDL) are expected in endemic communities. Both of these factors are critical in the continued transmission of KA, and act as barriers to achieving the elimination target. In an attempt to disrupt the anthroponotic transmission of Kala Azar (KA), KalaCORE designed and implemented an innovative pilot active case detection strategy targeting the population at risk at the village level.

2 Methods

219 villages from thirteen of the highest endemic blocks of Bihar and Jharkhand were selected based on village level analysis of two-year incidence rates (2014 and 2015), geolocation and operational feasibility. Selected villages underwent comprehensive house-to-house screening, with suspect cases were referred to 24 nodal 'diagnostic camps', which had capacity to diagnose and refer confirmed cases to the nearest treatment facility.

3 Results

A total of 1337 VL and 430 PKDL suspects were identified during house-to-house screening. Of these, 80% of suspect cases attended the diagnostic camps, mostly accompanied by front line workers. A total of 38 VL and 171 PKDL cases were confirmed positive at the camps. The cost per person screened was US$ 0.50, cost per suspect US$ 150, cost per confirmed case US$ 1350 and the cost per village screened US$ 1450.

4 Conclusions

This ACD approach was feasible and succeeded due to the participation of the front line workers. The strategy to shift from block level to village level risk was successful, with more specific populations-at-risk identified for screening. PKDL cases were as expected the highest number, although the pilot was conducted during the lowest annual period of reported KA cases. Although the cost per confirmed case was substantial, at this stage of elimination the relevance of cost-effectiveness is questionable. The strategy will be scaled up across a further 39 blocks at the start of the KA season in 2017 using a weighted 3-year incidence, with impact assessed in 2018.

C0602 VISCERAL LEISHMANIASIS ELIMINATION - WHERE DOES INDIA STAND?

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1 Background

Visceral Leishmaniasis (VL) also known as Kala-azar is endemic in the Indian subcontinent. In India, VL is at present endemic in 54 districts in the states of Bihar, Jharkhand, West Bengal and Uttar Pradesh. An estimated 130 million population is exposed to the risk of VL. The peak annual incidence of VL was seen in 1992, when 77102 cases and 1419 deaths were reported. The target date for elimination in India is by 2017.

2 Methods

The National Roadmap for VL Elimination (2014) document was developed for focused efforts at national, state, district and sub-district level. To achieve the target, programme is focusing both on case management and integrated vector management with effective IEC / BCC approach. Developmental partners are also helping the programme to improve access to and use of services, expanding the effectiveness, reach, training, community mobilization etc. Single day single dose treatment introduced during 2015. Incentive to patient for loss of wages and honorarium to Accredited Social Health Activist (ASHAs) has been provisioned. DDT has been replaced with Synthetic Pyrethroid for better community acceptance. District and Block level supervisory cadre are at place for supervision and monitoring. Programme has started diagnosis of all confirmed VL case for HIV test also. VL Health Management Information System (HMIS) has been generated on line.

3 Results

In 2007, the numbers of VL cases were 44533 and 203 deaths which were reduced to 8500 (81%) cases and 5 (98%) deaths in 2015. In 2016 as of November only 5484 cases reported with no deaths. 78% blocks have achieved less than one case per 10,000 population in 2015. Treatment with single day injection has improved treatment compliance upto 94%. Through active search and camps 403 positive VL cases and 303 new PKDL cases were treated in 2016. In 2015, 45 cases of HIV-VL co-infected were detected and treated. Similarly, in 2016, as on November 76 co-infected cases were treated.

4 Conclusions

With the concerted efforts, the number of VL cases are showing declining trend since 2012 in India. In 2015 there was a decline of 74% of VL cases and 94% reduction in deaths in comparison with the year 2011. The programme is of the view that VL cases will further reduce in 2017 as surveillance, case detection and treatment alongwith supervised monitoring and supervision has improved with the involvement of all the stakeholders and the target date of elimination is doable.

Friday, 19th of May
Greco Room D1-D2
9:30-11:00

Session 20. Clinical and experimental prophylactic and therapeutic vaccination. Clinical and experimental immunology

C0233 SYNTHETIC PEPTIDES DERIVED FROM KMP-11 PROTEIN INDUCE RESPONSE IN DENDRITIC CELLS FROM HUMAN NATURALLY EXPOSED TO LEISHMANIA SPP

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Universidad Nacional de Colombia Colombia

1 Background

Leishmaniasis is vector borne disease affecting about 1.3 million people every year worldwide. The profilaxis of leishmaniasis is more cost-effective compared with chemotherapy, therefore it is urgent the development of an effective vaccine for use in humans. *Leishmania* is a protozoa parasite which invades, and multiplicates inside macrophages, so the cellular immune response is essential in the elimination of intracellular amastigotes, specially Th1 profile with IFNγ production, directed by the initial contact between Dendritic Cells (DCs) through linear peptides anchored to HLA-DR, in order to activate T-lymphocytes. Therefore, this study focused on the identification of synthetic peptides as vaccine candidates against leishmaniasis.

2 Methods

Three Peptides derived from immunogenic KMP-11 (Kinetoplastid membrane protein 11) were identified and synthetized to be tested in human PBMC's (peripheral blood mononuclear cell) isolated from human voluntaries living in Leishmaniasis endemic areas in Colombia. Humans were divided in four clinical groups *i*) individual with active leishmaniasis (A), *ii*) Individuals cured with treatment (C), *iii*) Voluntaries with leishmanin test positive without history of leishmaniasis (L+) and *iv*) humans with leishmanin test negative (L-). Macrophages derived from PBMCs were treated with IL-4 (Interleukin 4) and GM-CSF (Granulocyte-macrophage colony-stimulating factor) in order to differentiated them to moDCs (monocyte-derived DCs). Those moDCs were stimulated with each peptide and then, there were analyzed cytokines produced in supernatants.

3 Results

When moDCs were stimulates with PHA (Phytohaemagglutinin), it induced the production of IL-8, IL-6 and TNFα in all individuals showing that the cell were competent to develop an immune response. Then moDCs displayed the induction of several cytokines specially TNFα, IL-6, IL-8, when they were stimulated with rKMP-11 in all the clinical groups including L- group. In addition, KMP-$11_{16\text{-}35}$, KMP-$11_{46\text{-}65}$ and KMP-$11_{76\text{-}90}$, peptide induced cytokine production in moDCs in individuals from all clinical groups expressing HLA-DR *01, *03, *04, *07, *08, *11, *13, *14, *15 and *16, showing the ability of KMP-11 derived peptides to act as antigens which triggers immune response in antigen presenting cells.

4 Conclusions

Peptides derived from *L. panamensis* KMP-11 protein have the ability to induce inflammatory cytokines in moDCs without a clear preference by a specific HLA-DR.

C0235 HOST PROTECTION AGAINST DRUG -SENSITIVE AND –RESISTANT LEISHMANIA DONOVANI INFECTION BY THERAPEUTIC COMBINATION OF MYCOBACTERIUM INDICUS PRANII (MW) AND HEAT INDUCED PROMASTIGOTES PROMOTES THE UP REGULATION OF FLT3+ PREDC LEADING TO IL-6 PRODUCING CD11C+ CDC

Somaditya Dey, *Debarati Mukherjee, Sirin Sultana Salma, Suvadip Mallick, Supratim Mandal, Aritri Dutta, Joydip Ghosh, Aabid Hussain, Chiranjib Pal*

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1 Background

The protozoan parasite *Leishmania* causes leishmaniasis threatening 350 million people worldwide. The major concerns of current chemotherapeutics are severe toxicity & emergence of resistance. The aim of this work is to design a successful adjuvant in combination with *Mycobacterium indicus pranii (Mw*) & heat induced *L. donovani* promastigotes (HIP) against experimental murine visceral leishmaniasis.

2 Methods

10 Balb/c mice, per group, were administered with subcutaneous injection of *Mw* (10^8 cells/ kg body weight) & HIP (50 µg/ kg body weight) for 7 days with a booster dose on 14th day after establishing infection. Organs were harvested for microscopic, flow cytometric & RT-PCR analysis to investigate the hypothesis.

3 Results

Mw+HIP effectively reduced the hepatic & splenic parasite burden of *L. donovani*-infected animals by inducing CD4+IFNγ+ T cells & reducing CD4+IL-10+ T cells, along with the upregulation of Th1 promoting cytokines (IL-6, TNF-α), chemokines (IP10, CCL4) & TLR2. Th1 responses, *in vitro*, depends on the cooperation & involvement of TLR2 & we speculated that the *Mw* + HIP combination therapy requires the cooperation of an integrated TLR~Cytokine~Chemokine loop. Dendritic cells (DCs) play a crucial role in determining the balance of CMI & humoral immunity by steering the T cell population towards a Th1 or Th2 response. We used this approach to identify IL-6 as a critical cytokine that mediate anti-*Ld* host protection. *Mw+HIP* induced the expansions of CD11c+CD11b+, CD11c+CD8α+ conventional splenic DCs & $CD11c^{interim}$ B220+PDCA1+ plasmacytoid DCs along with the upregulated expressions of IL-6 in treated animals. *Mw+HIP* restored the depleted bone marrow system, in vivo & could direct bone marrow common dendritic progenitor cells to preDC development, ultimately repopulating the IL-6 secreting splenic DCs. This novel combinational approach induced collateral host protective responses by diminishing the CD4+ CD25+ FoxP3+ splenic regulatory T cells along with the reduction in IL-10 & TGF-β level. Interestingly, *Mw+HIP* were also found effective against Miltefosine resistant-*Ld* (HePC-R) *in vitro* & *in vivo* as evidenced by the concomitant surge in the level of iNOS & limited expression of amastigote specific *Ld*-kDNA in macrophages.

4 Conclusions

We conclude that this novel combinational adjuvant eliminates parasite-induced immunosuppression by re-establishing the proinflammatory host immune responses.

C0302 THE YELLOW PROTEINS AND APYRASE OF PHLEBOTOMUS PAPATASI ARE CANDIDATES OF VACCINE AGAINST CUTANEOUS LEISHMANIASIS.

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1 Background

Several experiments demonstrate that the pre-exposure to saliva of phlebotomes confers protective immunity against leishmaniasis in murine models. Our prvious work in humans indicates that salivary proteins of *phlebotomus papatasi*, vector of *leishmania major*, elicit essentially IL-10-producing CD8+ T lymphocytes. Interestingly, blocking IL-10 enhanced the activation of IFN-g-producing CD+ T lymphocytes of the phenotype Th1. This is a great interest as it provides a new rationale for immunological approaches targeting the salivary components activating a Th1 immune response that could be useful for elaboration of potential vaccine candidates against human leishmaniasis.

2 Methods

Herein, we analyzed in humans the cellular immune response against fractionated salivary extracts of *phlebotomus papatasi* by studying proliferation and IFN-g production of magnetically separated CD4+ and CD8+ T lymphocytes. The significant proliferative response and IFN-g induction were demonstrated in CD4+ T lymphocytes stimulated with >30 KDa salivary fraction.
To test the cellular immune response to the immunogenic salivary proteins with a molecular weight of >30KDa, we usedNucleofection™technique to transfect peripheral blood mononuclear cells (PBMC) from immune donors with plasmids derived from the cDNA library from *P. papatasi* (Tunisian strain) salivary glands.

3 Results

We demonstrated that PBMC transfected with plasmids P36, P42 and P44 encoding respectively “apyrase” PpSP36 and “yellow proteins” PpSP42 and PpSP44, elicit IFN-g-producing CD+ T lymphocytes. Strikingly, yellow protein PpSP44 seems trigger the highest level of IFN-g secretion. Additionally, we used the recombinant form rPpSP44 of this protein to activate PBMC freshly isolated from another set of immune donors and evaluate its effect on proliferative response and IFN-g secretion.

4 Conclusions

Altogether, our results suggest that “yellow protein” PpSP44 constitute a potential vaccine candidate against human leishmaniasis.

C0565 DEVELOPMENT OF GENETICALLY MODIFIED LIVE ATTENUATED LEISHMANIA PARASITES AS POTENTIAL VACCINE CANDIDATE AGAINST VISCERAL LEISHMANIASIS

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1 Background

Currently no effective vaccine is available for potentially fatal visceral leishmaniasis (VL). We have developed a live attenuated *L. donovani* parasite vaccine candidate by deletion of centrin1 gene (*Ld*Cen1$^{-/-}$), which has been found to be safe, immunogenic and protective in animal models. In the present study, to identify the correlates of protection we have evaluated the immune responses generated by *Ld*Cen1$^{-/-}$ in human blood samples obtained from different clinical groups. Further, in order to develop another live attenuated vaccine candidate, we have selected a gene (A1) for deletion, which is highly expressed at the intracellular amastigote stage of the parasite.

2 Methods

Infectivity of *Ld*Cen1$^{-/-}$ was evaluated in human PBMCs differentiated macrophages. Cellular immune responses elicited by parasites was estimated in supernatants of PBMCsinfected with wild type or *Ld*Cen1$^{-/-}$ parasites using multiplex cytokine ELISA in healed VL (HVL, n=15), post kala-azar dermal leishmaniasis (PKDL, n=15), healthy (n=15) and VL (n=7) groups. Cellular source/s of cytokines was determined using flow cytometry. To delete LdA1 gene from *Leishmania* parasite, homologous recombination method was used and studies were carried out to see the effect of deletion on parasite growth, phenotype and infectivity.

3 Results

*Ld*Cen1$^{-/-}$ parasites infected human macrophages, as effectively as the wild type parasites. PBMCs obtained from individuals with a prior exposure to *Leishmania* (HVL and PKDL), strongly stimulated production of pro-inflammatory cytokines including, IL-12, IFN-γ, TNF-α, IL-2, IL-6 and IL-17. However, no significant stimulation was found in anti-inflammatory cytokines (IL-4 and IL-10). Significant increase was also found in IFN-γ secreting CD4+ and CD8+ T cells in PBMCs of HVL, with no increase in IL-10 secreting cells. Further, only single allele of A1 gene could be deleted (LdA1$^{+/-}$) as 2nd allele deleted parasite did not survive. The infectivity of LdA1$^{+/-}$ to macrophages was similar to the wild type and they showed reduction in growth, motility and size as compared to the wild type parasite.

4 Conclusions

*Ld*Cen1$^{-/-}$ has great potential as live vaccine candidates against VL since they elicit strong protective immune response in human PBMCs from HVL, similar to the wild type parasite infection, mimicking a naturally acquired protection following cure. LdA1 gene might play some essential role in *Leishmania* parasite life cycle and its complete deletion is not feasible.

C0643 TYPE I INTERFERON SIGNALLING SUPPRESSES ANTI-PARASITIC CD4+ T CELL RESPONSES DURING VISCERAL LEISHMANIASIS

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1 Background

Many pathogens, including viruses, bacteria, and protozoan parasites, suppress cell mediated immune responses through activation of type I Interferon (IFN-1) signalling. However the role of IFN-1 during *Leishmania donovani* infection causing visceral leishmaniasis (VL) is not well known. Here we report that IFN-1 play an important role in the pathogenesis of VL by impairing parasite clearance and suppressing pro-inflammatory cytokine production.

2 Methods

Mice lacking type-1 IFN signalling (B6.IFNαR1$^{-/-}$ mice) and wild type (WT) C57BL/6 mice were infected intra-venously with 2x10^{7} *L. donovani* amastigotes. Parasite burden was measured day 14, 28 and 56 post infections. To further study the impact of IFN-I on the immune response during leishmania infection, we next analysed serum cytokine levels in naive and infected WT and IFNαR1$^{-/-}$ mice. Intracellular staining and flow cytometry was performed to detect the CD4^{+} derived IFN-γ production. Peripheral blood mononuclear cells (PBMCs) from VL patients (before and after treatment) and endemic healthy controls were also collected to measure mRNA expression of IFN-1 related genes. Whole blood assay was deployed to measure the antigen specific immune response after IFN-1 signalling blockade.

3 Results

B6.IFNαR1$^{-/-}$ mice had enhanced pro-inflammatory cytokine production and better control of parasite burden in liver as well as spleen compared to wild type C57BL/6 mice. IFN-1 signalling suppressed the CD4+ derived IFN-γ production and prevented Th1 response from controlling parasite replication. Studies in VL patients supported these findings and showed enhanced accumulation of mRNA encoding type I IFN signature genes in peripheral blood mononuclear cells (PBMCs) that were reduced following successful drug therapy. Critically, we also showed using a whole blood assay, that blockade of type-1 IFN signalling enhanced antigen specific IFN-γ production, and that this response was HLA-II restricted.

4 Conclusions

Together, these results identify the type-1 IFN signalling pathways as a potential therapeutic target to treat VL by stimulating anti-parasitic CD4^{+} T cell responses.

C0845 EVALUATION OF PROTECTIVE IMMUNITY INDUCED BY LIVE ATTENUATED LEISHMANIA DONOVANI (LDCEN-/-) PARASITES IN PRESENCE OF ASYMPTOMATIC INFECTION

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1 Background

Currently there is no vaccine against visceral leishmaniasis (VL). Towards developing an effective vaccine, we have reported extensively on the immunogenicity of live attenuated LdCentrin$^{-/-}$ mutants in naive animal models. In VL endemic areas, asymptomatic carriers outnumber symptomatic cases of VL (9:1) and are considered to be a reservoir of infection. Vaccination of asymptomatic cases represents a viable strategy to eliminate VL. Immunological correlates of protection thus derived might have limited applicability in conditions where the immunized host has prior exposure to virulent infection.

2 Methods

To examine whether LdCen$^{-/-}$ parasites can induce protective immunity in experimental hosts that have low-level parasitemia from a previous exposure mimicking an asymptomatic condition, we infected C57Bl/6 mice with wild type *Leishmania donovani* parasites expressing LLO epitope (LdWT^{LLO} $10^{3,}$ i.v.). After 3 weeks when the mice had low level of parasitemia, were immunized with LdCen$^{-/-}$ parasites expressing 2W epitope (LdCen$^{-/-2W}$ $3x10^6$ i.v.) to characterize the immune responses in the same host. Antigen experienced CD4$^+$ T cells from the asymptomatic (LdWT^{LLO} infected) LdCen$^{-/-}$ immunized and other control groups were enriched using LLO and 2W specific tetramers followed by Flow cytometric analysis.

3 Results

Our analysis showed that comparable CD4$^+$ memory T cell responses (T central memory) represented by CD62Lhi, CCR7+, and IL-7R+ T cell populations were induced with LdCen$^{-/-}$ in both asymptomatic hosts and hosts that received LdCen$^{-/-}$ immunization alone. Upon restimulation with peptide, these T_{CM} cells differentiated into effector T cells. Upon virulent challenge, comparable reduction in splenic parasite burden was observed in both LdCen$^{-/-}$ and co-infection groups. Further, LdCen$^{-/-}$ immunization resulted in complete clearance of the pre-existing asymptomatic infection (LdWT^{LLO}).

4 Conclusions

Our results demonstrate that LdCen$^{-/-}$ immunization could be efficacious for use in asymptomatic VL individuals. Further, immunization with LdCen$^{-/-}$ could help in reducing the parasite burden in the asymptomatic cases and aid in controlling the VL.

C0964 IMMUNOTHERAPY OF CANINE LEISHMANIASIS BY PHOTODYNAMIC VACCINATION

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1 Background

A novel cell-mediated immunotherapy is being developed according to the *Leishmania* strategy of vaccine delivery (Chang et al., 2016 Parasit Vectors. 9:396) against difficult-to-cure diseases, e. g. canine leishmaniasis in Italy. The current clinical management of this disease there entails prolonged treatments of sick dogs for 30 days with heavy daily dosage of very toxic drugs (antimonials/miltefosine) followed by a daily maintenance dose of allopurinol for life. Still, relapses of the disease are frequent within the first year (Manna et al., 2015 Parasit Vectors. 8: 289).

2 Methods

With institutional IRB-approval and dog owner's consent, a direct observational open label trial was initiated for immunotherapy of 20 diseased dogs, of which 9 were each immunized with frozen photo- inactivated *Leishmania* at 10^7/0.1 ml and 11 similarly immunized after s.c. chemotherapy with meglumine antimoniate at 100 mg/kg/day for 30 days followed by allopurinol at 10 mg/kg/day for another 30 days. All dogs were assessed clinically for disease signs, hemato-biochemical profiles, anti-Leishmania antibodies by IFAT, and parasite loads of lymphnode aspirates by quantitative real-time RT-PCR of Leishmania DNA every 3 months for >3 years.

3 Results

Prognosis was improved for the group with immunotherapy after the initial 30 day-chemotherapy based on clinical scores and parasite loads assessed. The immunotherapy was found to stop relapse of the disease completely when used together with allopurinol and worked better than using allopurinol alone.

4 Conclusions

When applied appropriately, the immunotherapy appears to boost the feeble immunity expected to develop after chemotherapy. Work is on-going to see if it is robust enough to clear the infection completely from immunized dogs, and to enroll additional dogs for both prophylactic and therapeutic trials.

C1050 PHASE I AND II CLINICAL TRIAL FOR COMPARATIVE EVALUATION OF TOXICITY, IMMUNOGENICITY AND POTENCY OF LEISH-TEC®, LEISHMUNE®, KMP-11 AND LBSAP VACCINES AGAINST CANINE VISCERAL LEISHMANIASIS

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1 Background

Human and canine visceral leishmaniasis are highly prevalent in Latin American countries, especially in Brazil. The use of aprophylactic vaccine in dogs is an important control measure for both human and canine infection. Thus, this study aims to evaluate the toxicity, immunogenicity and potency of vaccines prototypes KMP-11 and LBSap, in comparison to the available commercial vaccines in Brazil, Leishmune® and Leish-Tec®, in a phase I and II clinical trial.

2 Methods

We designed a study where 35 dogs were classified into 5 groups: control; Leish-Tec; Leishmune; LBSap; KMP-11. A detailed and comparative analysis of toxicity vaccination was performed between vaccine doses. After complete vaccination protocol (T1) the dogs were challenged by intravenous route with *L. infantum* promastigotes and were followed up for 6 months (T2) where assay of immunogenicity and potency were performed.

3 Results

In the *in vitro* analyzes an increase in lymphoproliferative activity in LBSap and Leishmune was observed, occurring in LBSap an antigen-specific increase of $CD4^+$ and $CD8^+$ T-lymphocytes. The evaluating of the percentage of antigen-specific $CD4^+$ and $CD8^+$ lymphocytes producers of IFN-γ e IL-4 demonstrated an increase in both IFN-γ producing subpopulations in the LBSap, and it also showed an increase in IFN-γ producers in $CD8^+$ lymphocytes in the Leish-Tec. Our results of serological reactivity demonstrate that TRDPP® was able to distinguish among diseased and vaccinated dogs. After experimental challenge the dogs were followed for 6 months and clinical changes suggestive of *Leishmania* infection were observed, such as: apathy, lymphadenopathy and weight loss, in greater numbers in the control. In the parasitological evaluation of bone marrow by qPCR were observed reduction in parasite burden in all vaccines tested compared to control. However, in the group of dogs immunized with LBSap this reduction was more evident, being 47 times smaller.

4 Conclusions

Our results of toxicity indicate that the safety of adverse changes caused by immunizations are tolerable. Our immunogenicity results support the hypothesis of the vaccine process, in all vaccine groups, generating a protective immune response against the parasite and compatible with disease control. If we consider the most consistent immunological and parasitological findings against disease progression, the LBSap would be the most suitable for further research in phase III clinical trial vaccine in relation to KMP-11.

C1094 PROTECTION AGAINST VECTOR-BORNE VISCERAL LEISHMANIASIS BY A NOVEL REFINED SUBUNIT VACCINE

***Malcolm Duthie*[1]**, *Lais Pereira[2], MIchelle Favilla[1], Kimberley A. Hofmeyer[1], Steven J. Reed[1], Sonia Metangmo[2], Shannon Townsend[2], John D. Laurance[1], Alessandro Picone[1], Jeffrey Guderian[1], H. Remy Bailor[1], Aarthy C. Vallur[1], Hong Liang[1], Julie Vergara[1], Fabiano Oliveira[2], Randall F. Howard[1], Shaden Kamhawi[2], Rhea N. Coler[1], Jesus G. Valenzuela[2], Steven G Reed[1]*

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1 Background

Strategies to control visceral leishmaniasis (VL) have centered mostly on improving drug treatment regimens or reducing the sand fly-based transmission of the parasites, both of which have limitations. Patients may experience intolerance, side effects and toxicity during drug treatment, and the emergence of drug-resistant parasites would undermine the achievements made in improving drug delivery. Transient eradication of the sand fly vector is likely to provide only temporary abatement in the transmission cycle. Vaccination represents a sustainable long-term intervention strategy for the elimination of leishmaniases.

2 Methods

We recently advanced a single chimeric di-fusion protein (LEISH-F3; produced by contiguous expression of the NH and SMT genes) through phase I clinical trials. In this report we refined the F3 construct by adding a truncated version of the cysteine protease B (CPB; C) gene to render a tri-fusion protein, designated as LEISH-F3+, with potential for broader antigen recognition in *Leishmania*-affected regions. The truncation involved the targeted removal of amino acid sequences from CPB that were homologous to human sequences. We evaluated the ability of the LEISH-F3+ protein, formulated with the adjuvant GLA-SE, to protect against needle-inoculated *L. donovani* and *L. infantum* infection in mice and in an advanced experimental hamster model in which *L. donovani* parasites were inoculated by sand fly bites.

3 Results

Immune recognition was retained in the truncated form of the C component and its addition to LEISH-F3 did not diminish the protective capacity of immunization. With appropriate adjuvant formulation in GLA-SE, the LEISH-F3+ protein provided robust protection in mouse models of *L. donovani* and *L. infantum* infection. Importantly, immunization with LEISH-F3+/ GLA-SE afforded statistically significant protection against sand fly-borne infection of hamsters.

4 Conclusions

Our data support further evaluation of LEISH-F3+ as a vaccine candidate antigen and indicate the potential for a LEISH-F3+-based vaccine in VL-endemic regions.

Miradero Room

Session 21. Reservoirs

C0266 HIV INFECTION AND HUMAN COMPETENCE AS RESERVOIRS OF LEISHMANIA INFANTUM.

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1 Background

Visceral leishmaniasis (VL) caused by *Leishmania infantum* is a lethal zoonosis transmitted by sand flies. Although dogs are held as the main reservoirs, humans are also sources of infection. Nevertheless control policies are focused on dog culling sick people with VL are infectious and could contribute to disease transmission, especially those HIV-infected. Patients co-infected by HIV and *Leishmania*, in spite of treatment, can develop chronic infections with continuous parasite multiplication for several years. Although it has been shown that protease inhibitors used in HIV infection treatment can reduce in vitro the growth of *Leishmania sp.* it is likely that patients infected with HIV can be long-lasting and important infection sources of *L. infantum.*

2 Methods

Reservoir competence of patients with VL without and with HIV infection as well as of people asymptomatically infected with *Leishmania* was assessed by xenodiagnosis with the vector *Lutzomyia longipalpis*. Parasites were identified through optic microscopy and conventional polymerase chain reaction (PCR). The amount of *L. infantum* in the blood was obtained by quantitative PCR (qPCR).

3 Results

Among the 61 participants, 27 (44%) infected sand flies as seen by microscopy or PCR. Among the VL patients not infected with HIV xenodiagnosis was positive in 5 (25%) and among the patients co-infected by HIV 9 (45%) were positive when infectiousness was assessed by microscopy. Among the 19 asymptomatic patients four (21%) infected sand flies only evidenced by PCR. One (50%) asymptomatic patient with HIV had a positive xenodiagnosis by microscopy. 9/372 (2.4%) and 37/398 (9.2%) sand flies were infected when feeding in patients without and with HIV, respectively.

4 Conclusions

The study demonstrates that asymptomatic humans are capable of transmitting *L. infantum*, that ill persons with HIV infection are more infectious to sand flies, and that humans are more important reservoirs than usually thought. This fact may be considered when designing control policies for zoonotic VL. This study aslo demonstrated that xenodagnosis is better than qPCR alone as an indicator of infectivity.

C0857 SEROPREVALENCE OF LEISHMANIA INFANTUM, TOSCANA VIRUS AND SANDFLY FEVER SICILIAN VIRUS IN KENNELLED DOGS FROM PORTUGAL

Carla Maia[1], *Sulaf Alwassouf*[2], *José Manuel Cristónão*[1], *Nazli Ayhan*[2], *André Pereira*[1], *Herve Richet*[2], *Laurence Bichaud*[2], *Remi Charrel*[2], *Lenea Campino*[1]

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1 Background

Phlebotomine sandfly-borne diseases such as leishmaniases and phleboviruses are emerging threats to animal and public health worldwide. Leishmaniasis caused by *Leishmania infantum* is an endemic zoonosis in Portugal. Dogs are the main reservoir for human visceral infection. Toscana virus (TOSV) is also present in the country where it causes human infection and disease while serological data for Sandfly fever Sicilian virus (SFSV) is limited. The aim of this work was to evaluate the exposure to *L. infantum*, TOSV and SFSV in kennelled dogs from Portugal.

3 Methods

From May 2011 to May 2012, sera collected from 581 kennelled dogs from the Metropolitan area of Lisbon were tested for the presence of antibodies against *Leishmania infantum,* Toscana virus (TOSV) and Sandfly fever Sicilian virus (SFSV). Data on the gender, age, breed, fur, use of insecticides and clinical signs compatible with leishmaniasis were recorded.

4 Results

Seventy six (13.1%) dogs were seropositive for *L. infantum*, 327 (56.3%) for SFSV and 36 (6.2%) for TOSV. Six dogs were co-infected with *L. infantum* and TOSV, 51 with *L. infantum* and SFSV and 25 with TOSV and SFSV. One dog had antibodies to the three pathogens. *L. infantum* seroprevalence was significantly higher in pure breed dogs than in mongrel and in dogs with clinical signs while SFSV prevalence was significantly higher in males, in pure and crossed-breed dogs than in mongrel and in those not treated with insecticides. The seroprevalence of both viruses was significantly higher in dogs older than 7 years than in the ones with 1 to 7 years. A positive association was observed between the presence of antibodies to *L. infantum* and SFSV.

5 Conclusions

The obtained seroprevalence of *L. infantum* in kenneled dogs revealed that the parasite is still a serious concern for animal and public health while the detection of TOSV and SFSV antibodies indicates that both viruses are circulating in Portugal. The presence of antibodies to sandfly-borne pathogens in dogs, some of them of proven zoonotic concern, reinforces the need to implement efficient prophylactic measures to prevent infection and disease among vertebrate hosts including humans. In addition, further studies should evaluate if dogs might play a role in the natural cycle of TOSV and SFSV.

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C1038 LEISHMANIA INFANTUM-SPECIFIC PRODUCTION OF IFN-? IN STIMULATED BLOOD FROM OUTDOOR CATS IN ENDEMIC AREAS

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1 Background

Feline leishmaniosis (FeL) caused by *Leishmania infantum (Li)* is considered a rare disease in endemic areas whereas subclinical infections are common. Immune response plays an important role in driving the course of *Li* infection in humans and animals, however, feline immune response to *Li* infection has not yet been investigated. The aim of this study was to determine *Li* specific cellular immune response by means of the evaluation of interferon-g (IFN-g) production in stimulated blood from outdoor cats living in endemic areas (Sicily and Catalonia) and to compare it with clinical status, anti-*Li* antibody levels and retroviral infections.

2 Methods

102 cats were evaluated based on physical examination, *Li*-specific antibody level detection (quantitative ELISA) and FIV and FeLV infections. Heparin whole blood was stimulated with *Li* soluble antigen (LSA) and concanavalin A (ConA) and IFN-γ concentrations were measured by sandwich ELISA in supernatants obtained after 5 days of incubation. Statistical analysis was performed using Fisher's Exact Test, Kruskal-Wallis Test, Dunn's Multiple Comparisons Test, Mann-Whitney test and Wilcoxon Test.

3 Results

According to physical examination and retroviral testing, 63 cats were considered clinically healthy and 39 sick (17/39 with clinical signs compatible with FeL). Most of cats (n = 96) were *Li* antibody negative and six were positive (5.8%). Fourteen out of 100 cats (14%) had a retroviral infection (10=FIV; 4= FeLV). Twenty-five cats (24%) produced IFN-g after stimulation with LSA (LSA-IFNg) with a median of 214,2 pg/mL, while the majority of cats (95%) produced IFN-g after stimulation with ConA (ConA-IFNg) with a median of 2199 pg/mL. Values of IFN-g were significantly higher ($P < 0.0001$) after stimulation with Con A than with LSA. One out of 6 cats with positive serology produced IFN-g after stimulation with LSA. Moreover, LSA-IFNg producing cats had a significantly lower ($P=0.0148$) antibody level (median: 3,48 ELISA units (EU)) compared to LSA-IFN-g non-producers (median: 5,44 EU). Antibody level was however not correlated with IFN-g production. No significant difference was found in IFN-g production according to clinical status or retroviral infections.

4 Conclusions

As expected cats living in *Li* endemic areas are able to mount a *L. infantum* specific cell mediated immune response as previously described in other mammalian hosts.

C1183 CANINE VISCERAL LEISHMANIASIS: VALIDATION OF A CLINICAL RECORD BASED ON THE RESPONSE MODEL TO THE ITEM RESPONSE THEORY

Bruna Martins Macedo Leite[1], Manuela da Silva Solcá[1], Luciano Vasconcellos Pacheco[1], Leila Andrade Bastos[1], Lilia Carolina Carneiro da Costa[2], Leila Denise Alves Ferreira Amorim[2], Patrícia Sampaio Tavares Veras[1], ***Deborah Bittencourt Mothé Fraga[1]***

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[2]Instituto de Matem_tica, Departamento de Estat_stica, Universidade Federal da Bahia, Salvador Brazil

1 Background

The dog is the main urban reservoir of *Leishmania infantum* and may develop severe disease or present a subclinical form of canine visceral leishmaniasis (CVL). Dogs that develop disease symptoms may exhibit a broad spectrum of clinical signs varying in severity, making clinical diagnosis a challenging and complex task. Thus, our objective was to evaluate the validity of a standardized clinical record, containing clinical signs specific to CVL that employed a graded scoring method of intensity.

2 Methods

A cross-sectional study was performed in a sample of the canine population of an endemic area in Brazil. Clinical evaluations were performed, and each of the signs was recorded in the standardized clinical record. Dog was considered positive when tested positive in DPP®CVL, EIE®CVL, culture or qPCR. Logistic regression analysis was performed to evaluate the association between clinical signs and laboratory diagnosis of CVL. Each item of the canine clinical record was subjected to item response theory (IRT) analysis to evaluate the accuracy of the grades assigned.

3 Results

The prevalence of Leishmania infection was 38%. Among these, 94% presented at least one clinical sign. Three clinical signs (nasal hyperkeratosis, onychogryphosis and ear ulceration) were considered as significantly associated with laboratory detection of Leishmania infection in dogs ($p \leq 0.05$). Item response curves for each clinical sign on the clinical record was calculated using IRT analysis, distinguishing whether a certain clinical sign would be present in graded or binary form. Accordingly, ear crusting, lymphadenopathy, nasal hyperkeratosis, alopecia, nose depigmentation and onychogryphosis presented graded distributions with respect to clinical sign intensity, while furfuracea dermatitis, periocular dermatitis and ear ulceration exhibited binary distributions. Two clinical scores were calculated, one (complete) considering all of the items in the clinical record and another (simplified) considering only the three clinical signs associated with the outcome of CVL under multivariate logistic regression. The complete score showed better accuracy than the simplified one.

4 Conclusions

The results validated the standardized clinical record. The authors hope that the use of a standardized clinical record will aid researchers working with CVL to more accurately compare reported data, resulting in more homogenous datasets published in the literature, which would facilitate additional analysis.

C1419 EVALUATION OF THE TRANSMISSIBILITY AND INFECTION OF LEISHMANIA (VIANNIA) BRAZILIENSIS TO LUTZOMYIA LONGIPALPIS IN WILD AND SYNANTHROPIC RODENTS NATURALLY INFECTED IN ENDEMIC AREA OF NORTHEAST BRAZIL.

José Ferreira Marinho Jr[1], Juliana Figueiredo Costa Lima[1], Francisco Gomes Carvalho[1], Milena Paiva Cavalcanti[1], Jeffrey Jon Shaw[2], Orin Courtenay[3], ***Sinval Pinto Brandão Filho[4]***

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[4] Recife Brazil

1 Background

American Cutaneous Leishmaniasis (ACL) is a zoonosis considered a major public health problem due to its epidemiological complexity, where they participate in transmission cycles, various etiological agents, vectors and hosts. In the Northeast the predominant species involved with the American Cutaneous Leishmaniasis (ACL) is *Leishmania* (*Viannia*) *braziliensis*. In Pernambuco, in the Northeast of Brazil, 60% of the LTA cases are concentrated in the Zona da Mata region. Amaraji, a municipality located in this region, has presented an important incidence of LTA cases in recent years. This study aimed to evaluete transmissibility of *Leishmania (Viannia)* spp. infection to *Lutzomyia longipalpis* through xenodiagnosis in naturally infected wild and synanthropic rodents by real time PCR - target kDNA *Leishmania (Viannia)* spp.

2 Methods

Animals previously identified by capture-recapture, naturally infected by *Leishmania (Viannia)* spp., were submitted to xenodiagnosis.Xenodiagnoses were performed with *L. longipalpis* females coming from the laboratory of CPqAM / Fiocruz. Rodents were anesthetized and placed in cages with *L. longipalpis* by 1 hour. After feeding of sand flies, they were separated individually placed in a tube containing 70% ethanol for later DNA extraction and molecular test by real time PCR. Phlebotomine DNA were obtained on extraction using 5% Chelex solution.

3 Results

Five xenodiagnoses were performed (three with *Nectomys squamipes* and two *Rattus rattus*), using 407 females of *L. longipalpis*. Through real time PCR 61.43% (250/407) were positive.

4 Conclusions

The results of the phlebotomine *Lutzomyia longipalpis* species reinforce the permissive of this vector to the infection and transmission of *Leishmania (Viannia) braziliensis*.

C1482 FIELD EVALUATION OF RODENT BAIT TREATED WITH FIPRONIL FOR FEED THROUGH AND SYSTEMIC CONTROL OF PHLEBOTOMUS PAPATASI.

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1 Background

The sand fly *Phlebotomus papatasi* is the main vector of *Leishmania major*, etiologic agent of zoonotic cutaneous leishmaniasis (ZCL), which is endemic in North Africa, the Middle East, and Asia. In North Africa, *Meriones shawi* is one the main reservoir host of *L. major*. *Phlebotomus papatasi* populations are maintained in borrowing rodents such as *M. shawi*.

2 Methods

Fipronil-treated rodent baits were evaluated for systemic and feed through insecticidal activity against *P. papatasi* feeding on *M. shawi*.

3 Results

In the field, application of treated bait resulted in 80.0% reduction in the populations of *P. papatasi* up to 6 weeks after a single application of fipronil at a concentration of 0.005%.

4 Conclusions

This is the first study to demonstrate field efficacy of fipronil-treated rodent baits for *P. papatasi* control and the first study to evaluate this approach in *M. shawi,* a principal ZCL reservoir host. These results suggest that fipronil-treated rodent baits can be used to effectively reduce the populations of *P. papatasi* associated with *M. shawi* in ZCL endemic areas.

C1483 EPIDEMIOLOGY OF LEISHMANIA INFANTUM INFECTION IN WILD LAGOMORPHS IN ITALY

Stefania Zanet[1], *Mario Chiari*[2], *Elena Battisti*[1], *Paolo Tizzani*[1], *Anna Trisciuoglio*[1], *Lorenzo Taricco Carnaroglio*[1], *Antonio Lavazza*[2], *Ezio Ferroglio Ferroglio*[1]

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1 Background

The epidemiology of *L. infantum* in the Mediterranean basin has been altered due to profound changes in the ecology of phlebotomine sandfly and mammalian hosts. Wild lagomorphs have been recognized as sylvatic reservoir *Leishmania infantum.* In Italy native and introduced lagomorphs species are widespread and numerically relevant.

2 Methods

We tested by PCR, the spleen of 222 lagomorphs (n=10 wild rabbits *Oryctolagus cuniculus*, n=108 hare *Lepus europaeus* and n=104 Eastern cottontails *Sylvilagus floridanus*). The animals were culled during regular hunting activities between 2008 and 2014 and come from 10 different regions of Italy. A specific PCR protocol was used to a amplify a 145bp fragment of the kinetoplastid DNA of *L. infantum*. All positive amplicons were sequenced to confirm species identification.

3 Results

A total of 51 animals tested positive by PCR with an overall prevalence (P) of infection of 22.97 % (CI 95% 17.93-28.94). The highest prevalence was recorded in rabbits: P= 30% (CI95% 10.78%-60.32%) followed by *S. floridanus* with 28 of 104 animals infected and a prevalence of 26.92% (CI95% 19.33%-36.16%). The lowest prevalence was recorded in brown hare (P=18.52%; CI95% 12.32%-26.88%) with 20 infected animals out of 108.

4 Conclusions

L. infantum in widely present in lagomorphs, with prevalences similar to those of dogs and othercarnivores from the same study area. Despite not being statistically significant, the higher prevalence recorded in rabbits and cottontails can be attributed to the more limited home-range of these two species compared to *L. europaeus* which can favor the spread of infection as it better copes with the behavioral attitude of phlebotomine sandflies vectors. The role of lagomorphs as possible reservoir of infection needs to be accounted for, in prevention and control programs of zoonotic visceral leishmaniasis.

C1488 WHY VETERINARY EXPERTS HAVE RECOMMENDED THE USE OF ANTIMONIALS IN CANINE LEISHMANIASIS? THE CHRONOLOGY OF A DISASTER.

Lamothe Jacques

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1 Background

The VetLeish group has made recommendations concerning CL therapy. Glucantime® and Miltefosine are majour element of CL therapy in all their recommendations even if the use of the similar drugs in VL and in CanL had not been recommended by public healthe experts.

2 Methods

These recommendations are bases on two facts The superiority of the associaltion antimonial alloprurinol and the fact that the risk of transmitting resistant stains from reservoirs should not be an establihed scientific feature.

3 Results

An evidence-based review provided evidence for recommending the use of combination treatment but insufficient evidence for recommending the use of allopurinol alone.(Noli and Auxilia 2005). The problem is that this study is wrong due to both methodological and errors in the interpretation of study results. Its conclusions are based from a single publication with low level of evidence and whose results were contradictory with other study with a better level of evidence. Misinterpretations of other results studies were also found in this study.

The VetLeish group indicates in its princeps paper that the spreading theory (the transmission of resistant strains from a reservoir) is not valid , mentioning an inadequate reference. (Solano Gallego et al.2009)

4 Conclusions

It is importanty to open a discussion about these two questions .
Is there any evidence for the use of Glucantime® in CanL therapy ?
As vets is it ethic to ignore public health recommendations ?

C1560 DETECTION OF LEISHMANIA INFANTUM INFECTION IN RODENTS SPECIES OF CENTRAL MOROCCO.

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1 Background

In Morocco, *Leishmania infantum* cause cutaneous and visceral leishmaniasis. It is responsible for widespread visceral leishmaniasis in the North-Eastern slope of the Rif Mountains, the most active area of this form of the disease; but also for cutaneous leishmaniasis (CL) whichoccurs sporadically in the north of Morocco (Rioux et al., 1996; Rhajaoui et al., 2007; 2011).
L. infantum inMorocco is transmitted by *P. ariasi* as the main vector; *and P. perniciosus* and *P. longicuspis* suspected vectors (Guessous-Idrissi et al., 1997). The dog is considered as the main domestic reservoir of *L. infantum* in Morocco as well as in Mediterranean basin (Nejjar et al., 1998; Rhajaoui et al., 2011; Kahim et al., 2014).
However, L. infantum has been detected in several rodents species, which might serve as reservoir hosts, e.g. in Portugal (Helhazar et al., 2013), Italy (Pozio et al., 1981; Gradoni et al., 1983; Di Bella et al., 2003), Greece (Papadogiannakis et al., 2010), Saudi Arabia (Ibrahim et al., 1992), Spain (Morillas-Márquez et al., 1985; Portús et al., 2002; Navea-Pérez et al., 2015). With the aim to detect other potential reservoir of *L. infantum* in Morocco, eco-epidemiological study was carried out in central Morocco.

2 Methods

A total of 75 rodents were captured, using Sharman traps, from many localities of El Haouz province (44 specimens) and Essaouira province (31 specimens). Species identification was carried out morphologically according to Aulagnier and Thevenot (1986). Samples of skin (ear lobe, skin lesion) were harvested for parasite detection by PCR assays. To identify the *Leishmania* species, sequence of the internal transcribed spacer (ITS-1) products was analyzed

3 Results

For rodent species, 26 *R. rattus* and 18 *M. musculus* were collected in El Haouz province (58, 66%), while *R. rattus*, 11 *M. musculus* and 3 *R. norvegicus* were collected in Essaouira province (41, 33%). For parasite detection, three *R. rattus* and six *M. musculus* from El Haouz; and three *R. rattus*, two *M. musculus* and one *R. norvegicus* from Essaouira were affected by *L. infantum*.

4 Conclusions

We identified *L. infantum* in *M. musculus*, *R. rattus*, and *R. norvegicus* for the first time in Morocco. This cosmopolitan rodents live in close relationship with human dwellings, especially those in more precarious conditions, may have important implications in the transmission cycles of *L. infantum* in Morocco.

Greco Room D1-D2

Session 22. Early drug discovery. Animals models and genetic susceptibility. Drug resistance mechanism

C0132 NEW PARADIGMS FOR VISCERAL LEISHMANIASIS HIT DISCOVERY: HOW TO FIND NEW AND HIGH-QUALITY CHEMICAL STARTING POINTS FOR DRUG DISCOVERY.

Manu De Rycker

University of Dundee, on behalf of the DDU / GSK Kinetoplastid Drug Discovery Team UK

1 Background

One of the key challenges for visceral leishmaniasis (VL) drug discovery is finding new chemical starting points. Multiple approaches are currently in use, including target-based screening, cell-based screening in axenic systems and cell-based screening against intracellular amastigotes. The main issues are poor translation between the various assays and low hit rates against intracellular amastigotes. Here, we detail the development of a collection of new assays that, when introduced into a screening cascade, address many of these problems.

2 Methods

Several new screening assays for VL were developed, including assays that report cidal activity against promastigotes as well as amastigotes, an assay to measure rate-of-kill against intracellular amastigotes and a clinical isolates strain panel assay. Work to develop a higher sensitivity high-throughput screening assay was also carried out. In addition, we have established a cell-based reporter assay for protein turnover and explored alternative hit-finding strategies, such as pathogen hopping.

3 Results

Compound sets were screened in the various new assays to understand the respective assays' hit rates and sensitivity as well as the translation between the assays. Our results show that many factors affect the pool of hits identified, including life-stage, starting cell density, parasite replication rate and pH of the media. We also confirm previously demonstrated effects of host cell line and parasite strain on sensitivity in intracellular amastigote assays, and discuss how these can be exploited for hit discovery. In addition, we show that a pathogen hopping approach, using other members of the *Trypanosoma* genus as primary screening platform, is a valid approach to identify new hits for VL. Results from our protein turnover reporter assay demonstrate the usefulness of this type of assay to quickly identify phenotypic hits acting on this pathway.

4 Conclusions

Our work describes how new assays, screening cascades and hit-finding approaches can address the low hit-rate of current VL hit discovery approaches. VL drug discovery is seriously hampered by the paucity of good new chemical starting points and the work presented here shows promise to address this.

C0248 OPEN LAB AT GSK: EARLY DISCOVERY IN PARTNERSHIP TO COMBAT VISCERAL LEISHMANIASIS

Julio Martin Plaza

GSK Tres Cantos Spain

1 Background

Leishmaniasis is a poverty-associated disease that affects people across Africa, the Middle East, South America, and Asia. 200,000- 400,000 new cases each year and approximately 30,000 deaths are reported for Visceral Leishmaniasis (VL). An estimated 20 million individuals are infected with kinetoplastid pathogens, such as *Leishmania spp.* and *Trypanosoma spp*., resulting in extensive suffering and around 95,000 deaths per year. Of the 850 new therapeutic products registered in 2000–2011, only 5 (0.6%) were indicated for Neglected Tropical Diseases (NTDs), none of them being a new chemical entity (NCE) or vaccine. VL is included in the 2012 London Declaration signed by private and public partners to combat 10 NTDs by 2020 through coordinated action aimed at new World Health Organization goals. Partners pledge innovative, coordinated action aimed at new WHO goals. Open Innovation and partnership must be at the helm.

2 Methods

Not Applicable

3 Results

GSK is committed to global health and to discover innovative medicines that combat diseases of the development world (DDW). We do not seek commercial return, but access to medicine, and we pledge work hand-in-hand with public and private partners. The Open Innovation operational research at DDW lays on three pillars: Open Lab (providing access to our know-how and infrastructure), Open Source (sharing our data and assets with the worldwide research community) and Patent Pool (being more flexible with our Intellectual Property).

4 Conclusions

We will describe the portfolio of collaborative projects on VL and how we are leveraging Open Innovation and Open Lab in order to build a portfolio of assets, as well as unveiling new targets, mechanisms of action and chemical biology knowledge. The collaborative network which is emerging thanks to researchers and institutions is the pillar of this initiative. Expectation is that there will be synergies amongst partners, which otherwise had not happened. Ultimately, we envisage to contribute to the research community with new therapeutic targets and chemical tools which may become the source of future drug discovery programs.

C0253 DIPEPTIDYLCARBOXYPEPTIDASE INHIBITORS: NOVEL ANTILEISHMANIAL AGENTS FOR VISCERAL LEISHMANIALSIS

Neena Goyal

Lucknow India

1 Background

Visceral leishmaniasis (VL) is a neglected tropical disease. The lack of affordable therapy has necessitated the urgent development of new drugs that are efficacious, safe, and easily accessible to patients. With an aimed to identify new chemical entities, we focused on inhibitors of *Leishmania donovani* dipeptidylcarboxypeptidase (LdDCP), a novel drug target.

2 Methods

In present study, we evaluated in vivo antileishmanial efficacy of compounds belonging to (2Z,2¢Z)-3,3¢-(ethane-1,2-diylbis(azanediyl))bis(1-(4-halophenyl)-6-hydroxyhex-2-en-1-one) chemical series in golden hamsters, cronic visceral leishmaniasis model.

3 Results

Out of ten compounds, the Compound 1c exhibited excellent *in vivo* activity (>90%) against high grade of infection in hamster model. A nanoformulation of the lead compound (C1c-Dx-NP) was prepared that gave five folds dose benefit by intraperitoneal route and two folds benefit by oral route as compared to bulk compound.

4 Conclusions

These studies led to the identification of compound 1c as a preclinical candidate for further drug development as an oral treatment for VL

C0486 PRODRUGS FOR CUTANEOUS AND VISCERAL LEISHMANIASIS: STRUCTURE ACTIVITY RELATIONSHIP OF NITROHETEROCYCLIC COMPOUNDS ACTIVATED BY TYPE I NITROREDUCTASE

Juliana da Silva Pacheco[1], *Valter Viana Andrade Neto*[1], *Edézio Ferreira Cunha-Júnior*[1], *Debora Souza Santos Costa*[2], *Julio Cesar Ferreira Barcellos*[1], *Paulo Roberto Ribeiro Costa*[1], *Ayres Guimarães Dias*[2], *Eduardo Caio Torres Santos*[1]

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[2]*UERJ Brazil*

1 Background

Leishmaniasis treatment shows difficulties such as high cost, toxicity and resistance cases. The potential of nitro compounds in the treatment of these diseases has been rediscovered due to the knowledge of a class of enzymes known as nitroreductases (NTR) that catalyzes its reduction, enabling them, to be effective and generating more potent intermediates in parasite interior. In this present study, we evaluated the leishmanicidal activity of nitroheterocyclic compounds and their activation by *Leishmania infantum* type I NTR.

2 Methods

Promastigotes of *L. amazonensis* and *L. infantum* were incubated with fourteen nitroheterocyclic compounds up to 100 µM for 72 hours, viability was measured with resazurin to determine half of maximal inhibitory concentration (IC_{50}). Peritoneal murine macrophages were used to determine lethal dose (LD_{50}) and to antiamastigote activity, for both parasites species with the six most active compounds. ROS levels were measured fluorimetrically using H?DCFDA dye up to a 4 h kinetic. The NTR I gene of *L. infantum* was amplified, cloned and subcloned to obtain transfected promastigotes overexpressing the NTR I gene and cultured with different concentrations of nitroheterocyclic.

3 Results

Five of the compounds were able to inhibiting both species proliferation, with the highest effect on *L. amazonesis* growth. Among them, LQB 484 and LQB303 showed IC_{50} less than 12 µM for both *Leishmania* species. LQB 484, LQB303 and LQB304, showed to be the most effective in decreasing the infection of macrophages. We also evaluated the macrophage toxicity and the LD_{50} values were higher than 50 µM for LQB303 and LQB 484, indicating a selectivity index more than 20-fold. We only observed a significant increase in ROS production for LQB303AL. Also, promastigotes overexpressing the NTR I gene indicated that most of the compounds were capable of undergoing nitro reductions, highlighting the LQB303, about 11-fold more potent on overexpressing strain.

4 Conclusions

These results allow better understand the structural modifications made in the series of compounds LQB suggesting that the reduction of the nitro group in position 5 of the ring leads to activation of these compounds and some modifications in molecular structure, such as replacing thiophene by furan ring,makes them more sensitive compounds to enzymatic reduction and promising agents for the treatment of visceral and tegumentar leishmaniasis.

C0973 AN EXPLORATION OF THE PATHOGEN BOX COMPOUND COLLECTION AS ANTI-LEISHMANIAL LEADS

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1 Background

There is a pressing need to search for novel chemotypes to seed the drug discovery pipeline for parasitic diseases. To prime antiparasitic drug development, the Medicines for Malaria Venture has assembled a collection of 400 diverse drug-like compounds termed the Pathogen Box. Selected compounds are active in phenotypic screens against mycobacterial and parasitic diseases, including trypanosomatids. We used this collection to screen for drugs active against *Leishmania amazonensis*. To our knowledge, this study represents the first screen on this collection against any *Leishmania* parasites.

2 Methods

A modified intramacrophage alamar-blue-based assay was validated using gold standard antileishmanial drugs and was subsequently employed to identify the most potent-cytocidal compounds within the Pathogen Box against intracellular amastigotes of *Leishmania amazonensis*.

3 Results

Exploiting the utility of the alamar-blue assay, a simple colorimetric method to identify, and exclude, apparently less potent cytostatic compounds, 400 drug-like compounds from the Pathogen Box were subjected to an initial single-dose/single-time point triage assay with a 1μM concentration of drug for 72hrs. Of the 400 compounds, 21 compounds were prioritized for dose–response testing against axenic amastigotes. Following modification of a published intramacrophage alamar-blue-based assay, the potencies of these compounds were confirmed against intracellular amastigotes. Our best compounds within the Pathogen Box selected by MMV as antimycobacterial and antimalarial drugs showed cytocidal potency at nanomolar range against *L. amazonesis*, thus representing potential Target Product Profile for cutaneous leishmaniasis – a key hit selection criteria set by the DNDi. In addition, using LC–MS based metabolomics coupled with killing dynamics studies for selected compounds, here we report further characterization of our best hits against *L. amazonesis*.

4 Conclusions

The modified intramacrophage alamar-blue-based assay provides a simple and robust assay format for the determination of cytocidal potency of antileishmanial drugs. This work further highlights the potential of our best hits in the development of lead compounds against cutaneous leishmaniasis.

C1341 DRUG SEARCH FOR LEISHMANIASIS: A COMPUTATIONAL BIOLOGY APPROACH FOR HITS SELECTION.

Carlos Muskus Lopez[1], *Sara Robledo Restrepo*[1], *Carol Vanesa*[1], *Andres Florez*[2], *Stanley Watowich*[3], *Rodrigo Ochoa Deossa*[1]

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[3]*University of Texas Medical Branch-UTMB USA*

1 Background

The therapy for leishmaniasis has been practically invariant since 60 years and every day the panorama seems dark, due to increase drug resistance, cost and availability of the treatments and the mild and severe side effects associated with the treatment. In spite of some effort, there is not yet a very promising drug. Due to this, a project aimed to identify hits candidates was conducted at the PECET Lab by using massive virtual screening in collaboration with the World Community Grid (WCG) from IBM.

2 Methods

The structures of 53 *Leishmania spp*. proteins were downloaded from the Protein Data Bank (PDB) database and filtered to retain structures with resolutions higher than 3.0 A°, identified binding sites, and other metrics determined by the experimental technique (X-ray diffraction, NMR). Redundant molecules were excluded if the same protein structure was determined in different crystal forms or by different research groups. These proteins along with 10 different modelled structure (583 total structures) were docked against a compound library filtered based on drug properties from the ZINC database, using AutoDock Vina. Six of these compounds were evaluated *in vitro* using different human macrophage cell lines and GFP *Leishmania* parasites.

3 Results

Approximately 1000 compounds out of the almost 600.000 were detected by docking with good scores based on approximation of the free energy of binding. Interestingly, many of the docking hits were detected mainly against three proteins: The UDP-glucose pyrophosphorylase, dihydroorotate dehydrogenase (DHODH) and tyrosyl-tRNA synthetase. *In vitro* testing of six of the best compounds detected by docking against the DHODH, showed that three had acceptable activity against *Leishmania* parasites, and one had a strong activity to kill the parasites without affecting the human-derived cell lines.

4 Conclusions

It was possible to identify after a massive in silico drug search initiative a set of compounds with potential activity against *Leishmania*. After an initial *in vitro* screen of eight of the compounds, it was possible to detect a promising hit and other additional three hits with moderate activity. However, these compounds might be susceptible of optimization to increase their activity. In addition, new compounds from the in silico search can be also evaluated *in vitro*, and the hits with good selectivity index should be evaluated *in vivo*.

C1345 SEARCHING FOR LEISHMANICIDAL AGENTS: PHOSPHODIESTERASES AS POTENTIAL DRUG TARGETS FOR ANTI-LEISHMANIA THERAPY

***Carmen Gil*[1]**, *Victor Sebastián[1], Titilola Kalejaiye[2], Julianna Siciliano de Araújo[3], Marcos Meuser Batista[3], Nuria E. Campillo[1], Ana Martinez[1], Nazaré Correia Soerio[3], Rob Leurs[4], Harry de Koning[2], Louis Maes[3]*

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[4]Division of Medicinal Chemistry, Vrije Universiteit Amsterdam The Netherlands

1 Background

The available treatments for leishmaniasis are less than optimal due to limitations such as the toxicity and/or the emergence of resistant strains. Discovery and development of novel effective and lower toxic drug candidates are still required. Cyclic nucleotide specific phosphodiesterases (PDEs) of *L. major* have been postulated as potential drug targets [*BMC Microbiology* 2006, 6:25]. The structural similarity between human and protozoan PDEs together with our expertise in the human field with cAMP-specific PDEs inhibitors, led us to select human PDE inhibitors as pharmacological tools to validate these leishmanial targets.

2 Methods

Compounds were evaluated *in vitro* on *L. infantum* intracelullar amastigotes and for metabolic stability using mouse liver microsomes. Induction of elevated levels of cAMP was used as an indirect way to measure PDE inhibition. The efficacy of two 'hits' was tested in the Balb/c mouse *L. infantum* model after oral treatment at 50 mg/Kg b.i.d. for 5 days and evaluation of the parasite burdens in the target organs liver, spleen en bone-marrow.

3 Results

The two 'hits' that were chemically related to human PDE inhibitors showed potent and selective *in vitro* activity against *L. infantum* intracellular amastigotes. In addition, both were metabolically stable and moderately reduced tissue burdens after oral treatment. Increased cAMP levels were measured in *L. infantum* promastigotes upon incubation with the PDE inhibitors.

4 Conclusions

One of the 'hits' is able to kill *Leishmania* by increasing levels of cAMP and hence deserves further investigations on SAR and PK-PD.

This work was supported by the EC 7th Framework Programme FP7-HEALTH-2013-INNOVATION-1, PDE4NPD (no. 602666).

C1400 IN SILICO IDENTIFICATION CONFIRMED BY BIOCHEMICAL AND BIOLOGICAL VALIDATION OF A SERIES OF ANTI-LEISHMANIA MOLECULES

Emna Harigua Souiai[1], *Yosser Zina Abdelkrim*[1], *Imen Bassoumi-Jamoussi*[1], *Ons Zakraoui*[1], *Guillaume Bouvier*[2], *Khadija Essafi-Benkhadir*[1], *Josette Banroque*[3], *Nathan Desdouits*[2], *Helene Munier-Lehmann*[2], *Mourad Barhoumi*[1], *Kyle N. Tanner*[3], *Michael Nilges*[2], *Arnaud Blondel*[2], *Ikram Guizani*[1]

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1 Background

Leishmaniases are neglected parasitic diseases in spite of the major burden they inflict on public health. The identification of novel drugs and targets constitutes a research priority. For that purpose we used *Leishmania* initiation factor 4A (LeIF), an important translation initiation factor as a potential drug target.

2 Methods

Modeller was used to generate two conformation models of LeIF. Both structtures were validated and analyzed through molecular dynamic simulations. Resulting trajectories permitted relevant pockets identification. Selected pockets were targeted through a virtual screening of a diverse chemical library. A selection protocol was developed to identify potential inhibitors of LeIF. Selected molecules were tested on the ATPase activity of LeIF and eIF4AMus using a colorimetric assay. Validated inhibitor were used as a bait to search for chemical analogs and Tanimoto distance was used to quantify chemical similarity. Promastigote viability and cytotoxicity of each studied compound were determined by MTT assay. PMA-differentiated THP-1 derived macrophages were used for testing the susceptibility of intracellular amastigotes. Light microscopy was used to assess the effect of compounds in intracellular L.infantum growth inhibition.

3 Results

A model of the substrate-free form of LeIF was validated and two potential binding sites could be identified. Virtual screening targeting both pockets was performed and 305 molecules were selected. We tested these molecules for their effects on the ATPase activity of LeIF and its mammalian homolog. Four samples caused inhibition of this activity. Detailed kinetic study could be performed on the most potent compound (**C208**), having a half maximal inhibitory concentration of 150 µM for 1 µM of protein. Chemical screens for analogs led to the identification of 36 analogous molecules, out of which two could demonstrate inhibition of LeIF ATPase activity. In vitro effects of C208 and its analogs on *L.infantum* parasites reduced parasite viability in a dose-dependent manner. The concentration that causes 50% reduction in survival compared to the control was estimated to 5 µM for amastigotes and 3 µM for promastigotes. Cytotoxicity assay of C208 and its analogs on PMA-differentiated THP-1 cells showed negligible effects.

4 Conclusions

Compound **C208**and its analogs constitute a novel route for anti-*Leishmania* drug design. Further work is ongoing to understand the mode of binding and interaction between LeIF and its inhibitors.

C1372 THE COMMITMENT TO DEVELOP DRUGS FOR LEISHMANIASIS IN COLOMBIA

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1 Background

Drug development process requires a comprehensive strategy that may contribute in cost reduction, shorten deadlines, and increase the likelihood that a candidate will be successful. That is why in the last decade the PECET has resolutely undertaken to work on all fronts that lead to obtaining of a drug or therapeutic alternative for leishmaniasis. Here we present the strategy that has been used and the progress made so far in this process.

2 Methods

The strategy was based on the creation of public-private partnership. From the technical point of view, the key points were: i) identification of the optimal bioassays for biological activities. ii) identification of Hit and Lead compounds, iii) identifying the "pharmacophore" and improvement of interactions with the target. iv) determination of toxicological profile, and therapeutic response in the cutaneous and visceral leishmaniasis experimental model. v) clinical studies of pharmacodynamics/pharmacokinetic, safety and efficacy of drug candidates. From the logistic point of view, key points have been i) identification of the target population, intellectual property, protection of development, among others. ii) identifying the attributes of the product. iii) defining the market and related issues according to the countries where drugs will be commercialized and iv) manufacturing process.

3 Results

In 2009 was born CIDEPRO, an alliance between local universities and pharmaceutical industries, with the purpose of combine efforts to eliminate the barrier of knowledge fragmentation and dispersed resources. Although many collaborative works have been done, working from discovery to the clinical trial phases, some of products with advanced studies are: a topical formulation based onof a mixture of calendula, yanten and tara (Alyeyuba®) which is being evaluated in phase I. A nanoformulation of hypericin for photodynamic therapy and an oral lipidic nanoformulation of an halomethylated quaternary ammonium salt; both formulations have already completed the preclinical studies. And finally, there is also a topical formulation of a mixture of natural sapogenin plus two synthetic hizdrazones that is in preclinical phase of evaluation.

4 Conclusions

The challenge of developing a drug that may serve as an alternative for the treatment of cutaneous leishmaniasis has been a succesfull experience that has allowed the implementation of high quality methodologies and infrastructure.

Miradero Room

Session 23. Control programs. National and international funding organizations

C0288 ADDRESSING THE EMERGENCE AND SPREAD OF LEISHMANIASES IN THE BORDERS OF ARGENTINA, BRAZIL AND PARAGUAY + URUGUAY + BOLIVIA

Oscar Daniel Salomon[1], *Vanete Thomaz Soccol*[2], *Nilsa Gonzalez Britez*[3], *Gabriela Willat*[4], *Lineth Garcia*[5], *Zaida Yadon*[6]

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[6]*PAHO United States of America*

1 Background

This project studied biological, environmental and social drivers associated with leishmaniases, firstly in the border of Argentina (Ar), Brazil (Br) and Paraguay (Py) from 2014, later Uruguay (Uy) due to the emergence of canine visceral leishmaniasis (cVL) in 2015, and Bolivia (Bo) after the report of VL vectors close to Argentinean border in 2016.

2 Methods

Biological variables: Vectors, dogs and environmental variables were sampled on sites based on grids of 400 x 400 m in urban-periurban houses, and periurban-rural-forest transects. Three nights mini-light trapping and samples from the 5 closer dogs were taken in a transversal survey, and a seasonal sub-sample. Social variables: In-depth and semi-structured interviews to key informants and community.

3 Results

A) *Lutzomyia longipalpis* (Ll) prevalence in warmer season, in urban peridomestic and indoor habitats, but spreading to rural areas. *Nyssomyia whitmani* (Nw) remains in sylvatic-rural stratum, and 'green urban patches', persistent in colder seasons. B) *Leishmania infantum* and *L. braziliensis* are the main parasites infecting indoor and outdoor phlebotomine and dogs. C) Transmission scenarios categorization: i) colonized-broad urban distribution of Ll, cVL prevalence rates of 22-26% increasing along time, the clustering of Ll and cVL could be not overlapped (Br, Ar foci), ii) incipient colonization- Ll and cVL restricted to small overlapped clusters, cVL general prevalence up to 4% (Ar, Br localities close to (i), Ur, Py, Bo); iii) Nw without Ll, cVL imported cases, cutaneous leishmaniasis risk. D) The disparate perceptions of public and private practitioners (human and animal health), protectionists and householders, about human-dog inter-specific relationships conditioned the dog ownership and dog-market practices, and the cVL interventions effectiveness.

4 Conclusions

Recent VL foci allow the modelling of risk dynamic maps ponderating biological, environmental and social drivers (in progress), at different spatial levels inferred from the results, city-foci and within foci (400 x 400 m and domestic units). The project design and results promoted the implementation of Br-Parana State and Ur VL programs, Py and Bo VL awarness in new areas, Ar agreement between public health and private practitioners about cVL management.

Financial Support IDRC-Canada Project #107577, Ministry of Health of Argentina, Brazil, Paraguay and Uruguay; TDR/PAHO Small Grants Programme Bo-Ar border.

C0299 ADDRESSING THE EMERGENCE AND SPREAD OF LEISHMANIASES IN THE BORDERS OF ARGENTINA, BRAZIL AND PARAGUAY + URUGUAY + BOLIVIA: THE ARGENTINEAN CASE

Oscar Daniel Salomon[1], *María Soledad Santini*[2], *María Gabriela Quintana*[3], *Domingo Javier Liotta*[3], *Andrea Veronica Mastrangelo Salomon*[4]

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1 Background

Argentina node of the project network focused the study in the border with Brazil and Paraguay, an area with urban, rural, and natural forest environments. This region reported steady cutaneous leishmaniasis (CL) cases, but with outbreaks in the last twenty years with *Nyssomyia whitmani* (Nw) as vector in ecotones, while visceral leishmaniasis (VL) and urban *Lutzomyia longipalpis* (Ll) were first reported there during year 2010.

2 Methods

Biological variables: Vectors, dogs and environmental variables were sampled on sites located in the 'worst peridomestic scenarios' in each cell of a grid of 400 x 400 m (Puerto Iguazu-PI, Puerto Libertad-PL), and urban periphery-rural-forest transects. Three nights mini-light trapping and samples from the 5 closer dogs were taken in a transversal survey, and a seasonal sub-sample. Social variables: Participant observation of inter-specific relationships and semi-structured interviews to key informants and community.

3 Results

A) Transmission scenarios: i) Ll broad urban distribution, canine VL (cVL) prevalence 22-26%, clustering of Ll and cVL not overlapped (PI), ii) Ll and cVL restricted to small overlapped clusters, cVL general prevalence 2-4% (PL); iii) Nw without Ll, cVL imported, CL risk (transects). B) Spatial segregation between Ll-urban and Nw-urban periphery/rural. C) Ll abundance associated with 'urban' variables in 'green-patched' environments, blood source availability, and closeness to water/wet areas. D) Persistence in time 'hot spots' of Ll abundance and force of infection. E) Disparate perceptions and demands of public and private practitioners (human and animal health), protectionists and householders, about human-dog inter-specific relationships that conditioned the dog ownership and dog-market practices, as the cVL interventions effectiveness.

4 Conclusions

The modeling of the environmental and social drivers of sites with persistent high-risk transmission is contributing to develop a new risk assessment system, and to propose intervention measures for different spatial scales. Then, the discussion with the social actors in order to implement an actual surveillance-action strategy will use as input the knowledge of the different dog-human perspectives. Further, the project design and results promoted an agreement between public health and private practitioners about cVL management for the whole country. Financial Support IDRC-Canada Project #107577-001, Ministry of Health of Argentina

C0489 SISLEISH: A MULTI-COUNTRY STANDARDIZED TOOL FOR MONITORING LEISHMANIASIS STATUS IN THE AMERICAS.

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1 Background

Leishmaniasis is a serious and growing public health issue in the Americas and due to its high complexity and diversity is a challenge for surveillance and control. In 2012, the Pan American Health Organization/World Health Organization (PAHO/WHO) created the Regional Information System of Leishmaniasis (SisLeish) with the main objective of providing updated information for general knowledge and improvement of Surveillance Systems, by maintaining a consolidated and analysed database.

2 Methods

SisLeish is a dynamic web page system with specific modules for demographic data, clinical, diagnostic criteria, confirmed cases, species of *Leishmania* and involved vectors. The system is based on epidemiological and operational indicators, such as density and incidence rates, "number of cases" and the composite indicator (combination of the others). It has restricted access to PAHO/WHO and countries of the Region, who are able to enter data from the previous year and visualize the output information, which is available at the first and second administrative level.

3 Results

In 2013, 16 countries entered their cutaneous leishmaniasis (CL) data, where 11 entered at the second administrative level (2adm), and 73.3% of the cases were confirmed by laboratory diagnosis (LD), 7.35% by clinical-epidemiological diagnosis (CED) and 19.35% were unknown, and as far as monitoring the cases, 66.6% of the patients had unknown follow up. In 2016, 18 countries entered CL data, where 17 at the 2adm, 87.6% of the cases were confirmed by LD, 9.2% by CED, 3.2% were unknown, and 46.8% had unknown follow up. The surveillance, control and assistance module was created in 2015 to monitor Programs management and to be compatible with the WHO's global database, initially with an adhesion of only 2 countries, whereas in 2016, 10 countries reported to this module. Currently, the system is under maintenance to enhance its functions and to provide public access.

4 Conclusions

There was an improvement of data entry and adhesion to the system from 2012-2016. It has stimulated several Programs to advance their surveillance system, and has been used by countries without an information system. SisLeish allows analysis and monitoring of leishmaniasis, resulting in knowledge of distribution and tendency of the disease. Therefore, it is imperative to stimulate countries to continue reporting data, so it can remain as an important surveillance tool in order to guide and implement actions.

C0500 LEISHMANIASES IN THE AMERICAS: EPIDEMIOLOGICAL STATUS, ADVANCES AND CHALLENGES FOR THE MANAGEMENT OF PROGRAMS TO STRENGTHEN THE SURVEILLANCE AND CONTROL

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1 Background

In the Americas, leishmaniases (LEISH) are present in 20 countries, and are a public health issue due to morbidity of its severe clinical forms (SCF), mortality and wide distribution. The World Health Assembly, Resolution WHA 60.13, 2007, stablished a commitment between the countries to strengthen the surveillance and control (SC) of LEISH, reinforced by the Pan-American Health Organization/World Health Organization (PAHO/WHO) through Resolutions CD49.R19, 2009 and CD55.R15, 2016. This abstract shows current epidemiological status, advances and challenges based on the Region's commitment.

2 Methods

Data from 2001-2011 was collected directly from the countries by PAHO/WHO, and data from 2012-2015 is available in the Regional Information System of Leishmaniasis (SisLeish). The actions were established by Regional and National Programs, representing a joint effort of the Global Program, PAHO/WHO, experts, partners, and countries' authorities.

3 Results

From 2001-2015, 17 endemic countries reported 843,052 cases of cutaneous and mucosal leishmaniasis (CML) with an annual average of 56,203 cases. 692,590 of the cases (82.1%) were registered in Brazil and Andean countries. 32,000 of the cases (3.8%) were mucosal leishmaniasis (ML); however, 95% were reported by Bolivia, Brazil, Paraguay and Peru. Also, there were 57,462 cases of visceral leishmaniasis (VL), while 95.8% were reported by Brazil. The fatality rate (FR) ranged from 3.1% (2001) to 8.38 (2015). The main achievements include: creation of SisLeish; update of treatment recommendations; inclusion of treatment drugs into PAHO Strategic Fund; standardization of dog management, vector and diagnostic techniques and implementation of the Regional Foreign Assessment Program for Microscopic Diagnosis; definition of indicators for risk stratification; elaboration of the LEISH Plan of Action (LPA), 2017-2022, a self-learning on-line course, and capacity-buildings to strengthen diagnosis, treatment, vector control, and SC. Nonetheless, there are still many challenges ahead, such as implementation of the LPA, strengthening and maintenance of the service organization and infrastructure, SC sustainability, and assured access to diagnosis and treatment.

4 Conclusions

Despite the progress so far, VL FR is still high and the SCF of CML still represents a challenge to case management. It is necessary to combine efforts to achieve the goals of the LPA, to reduce the morbidity and mortality of this disease.

C1019 ZOONOTIC VISCERAL LEISHMANIASIS (ZVL) IN EUROPE: A SURVEY AMONG SPANISH AND FRENCH VETERINARIANS ON THE AWARENESS OF THE SPREAD AND CONTROL OF ZVL

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1 Background

Zoonotic visceral leishmaniasis (ZVL) is transmitted by female sandflies that may infect dogs, humans, and wildlife. In the last decade the disease prevalence has increased fivefold in several parts of southern Europe, where currently an estimated 2.5 million dogs are infected. The main drivers of transmission are the increase in sandfly distribution due to climate change and the traveling and migration of dogs. The European Union (EU), the World Health Organization (WHO), the European Food Safety Authority (EFSA), and the European Scientific Counsel for Companion Animal Parasites (ESCCAP) among others have created international guidelines, providing protocols describing intervention strategies to control the spread of ZVL in Europe. However, it remains unclear whether these guidelines are being implemented in the field to control the further spread of ZVL.

2 Methods

In this study we developed a survey consisting of 24 questions that we distributed via online platforms and through multiple mailing lists to reach practicing veterinarians in Spain and France. The questions tested their 1) awareness of the spread and public health risks of ZVL in Europe, 2) awareness of the international guidelines, 3) type of protocol used when suspecting and confirming a ZVL case, and 4) their reporting's of confirmed cases.

3 Results

889 veterinarians participated in the survey of which 616 were Spanish (64%) or French (36%). Even though 60% of them were aware of the current increase of ZVL in Europe, 70% were not aware of any guidelines. However, most of their preventive and treatment actions were in line with the intervention strategies as advised in the guidelines' protocols, apart from the fact that only 12% of veterinarians would report a confirmed case, including notifying colleagues within the same practice.

4 Conclusions

Since the lack of reporting and monitoring of the ZVL infections, we suggest that an easy online international network is founded where both veterinarians as well as general practitioners report confirmed cases of ZVL. This will be crucial for monitoring, control and preventing the further spread of ZVL in Europe at the regional, national and international level.

C1499 STRENGTHENING THE TRANSDISCIPLINARY HEALTH TEAM AND ITS IMPACT ON VISCERAL LEISHMANIASIS CONTROL NUEVA ESPARTA - VENEZUELA

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1 Background

In the framework of the Visceral Leishmaniasis (VL)Research Project . In 2010-2016, in endemic communities of Diaz Municipality, Nueva Esparta state, the educational component is integrated as a space to generate changes towards the transformation of health teams as Facilitators to the communities, with the purpose of positively impacting the indicators of the endemic behavior of VL in the area. Objective: To develop an educational program that strengthens the health team as facilitators of the control program of visceral leishmaniasis in the endemic communities of the Municipality Diaz. State Nueva Esparta.

2 Methods

An educational program was applied with the conceptual vision of the paradigm: to train to transform, in the same one emphasizes the integral formation, the commitment and the responsibility of the team in the management of the project before the communities. The strategy for its operationalization was a training plan with the ecohealth principles in different areas including: effective communication, clinical, epidemiological and immunological aspects of visceral leishmaniasis, pet care, introduction to georeference and focus group methodology; Through the use of techniques of group dynamics and with a process of evaluation of input, process and results.

3 Results

The trend is aimed at updating and strengthening the team around the knowledge and technologies that directly impact project management in endemic communities, including the importance of effective communication tools allowing better psycho-affective and professional integration of the project team. Update on clinical, epidemiological and immunological diagnostic techniques and advances in LV research, management of georeferencing as a platform in epidemiological education and surveillance of endemic diseases through focal group networks. There was a reduction in the incidence rates of canine and human LV of 30 and 70%, respectively, in the period studied.

4 Conclusions

The strengthening of health teams through community education and ecohealth principles provides a basis for the sustainability of programs to control visceral leishmaniasis. Financed by CDCH UCV PSU09-7878 MS; MPPCTI, PEII No. G-20012000976 MS

C1868 ADDRESSING THE EMERGENCE AND SPREAD OF LEISHMANIASES BETWEEN BORDERS OF ARGENTINA, BRAZIL AND PARAGUAY + URUGUAY + BOLIVIA: THE PARAGUAYAN CASE.

Antonieta Rojas de Arias[1], *Nilsa González*[2], *Monica Ruoti*[1], *Andrea Gimenez*[3], *Oscar Salvioni*[1], *Enmanuel Céspedes*[2], *Jorge Miret*[4], *Nidia Martinez*[5]

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1 Background

In the border region of Argentina (Ar), Brazil (Br) and Paraguay (Py), since 2014 a multi-country project was performed to identify the main biological, environmental and social drivers associated with the risk of transmission of visceral Leishmaniasis (VL) and Cutaneous Leishmaniasis (CL). Later Uruguay and Bolivia have been incorporated. In Paraguay VL has established itself as a serious public health problem.

2 Methods

This study was carried out in three districts of Alto Parana. The biological components, involves three-night sampling with sand-fly light traps and samples from the 5 closer dogs to the trap were taken of the different areas on grids of 400 x 400 m.in a transversal survey and a longitudinal sub-sample. Social variables: In-depth and semi-structured interviews were applied on 126 families, belonging to Pte. Franco district.

3 Results

This is the first finding and dominance of *L. longipalpis* (73%) in urban and peri urban sites of Alto Paraná. *Nyssomyia whitmani* (22%) and other vectors remain in peri-urban areas. All species are frequent in warmerseasons. *L. infantum is* the main parasites infecting sand-flies (12%) and dogs (4.3%) from two districts. An 8% of the inhabitants consider LV as a problem in the area, 73% of the community knows the leishmaniasis, and however they do not know the mode of transmission. Villagers know the disease-dog relationship but do not the risk.

4 Conclusions

The project identified new risk zones in the border area of Alto Parana, based on the first report of the presence of *L. longipalpis* and canine LV in the area. Small foci were detected, very restricted to areas near the Paraná River: President Franco (rural-urban) and Ciudad del Este (urban). Although the community mentioned hearing about leishmaniasis, it had no knowledge of the risk of infection. These results allow us to infer the risk of future emergencies in the area, so it is necessary to increase epidemiological surveillance and health education for the control of leishmaniasis.

Financial Support IDRC-Canada Project #107577, PAHO

Greco Room D1-D2
15:00-16:30

Session 24. Vector biology and leishmania-sand fly interactions

C0331 LEISHMANIA ATTACHMENT IN PERMISSIVE SAND FLIES: CHARACTERIZATION OF MIDGUT MUCIN AND ITS INVOLVEMENT IN THIS INTERACTION.

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1 Background

Promastigote binding to the sand fly midgut is a crucial step in *Leishmania* development in the vector. Laboratory studies on sand fly competence to *Leishmania* parasites distinguish two sand fly groups: several species are termed "specific/restricted" vectors as they support the development of a single *Leishmania* species, while the others belong to so-called "permissive" vectors susceptible to a wide range of *Leishmania* species. In the specific vector *Phlebotomus papatasi,* promastigotes of *L. major* attach to the midgut epithellium using an abundant surface molecule lipophosphoglycan (LPG). On the other hand, the molecular background to attachment in permissive vectors such as *Lutzomyia longipalpis* has remained unclear.

2 Methods

L. major lines deficient in LPG were tested for their ability to develop in permissive and specific vectors. In parallel, midgut glycoproteins were detected in several sand fly species and a combination of biochemical, molecular and parasitological approaches were used to characterize the properties of an O-linked glycoprotein from the *L. longipalpis* midgut.

3 Results

LPG-deficient mutants of *L. major* developed well in all permissive sand fly species tested. Repeated findings of O-linked glycoproteins in the midgut of permissive vectors led us to characterize such a glycoprotein from *L. longipalpis*. This molecule has an apparent molecular weight 45–50 kDa by SDS-PAGE, and corresponds to a putative 19 kDa (non-glycosylated) protein with unknown function detected in a midgut cDNA library of *L. longipalpis.* We produced the corresponding recombinant protein (rLuloG), used this to raise anti-rLuloG antibodies, which localized the native glycoprotein on the epithelial midgut surface of *L. longipalpis*. Although we could not block *Leishmania* attachment to the midgut with anti-rLuloG during sand fly infections, we demonstrated strong binding of rLuloG to surface of *Leishmania* promastigotes.

4 Conclusions

We found that LPG is not required for *Leishmania* attachment in the midgut of permissive vectors and we characterized a novel O-glycoprotein from *L. longipalpis*. This protein has mucin-like properties, it is GPI-anchored in the membrane of enterocytes and localized on the luminal side of the midgut epithelium. A recombinant form of the protein binds to *Leishmania* parasites *in vitro*. Our data support strongly the view that LuloG participates in *Leishmania* attachment to the *L. longipalpis* midgut and thus is important for parasite transmission.

C0813 PREPARING TO BREAK THE VL TRANSMISSION CHAIN: INTEGRATING DATA FROM VECTOR CONTROL, SEROLOGICAL SURVEILLANCE AND PH. ARGENTIPES GUT MICROBIOTA TO EVALUATE INTERVENTION EFFICACY.

Geraldine Foster[1], *Gala Garrod*[1], *Lee Haines*[1], *Rinki Deb*[1], *Pradeep Das*[2], *Sridhar Srikantiah*[3], *Ian Goodhead*[4], *Michael Coleman*[1]

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1 Background

As India moves closer to reaching its visceral leishmaniasis (VL) elimination goal, attention turns toward characterising the persistent, low-level transmission that continues to occur. Evaluating vector control intervention efficacy, understanding vector biting frequency in the presence of vector control, and describing molecular differences between and among vector populations all contribute to understanding and containing the sources of persistent transmission.

There is currently no integrated dataset available that combines each of the above assessments to allow detailed analysis of transmission dynamics. We used existing entomological sentinel sites in Bihar State, India as a base for collating data on entomological abundance, quality assurance (QA) of indoor residual spraying (IRS), *Phlebotomus argentipes* bite exposure pre- and post-IRS, and characterisation of gut microbiota.

2 Methods

The study was split into three arms: DDT IRS, pyrethroid IRS and no IRS (control). Entomological assessments were performed using miniature CDC light traps. 5ml peripheral blood samples were collected from householders living in houses with light traps installed. Serum was frozen at -80C to allow simultaneous assessment of pre- and post-IRS samples. Pre-IRS sampling occurred one day prior to IRS; post-IRS sampling after 10 weeks. IRS QA was performed using Insecticide Quantification Kits. For gut microbiota assessments, female and male *Phlebotomus argentipes* were transferred to Liverpool School of Tropical Medicine for 16S sequencing.

3 Results

IRS QA showed an average pyrethroid concentration of 13.1 mg/m^2, against a target concentration of 25 mg/m^2. No significant decrease in *Ph. argentipes* abundance in sites with DDT IRS was seen ($p = 0.237$). Significant decreases in abundance in sites with pyrethroid IRS were observed ($p = 0.003$). 100 participants in each serum study arm were enrolled pre-IRS. Post-IRS samples were collected from 45/100 (DDT), 81/100 (pyrethroid), and 79/100 (control) participants, respectively. 900 female and 100 male *Ph. argentipes* were collected for gut microbiota assessment.

4 Conclusions

Breaking the VL transmission chain requires integrated data that covers the quality and insecticide of IRS, entomological abundance assessments, human antibody response to *Ph. argentipes* bites and molecular differences between and among vector populations.

C0987 EVALUATION OF THE MECHANISM OF LEISHMANIA PATHOGENESIS AFTER INFECTED SAND FLY BITES HIGHLIGHTS THE EFFICACY OF GENETICALLY MODIFIED LIVE ATTENUATED LEISHMANIA VACCINES AGAINST VECTOR CHALLENGE

Ranadhir Dey[1], *Hamide Aslan*[2], *Fabiano Oliveira*[3], *Claudio Meneses*[3], *Amritanshu Joshi*[1], *Lais Pereira*[3], *Shannon Townsend*[3], *Anderson Guimaraes Baptista Costa*[3], *Parna Bhattacharya*[1], *Waldione de Castro*[3], *Tiago Donatelli Serafim*[3], *Iliano Coutinho Abreu*[3], *Philip Castrovinci*[3], *Robert Duncan*[1], *Shaden Kamhawi*[3], *Jesus Valenzuela*[3], *Hira L. Nakhasi*[1]

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1 Background

During natural transmission, an infected sand fly deposits parasites along with saliva and vector-derived components of the infectious inoculum. The enhanced virulence observed in sand fly-mediated transmission has been attributed to a sustained recruitment of neutrophils. The mechanism underlying this phenomenon remains unknown. In addition, previous studies have demonstrated that several experimental *Leishmania* vaccines protected mice against needle-challenge but failed against vector-mediated challenge. These studies clearly demonstrate that a vector-initiated infection is crucial for understanding pathogenesis, immunity and vaccine evaluation for leishmaniasis.

2 Methods

Mice ears were exposed to *L. donovani*-infected sand flies. Cytokines were measured by real time PCR and ELISA. Hamsters were immunized with LdCen-/- and rLJM19 and challenged with *L. donovani*-infected sand flies.

3 Results

Vector-transmission of *L. donovani* reproduced the intense accumulation of neutrophils previously observed at bite sites following *L. major* transmission, and resulted in a 100-fold increased IL1β induction compared to needle injected parasites. Further, our studies demonstrated that IL1β is the main cause of neutrophil persistence following sand fly-transmission, and revealed an important role for neutrophils in *L. donovani* dissemination. Importantly, neutralizing the effect of IL1β by blocking IL1R abrogated neutrophil recruitment after infected bites and abolished parasite visceralization. To address whether live attenuated parasites can protect against the enhanced virulence of a sand fly-initiated infection, hamsters were immunized with LdCen-/- and sand fly salivary gland protein rLJM19. Five weeks post immunization hamsters were challenged with *L. donovani*-infected sand flies. We monitored these hamsters for up to 14 months post-challenge. During this period 50% of hamsters from the non-immunized group developed severe VL and died. None of the hamsters from the immunized group developed any VL symptoms. The protection observed by survival of immunized hamsters was corroborated by a significantly lower splenic and hepatic parasite burden compared to non-immunized animals.

4 Conclusions

The study demonstrates that *LdCen-/-* immunization is efficacious in controlling VL initiated by the sand fly vector. Further, these studies highlight the importance of pre-clinical evaluation of *Leishmania* vaccines against a sand fly challenge before proceeding to clinical studies.

C1138 GENE EDITING IN SAND FLIES BY CRISPR/CAS9 TECHNOLOGY

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1 Background

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) along with the CRISPR associated proteins (Cas) are part of the bacteria immune system against viral infections. Recently, this technology has been modified for genome engineering and it has rapidly emerged as a very effective tool for gene editing. In the medical entomology field, there are already interesting data on gene editing in mosquitoes. However, no attempts of gene editing have been reported in sand flies, the vectors of Leishmaniasis. Here, we present a proof of concept experiment of CRISPR/Cas9 technology in sand flies.

2 Methods

DNA sequences of kynurenine monooxygenase (KMO) and YELLOW genes, responsible for black eye pigment and brown body color respectively, were inspected in search of NGG sequences. Single guide RNAs (sgRNA) were produced by PCR with the complementary primers containing the T7 promoter, the gene specific and the Cas9 binding sequences and were *in vitro* transcribed using the Megascript T7 kit (Ambion). An *in vitro* assay was developed to test the individual activity of each sgRNA. A mixture of 6 sgRNA for each target gene and Cas9 mRNA was injected in *Lutzomyia longipalpis* early stage embryos (less than 1 after oviposition) using a FemtoJet microinjector (Eppendorf). The first generation (G0) was reared and crossed to wild type sand flies. G1 was intercrossed and G2 were screened for a visual phenotype.

3 Results

All sgRNA showed high activity *in vitro*. 539 and 422 embryos were microinjected for KMO and YELLOW experiment, respectively. The G0 hatching rate was 11.4% and 14.22% for each group. After microinjection of sand fly embryos with Cas9 and specific sgRNA a visual phenotype was observed in the second generation of insects (5,244 and 6,314 *Lu. longipalpis* were screened for KMO and YELLOW experiments, respectively). This visual phenotype corresponds to the homozygous individuals coming from the heterozygous intercrosses on the G1.

4 Conclusions

Here we report the first demonstration of gene editing in sand flies by CRISPR/Cas9 technology in an experiment targeting the KMO and YELLOW genes. Gene editing in sand flies is feasible and will allow us to do loss of function studies with salivary genes to better understand the role of saliva in blood feeding process and parasite transmission.

C-1349 MICRO AND MESO SCALE ENVIRONMENTAL DETERMINANTS OF LUTZOMYIA LONGIPALPIS AND NYSSOMYIA WHITMANI ABUNDANCE, MAIN VECTORS OF VISCERAL AND CUTANEOUS LEISHMANIASIS IN PUERTO IGUAZ&UACUTE;, ARGENTINA

***Marìa Gabriela Quintana*[1]**, *Marìa Soledad Santini[2], Regino Cavia[3], Mariela Martìnez[1], Marìa Soledad Fern·ndez[3], Domingo Javier Liotta[4], Magali Giuliani[1], Sofia Lorian Moya[1], Oscar Daniel Salomón[1], and Project Team IDRC[1]*

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1 Background

The study area includes the localities of Puerto Iguazú and Puerto Libertad, northeast Argentina. The area reported outbreaks of cutaneous leishmaniasis (CL) since the ´90s, with presence of *Nyssomyia whitmani* (Nw) as the main vector related to unmodified environments and its ecotones. Regarding to visceral leishmaniasis (VL) urban reports started in 2010 with *Lutzomyia longipalpis* (Ll) as the main vector.

2 Methods

We divided the urban and periurban environments of both cities into a grid of 400 m2, with 55/15 samples sites respectively. In rural areas and natural forests we set 12/13 sample sites. The selection criterion for all sites was the 'worst scenario'. In each site, REDILA traps were set for three consecutive nights, and a total of 48 meso and microscale environmental characteristics surveyed simultaneously. The proportion covered by six landcover classes were estimated for different surface areas (between 0.27 and 20.25 hectares) arround each sampled site. The best area was selected comparing the deviance explained by adjusted Generalized Linear Models. To evaluate the posible determinants for each vector we developed a zero-inflated negative binomial regression model. We analyzed females for detection of *Leishmania infantum* DNA, products were purified and sequenced for identity analysis with BLASTm.

3 Results

Ll abundance was better explained by the land cover characteristics of the area of 20.25ha, and for Nw the areas of 1.05ha and 20.25ha. The significant variables for the 'zero-inflated' component of Ll were the presence of chicks (micro scale) and Normalized Difference Water Index (meso scale), while the abundance of Ll was related to A) Mesoscale: drinking water network, garbage collection, sewer and B) Microscale: availability of differents blood sources (presence of hens, dogs or both). Analisys for Nw are in progress. *Leshmania infantum* DNA was detected in 7/369, 5 Ll, 1 Nw and 1 *Micropygomyia quinquefer* (first detection).

4 Conclusions

Lutzomyia longipalpis has urban distribution related to urbanization indicators, food presence and water bodies in the closeness. *Nyssomyia whitmani* (preliminary) has preference for areas with higher vegetation presence, in mixed landscapes (periurban, rural, natural areas). The selection of the better scale will allow to design appropriate control strategies according to each species.

Financial Support IDRC-Canada Project #107577-001, Ministry of Health of Argentina

C1363 MICROBIOTA IN THE LUTZOMYIA LONGIPALPIS MIDGUT ARE ESSENTIAL FOR SURVIVAL OF LEISHMANIA INFANTUM

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1 Background

The vector-borne disease leishmaniasis is transmitted to humans by phlebotomine sand flies. Development of the *Leishmania* spp. protozoa to infective metacyclic promastigotes in the insect gut is an essential prerequisite for transmission. We hypothesized that vector gut microbiota influence the development of virulent parasites.

2 Methods

We investigated midgut bacterial microbiomes of the sand fly *Lutzomyia longipalpis* with or without *Leishmania infantum* infection through 16S rDNA sequencing of DNA from isolated midguts. Conditions included sand flies fed on sucrose, blood, or blood infected with *L. infantum*. Experiments included three groups of 20 midguts for each condition dissected on days 0, 1, 3, 6, 9, and 12 of the experiment. Three independent experiments were performed.

3 Results

Sucrose fed sand flies contained a highly diverse, stable midgut microbiome. Blood feeding caused a decrease in community richness, which eventually recovered. However bacterial richness progressively decreased in *L. infantum*-infected sand flies. *Acetobacteraceae* became dominant and *Pseudomonadaceae* diminished coordinately as metacyclics developed. Importantly, antibiotic-mediated perturbation of the midgut microbiome just after blood meal digestion rendered sand flies unable to support parasite growth and consequent development to infectious metacyclic forms. This occurred in the absence of fungal overgrowth measured by 18S qPCR.

4 Conclusions

These data suggest that the sand fly midgut microbiome plays a critical role in *Leishmania* growth, facilitating differentiation within the sand fly vector prior to disease transmission.

C1464 AN INTERGRATED OVERVIEW ON THE MIDGUT BACTERIAL FLORA COMPOSITION OF PHLEBOTOMUS PERNICIOSUS, A VECTOR OF ZOONOTIC VISCERAL LEISHMANIASIS IN THE WESTERN MEDITERRANEAN BASIN.

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1 Background

Leishmania developmental within the sand fly vector takes place in the lumen of the insect's digestive tract, and in the presence of an abundant digestive microbiome. The composition of the microbiome is thought to be under the influence of a set of factors, including the nature of the host and its environment. It has been demonstrated that the insect gut microbiome can influence the development of several human pathogens, including *Plasmodium falciparum*. For sand flies and *Leishmania*, understanding the interactions between the parasite and the microbial environment of the vector midgut can provide new tools to control *Leishmania* transmission.

2 Methods

Females *P. perniciosus* from laboratory or collected from the field during the period from July to October 2011. Midguts dissected from laboratory reared and field collected *P. perniciosus* females were analyzed by culture dependent and independent methods.

3 Results

A total of 441 and 115 cultivable isolates assigned 30 and 11 phylotypes from field-collected and colonized *P. perniciosus*, respectively. Seasonal bacteriome variations were observed, with a nearly monthly specific bacterial composition. In parallel, a compilation and a meta-analysis of all available data concerning the bacteriome of Psychodidae, both *Phlebotomus* and *Lutzomyia* was performed and compared to *P. perniciosus* data obtained herein. This integrated analysis highlighted the substantial divergences between Old and New world sand flies with regards to the midgut microbiome present in these insects, and the highly specific associations observed between Old World sand fly species and the commensal bacteria they harbor.

4 Conclusions

Our results pinpoint the need for a more exhaustive understanding of the bacterial composition and microbiome dynamic in phlebotominae sand flies in order to potentially capture the role of midgut bacteria during *Leishmania* development and transmission by sand fly vectors. The occurence of *Bacillus subtilis* in *P. perniciosus* and in at least two other sand fly species studied so far strongly suggest that this bacterial species is a potential candidate for paratransgenesis or biocontrol approaches at controlling sand fly populations and preventing *Leishmania* transmission.

C1480 AN OVIPOSITION PHEROMONE ASSOCIATED WITH CONSPECIFIC EGGS IS AN ATTRACTANT AND STIMULANT TO GRAVID PHLEBOTOMUS PAPATASI FEMALES

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1 Background

The chemical ecology of *Lutzomyia longipalpis* the South American vector of *Leishmania chagasi infantum* has been extensively studied and this species complex is known to produce both sex and oviposition pheromones. By contrast very few studies on the pheromones of Old World sand flies has been performed.

2 Methods

The bioassay was performed according to the method described by ElNaim and Ward (1991). Gravid females were transferred into Nalgene plastic pots to oviposit. The base and walls of the oviposition pots were lined with plaster of Paris. After drying the plaster layer was divided into two halves, one designed for test site and the second half for control site. Each oviposition site was demarcated, using sharp forceps, into eight 1x1 cm units bound by grooves 3 mm deep. Before use, all the pots were kept moist by distilled water and then placed under tissue paper to eliminate the excess of water.
When females *P. papatasi* lay, eggs were transferred with dissecting needle and placed into the grooves in the 'test' site and the 'control' site was left blank. During each experiment 30 gravid females were released in different oviposition pots and left in incubator in complete darkness at 29-30°C and at 95% RH for 4 days. One day after oviposition, all eggs laid in 'test' and in 'control' sites were counted.
Furthermore, we tested the oviposition response to washed eggs. In this experiment we prepared egg's extract in hexane from batches of 160 eggs and placed on filter paper in 'test' sites pots. Five days later, all eggs laid in 'test' site and in 'control' site were counted. Experiment was replicated twelve times. Mann-Whitney test was used to compare between number of eggs laid on test and control sites

3 Results

Female *P. papatasi* laid a significantly greater number of eggs on sites with pre-existing eggs (N = 160) than on sites without eggs. When smaller numbers of pre-existing eggs less than 160 (80 eggs) were used, no significant differences between the mean numbers of eggs laid on test and control sites were detected. Furthermore, when extract was prepared in hexane from batches of 160 eggs and placed on filter paper in test pots, a significant increase in the number of eggs laid compared to the controls was found.

4 Conclusions

Together, these results strongly suggest the presence of an oviposition pheromone associated with conspecific eggs of *P. papatasi* females.

C1823 INFLUENCE OF THE GUT BACTERIAL MICROBIOTA ON LEISHMANIA MEXICANA TRANSMISSION BY SAND FLY BITE.

Emilie Giraud[1], *Jakob Hurst*[2], *Martin Oihane*[3], *Al-Sharook Omar*[1], *Crainey Lee*[4], *Matthew Rogers*[1]

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[4]*Instituto Leonidas e Maria Deane Brazil*

1 Background

Leishmania, the causative agent of human leishmaniasis, are transmitted during the blood-feed of a female phlebotomine sand fly. In sand flies *Leishmania* parasitise the gut, where they are exposed to other microorganisms. The source of this microbiota could be highly variable when one considers that sand flies develop as larvae in organic-rich rodent burrows, feed on sugar sources, such as nectar and aphid honeydew, as adults and take bloodmeals from a wide variety of vertebrate hosts.

2 Methods

The aim of the present study was to analyse the sand fly gut microbiota of newly emerged adults by 454 pyrosequencing and by a real-time quantitative PCR and to identify the ability of these microorganisms to influence *Leishmania* growth in vector and transmission to mammalian hosts.

3 Results

This study revealed two distinct, diet-independent clusters of bacteria into sand fly guts which show a predominance or absence of *Ochrobactrum* in Cluster 1 and Cluster 2, respectively. At the same time a real-time quantitative PCR based method was developed to analyse both parasite and bacterial transcripts on the same gut. Using the *Leishmania* gene target *ssrRNA* and the metacyclic-specific transcript *sherp* we could confidently differentiate metacyclic (infectious forms) and non-metacyclic parasites. Our results with individual infected sand fly bites show that the majority of flies in Cluster 1 (High quantity of *Ochrobactrum*) transmit a higher number of parasites with a lower percentage of metacyclics compared to flies in Cluster 2 (Low quantity of *Ochrobactrum*).

4 Conclusions

These results describe the influence of *Ochrobactrum* on the life cycle of *Leishmania* parasites in sand fly guts and on the dose and quality of *Leishmania* transmitted into bite sites.

Miradero Room
15:00-16:00

Session 25. Leishmania-HIV co-infection

C0287 MUCOCUTANEOUS LEISHMANIASIS AND HIV CO-INFECTION: POST-MORTEM DIAGNOSIS OF LEISHMANIA (LEISHMANIA) INFANTUM CHAGASI BY ITS-1 RFLP

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1 Background

A co-infected (HIV) patient diagnosed in the past withmucocutaneous leishmaniasis presented hoarsenessand oropharyngeal lesions, aside from episodes of cough, fever and weight loss referred to in the last months. In addition, the abdominal ultrasonography presented a splenomegaly. Due to the worsening of hoarseness, a larynx biopsy was performed and amastigotes of *Leishmania* were found in the histological examination. But because of a pancytopenia and the presence of *Leishmania* amastigotes in stained smears from a bone marrow aspirate, it has been concluded that a visceralization of the mucocutaneous leishmaniasis had occurred, mainly due to the presence of HIV. Nevertheless, the positivity of rk39 (specific for *donovani* complex), and the fact that the patient was from a known endemic area for both, cutaneous (CL) and visceral leishmaniasis (VL), led us to think about the possibility of a presence of a specie which is commonly responsible of VL.

2 Methods

A tiny stored paraffin-embedded larynx biopsy (post-mortem),obtained from the pathology laboratory, was submitted to three conventional Polymerase Chain Reaction (PCR) assays using the following targets: the kDNA (primers K20/K22 and RV1/RV2); internal transcribed spacer 1 (ITS1) from the parasite rDNA gene (primers LITSR and L5.8S), and after PCR-ITS1-RFLP (Restriction Fragment Length Polymorphism) analysis.

3 Results

After the definition of the genus and subgenus of *Leishmania* by kDNA-PCR with primers K20/K22 and RV1/RV2, respectively, the *Leishmania (L.) infantum chagasi* specie was identified by the ITS1-PCR-RFLP

4 Conclusions

The identification *of L.* (*L.*) *infantum chagasi* took place only *post-mortem*. Nevertheless, ITS1-RFLP identified *L. (L) chagasi* as the etiological agent in this patient, therefore corroborating the finding of serology (positive rK39), an immunochromatographic test that is specific for the *Leishmania donovani* complex, which includes *L.* (*L.*) *infantum.* In this report, the unequivocal identification was carried out by rK39 immunochromatographic test and ITS-PCR-RFLP, and these techniques are suitable to be applied in developing countries.

FINANCIAL SUPPORT
This research project was fully supported by FAPESP (Fundação de Amparo à Pesquisa no Estado de São Paulo), grant number 2010/50304-8 awarded to Dr. Lucia Maria Almeida Braz.

C0414 TREND IN CUMULATIVE CASES AND MORTALITY RATE AMONG VISCERAL LEISHMANIASIS PATIENTS IN EASTERN SUDAN: A 14-YEAR REGISTRY, 2002-2015

Gamal Khalid Adam Yousif

Faculty Of Medicine Gadarif Univercity Sudan

1 Background

Leishmaniasis is one of the neglected infectious diseases of the world. This disease largely affects individuals of low socio-economic level, mainly in developing countries.

2 Methods

The aim of this study was to investigate the cumulative number of cases, case fatality rate, and trend in incidence and mortality rate of visceral leishmaniasis (VL) in Eastern Sudan. A retrospective descriptive study was performed through analysis of the database managed by the Ministry of Health in Gadarif State, which includes all cases of VL.

3 Results

.Over the 14-year study period (2002–2015), a total of 51 773 patients were registered in Gadarif State with clinical and laboratory evidence of proven VL. The trend in VL was considerably higher over the years 2003–2005 as compared to the last 5 years of 2011–2015. The highest fatality rate was observed in 2002 (4.8%) and it had declined in 2014 (1.1%) and 2015 (1.7%). Rural residence was statistically associated with death related to VL ($p = 0.021$).

4 Conclusions

This study showed a high number of cumulative cases of VL in Eastern Sudan. The fatality rate was found to be highest among rural residents and has declined in the last 5 years. Thus immediate interventions are needed in terms of health education and the implementation of preventive measures, with a specific focus on people residing in rural areas.

C0719 PREVALENCE AND RISK FACTORS OF ASYMPTOMATIC LEISHMANIA INFECTION IN HIV-INFECTED INDIVIDUALS IN NORTH WEST ETHIOPIA

***Johan van Griensven*[1]**, *Saskia Van Henten[1], Bewketu Mengesha[2], Mengistu Endris[2], Said Abdellati[1], Dorien Van Den Bossche[1], Lieselotte Cnops[1], Wim Adriaensen[1], Wondimu Asefa[3], Tesfa Simegn[3], Dagnachew Debasu[4], Yonas Gedamu[4], Florian Vogt[1], Ermias Diro[5]*

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[2]University of Gondar, Ethiopia
[3]Metema hospital, Ethiopia
[4]ITM, Ethiopia
[5]University of Gondar, Belgium

1 Background

In endemic regions, asymptomatic *Leishmania* infection is common. HIV patients are at increased risk of progression to symptomatic disease (visceral leishmaniasis (VL)), with high mortality. Thus, certain HIV patients might benefit from prophylactic treatment strategies for VL by preventing progression to disease. However, before evaluating such strategy, the prevalence, markers and determinants of asymptomatic infection need to be determined.

2 Methods

We conducted a cross-sectional analysis, consecutively enrolling HIV-infected adult patients on follow up at the HIV care clinic of a district hospital in a VL-endemic area in North-west Ethiopia from September 2015 to August 2016. Those with VL at or within five years before enrolment were excluded. Asymptomatic *Leishmania* infection was defined as positivity for any of the *Leishmania* markers tested: Direct Agglutination Test (DAT), rK39-Rapid diagnostic test (RDT), and urine antigen (KAtex) test. Socio-demographic information, information related to *Leishmania* exposure, and clinical information was collected to study determinants of infection. Risk factors for asymptomatic *Leishmania* infection were determined using logistic regression.

3 Results

A total of 504 patients were included. The median age was 38 years (interquartile range (IQR) 30-44) years, 61% were male. Most were farmers (37%) or daily labourers (23%); 55.0% (271/496) slept outside regularly, 3.2% (16/502) had a VL case in the household ever, 5.4% (27/499) had a VL case in the neighbourhood. The median time of residence in a VL-endemic area was 17 years (IQR 10-25). A history of VL was present in 2.6% (13/503). At enrolment, most (96%) were on antiretroviral treatment (ART) - for a median of 4 years (IQR 2-6); the median CD4 count was 380 cells/µL (IQR 254-521). The prevalence of asymptomatic *Leishmania* infection was 9.5% overall, 8.3% for rK39, 2.2% for DAT, and 0.8% for KAtex. The highest prevalence (15%) was seen in males aged 28-48 years. Independent risk factors were being male (odds ratio (OR) 4.0; 95% confidence intervals (CI) 1.7-9.3), not being on ART (OR 3.5; 95% CI 1.1-11.6) and a history of VL (OR 8.7; 95% CI 2.6-28.9).

4 Conclusions

The prevalence of asymptomatic *Leishmania* infection was high, justifying further longitudinal studies to determine which patients are at the highest risk of progression to VL. Ultimately, this could result in a VL screen and treat strategy, with those at highest risk receiving VL prophylaxis.

C1232 VISCERAL LEISHMANIASIS AND HIV CO-INFECTION IN NORTHWEST ETHIOPIA: UPTAKE OF ANTIRETROVIRAL TREATMENT AND BURDEN OF DISEASE AMONGST PATIENTS ENROLLED IN HIV CARE

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[1]*ITM Antwerp Belgium*
[2]*Metema hospital Ethiopia*
[3]*University of Gondar Ethiopia*

1 Background

Program operational aspects have been largely ignored related to visceral leishmaniasis (VL)-HIV co-infection in East-Africa. While all VL cases should start antiretroviral treatment (ART), studies on ART uptake are missing. A complementary strategy to decrease VL-HIV burden/mortality entails preventing VL in HIV patients living in VL-endemic regions. However, there are no data on how common VL is observed in individuals enrolled in HIV care in such regions. The aim of this study was to 1) document the proportion of VL diagnoses that occur in previously diagnosed HIV-patients - versus VL and HIV diagnosed concurrently; 2) for both groups: uptake of ART and cotrimoxazole secondary prophylaxis (CPT).

2 Methods

We conducted a retrospective cohort study including all VL-HIV coinfected adults at the Leishmania center at the Gondar referral hospital (2010-2015) and at a district hospital (2010-2014) in North-West Ethiopia.

3 Results

We included 112 patients at the referral hospital and 58 at the district hospital. The median age was 30 years, 98% were male. The proportion of VL-HIV coinfected patients with VL diagnosed while enrolled in HIV care was 56% (63/112) and 19% (11/58) at the referral/district hospital respectively. In this patient group, the median CD4 count at VL diagnosis was 45 cells/µL and 248 cells/µL at the referral/ district hospital respectively. 76 % (56/44) were on ART at VL diagnosis. Ten (13%) patients died during admission. Uptake of ART and CPT was high.

The remaining 96 (56%) patients had both infections diagnosed concurrently, with a median CD4 count at VL diagnosis of 56 cells/µL and 143 cells/µL at the referral/district hospital respectively. Nineteen (20%) patients died during admission. Amongst cured patients, CPT uptake was 89% and 72% at the at the referral/district hospital, respectively; documented ART uptake was 67% and 36% at the referral/district hospital respectively. For the entire patient group, overall ART uptake was 61% and 28% at the referral/district hospital respectively. At the district hospital, documented uptake was especially low in referrals.

4 Conclusions

A substantial proportion of VL-HIV cases occur amongst patients in regular follow-up for HIV care, requiring further evaluation of preventive strategies. Amongst newly diagnosed VL-HIV coinfected patients, there was a low rate of documented ART initiation, especially at district level. The reasons, including poor data collection/referral systems, should be assessed.

C1541 COMPARATIVE STUDY OF VISCERAL LEISHMANIASIS IN IMMUNOCOMPETENTS, HIV PATIENTS AND PATIENTS UNDER IMMUNOSUPRESSIVE THERAPHY DURING THE LARGEST COMMUNITY OUTBREAK OF LEISHMANIASIS IN EUROPE (FUENLABRADA, SPAIN): CLINICAL MANIFESTATIONS, EVOLUTION, AND IMMUNOLOGICAL RESPONSE

Alicia Castro Gordon[1], Belén Matía Hernando[1], Eugenia Carrillo[2], Laura Botana[2], Laura Fernández[2], Laura Molina[1], Ana Ibarra-Meneses[2], Jesús García-Martínez[1], Carmen Sánchez[2], Jerónimo Jaquetti[1], Luis Horrillo[1], Isabel García-Arata[1], Jose Manuel Ruiz Giardin[1], Javier Moreno[2], ***Juan San Martín López[3]***

[1]Hospital Universitario de Fuenlabrada Spain
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[3]Las Rozas España

1 Background

Clinical outcomes of immunocompetent and immunocompromised patients with visceral leishmaniasis (VL) are known to be different. Largest leishmaniasis outbreak in Europe was declared since June 2009 in Fuenlabrada (Madrid, Spain). Our aim was to describe our experience with VL in immunocompetents (IC), HIV and patients under immunosuppressive treatments (corticoids, methotrexate and anti-TNF)(IS). We have included in the study SLA-cell proliferation test comparison, that has been tested as marker of specific cellular immunity for VL.

2 Methods

From June 2009 (outbreak start) we prospectively collected variables regarding clinical presentation, diagnostic procedures, treatment and follow up of patients with VL. Since January 2013 patients were recruited for PCR leishmania, serology (IFAT) and SLA-cell proliferation test in active leishmaniasis vs cured patients

3 Results

110 patients have been diagnosed of VL: 14 HIV, 15 IS and 81 IC. Fever were less frequent in HIV patients (62%), versus 98% IC group and 93% in IS group (p=0.001). IS group presented a lower hemoglobin media (8.9 gr/dl) vs. 10 gr/dl of the other two groups (ANOVA p=0.014), and a lower media of platelets counts (69333/ml)(p=0.009). HIV group did not raise any of the acute phase reactants (p=0.001). In relation to the diagnostic techniques, we did not find many differences in between the groups. The rapid test with rK39 dipstick showed low sensibility in all groups, but had a positive predictive value of 97%. There were 8 relapses in IC (10%), 5 in HIV (38%) and 3 in IS (20%). SLA-cell proliferation test was lower in HIV patients (0.95) with active VL than IC (1.55) and IS (2.1) (p=0.03) but all groups improved (all above 5.0) when cured, without differences between groups.

4 Conclusions

In TARGA era, our HIV patients showed a less virulent form of VL than IC an IS patients. Hematological findings in IS must not be blamed to immunosuppressive drugs and high index of suspicion for VL must be maintained. Serological results shown not differences between groups. We had a high index of relapses even in IC with usual doses.

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Greco Room C1-C2

Session 26. Early drug discovery. Animal models and genetic susceptibility. Drug resistance mechanism

C0205 STRUCTURE-BASED DESIGN OF NOVEL DIHYDROOROTATE DEHYDROGENASE INHIBITORS IN LEISHMANIA AND IN VITRO VALIDATION

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1 Background

The classical treatments for diseases caused by trypanosomatids such as leishmaniasis are associated to severe side effects, including death. Furthermore, drug resistant parasites are a major health problem in different endemic countries. For those reasons, there is an urgent need for new, safe and inexpensive anti-trypanosomatid drugs. One strategy is through the structure-based design of inhibitors against essential molecular targets. Public repositoriescurrently contains more than 150 experimentally resolved trypanosomatid protein structures that could be used for virtual screening approaches. Our study focuses in one enzyme, the dihydroorotate dehydrogenase from *Leishmania major*,which is associated to the *de novo* pyrimidine synthesis pathway and has structural differences that can be exploited for drug discovery purposes.

2 Methods

The dihydroorotate dehydrogenase protein crystal (PDB:4EF8) was used to find novel active compounds through a virtual screening approach combining different structure-based methodologies. First, a molecular dynamics production simulation of 1 nanosecond was run and a set of 100 proportionately separated snapshots were obtained. Then, a molecular docking was run between each protein state and a library of ~600K drug like molecules. The best ranked complexes were followed up by a refinement stage of the binding score based on posterior molecular dynamics simulations and a MM-PBSA protocol. The final list of compounds were validated through *in vitro* assays against a cultured strain of *Leishmania panamensis*.

3 Results

After obtaining the average docking score across the trajectory snapshots, a set of 20 promising molecules were prioritized and re-ranked according to the MD analysis and the MM-PBSA protocol. In particular, the effect of the solvent within the protein-ligand complex was analyzed based on the interactions created after visual inspection. In the end, 6 molecules were selected and purchased for *in vitro* tests of cytotoxicity and biological activity. In particular, one compound showed a selectivity index greater than 285, with almost 90% inhibition of the parasite under the experimental conditions.

4 Conclusions

The inclusion of complementary structure-based methodologies for the design of novel inhibitors against a key enzyme such as the dihydroorotate dehydrogenase have demonstrated potential efficacy at *in vitro* level. The selected candidates will be further optimized and tested under *in vivo* conditions.

C0207 IN VIVO EFFECTIVENESS AND IMMUNOMODULATORY EFFECTS OF THE ANTIDEPRESSANT SERTRALINE ENTRAPPED INTO NANOLIPOSOMES: AN EXPERIMENTAL STUDY AGAINST LEISHMANIA INFANTUM

Andre Gustavo Tempone[1], *Thais Alves Costa-Silva*[1], *Andres J Galisteo Jr*[2], *Leandro Ramos Souza Barbosa*[2]

[1]*Instituto Adolfo Lutz Sao Paulo Brazil*
[2]*University of Sao Paulo Brazil*

1 Background

Visceral leishmaniasis (VL) affect more than 1.5 million people with limited and toxic therapeutic options; the search for new and safe alternatives is needed. Considering Drug Repositioning as a successful and cost-effective approach for neglected diseases, we selected the FDA-approved antidepressant sertraline to evaluate its antileishmanial effect. Sertraline was entrapped into nanoliposomes containing phosphatidylserine (nLP-SERT) and its effectiveness was studied in a VL murine model.

2 Methods

Sertraline was entrapped in liposomes using the film hydration method and quantified using High-Performance Liquid Chromatography. Dynamic light scattering (DLS) and ζ-potential were applied in order to study the physico-chemical properties of nLP-SERT. Male BALB/c mice were infected with *Leishmania infantum* (MHOM/MA67ITMAP263) and 5 days post infection, animals were treated subcutaneously at 0.3, 1, 5 and 15 mg/kg for 10 days. Empty liposomes (free of drug) were used as controls. The parasite burden was analyzed in liver using the limiting dilution assay. The serum levels of cytokines (IL-4, INF-γ, TNF-α, IL-6, IL-10 e IL-12p70) of nLP-SERT-treated animals were analyzed by the CBA kit (Thermo-Fisher) using flow cytometry.

3 Results

Our results demonstrated that nLP-SERT was highly effective at 0.3 mg/kg and 1 mg/kg, reducing the parasite burden in liver by 72% (SEM 5.01; $p< 0.05$) and 89% (SEM 6.1; $p< 0.05$), respectively. At higher doses, an unusual effect was observed; at 5 mg/kg the parasite burden was reduced by 71% (SEM 15.9), a similar reduction to that observed at 0.3 mg/kg. At 15 mg/kg, the parasite burden was 2-fold higher than the untreated group. At the therapeutic dose, the IL-6 levels in serum of animals treated with nLP-SERT at 1 mg/kg were reduced by half ($p<0.05$) when compared to untreated group, but at 15 mg/kg, these levels increased about 4-fold ($p<0.05$) when compared to the untreated animals. The levels of MCP-1 were increased ($p<0.05$) in both doses of nLP-SERT (1 and 15 mg/kg). Physicochemical studies confirmed a negatively charged nanoformulation with vesicles of 127 nm of diameter.

4 Conclusions

At a therapeutic dose (1 mg/kg), nLP-SERT demonstrated a safe and good in vivo efficacy, also reducing a disease exacerbatory cytokine (IL-6) and increasing MCP-1 levels. At a 15-fold higher dose, nLP-SERT was harmful, disturbing the immune system and affecting the parasite burden. Supported by FAPESP 2015/23403-9 and 2015 15822-1.

C0383 VALIDATION OF A NEW BALB/C MOUSE/L. GUYANENSIS FOOTPAD MODEL AS PART OF A TIERED IN VIVO SCREENING PARADIGM FOR DEVELOPMENT OF DRUGS TO TREAT CUTANEOUS LEISHMANIASIS

Diana Caridha, *Mozna Khraiwesh, Brian Vesely, Franklin Ngundam, Kirk Butler, Susan Leed, Erica Penn, Norma Roncal, Kenda Lawrence, Rick Sciotti, Lisa Read, Max Grogl, Chuck Bane, Chad Black, Mara Kreishman-Detrick, Mark Hickman*

WRAIR, USA

1 Background

Recent reviews on in vivo animal models for CL emphasize the need for in vivo models with clinical similarities to the human form of CL that ensure positive correlation between in vitro potency and in vivo efficacy before beginning human clinical trials. There are very few examples of L. guyanensis-rodent animal models in the literature. The Leishmania Team at the Walter Reed Army Institute of Research uses a gated tier-testing paradigm to progress compounds with in vitro potency and good physicochemical properties into a first tier lesion suppression efficacy model in BALB/c mice, followed by a second tier lesion cure model in BALB/c mice and Golden Syrian Hamsters infected with L. major. In order to provide for testing models with different virulence profiles, promising lead compounds are further tested in a third and final-tier footpad cure model in BALB/c mice infected with transgenic New World L. guyanensis parasites.

2 Methods

Drug treatment in BALB/c mice starts two weeks post infection, when the differences between infected and uninfected footpad thickness become statistically different and well before the *L. guyanensis* parasites spread to the lymph nodes.

3 Results

L. guyanensis parasites spread to the draining lymph nodes approximately 20 days post infection. Administration of known antileishmanial compounds such as the three formulations of Amphotericin B, Pentostam, Pentamidine, Paromomycin, Allopurinol, Sinefungin and Sitamaquine significantly reduces the footpad thickness in BALB/c mice infected with *L. guyanensis*. Anti-inflammatory compounds reduce the footpad thickness but not the parasite load.

4 Conclusions

In this newly validated model we can reach an accurate conclusion on the efficacy of a candidate compound in 40 days (compared to > 70 days for the base of the tail lesion cure model), which maximizes the number of drugs that can be tested and minimizes animal pain and suffering. Additionally, use of the *in vivo* imaging technology provides a tool for distinguishing between antileishmanial and anti-inflammatory compounds.

C0501 A HOLISTIC APPROACH TO THE DEVELOPMENT OF TOPICAL TREATMENTS FOR CUTANEOUS LEISHMANIASIS

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1 Background

As recommended drugs for cutaneous leishmaniasis (CL) show limited effectiveness, renewed effort is needed to develop affordable, safe and potent drugs that provide options for oral or topical administration. Challenges include the discovery of compounds that have (a) activity against a range of *Leishmania spp.*, (b) skin disposition following oral or topical delivery, and (c) stability in zone 4 conditions. We focus on topical formulations.

2 Methods

In a step wise process, we determine:

- Compound potency against 5/6 species of *Leishmania*
- The permeation of compounds across un-and infected skin using *in vitro* and *in vivo* models, during which we have characterized (i) skin histology (ii) the integrity of the skin barrier by measuring the transepidermal water loss (TEWL) (iii) permeation of drugs with differing lipophilicity using Franz diffusion cells (FDC). Caffeine and ibuprofen (controls) and amphotericin B, paromomycin and buparvaquone were tested.
- Impact of formulations using miltefosine as an example, with different solvents and solvent combinations, through mouse skin using FDC. The topical formulations with the highest drug permeation or skin disposition were tested in mice infected with *L. major*.
- *In vitro* ADME evaluation of the most potent PK appropriate compounds precede *in vivo* studies in a mouse model.

3 Results

- Histology intact infected skin showed disruption of the skin structure and barrier. Both the increase in TEWL values as the lesion size progresses and the enhanced permeation for ibuprofen (2x), caffeine (66x), buparvaquone (0.2x) and paromomycin (548x) in infected compared to uninfected skin confirm this.
- The overall permeation of miltefosine through and into the skin was low (<4%) regardless of the solvents used. This was reflected in limited antileishmanial activity of the drug formulations when applied topically on infected skin *in vivo*.
- An approach to lead compound selection that included antileishmanial activity and the evaluation of 'drug-like' properties to optimize compound and formulation design for *in vivo* evaluation.

4 Conclusions

An in-depth understanding of how CL affects the physiology of the skin and drug exposure upon local administration is key to the development of a topical treatment against CL. More efforts are needed to screen new compounds against a panel of *Leishmania spp.* and pharmacokinetic parameters relevant to the targeted route of administration need to be included early on in the selection process to increase success rate.

C0533 PHARMACODYNAMICS OF THREE NOVEL ANTILEISHMANIAL LEAD SERIES AGAINST LEISHMANIA INFANTUM AND L. DONOVANI: IN VITRO 'TIME-TO-KILL' ASSAY

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1 Background

Current antileishmanial treatment regimens show particular limitations such as toxicity, treatment failure and drug-resistance issues, hence stressing the need for novel drugs. The Drugs for Neglected Diseases initiative (DNDi) is currently developing oxaboroles, aminopyrazoles and nitro-imidazoles as novel antileishmanial 'lead' series. To better understand the pharmacodynamics, an *in vitro* 'Time-to-Kill' (TTK) experiment was performed.

2 Methods

The TTK assay on intracellular amastigotes of the laboratory strains *Leishmania donovani* (MHOM/ET/67/L82) and *L. infantum* (MHOM/MA(BE)/67/ITMAP263) was performed with 6 representative compounds of the three chemical series and 4 reference drugs (miltefosine [MIL)], paromomycin [PMM], pentavalent antimony [Sb^V] and amphotericin B [AMB]). The in-test drug concentration was $5*IC_{50}$. TTK was defined as the minimal exposure time needed to eliminate all viable intracellular amastigotes. Activity and cytotoxicity were evaluated by the standard microscopic Giemsa assay in parallel with the promastigote back-transformation assay.

3 Results

MIL displayed the fastest cidal activity compared to the other reference drugs with a TTK of 72h compared to 168h, 288h and 192h for respectively PMM, SB^V and AMB. All selected DNDi lead compounds showed similar or better TTK than the reference drugs, with the nitro-imidazoles showing the fastest cidal action (TTK ≤72h). The results were fully comparable for both *Leishmania* species, except for the aminopyrazoles which could not reach a TTK within the 15 days of drug-exposure for *L. donovani*.

4 Conclusions

The DNDi lead compounds showed promising results as their TTK was similar or better than the reference drugs. The nitro-imidazoles reveal to be the more promising series with regard to TTK. Implementing multiple strains in the TTK assay is of importance as there might still be some species and/or strain-dependent effects, as observed for the aminopyrazoles.

C0702 USING ATP AFFINITY CHROMATOGRAPHY FOR LEISHMANIA DRUG TARGET DECONVOLUTION

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1 Background

In the past, most anti-leishmanial drugs were obtained by phenotypic screening on repurposing without optimization towards their targets, which led to important side effects. It is therefore crucial to identify the target of compounds to improve their selectivity. However, one of the major problems for pharmaceutical industry is to prioritize hit compounds for further development following high throughput screening. We thus developed an approach allowing the easy selection of compound that target ATP-binding based on their target specificity.

2 Methods

We used a proteomic approach combining ATP affinity chromatography, competition assays, 2D-DiGE and mass spectrometry (MS) to evaluate the specificity and discover the targets of kinase-biased hit compounds. These compounds were obtained after screening against casein kinase CK1.2 or *L. amazonensis* infected primary macrophages.

3 Results

We established a method based on ATP affinity chromatography to capture all the functional ATP binding proteins present in parasite protein extracts. We identified 1200 putative ATP-binding proteins (ATPome), including 80 kinases. Next, we combined the method with competition assays and 2D-DiGE to evaluate compounds specificity and reveal the drug targets based on the absence of proteins following competition. As a proof of principle, we tested two compounds: 5'Iodotubercidin (general kinase inhibitor) and D4476 (specific inhibitor). We observed numerous proteins absent or reduced in abundance when testing 5'Iodotubercidin, which indicates that many proteins were competed, thus demonstrating pleiotropic compound action. In contrast, only few proteins were competed with D4476, suggesting a more specific action. These finding confirmed the efficacy of the method. Finally we applied our approach on our four hit and validated them as specific compounds.

4 Conclusions

This robust, reproducible and native method allowed us to obtain the first ATPome in trypanosomatids and to develop an innovative drug target deconvolution method that can easily be applied to other pathogens or diseases such as cancer. Indeed, this biochemical method using ATP affinity chromatography coupled with 2D-DiGE analysis to determine the specificity of various compounds is quick, visual and semi-quantitative. It allowed us to prioritize some of our compounds based on their specificity toward their targets, which are currently determined by MS analysis.

C0854 IN VIVO IMAGING MODELS FOR VISCERAL AND CUTANEOUS LEISHMANIASIS

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1 Background

The development of new drugs against neglected tropical diseases (NTD) caused by kinetoplastids has for several years been hampered by profit-driven R&D. Through the efforts of WHO, DNDi and public-private partnerships the last decade has seen an increase in NTD drug discovery efforts with access to larger compound libraries and the chemical expertise for hit-to-lead optimization. This is yielding many new anti-parasitic compounds bringing with it the challenge of better compound prioritization at the discovery phase. Good murine models that provide reproducible results and predict clinical efficacy remains lacking particularly for leishmaniasis. We have previously developed an *in vivo* live murine imaging model for stage 2 human African trypanosomiasis (HAT) that report the presence of parasites through detection of *Trypanosoma brucei* expressing red-shifted luciferase (Myburgh *et al*, 2013 PLoS Neg. Trop. Dis. 7, e2384). This imaging approach has now been extended and modified to models of visceral and cutaneous leishmaniasis.

2 Methods

Clinical isolates of *L. donovani* from Africa and India, *L. infantum* from Brazil and *L. mexicana* have been modified to express red-shifted firefly luciferase - this improves light penetration from deeper tissue, allowing for better sensitivity and detection of parasites in lower density sites. We have monitored parasite distribution and burden in live mice using real-time longitudinal imaging. This is combined with *ex vivo* imaging of organs, limited dilution assays (LDA) and qPCR for accurate determination of parasite burden at endpoints.

3 Results

In vivo imaging identified genetically modified strains that retained virulence and emitted bioluminescence *in vivo*. Bioluminescent parasites were detected in target organs such as liver, spleen and bone-marrow for visceralizing strains, and in cutaneous lesions for *L. mexicana*. Parasite distribution and burden was verified by *ex vivo* imaging, LDA and qPCR. Our method is being validated using known leishmanicidal drugs and is being used to test efficacy of novel experimental compounds.

4 Conclusions

The imaging method enables monitoring of drug effects over time, the development of time-kill data *in vivo* and a reduction in the number of animals used.

C1151 FLAVONOL-LIKE COMPOUNDS IDENTIFICATION AS ANTILEISHMANIA AGENTS: CHEMISTRY, BIOLOGY AND TARGET STUDIES.

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1 Background

Current drugs in therapy against Leishmanisis show limited efficacy and drug resistance effects, therefore new drugs are urgently needed. A phenotypic approach was applied as a useful tool for drug discovery with the advantage of identifying compounds which are active against the whole cell. Among a library of natural products, flavonols such as Fisetin and Quercetin turned out to be potent antiparasitic compounds. Recently, we reported the antiparasitic activity of a library of classical flavonols (Borsari C et al JMedChem2016). Thus, the chromen-4-one moiety was confirmed a promising scaffold for the development of antiparasitic compounds. In the present work, we identifed a series of flavonol-like compounds and studied the biological profile and targets.

2 Methods

A libraryof 22 flavonol-like compounds was syntehsized, medium throughput screening performed.
Early ADME toxicity properties were assessed to ensure a safe profile.
Snapshot PK studies were performed and cyclodestrin improved the short half-life of the lead compound.
Further derivatives have been synthesized showing improved activities.
Target identification proteomic studies were used such as Mass Spectrometry and gel electrophoresis(GE)-based fluorescence spectroscopy.

3 Results

In the present study, a novel library of 22 flavonol-like compounds was synthesized and biologically evaluated. Compound CB80 showed an antileishmanial activity comparable to that of Miltefosine (EC50 vs L. infantum H80 = 1.9 μM, Milte = 3.2 μM). Early ADME toxicity tudies showed a safe profile. Snapshot PK studies were performed and cyclodestrin were used to improve the short half-life of the lead compound. Further derivatives have been synthesized showing improved activities. Target identifications studies using differential Mass Spectrometry appoaches combined with GE studies showed specific proteins modlutaion different from currently known protein targets. Drug resistance studies ithrough a genomic approach showed no drug resistance nd efficacy in Miltefosine resistant strains.

4 Conclusions

CB80, a flavonl-like compound, was identfiied as a promising anti-leishmania agent. It showed comparable activity with Miltefosine, no early toxicity effects. Proteomic studies and target identification process provide evidence of novel targets. CB80 shows no drug resistance development and is effective against anti-Leish drug resistant strains, including Miltefosine. Short half-life observed, suggested the synthesis of optimized compounds.

C1707 DISCOVERY OF ANTI-LEISHMANIAL COMPOUNDS FROM NATURAL SOURCES

Carolina Borsoi Moraes[1], *Laura M. Alcantara*[1], *Bethlehem Kebede*[2], *Markus Wolf*[3], *Deepa Karunakaran*[2], *Thalita C. S. Ferreira*[1], *Vanessa Fontana*[1], *Caio H. Franco*[1], *Claudia P. Bertolacini*[1], *Asaad K.M.A. Ahmed*[1], *Sheraz Gul*[3], *Lucio H. Freitas-Junio*[4], *Stephen Wrigley*[2]

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[4]*Instituto Butantan Brazil*

1 Background

There is an unmet demand for the discovery of new chemical entities for the treatment of leishmaniasis. Natural products provide a vast range of bioactive chemical diversity, and many anti-infective agents have a natural origin, including some antiparasitic drugs. The aim of this work was to identify untapped sources of novel antileishmanial compounds by exploring libraries of samples produced from fungal fermentations and African medicinal plants, both rich resources of secondary metabolites.

2 Methods

Two natural product libraries were assembled: the MycoDiverse™ library comprised 11,000 extracts from fermentations of both higher and entomogenous fungi; and a second library composed of 3,300 HPLC fractions generated from extracts of Sudanese medicinal plants. Both libraries were screened against *Leishmania infantum* in a high content screening assay. After hit de-replication step, the most active samples were prioritised by dose response profiling and the best hits subjected to a two-stage assay-guided fractionation process, followed by scaled-up production of active fractions for purification and structure elucidation of active compounds.

3 Results

The screening and assay-guided purification process has resulted in the identification of several compounds with promising antileishmanial activity of both fungal and plant origin. Structure elucidation of novel compounds is ongoing. Other compounds identified, such as diphyllin and carnosic acid derivatives from the plant extracts represent compound classes previously associated with anti-leishmanial activity, while the fungal cyclic depsipeptide cardinalisamide A has recently been reported as an antitrypanosomal agent. Several of the most promising compounds show a hit-like activity profile, with EC_{50}s below 10 µM and high efficacy (> 90%), and low cytotoxicity against THP-1 host cells. Test of novel hits against other *Leishmania* species and other protozoan parasites is under way.

4 Conclusions

Screening libraries of natural sources can be a rich source of new chemical entities for drug discovery. The molecules identified show hit-like profiles and are important starting points for the discovery of new antileishmanials.

This project has received funds from the New Medicines for Trypanosomatidic Infections (NMTrypI), Grant Agreement no. 603240 (http://www.nmtrypi.eu/)

Saturday, 20th of May
Miradero Room
09:30-11:00

Session 27. Control programs. National and international funding organizations

C0177 RISK FACTORS AND PREDICTION OF ZOONOTIC VISCERAL LEISHMANIASIS DISPERSION

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1 Background

Visceral leishmaniasis (VL) is an importante zoonosis transmitted by sandflies. In Brazil it represents a serious public health problem, and domestic dogs are consider the main parasite reservoir. The disease is expanding geographically in that country, and in São Paulo State it is spreading from northwest to sautheast, since its first human case in 1998. Some factors can contribute to this event, as the construction and proximity of Bolívia-Brazil gas pipeline, the use of Marechal Rondom Highway, socioeconomic conditions, climatic characteristics, and ecological imbalances. We developed a Bayesian model to analyze the temporal-geographic spread of vector and infected hosts in São Paulo State, in order to identify risk factors and and predict the VL dispersion.

2 Methods

We integrate data of the VL vector and infected dogs and humans in São Paulo municipalities since 1998 until 2013 through an innovative spatial temporal Bayesian model in conjunction with geographic information system. This model is used to infer drivers of invasion process and predict the future progression of VL through the State.

3 Results

It was observed that the vector dispersion was affected by the invasion pressure index (wich takes into account vector presence in nearby municipalities at the previous time step) and proximity to the gas pipeline. The proximity to the Highway and the vector presence affected the infected dogs, hence suggesting that climate changes could contribute to the disease spreading. Factors associated to the economy and altitude do not presented significant association with the disease expansion. The infected dogs together the vector presence affected the dispersion of infected humans, thus to avoid the increasing of human cases we suggest that the actions to prevent and control the VL in São Paulo State should still focus in reduction the vector -dog contact in endemic regions, and also in areas with potential risk to the VL agent insertion. Our prediction for VL vector and infected dogs and humans are indicating that their dispersion will be in direction where the risk factors are present in the State.

4 Conclusions

Identify risk factors and areas of high risk for the introduction of vector which transmit the VL agent and the infected hosts enable more geographically target prevention and control measures, thus contributing to public and animal health policy-making. Our model also can be applied in other areas where this zoonotic disease is spreading.

C0203 THE IMPORTANCE OF SYSTEMS ECOLOGY: IMPLICATIONS OF VECTOR-BASED FIELD STUDIES AND QUANTITATIVE ANALYSIS OF VECTOR CONTROL PROGRAMS.

David Poche[1], *Rajesh Garlapati*[2], *Zaria Torres*[3], *William Grant*[4], *Hsiao-Hsuan Wang*[4], *Shanta Mukherjee*[2], *Tyler Clarke*[3], *Aliana Norris*[3], *Lindsey Briley*[3], *Daniel Somers*[3], *Gregory Franckowiak*[3], *Rahul Chaubey*[2], *Tahfizur Rehman*[2], *Vinod Kumar*[2], *Epco Hasker*[5], *Richard Poch*[3]

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1 Background

Visceral leishmaniasis (VL) is a deadly parasitic protozoan, transmitted by phlebotomine sand flies. More incidence of VL occur in Bihar, India than any other region in the world. Indoor residual spraying (IRS) is currently and historically the only vector control practice performed in this region. Recent findings, regarding important aspects of sand fly ecology and common human social practice in villages in Bihar, suggest that supplementary vector control strategies, such as the application of systemic insecticides, may need consideration if VL is to be eliminated.

2 Methods

We discuss a quantitative sand fly model, two vector-related field studies investigating vegetation preference and lateral flight competence, and one house-by-house Bihari-village survey to determine sleeping habits of VL patients. The purpose of each study was to: a) model the dynamic relationship between fipronil-based cattle treatment and the *Phlebotomus argentipes* lifecycle, b) revisit previously published results suggesting adult *P. argentipes* to be arboreal, resting on outlying plants, c) monitor the short-term lateral flight distance of adult *P. argentipes.* and d) determine common sleeping practices amongst Bihari villagers at risk of sand fly bites.

3 Results

The above studies suggested that a) fipronil-based treatment has potential to reduce adult sand fly populations noticeably (95-97%) if performed 3-6 times per year, b) a proportion of sand flies are indeed arboreal and show a preference for banana plants (63%) over other vegetation types, and female Palmyra palm trees (63%) over male palms, c) most sand flies fly only short distances (~20m) with maximum distances rarely >150m, and d) >95% of villagers surveyed sleep outside at least part of the year (particularly during the hottest months).

4 Conclusions

These results suggest systemic insecticides could serve as a useful addition to the current IRS control programs, which due to the considerable number of sand flies and humans present outdoors during the night, may not have the maximal, desired impact on VL transmission. We do not argue against the use of IRS, but rather suggest that using systemic insecticides to target sand flies feeding on cattle, in conjunction with IRS to control indoor resting sand flies, increases the likelihood of successfully reducing VL instances. Further studies regarding vector ecology and human social practice will be needed to further shed light upon this complex system of interest.

C0998 IDENTIFICATION OF BOUNDARY SHIFTS FOR CAUSATIVE AGENTS OF CUTANEOUS LEISHMANIA FOCI IN NORTHEASTERN IRAN BY MOLECULAR AND IMMUNOLOGICAL TECHNIQUES

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1 Background

Cutaneous Leishmaniasis (CL) is one of the most important parasitic skin diseases which is endemic in many parts of Iran.

Khorasan-Razavi province, north east of Iran, has been known as an old important center for both forms of ZCL and ACL. Mashhad, Torghabeh-Shandiz, Neishabur and Sabzevar are endemic for ACL, while ZCL has been reported from Sarakhs, Lotfabad and Daregaz.

This study aimed to investigate on *Leishmani*a causative agents by new molecular and Immunological methods in different cities of this province which is essential to control and reservoirs combating.

2 Methods

This research was performed on more than 600 people with confirmed CL by direct slide examination. ELISA, electrophoresis isoenzyme, RAPD PCR and PCR are the selected methods to diagnosis the agents of CL in Mashhad, while PCR technique was only applied for Torghbeh-Shandiz, Neishabur, Sabzevar, Sarakhs, Lotfabad and Daregaz.

3 Results

Based on available data obtained from these methods, both *Leishmania* species caused CL, *L.tropica* and *L.major*, were observed in all the cities investigated, except of Torghbeh-Shandiz and Neishabur that have remained as ACL foci.

4 Conclusions

These emerged epidemiological changes in ZCL foci may be due to migration and travelling from neighboring countries such as Afghanistan, Turkmenistan and Iraq, rural to urban habitation, a rise in transportation rate to other parts of the country and even other countries in last decades. Furthermore, irregular constructions in cities, piling building debris and litters near residential areas which lead to increase of reservoirs of *L. major* and also carriers of both species.

C1268 VISCERAL LEISHMANIASIS CONTROL IN SOMALIA: ACHIEVEMENTS IN A CHALLENGING SCENARIO (2013-2015)

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1 Background

Visceral leishmaniasis (VL) due to *L. donovani* is endemic in war-torn Somalia. Diagnosis and treatment mainly rely on three treatment centres located in Bakool and Bay regions. We aim at describing the epidemiologic and clinical features of VL cases that were attended to in such centres between January 2013 and December 2015.

2 Methods

Our analysis is based on data routinely collected through a standardized template submitted on a monthly basis by the treatment centres to the central level of the National Control Programme and the WHO. The following variables were included: date of visit to the clinic, place of residence (region, district, village), patient category (primary, relapse), age, gender, number of months sick before clinic visit, Z-score (for children <5 y/o), rapid test (rK39) results, treatment regimen, treatment outcome. Descriptive analysis of primary VL cases was performed using Stata v13 and cases with identifiable villages were mapped using QGIS.

3 Results

Between January 2013 and December 2015, 3112 patients were tested for VL, 2927 were primary cases, 2 cases were relapses and 183 did not have information on patient category. Our analysis focuses on the primary VL cases. Most of these cases were male (61.3%) and below the age of 5 (60.2%). Among those <5 y/o with information available on nutritional status, 75% were regarded as suffering from moderate or severe malnourishment. On average, individuals spent 3.2 months sick before seeking treatment in these clinics. Overall, 78.5% of the cases tested rK39 RDT-positive and 16.9% tested negative but were considered VL cases based on clinical features. Regarding treatment regimen, almost 80% of the patients were treated with monotherapy, either sodium stibogluconate or meglumine antimoniate, and approximately 20% were administered a combination therapy with either medicines plus paromomycin. Among the patients whose treatment outcome was recorded, 97% of them were cured and 1.1% of them died. Regarding source of infection, 68% of cases were mapped at village level and originated from 292 villages. The most represented villages were Dhanaawe (6%) and Mada (5%) in the district Tieglow.

4 Conclusions

Our data show that in spite of the ongoing humanitarian crisis, the country is able to offer access to VL health services and manage the relevant epidemiological data. This is a major contribution towards the establishment of an adequate public-health response to VL.

C1277 STRENGTHENING LEISHMANIASIS SURVEILLANCE BY IMPLEMENTING AN ONLINE INTEGRATED PLATFORM FOR NEGLECTED TROPICAL DISEASES SURVEILLANCE AND CONTROL

***Lise Grout**, José Antonio Ruiz-Postigo, Marc Garnica, Mar Velarde-Rodriguez, Maya Allan, Mona Osman, Imane El Mghari, Daniel Argaw*

World Health Organization. Geneva, Switzerland

1 Background

Effective disease control needs an effective disease surveillance. Early case detection, prompt treatment and surveillance is critical for the neglected tropical diseases requiring case-management (CM/NTDs), including leishmaniasis. WHO has been working on the development and implementation of an online integrated surveillance platform for CM/NTDs for a more efficient and sustainable health information system.

2 Methods

The tool selected is the DHIS2 (District Health Information System, version 2), which is a flexible, open-source information system, for reporting, analysis and dissemination of health data, with visualization features including GIS, charts, and tables. DHIS2 has currently been implemented by aroun 50 countries, some of them being highly endemic for leishmaniasis.
In 2016 the minimum data and indicators to be collected and monitored have been standardized. Retrospective data has been imported in the online platform. Excel templates have also been developed to easily integrate data shared by countries that are not using DHIS2.

3 Results

Aggregated retrospective data has been imported for all the countries and individual retrospective data has been imported for Somalia and Morocco and dashboards have been created to monitor trends and share interpretation of computed indicators.
Four workshops has already been organized to present the online platform, discuss the standardized forms and indicators, and get feedback on the tool before the finalization of the first version by the end of 2016. In 2017, capacity will be built at all the levels of the surveillance systems, from health facilities to global level. The first countries planned to be trained for prospective data collection in the first quarter of 2017 were Kenya, Somalia and Uganda.

4 Conclusions

At national level, this online tool will strengthen the national health information systems, the disease surveillance at health facility level, and therefore strengthen the evidence-based decision making process. It promotes and improve the data standardization, collection, analysis and dissemination at national, regional and global level. At global level, this data warehouse eases the collection of good quality data, the trends analysis and the monitoring and evaluation of all the CM/NTDs in a single place. This platform will contribute to the process of validation or verification of the elimination goals.

C1690 ACCESS TO ANTILEISHMANIAL DRUGS IN THE AMERICAS: CHALLENGES AND OPPORTUNITIES

Carolina Batista[1], *Joelle Rode*[2], *Mady Malheiros Barbeitas*[1], *Marina Certo*[1], *Samantha Y. O.B Valadas*[3], *Nora Giron*[3], *Ana Nilce Silveira Maia-Elkhoury*[3]

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1 Background

Leishmaniasis is a neglected disease with different clinical forms. In the Americas, cutaneous leishmaniasis (CL) is endemic in 18 countries. According to PAHO/WHO (Pan American Health Organization - World Health Organization), 797,849 cases were reported between 2001 and 2014, with 75% concentrated in Brazil, Colombia and Peru. Visceral leishmaniasis is present in 12 countries in the region with 48,720 reported cases from 2001 to 2014, with 96.4% concentrated in Brazil. Presently, access and availability of antileishmanial drugs remain a challenge in the region. This research presents a critical assessment of the main access barriers for leishmaniasis in the Americas.

2 Methods

This study used the Frost & Reich access framework based on four axes: Architecture, Availability, Affordability and Adoption. Desk review was conducted in order to understand the main public health needs and gaps that ought to be addressed.

3 Results

The current drugs for leishmaniasis treatment not only present important toxicity and limited tolerance, but also face several access barriers. The number of suppliers is limited and production relies on demand, resulting in a high cost of treatments when purchased individually by countries. Meanwhile, some medicines are not included in many national lists of essential drugs and are not registered in many countries, making the purchase process difficult. As there are specific regulatory requirements and barriers in each country, drugs are often acquired and distributed through the PAHO Strategic Fund, which guarantees quality and reduces costs due to the consolidation of demand. Moreover, there is a lack of trained health personnel, of political representation of patient groups, and of advocacy campaigns. Furthermore, there is a need for a quick diagnostic tool for CL and for strengthening of surveillance systems and patient management. The PAHO/WHO recommendations are not fully adopted and patients are not aware of treatment options, while adverse drug reactions remain underreported.

4 Conclusions

Despite the progress achieved, there is still a need for new approaches and strategies to improve access to antileishmanial drugs in the Americas. It is important to promote regional consolidation of demand, and to negotiate better prices and supply system; as well as to include available treatments in national pharmacovigilance programs and promote their impact and appropriate use through the national health systems.

C1847 CLINICAL MENTORING BY MOBILE TEAMS - A NEW APPROACH TO VISCERAL. LEISHMANIASIS CARE IN RESOURCE-CONSTRAINED SETTINGS IN EAST AFRICA

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[6]*MSF The Netherlands*

1 Background

KalaCORE is a DFID-funded initiative aiming to reduce health impact and economic burden of visceral leishmaniasis (VL) in 6 highly endemic countries by ensuring access to early diagnosis and complete treatment. In Africa, VL puts high demands on the health system due to the complex diagnostic tree, long treatment courses requiring hospitalisation and frequent clinical complications due to the high prevalence of co-morbidities: malnutrition, anaemia, diarrhoea, pneumonia, malaria, HIV, and tuberculosis. VL occurs in settings where a lack of well-trained personnel and a high staff turn-over are common.

2 Methods

Clinical mentoring teams consisting of a clinician, one or two nurse(s) and a laboratory technician are deployed in South Sudan and Ethiopia to visit VL treatment facilities (implementation in Sudan is planned). Additionally, a phone number ('hotline') is available 24/7 to health workers for clinical and other queries.

3 Results

In South Sudan, two teams responded to outbreak rumours and provided VL care in remote and often highly insecure settings where in some cases barely the most basic health services were available. In 95 mentoring visits between February 2015 and October 2016, 60 facilities were supplied with VL drugs and/or diagnostics where needed, and bedside teaching and training addressed clinical skill gaps for more than 150 health workers.

In Ethiopia, three teams performed 44 mentoring visits to 18 health facilities in 5 endemic regions between June and November 2016. Clinical case reviews, ward rounds, quality control of laboratory tests and interviews of health staff were performed with the aid of standardized checklists. Shortcomings were found in all aspects of care: clinical and laboratory practices, nursing, reporting, VL supply gaps and absence of guidelines, SOP's and diagnostic and clinical algorithms. Findings were discussed on-site and bedside mentoring and training were provided where needed. Problems were documented in detail and followed up in next visits.

4 Conclusions

Clinical mentoring improves diagnostic and clinical skills of field staff in remote and resource-poor settings, addresses stock gaps and is expected to positively impact patient care and treatment outcome, It is therefore essential for strengthening VL control programs in the East African setting. Sustainability can be achieved by eventually identifying local clinical mentors within health facilities, similar to WHO's recommendations for clinical mentoring in HIV programs.

C1894 VISCERAL LEISHMANIASIS TREATMENT ACCESS - THE REALITY ON THE GROUND IN SUDAN

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1 Background

The KalaCORE project is a DFID-funded initiative to eliminate and control visceral leishmaniasis (VL) in Bangladesh, India, Nepal, Ethiopia, South Sudan and Sudan. The goal is to reduce the health impact and economic burden of VL by ensuring access to early diagnosis and complete treatment. In Sudan, the roll out of the VL Control Program is hindered by a lack of readiness of the hospitals diagnosing and treating VL.

2 Methods

Thirty-six hospitals in 10 states were visited and assessed via a tool developed by KalaCORE for collecting information on the readiness of health facilities for the provision of VL services.

3 Results

24 out of 36 (67%) of hospitals experienced stock-out of first line VL drugs in the 3 months previous to the visit, and in 29 out of 36 (81%) hospitals no trained staff member (trained in VL in the year previous to the visit) was present. In only one facility the laboratory was fully equipped for VL diagnosis and in most (34) hospitals the wards did not meet basic standards for clinical care of VL. Due to the lack of training, there was low awareness of the national VL protocol and malpractices in diagnosis and treatment were observed. In many cases, patients had to pay for diagnostic tests and VL drugs. The burden of cutaneous leishmaniasis (CL) in North and South Darfur and North and South Kordofan was much higher than previously thought, due to incomplete reporting. As a result of the assessment, several urgent measures were put in place by the Ministry of Health, supported by KalaCORE: immediate on-site training for senior medical doctors in each hospital; a VL 'hotline', a phone number reachable 24/7 for urgent clinical and other questions; immediate distribution of VL drugs and rapid diagnostic tests to be provided for free to patients; an urgent training for lab technicians in high-burden hospitals; an increase of provisions for cutaneous leishmaniasis; an orientation on VL for the State Coordinators; a rapid rehabilitation of the VL patient ward in Um El Kher hospital in Gedaref State.

4 Conclusions

Roll out of the VL control program in Sudan is dependent on the success of all aspects of implementation: an effective supply system for drugs and diagnostics, provided for free to patients and involving, educating and motivating health workers and other stakeholders on the ground.

C0849 A CLINICAL SEVERITY SCORING SYSTEM FOR VISCERAL LEISHMANIASIS IN HIV-NEGATIVE PATIENTS IN SOUTH SUDAN

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1 Background

South Sudan is one of the most endemic countries for visceral leishmaniasis (VL), and is frequently affected by large epidemics. In resource-limited settings, clinicians require a simple clinical tool to identify VL patients who are at increased risk of dying, and who need specialised treatment with liposomal amphotericin B and other supportive care. The aim of this study was to develop and validate a clinical severity scoring system based on risk factors for death in HIV negative VL patients in South Sudan.

2 Methods

A retrospective analysis was conducted of data from a cohort of 6,633 VL patients who were treated in the Médecins Sans Frontières (MSF) hospital in Lankien between July 2013 and June 2015. Risk factors for death during treatment were identified using multivariable logistic regression models, and the regression coefficients were used to develop a severity scoring system. Sensitivity and specificity of score cut-offs were assessed by receiver operating characteristic (ROC) analysis.

3 Results

In multivariable models, risk factors for death in adult VL patients were: anaemia (odds ratio (OR) 4.46 (95% CI 1.58-12.6) for Hb <6g/dL compared with ≥9g/dL), nutritional status (OR 4.84 (2.09-11.2) for BMI <13 kg/m^2 compared with ≥16 kg/m^2), weakness (OR 4.20 (1.82-9.73) for collapsed compared with normal weakness), jaundice (OR 3.41 (1.17-9.95)), and oedema/ascites (OR 4.86 (1.67-14.1)). For children and adolescents the risk factors were: age (OR 10.7 (6.3-18.3) for age <2 years compared with 6-18 years), anaemia (OR 7.76 (4.15-14.5) for Hb <6g/dL compared with ≥9g/dL), weakness (OR 3.13 (22.8-105.2) for collapsed compared with normal weakness), and jaundice (OR 12.8 (4.06-40.2)).The multivariable prediction model based on the regression coefficients for each of the risk factors resulted in a severity scoring with a predictive ability of 74.4% in adults and 83.4% in children and adolescents.

4 Conclusions

Our evidenced-based severity scoring system demonstrated sufficient predictive ability to be operationalised as a simple clinical tool for field clinicians for rational allocation of treatment to VL patients in South Sudan.

POSTERS

Tuesday, May 16th

Genetics, taxonomy and omics. Tools and reverse genetics

C0083 REVEALING THE MECHANISM OF COMPOUNDS WITH ANTI-LEISHMANIAL ACTIVITY: METABOLOMICS AS A TOOL IN THE DRUG DISCOVERY PIPELINE

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1 Background

With over 20,000 deaths and 300,000 new cases reported annually, visceral leishmaniasis is a serious infectious disease. As resistance to available drugs continues to rise, there is a huge requirement to improve treatment. Understanding the mechanism of action (MoA) of currently available treatments offers a means to explore *Leishmania* metabolism, understand the (in)effectiveness of current treatments and highlight suitable targets for novel treatments. Metabolomics offers an ideal approach to study drug MoA, especially for compounds where the target is unknown or where a polypharmacy effect is suspected. In this research, a set of 25 compounds from GlaxoSmithKline's 'Kinetoboxes' have been used to treat *Leishmania donovani* axenic amastigotes and analysed using metabolomics to cluster their MoAs.

2 Methods

A method for the analysis of single intracellular extracts on reversed-phase liquid chromatography mass spectrometry (RP-LC-MS) and capillary electrophoresis mass spectrometry has been developed. This methodology involved the optimisation of sample preparation and metabolite extraction as well as analytical methods, which were validated in a set of replicate parasite samples before being applied in the large-scale study of anti-leishmanial compounds.

3 Results

Different clustering approaches have enabled the identification of metabolic effects of different groups of the compounds on *Leishmania*. From the analysis of RP-LC-MS data representing effects on the lipidome, four main classes of lipids were found to be significantly affected by the drugs. These were sterols, phospholipids, fatty acids and sphingolipids. With respect to the more-polar metabolome, CE-MS data revealed key pathways distinguishing different compounds that included arginine/proline, histidine, lysine and methionine metabolism.

4 Conclusions

A novel approach for the simultaneous analysis of 25 compounds with anti-leishmanial activity has been demonstrated, highlighting the potential of metabolomics in the drug discovery pipeline. This methodology has the potential not only to study treatments for leishmaniasis or other kinetoplastid diseases but an array of different diseases for which novel treatments are required. This is a relatively low cost but information rich approach to determine MoAs of different compounds that have been shown to be largely unrelated to drug structure, and to develop the knowledge required to select compounds for further advancement in the drug discovery pipeline.

C0157 NULL MUTANT PREPARATION OF IRANIAN STRAIN OF LEISHMANIA MAJOR (MRHO/IR/75/ER) P27GENE KNOCKOUT AND EVALUATION OF ITS SAFETY IN BALB/C MICE

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1 Background

Cutaneous leishmaniasis (CL) is one of the world health problems. *Leishmania major* (*L .major*) is the etiological agent of zoonotic cutaneous leishmaniasis (ZCL). Promastigote and amastigote are two morphological forms of *Leishmania* parasites that express different antigens. p27 is an important gene encoding cytochrome c oxidase (COX) component. p27 gene expresses a 27 kDa protein that essential role in ATP synthesis. It was more abundantly expressed in amastigote and metacyclic promastigote forms of *Leishmania* parasites. This study aimed to produce *L. major* deficient in the p27 gene (Lmp27$^{-/-}$) mutant and its safety assay in BALB/c mice.

2 Methods

The molecular constructs were cloned in *Escherichia coli*, Top 10 strain and confirmed by various molecular methods. Amastigote forms of *L. major* were obtained through cell culture. The construct were transfected to amastigotes by electroporation. Double knockout *L. major* prepared through homologous recombination and clonal selection was done with adding different concentration of Hygromycin B. Final confirmation done by southern blotting. To evaluate safety, Lmp27$^{-/-}$ mutant inoculated at the base of BALB/c mice tails. Any side effect at injection site and parasite burden in spleen and liver of infected mice were measured and compared with positive control (Inoculated wild type strain).

3 Results

PCR and southern blotting methods confirmed that two alleles of the p27 gene in *L. major* were successfully removed and null mutant promastigotes of *L. major* are able to survive culture media, although their proliferation and differentiation are so slowly than wild type of the *Leishmania* parasite. Also evaluation of the prepared mutant in BALB/c mice has shown that Lmp27$^{-/-}$ mutant was not able to produce any skin lesion and *Leishmania* infection in the liver and spleen of the inoculated BALB/c mice 12 weeks after inoculation.

4 Conclusions

In conclusion, the produced p27 gene (Lmp27$^{-/-}$) mutant of *L. major* is safe in BALB/c mice, but require further improvement for vaccine application.

C0176 GENOMICS OF LEISHMANIA PANAMENSIS: INSIGHTS INTO THE UNIQUE GENETIC FEATURES OF THE L. (VIANNIA) SUBGENUS

Alejandro Llanes, Carlos Mario Restrepo, Ricardo Lleonart

INDICASAT AIP Panama

1 Background

Pathogenic *Leishmania* species are separated into two subgenera, *Leishmania* (*Leishmania*) and *L.* (*Viannia*). *L.* (*Viannia*) species are exclusive to Central and South America and cause predominantly cutaneous leishmaniasis, but parasites may occasionally migrate to the nasopharyngeal area, leading to the more severe mucocutaneous presentation. The first reference genome for a *L.* (*Viannia*) species, *L. braziliensis*, was published in 2007. Authors reported several genetic features apparently unique to this species, such as the presence of potentially active mobile elements and an RNA interference pathway. Here we go deeper into the unique genetic features of the *Viannia* subgenus by sequencing the genome of *L.* (*Viannia*) *panamensis*.

2 Methods

Genomic DNA from our PSC-1 reference strain of *L. panamensis* was sequenced by using the Roche's 454 and Illumina platforms. Reads were assembled *de novo* with Newbler and the assembly was improved with PAGIT tools. Annotation of pseudochromosomes was performed by combining automatic transfer of gene models from the *L. braziliensis* genome with *de novo* gene prediction, followed by manual revision. Analysis of protein-coding genes was done with OrthoMCL, Interproscan and Blast2GO. Illumina reads were aligned to the annotated pseudochromosomes with SMALT and the alignments were used for chromosome somy/gene copy number estimation with Samtools.

3 Results

We sequenced and characterized the genome of a second *L.* (*Viannia*) species, *L. panamensis*. Against a background of conserved gene content and synteny, we found 61 protein-coding genes shared by *L. braziliensis* and *L. panamensis*, but apparently not present in *L.* (*Leishmania*) species. Some of these possibly *Viannia*-specific genes appear to be involved in modification of surface glycoconjugates and resistance to oxidative stress, functions that are associated with the interaction of parasites with host/vector tissues and their survival within host macrophages. As previously reported for *Leishmania*, we observed several examples of genetic amplification in the PSC-1 genome, including variations in chromosome somy, possible episomal amplifications and specific gene arrays.

4 Conclusions

Sequencing the genome of *L. panamensis* allowed us to report distinctive genetic features shared by at least two *L.* (*Viannia*) species. Some of these features may contribute to the unique phenotypic characteristics of *Viannia* parasites, including their enhanced adaptive capacity and metastatic ability.

C0236 COS-SEQ AMASTIGOTE: NOVEL STAGE-SPECIFIC GENES INVOLVED IN DRUG-RESISTANCE

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1 Background

Increasing drug resistance towards first line antimony-derived compounds (Sb^V) has forced the introduction of novel therapies in leishmaniasis endemic areas including amphotericin B (AmB) and miltefosine (MF). However, their use is threatened by the emergence and spread of drug-resistant strains. In-depth knowledge of resistance mechanisms will help to design new therapeutic strategies and to overcome treatment failure. In order to discover stage-dependant resistance genes, which could be more relevant to the clinical disease, we have updated our Cos-seq approach through the introduction of macrophage infections in the pipeline.

2 Methods

A *L. infantum* population complemented with a *L.-infantum* cosmid library was submitted to increasing concentrations of MF, AmB and Sb^V in experimental infections of THP-1 cells. For each step of selection, amastigotes were extracted and cosmids were isolated. Reads were mapped to *L. infantum* genome using BWA. Enriched genes were then analyzed using RSEM and edgeR and grouped according to their enrichment pattern (Trinity). Untreated controls were used to normalize gene enrichment. Cosmids harboring predicted drug-resistance genes were recovered from the cosmid pool and re-transfected in sensitive *L. infantum* to confirm their phenotype in THP-1 infections. Genes were validated using single gene overexpression in wild-type parasites and/or gene disruption by using a novel CRISPR-Cas9 method.

3 Results

Cos-seq screen in amastigotes revealed 4 highly enriched loci for AmB (Chr 16, 26, 29 and 32), 6 for Sb^V (Chr 4, 6, 10, 16, 23 and 26) and 7 for MF (Chr 7, 10, 16, 29, 31, 32 and 33). Of these, 12 cosmids have been recovered and tested for resistance in promastigotes and amastigotes. Despite most of the them were exclusively involved in drug resistance in amastigotes, Sb^Vcosmid related to Chr 6 was responsible for resistance in both promastigotes and amastigotes. Gene LinJ.06.1010 was identified as responsible of this phenotype within this cosmid. This new gene is a leucine-rich repeat protein that seems to be involved in multidrug resistance phenotypes in *Leishmania*. MF and AmB highest levels of resistance were linked to Chr 10 and 16 (LinJ.16.0490), respectively.

4 Conclusions

This study has revealed the power of the Cos-seq approach to discover novel drug-resistance genes that are specific to the clinically relevant stage of *Leishmania*, as well as a novel stage-independent gene responsible for multiresistant phenotypes.

C0239 DISCOVERY AND DEVELOPMENT OF POLYMORPHIC MICROSATELLITES FROM LEISHMANIA PANAMENSIS USING NEXT GENERATION SEQUENCING, AND PRELIMINARY POPULATION GENETICS ANALYSIS OF FIELD ISOLATES CIRCULATING IN CENTRAL PANAMA.

Carlos Mario Restrepo Arboleda, *Alejandro Llanes, Carolina De La Guardia, Ricardo Lleonart*

INDICASAT AIP Panama

1 Background

The parasite *Leishmania panamensis* is the main cause of leishmaniasis in Panama. The disease is largely uncontrolled, with a rising incidence and no appropriate control measures. While microsatellites are considered some of the best genetic markers to study population genetics and molecular epidemiology in these and other parasites, none has been developed for *L. panamensis*.

2 Methods

We used the genome of *L. panamensis* strain PSC-1 for high-throughput microsatellite screening. MSATCOMMANDER v1.08 was used to screen the chromosome sequences for microsatellite motifs and for designing primers for each detected locus. Candidates for synthesis were selected by using several Perl scripts developed in-house. Microsatellite alleles were detected on a Genetic Analyzer 3130. Electropherograms were analyzed using GeneMarker v2.20. We have evaluated the sensitivity and specificity of the panel based on a sample of 27 isolates obtained from cutaneous leishmaniasis patients from central Panama and several reference species from both *L. (Leishmania)* and *L. (Viannia)* subgenera. The genetic equilibrium was assessed both intra- and inter-loci, while the reproductive mode was evaluated using several tests.

3 Results

The new set of microsatellites is composed of seventeen loci, mainly spanning trinucleotide or longer motifs. The new microsatellite panel shows high polymorphism and sensitivity, as well as good specificity showing no cross-reactivity with human DNA or with DNA from *Lutzomyia* sp. In general, our microsatellite panel showed good transferability to species of the *L. (Viannia)* subgenus. In contrast, there was a generalized lack of transferability for species of the *L. (Leishmania)* subgenus, with most of the markers giving blank genotypes or very low signals with out of range peaks. The preliminary data described here for *L. panamensis* suggest extensive departure from Hardy-Weinberg proportions, significant linkage disequilibrium and strong deficit of heterozygotes. Several recombination tests involving multilocus linkage disequilibrium and a phylogenetic approach allowed rejection of frequent recombination in our dataset.

4 Conclusions

The genome-wide strategy described here proved to be useful to identify and test new polymorphic microsatellite loci in *Leishmania*. The new panel of polymorphic microsatellites is a valuable contribution to the existing molecular markers for the study of genetic structure and other aspects of this important species.

C0314 GENETIC ANALYSES OF NUCLEAR-KINETOPLAST GENOMES IN NEW WORLD LEISHMANIA PARASITES ALONG THE ANDES REVEAL SPECIES DIVERSITY AND PATTERNS OF ALLOPATRIC SPECIATION

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1 Background

New World leishmaniasis is caused by parasites that show an extensive diversity from an evolutionary, ecological and clinical point-of-view. The two main species of *Leishmania* encountered in Peru illustrate this diversity. *L. braziliensis* is endemic in the Amazonian lowlands East of the Andes and could lead to severe muco-cutaneous lesions. In contrast, *L. peruviana* is mainly found on the Western slopes of the Andes and mostly causes mild cutaneous lesions. Early molecular karyotype work revealed patterns of genome erosion in *L. peruviana* that could possibly explain the decrease in pathogenicity. Here we analysed in detail the nuclear and kinetoplast genomes of both species in order to understand the implication of genomic variability in speciation and clinical outcome.

2 Methods

We have sequenced the nuclear and kinetoplast genomes of 116 *L. braziliensis* and *L. peruviana* isolates that were collected from patients presenting (muco-)cutaneous lesions in Peru and Bolivia.

3 Results

SNP calling revealed a total of 237,721 variable sites in 67 core *L. braziliensis* isolates, while only 32,110 variable sites were detected in 29 *L. peruviana* isolates. The genomes of L. peruviana were almost devoid of heterozygous sites, resulting in long tracts of homozygosity. Phylogenomic and population genomic analyses revealed strong population substructure in *L. peruviana* that matched biogeographical regions present along the Western slopes of the Andes. We found that the most northern *L. peruviana* population diverged firstly from *L. braziliensis*, followed by a southbound colonization of *L. peruviana* into each biogeographical region. Analyses of kinetoplast maxicircles revealed a discordant pattern of introgression, suggesting multiple occurrences of hybridization within both species. Finally, a large variation of chromosome and local copy number was observed among all isolates, with a significant number of small partial duplications (200-500bp) and deletions (100-800bp). This was accompanied by expansion/contraction of genes within several tandem arrays, accounting for genome erosion.

4 Conclusions

Our results confirm an allopatric speciation event of *L. peruviana* following colonization of the Western slopes of the Andes through the Porculla pass in the North of Peru, resulting in serious losses of genomic variability (i.e. founder effect).

C0362 COMPARATIVE GENOMICS OF CANINE-ISOLATED L. (LEISHMANIA) AMAZONENSIS FROM AN ENDEMIC FOCUS OF VISCERAL LEISHMANIASIS IN GOVERNADOR VALADARES, SOUTHEASTERN BRAZIL

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1 Background

Leishmaniasis is a highly diverse group of diseases caused by kinetoplastids of the genus *Leishmania*. Among the causative species in Brazil, *Leishmania (Leishmania) amazonensis* is an important etiological agent of human cutaneous leishmaniasis that accounts for more than 8% of all cases in endemic regions. Although *L. (L.) amazonensis* is generally found in the north and northeast regions of Brazil, recent reports indicate that this species has expanded to other areas. Here, we report the first isolation of *L. (L.) amazonensis* from dogs with clinical manifestations of visceral leishmaniasis in Governador Valadares in the southeastern Brazilian State of Minas Gerais where *L. (L.) infantum* is also endemic.

2 Methods

Lymph node aspirates were collected between 2008 to 2015 from domestic dogs with clinical VL symptoms from Governador Valadares. Parasites were isolated and genomic DNA was used to generate 350bp libraries. These libraries were then used to produce 100 bp paired-end reads by Illumina HiSeq. Reads were mapped with Bowtie2 into the *L. (L.) infantum JPCM5, L. (L.) mexicana U1103 and L. (L.) amazonensis M2269* reference genomes followed by SNP calling with Mpileup. Filtered SNPs where used for PCA and phylogenetic analysis whereas mapping information for gene and chromosome copy number variation.

3 Results

Our results show that most *L. (L.) amazonensis* chromosomes are disomic in contrast to other *Leishmania* species where genome plasticity and mosaic aneuploidy is a common trait. Gene copy number variations in relation to *L. (L.) mexicana* revealed species-specific expansions in proteins involved in cell differentiation, cellular trafficking and parasite host interaction. We also found evidence that the presence of *L. (L.) amazonensis* in southern Brazilian regions correspond to an ancient dispersion event rather than a recent introduction of this species, with a common ancestor with the northern strains 80,000 years ago.

4 Conclusions

To our knowledge, this article presents the first report of *L. (L.) amazonensis* in Governador Valadares and shows a potential risk for current control efforts in the area that have been designed considering only the presence of *L. (L.) infantum*. Given the different clinical presentations of human infections with *L. (L.) amazonensis* and *L. (L.) infantum*, surveillance and control activities in this region should consider the presence of *L. (L.) amazonensis* and address putative vectors for this species and the risks of co-infections in human subjects.

C0374 EPIDEMIOLOGICAL ANALYSIS OF L. TROPICA STRAINS AND CLINICAL SLIDES FROM SYRIAN AND TURKISH PATIENTS USING MULTILOCUS MICROSATELLITE TYPING (MLMT)

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1 Background

Turkey is located in an important geographical location by being the link between Asia and Europe in terms of epidemiology of vector-borne diseases. In Turkey, cutaneous leishmaniasis caused by *Leishmania (L.) tropica* is one of the major public health threats and according to the Ministry Health of Turkey, 45% of CL patients originate from ?anl?urfa province located in southeastern region of Turkey. First cutaneous leishmaniasis (CL) case reported in 1883 and 46.003 cases recorded between 1990-2010 in Turkey. Herein, we aimed to identify MLMT profile of *L. tropica* using the strains and slide samples obtained from Turkish and Syrian patients to reveal the epidemiological status of CL caused by *L. tropica* in Turkey

2 Methods

A total of 38 previously isolated and cryopreserved strains and 20 slide samples, both were obtained from Turkish and Syrian patients, were included in the present study. Genomic DNA was isolated using Qiagen DNeasy® Blood & Tissue Kit and multilocus microsatellites typing (MLMT) was performed using 12 highly specific microsatellite markers. Repeat numbers for each locus were recorded and Delta K (ΔK) calculation and Bayesian statistics was used for determining the population structure.

3 Results

Delta K calculations were showed three main populations (POP A, POP B and POP C) among all samples. Further analysis was revealed three subpopulations both for POP B and C, while no subpopulation was identified for POP A. One of *L. tropica* strain was viscerotropic, while the rest were dermatotropic. Descriptive analysis per populations (K:3) was also performed and population B was found to be the most heterogeneous group by having the highest number of alleles (3.00) and H_e (0.466) values and H_o (0.139) values and the mean number of alleles per populations was 2.3.

4 Conclusions

Findings of the present study suggest that, the epidemiological status of *L. tropica* is more complicated than expected and compared to previous studies. A new population, which comprised of Syrian *L. tropica* samples, was firstly reported for Turkey and data available here will provide epidemiological knowledge to further studies.

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C0440 LEISHMANIAC´S GUIDE TO GENOME ANALYSIS

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1 Background

Next generation sequencing technologies made it possible to explore *Leishmania* genomes, from single strains to natural populations. These studies highlighted the important plasticity of *Leishmania* genomes, among others through episomal/intra-chromosomal amplification and massive aneuploidy. Recent results also showed dramatic changes in genome structure during the life cycle and among individual cells (mosaic aneuploidy). This plasticity can hinder the proper identification of ploidy, copy number variations and base polymorphisms. In addition, technical biases in various sequencing steps and misinterpretations of sequence data can lead to erroneous conclusions. We describe how to avoid typical pitfalls in the *Leishmania* genome exploration.

2 Methods

We used two *Leishmania donovani* BPK282 reference genomes created using 454 Life Sciences Genome Sequencer FLX Titanium and SMRT WGS from Pacific Bioscience (PacBio) RSII. We evaluated four Illumina sequencing platforms, and Nextera XT and TruSeq SBS Kit v3-HS DNA library preparation kits.

3 Results

PacBio assembly improved the quality of the reference genome which increased the accuracy of genetic variant calling. The type of Illumina sequencing platform did not affect the accuracy significantly. DNA preparation kits, however, significantly affected the results. Genome diversity discovery was improved by taking the presence of frequent aneuploidy into account and by using data correction methods developed for single cell sequencing.

4 Conclusions

We demonstrate that DNA sequencing preparation kits and analysis methods can have significant impact on the accuracy of genome diversity analysis. We provide some practical guidelines for researchers who like to jump in or pursue *Leishmania* genome exploration.

C0470 THE 60KDA HEAT SCHOCK PROTEINS OF LEISHMANIA DONOVANI

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1 Background

Leishmania parasites must adapt to the changing environments of arthropods and mammals without the benefits of regulated transcription. This makes post-transcriptional events, i.e. protein synthesis and folding more important for regulation. For this, *Leishmania* spp express a wide array of chaperone protein families. One of those is type I chaperonin family, i.e. 60 kD chaperonins (CPN60), which are highly conserved throughout the *Leishmania* spp. There are four known CPN60 genes. CPN60.1 expression was below detection, while CPN60.2 (Schlüter et. al., 2000) and CPN60.3 (Silverman et. al., 2010b) were detected on the protein level. The latter two were also found to be part of the immune-modulatory exosomes, but little more is known about the role of CPN60 proteins in *Leishmania*. The aim of this study is to analyse the role of each of the four CPN60 genes in the *Leishmania* life cycle.

2 Methods

The encoding genes were replaced in *Leishmania donovani* using homologous recombination. Resulting null mutants were phenotypically analysed for specific changes, such as growth under different conditions, morphology and infectivity. For essential genes, the use of inducible diCre recombination is under way.

3 Results

CPN60.4 and CPN60.2 are non-essential genes in *L. donovani* promastigotes. While CPN60.2 null mutants did not show any specific phenotype under normal growth conditions, the adjacent gene for CPN60.3 (83% identity) was found to be essential in promastigotes. A CPN60.1 null mutant could also not be obtained so far. The phenotype of the viable null mutants during their intracellular stage is currently under investigation.

4 Conclusions

The comprehensive analysis of the null mutants for individual CPN60 genes will clarify their roles in the *Leishmania* life cycle.

C0471 MLTEFOSINE RESISTANCE IN MILTEFOSINE ADAPTED PROMASTIGOTES OF LEISHMANIA DONOVANI FROM NEPAL: GENOMIC, METABOLOMIC AND LIPIDOMIC CHANGES

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1 Background

Visceral leishmaniasis (VL) is a neglected tropical disease responsible for at least 40,000 deaths/year, the majority of which occur in the Indian subcontinent. There are a limited number of drugs available for chemotherapy of VL and development of drug resistance limits their clinical efficacy. In this study, we selected MIL resistant parasite (MIL-R) by growing parasites, with different inherent susceptibilities to trivalent and/or pentavalent antimony (Sb), in increasing levels of MIL. We compared the genome, metabolome and lipidome of wild-type (WT) strains and MIL adapted Sb resistant or Sb susceptible lines, to comprehensively evaluate the underlying mechanisms associated with MIL-R and whether inherent Sb resistance influenced results.

2 Methods

The IC50 of promastigotes and intracellular amastigotes was determined for MIL-R parasites and their corresponding WT. The genome of WT, parental MIL-R parasites and their MIL-R clones were compared using high depth genome sequence analysis. The metabolomics and lipidomic profile of promastigotes parasites was determined by separating lipids and metabolites using HPLC and mass spectrophometry to identify components present. The effect of MIL-R on MIL uptake was determined by quantifying MIL levels within parasites cultured in the presence of MIL for different time intervals (0-120 mins).

3 Results

The IC_{50} values of Sb S and Sb R strains did not increase until parasites were adapted to 12.2 µM MIL. Twenty-three metabolites were significantly upregulated in Sb S MILR clones compared with the Sb S WT parent. In contrast, only 12 metabolites were significantly different in a Sb R MIL R clone compared with the Sb R WT, with 9 being upregulated and 3 being down regulated compared to the WT. Nine metabolites involved in lipid metabolism were found to be the affected by selection of MIL resistance between the two strains and six were involved in the Kennedy metabolic pathway. Genome analysis indicated that the LdMT gene is important in MIL-R.

4 Conclusions

This study highlighted that adaptation to MIL first altering chromosome copy number and then induced a mutation in the LdMT gene when MIL selection pressure was increased. The results indicated that these changes were paralleled by metabolic and lipid changes which focused on the Kennedy pathway. There were more metabolite changes occurring in the MIL-R Sb S strain compared to the MIL-R Sb R strain, suggesting that Sb resistance was associated with a faster development of MIL resistance.

C0485 THE GENETIC BASIS FOR THE NATURAL ATTENUATION OF LEISHMANIA DONOVANI.

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1 Background

One of the central questions in *Leishmania* research is why some species cause cutaneous infections but others such as *Leishmania donovani* cause fatal visceral disease. Interestingly, *L. donovani* causes both visceral and cutaneous leishmaniasis in Sri Lanka.

2 Methods

L. donovani clinical isolates from Sri Lanka were isolated from a cutaneous leishmaniasis (CL) and from a visceral leishmaniasis (VL) patient and subjected to genome sequencing to identify genetic determinants associated with *Leishmania* tropism. A biological approach was also taken by performing Gain-of-Function (GOF) selection experiments where the attenuated CLstrain was repeatedly passaged through the spleen of mice over several months to selected forstrains with GOF for survival in the spleen. The regions of genetic change in the CL, VL and GOF strain were compared to identify sequences associated with survival in visceral organs. CRISPR- Cas was then used to perform functional genomic analysis to investigate the biological significance of the identified genetic differences.

3 Results

The CL isolate was severely attenuated in visceral infection in experimentally infected mice, while the VL isolate was highly virulent, demonstrating that these isolates were phenotypically different. Genome sequencing and transcriptome analysis revealed that these *L. donovani* strains were different, but were more similar to each other than to the reference *L. donovani* strain from Nepal. There were no gene deletions or pseudogenes specific to the CL isolate. Among the identified genetic differences, the A2 gene family had a higher copy number in the VL than the CL isolate and there were numerous SNPs. Experimentally increasing A2 expression in the CL isolate promoted parasite survival in visceral organs and removal of A2 genes using CRISPR-Cas resulted in a severe attenuation in visceral organs but not growth in culture. Among the SNPs identified, an amino acid change in the active site of the ras like RagC GTPase (R231C) enzyme in the TOR pathway also appears to have contributed to the attenuation of the CL parasite. Further analysis of the GOF strain will be presented.

4 Conclusions

Comparison of these closely related *L. donovani* parasites is an effective way to identify proteins required for visceral disease and also identifies mechanisms associated with attenuation through genetic change. This has far-reaching implications understanding parasite virulence and possibly for the development of attenuated vaccine strains.

C0519 DEVELOPMENT OF POLYMORPHIC MICROSATELLITE MARKERS FOR THE SAND FLY PHLEBOTOMUS PAPATASI (DIPTERA: PSYCHODIDAE)

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1 Background

Phlebotomus papatasi is a widely distributed sand fly species in different tropical and sub-tropical regions including the Middle East and North Africa. It is considered an important vector that transmits Leishmania major parasites, the causative agents of cutaneous leishmaniasis. The development of microsatellite markers for this sand fly vector is of high priority to understand its population structure and to monitor its geographic dispersal.

2 Results

Fourteen polymorphic microsatellite markers were developed with simple di-, tri- and tetra nucleotide repeats. The F statistics calculated for the 14 markers revealed high genetic diversity; He ranged from 0.407 to 0.767, while Ho was slightly lower and ranged from 0.083 to 0.514. The number of alleles sampled fall in the range of 9 - 29. No significant linkage disequilibrium was detected and high values for inbreeding coefficient (Fis) are likely due to inbreeding.

3 Conclusions

The development of these novel functional microsatellites enable high resolution of P. papatasi populations and opens a path for researchers to perform multi locus-based population genetic structure analyses as part of the efforts to understand the population dynamics of this vector in different countries, an important global strategy for understanding the epidemiology and control of leishmaniasis.

C0542 IDENTIFICATION OF NEW CANDIDATE RAPD MARKERS FOR TYPING LEISHMANIA DONOVANI COMPLEX

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1 Background

Leishmania donovani has been associated with different clinical forms. Identification of a genetic marker that can differentiate between L. donovani isolates from different clinical forms will strengthen the epidemiological studies and enable development of effective treatment and control programs. The current study aimed to identify new genetic markers for characterization of Sudanese L. donovani isolates using RAPD-PCR and DNA sequencing.

2 Methods

A cross sectional lab based study was conducted using 30 Leishmania parasite isolates from the cryobank of the immunobiology laboratory, originally collected from Gadaref , Khartoum and Northern states. Thirty *Leishmania* isolates used in this study were cultured from visceral, mucosal and cutaneous leishmaniasis patients. Parasite DNA was extracted using Phenol chloroform extraction method. kDNA PCR was done using genus specific primers for mini - circle kinetoplast (kDNA). Isoenzyme analysis was done for 13 enzyme loci. RAPD-PCR was used to amplify the DNA of the selected leishmania isolates using two primers OPA10 and OPB12. PCR products from parasite isolate representative of the three leishmania zymodemes *L.donovani* (MON18), *L.infantam* (MON30)and *L.archibaldi* (MON82), were sequenced commercially and the sequences were analysed using NCB-BLAST, restriction sites were predicted using Webcutter, Multiple sequence alignment and phylogenetic tree analysis was performed using Mega 6 software.

3 Results

PCR amplification of the kDNA identified 29 isolates as *L.donovani* and one isolate as *L.major*.Eleven DNA fragments sequences obtained and mapped on the databases.Blast results showed that the three of them were homologous to lysyl-tRNA synthetase gene within chromosome 15.While alignment result showed variation within the gene sequence between the three species. The analysis of the polymorphic region using webcuter suggested three restriction enzymes to be used in the genotyping of the isolates.The restriction enzymes were BglI,EcoRV and Eco321 showed different cutting patterns among the different species.The constructed phylogenetic tree depicted the relationship between the isolates.

4 Conclusions

Genetic differences between *leishmania donovani* zymodemes were detected using RAPD PCR and sequencing.The amplicon 1300b represented different sequences of the lysyl-tRNA synthestase gene, and could be used as a new genetic marker to categorize members of *lieshmania donovani* complex.

C0573 MODULATION OF ANEUPLOIDY IN LEISHMANIA DONOVANI DURING ADAPTATION TO DIFFERENT IN VITRO AND IN VIVO ENVIRONMENTS, AND ITS IMPACT ON GENE EXPRESSION

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1 Background

Aneuploidy is deleterious in multicellular organisms, but tolerated and potentially beneficial in unicellular organisms, including pathogens. *Leishmania* is emerging as a new model for aneuploidy: *in vitro* cultivated strains are highly aneuploid, with inter-strain diversity and intra-strain mosaicism. The alternation of two life stages in different environments (extra-cellular promastigotes and intra-cellular amastigotes) offers a unique opportunity to study the impact of environment on aneuploidy and gene expression, in absence of regulated promotors.

2 Methods

We sequenced the whole genome and transcriptome of *Leishmania donovani* strains, throughout their adaptation to *in vivo* conditions mimicking natural vertebrate and invertebrate host environments: golden hamster and *Phlebotomus argentipes* or *P. orientalis*, respectively.

3 Results

Nucleotidic sequences were quasi-unchanged within a strain, in contrast to aneuploidy. High in promastigotes, *in vitro*, aneuploidy dropped significantly in hamster amastigotes, in a progressive and strain-specific manner, but it was also accompanied by the emergence of new polysomies (among others on chr8 and 10). After exposure to the insect environment, smaller yet consistent karyotype changes were detected. Changes in chromosome copy number were correlated with the corresponding transcript levels, but additional aneuploidy-independent regulation of gene expression was observed. This concerned stage-specific gene expression, downregulation of the entire chr31, and selected genes on polysomic chromosomes, and up/down regulation of a complete polycistronic unit in chr5 (all snoRNAs).

5 Conclusions

Aneuploidy changes in *Leishmania* are likely adaptive and exploited to modulate gene dosage and expression; they are well tolerated, but a compensatory control mechanism may limit over-expression of specific genomic regions. Our model should allow exclusive studies on aneuploidy's impact on molecular adaptations and cellular fitness.

C0591 CONSENSUS MULTILOCUS SEQUENCE TYPING (MLST) SCHEME FOR WORLDWIDE LEISHMANIA SPECIES CLASSIFICATION

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1 Background

In leishmaniasis the differentiation of the parasites is relevant to eco-epidemiology, clinical diagnosis, and management of patients. Molecular identification of *Leishmania* parasites has been addressed using different kinds of techniques, where DNA based approaches offer multiple possibilities such as distinction among different isolates, their assignment to taxa, detection of *Leishmania* parasites, and detection of intra-specific diversity, and so on.

2 Methods

Twenty-seven gene fragments, previously published by other authors, were analyzed by *in silico* approaches for 14 *Leishmania* spp. A total of 35.2% of polymorphisms were observed. A scheme optimization was carried out by testing all possible combinations among the genes (MLSTest 1.0). Monophyly of the groups and branch support were maximized in order to select the best 15 gene combination. Primers were designed and amplified over 25 reference strains of the main *Leishmania* spp. for the final selection of 3 and 5 gene combination. Maximum likelihood and Neighbor-Joining trees were constructed in order to identify variation among different strains (MEGA 6). Congruence among trees was evaluated by using Templeton test.

3 Results

From the *in silico* approaches a global DP was calculated (0,994) and 15 gene candidates were selected: *ASAT, GPI, 6PGD, PPA, MKK, ICD, MPI, G6PD, PGM, HX, ACON, ALAT, ENO, PMM* and *Ch36-1190*. Those genes exhibited the best conditions for monophyly and branch support for every complex and species. All the genes were successfully amplified and two different MLST schemes were selected. The first scheme consists on: ENOL, PPA, GPI, 6PGD and HX. Meanwhile, the second MLST scheme was based on: ACO, ENOL and PPA. Both schemes exhibited good bootstrap values and showed the same phylogenetic relationships than the tree constructed with the 15 analyzed loci.

4 Conclusions

To our knowledge, this is the first attempt to obtain a consensus MLST scheme from all the proposed gene targets used until nowadays. These two levels of MLST proposed in the current work will be called as a nested Multilocus Sequence Typing. Further analysis including more strains will be done.

C0638 STAGE-SPECIFIC REGULATION OF PROTEIN ABUNDANCE IN L. DONOVANI BONA FIDE AMASTIGOTES AND VIRULENT PROMASTIGOTES.

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1 Background

Leishmania survival and pathogenicity depends on the parasite's adaptation to different host environments through stage differentiation of promastigotes within the sand fly, and of amastigotes inside mammalian host cells. The biology of stage differentiation has been previously analyzed using *L. donovani* promastigotes from long-term culture, and derived axenic amastigote-like parasites. However, the loss of virulence in these parasites and the important differences in biology between axenic and *bona fide* amastigotes primed us to investigate *Leishmania* stage differentiation under more physiological conditions. We applied systems-wide analyses at transcriptomic and proteomic levels to compare *bona fide* tissue-purified amastigotes obtained from infected hamsters with culture-derived virulent promastigotes shortly after *in vitro* conversion.

2 Methods

We combined High-throughput Sequencing of RNA with label-free quantitative proteomic to reveal *L. donovani* stage-specific expression profiles and gain new insight into post-transcriptional and post-translational regulatory mechanisms.

3 Results

RNAseq and Gene Set Enrichment Analyses identified significant increased transcript abundance for 387 genes in amastigotes with enrichment in functions for RNA binding and RNA processing, and for 991 genes in promastigotes showing enrichment in proteins with proteolytic and glycolytic activities. A total of 3805 proteins were quantified and significant increase in stage-specific abundance was observed in amastigotes and promastigotes for 561 and 1252 proteins, respectively. Proteomic and transcriptomic data comparison reveals a higher dynamic range for protein abundance with mean fold changes ranging from -2 to 2.9 versus -1.3 to 1.65 for transcript abundance. This comparison allowed us to distinguish two regulatory groups: (i) genes with a stage-specific increase or reduction that correlated in both transcript and protein abundance, suggesting regulation by RNA stability and (ii) genes with uncorrelated RNA and protein abundance in one of the stages, suggesting regulation at the level of translational efficiency or protein turnover.

4 Conclusions

This first systems-wide comparison of *L. donovani* tissue-derived amastigotes and virulent promastigotes reveals protein turnover as an important stage-specific regulatory mechanism.

C0699 GEOGRAPHICAL DISTRIBUTION AND EPIDEMIOLOGICAL CHARACTERIZATION OF LEISHMANIASIS IN THE MAGHREB THROUGH MULTILOCUS ENZYME ELECTROPHORESIS (MLEE) OF 674 LEISHMANIA STRAINS.

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1 Background

Leishmaniasis remains a major public health problem in the Maghreb's countries where several forms are endemically presents. Identification of the causative parasitic species is essential to determine the geographic distribution of each form in order to suitable control measures. MultiLocus Enzyme Electrophoresis (MLEE) is the gold standard method for epidemiological study of *Leishmania* species

2 Methods

A series of 674 *Leishmania* strains from, Algeria Tunisia Morocco and Libya, collected from different sources (humans, domestic and wild canines, rodents and phlebotomine sandflies) were identified by using MultiLocus Enzyme Electrophoresis (MLEE) at the National Center of *Leishmania* in Algeria. The isoenzymatic identification was performed by using 15 enzyme systems according to Rioux methods. The collected data were statistically analyzed.

3 Results

The enzymatic polymorphism is important in strains isolated from VL and sporadic CL. 8 zymodemes of *L.infantum* are detected*:* MON-1 (64.55%), MON-24 (24.86%), MON-80 (8.20%), MON-34 (1.06%), MON-77, MON-27, MON-33 (0.26%) and MON-78 (0.53%). All strains isolated from zoonotic CL were identified as *Leishmania major* MON-25. However, chronic cutaneous leishmaniasis is represented by 02 zymodemes of *L.*killicki (syn. *L. tropica)*: MON-301 and MON-8. The visceralization of some dermotropic Algerian and Tunisian strains was also observed (MON-24 (2.56%) and MON-25 (1.4%) in Algeria, MON-24 (3.91%) in Tunisia). Otherwise some visceral strains revealed an atypical polymorphism.

4 Conclusions

The limitation of discriminatory power of MLEE in these caseshighlighted the need of other molecular tools like Multilocus Sequence Typing (MLST) and Multilocus Microsatellite Typing (MLMT) to identify these atypical strains.

C0742 DEVELOPMENT OF A COMPUTATIONAL MODEL FOR VISCERAL LEISHMANIASIS DRUG DEVELOPMENT USING TRANSCRIPTOMIC AND IMMUNOLOGICAL DATA

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1 Background

Leishmania donovani and *Leishmania infantum* are responsible for a significant health burden worldwide, with 200,000 to 400,000 new cases of visceral leishmaniasis reported annually; however many aspects of the host-parasite interplay during infection have yet to be elucidated. CRACK-IT is an interdisciplinary project that brings together immunological, pharmacological and transcriptomic data in an attempt to understand host-parasite dynamics during infection and following treatment. Based on a published Petri net model of the host response during hepatic infection (Albergante et al *PLoS Comp. Biol*. 9(11):e1003334), we are constructing a new multi scale model of splenic infection that is expected to significantly reduce the numbers of animals required for pre-clinical evaluation of drug combination therapies (including immuno-chemotherapy).

2 Methods

The spleen is a non-resolving site of infection in murine models of visceral leishmaniasis and may better reflect immunological changes at the onset of treatment in humans than the liver. Hence, CRACK-IT is primarily focused on immune and drug interactions in the spleen. Mice were infected with either *Mycobacterium bovis* BCG or *Leishmania donovani*, infections that generate similar immunological responses, and administered either liposomal Amphotericin B or miltefosine at standard curative doses for *L. donovani* infection. Spleen samples from individual mice were analysed by RNAseq and the data analysed to define transcriptomic changes associated with drug treatment in the presence or absence (BCG) of microbicidal activity.

3 Results

Our hypothesis is that host differentially expressed (DE) genes unique to *L. donovani* infections will represent responses associated with altered pathogen load, whereas those common to both infections are more likely to correspond to immunological changes caused by or initiated by drug administration alone. We will focus discussion on ~450 DE genes identified to date, reporting on subsequent pathway analysis.

4 Conclusions

The CRACK-IT project encompasses a series of experiments, each exploring a key aspect of *Leishmania* infection and the response to treatment, and each providing data for parameterisation of a multiscale computational model. We believe understanding these confounding drug interactions is crucial for the integration of pathway level information into developing biologically robust models.

C0769 NOVEL MOUSE MODELS FOR RESISTANCE AND SUSCEPTIBILITY TO LEISHMANIASIS

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1 Background

Human leishmaniasis is characterized by a great diversity of manifestations. Mice provided models for many of them, but it has been believed that they cannot reflect some human clinical characteristics, such as the frequently observed mixed cytokine response to infection. Nitric oxide (NO) induction is also more difficult to demonstrate in humans than in rodents. In order to extend the range of models for the diverse phenotypes of human leishmaniasis, we analyzed previously untested strains O20 and B10.O20.

2 Methods

Response to *Leishmania major* was analyzed 8 weeks after infection. Cell infiltration to organs was studied in histological hematoxylin-eosin stained sections. Parasite load was determined by PCR-ELISA. Cell subpopulations in spleen were quantified by flow cytometry. Splenocytes and peritoneal macrophages were stimulated *in vitro* with soluble *Leishmania* antigen (SLA). Cytokines were quantified by ELISA. NO production was measured by Griess reaction.

3 Results

We describe novel unexpected phenotypes of leishmaniasis in strains O20 and B10.O20. B10.O20 was derived from the resistant strains C57BL/10 (B10) (96% of genome) and O20 (4% of genome) and surprisingly it is susceptible to *L. major*, as it exhibits large skin lesions, high parasite numbers in skin and lymph nodes, and a massive infiltration of $CD11b^+CD193^+$ and $CD11b^+Gr1^+$ cells into spleen. Surprisingly, after stimulation with SLA, splenocytes of B10.O20 produce significantly higher level of all Th1, Th2 and Th17 cytokines than both B10 and O20 strains, which indicates a chronic inflammation with imbalance of several axes of immune response.The highly resistant O20 mice also differ from all resistant strains tested until now, as their splenocytes did not respond to SLA by cytokine production and their macrophages did not produce NO, suggesting a novel mechanism of resistance.

4 Conclusions

Our data address and emphasize the need for using more mouse strains to analyze disease susceptibility in order to better reflect the phenotypes in human population. The responses of strains B10.O20 and O20 to *L. major* revealed novel disease phenotypes that have not been observed before in mouse, but were seen in several clinical studies, thus generating a more complete mouse model of leishmaniasis. We have observed that a small percentage of genes of resistant strain O20 on background of resistant strain B10 induces high susceptibility to *L. major.*

C0775 COMPARATIVE ANALYSIS OF THE PROTEOME OF LEISHMANIA (VIANNIA) BRAZILIENSIS NATURALLY RESISTANT OR SUSCEPTIBLE TO NITRIC OXIDE

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1 Background

Leishmania (Viannia) braziliensis is associated with various clinical outcomes of American Tegumentary Leishmaniasis (ATL) including self-healing localized cutaneous lesions, multiple disseminated lesions and metastasis to oropharyngeal mucosa. The production of cytokines, reactive oxygen species (ROS) and nitric oxide (NO) by host macrophages normally leads to the destruction of phagocytosed parasites. However, some parasite strains have been reported as resistant to that microbicide mechanism. The *L. braziliensis* strains used in this study were previously characterized as being naturally resistant or susceptible to NO. The resistant strain was also related to higher number of lesions than the susceptible strain, as well as to resistance to pentavalent antimony. However, the molecules and/or metabolic pathways of the parasite that contribute to such resistance are unknown. The aim of this work is to identify molecules associated with NO resistance that ultimately may be related to the different clinical manifestations caused by these polar strains.

2 Methods

Using qPCR and label-free mass spectrometry-based proteomics analyses, we conducted a comparative quantitative analysis of genes and proteins involved in metabolizing ROS and NO in these strains.

3 Results

L. braziliensis strains exhibit significant differences of NO-resistance *in vitro*, but not against ROS. Genes of the polyamines and the pteridines pathways and genes coding antioxidant enzymes were differentially expressed along the growth curve of the strains, as well as exhibited altered expression when parasites were exposed to treatment with NO. Statistical analysis of quantitative differences between the proteomes of the resistant and susceptible strains, treated or not with NO, revealed that they have significant differences (i) in their total protein content per cell; (ii) in the abundance of proteins involved in replication, transcription, translation, mitochondrial homeostasis and NO metabolism (iii) in the abundance of membrane transporters associated with resistance to antimony; and (iv) in the abundance of kinases and phosphatases.

4 Conclusions

Together, our results suggest that natural resistance to NO involves a differentiated cell proliferation, rapid and efficient stress response to NO in terms of protein synthesis and signaling, and a mitochondrion resilient to nitrosative stress that contribute to the phenotype of natural resistance against the response mediated by NO.

C0797 COMPREHENSIVE SELECTION AND EVALUATION OF REFERENCE GENES FOR BALB/C MICE SPLEEN SAMPLES IN THE LEISHMANIA INFANTUM INFECTION MODEL

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1 Background

The interaction of *Leishmania* with BALB/c mice induces dramatic changes in transcriptome patterns in the parasite, but also in the target organs (spleen, liver…) due to its response against infection. Real-time quantitative PCR (qPCR) is an interesting approach to analyze these changes and understand the immunological pathways that lead to protection or progression of disease. However, qPCR results need to be normalized against one or more reference genes (RG) to correct for non-specific experimental variation. The development of technical platforms for high-throughput qPCR analysis, and powerful software for analysis of qPCR data, have acknowledged the problem that some reference genes widely used due to their known or suspected "housekeeping" roles, should be avoided due to high expression variability across different tissues or experimental conditions.

2 Methods

In this paper we evaluated the stability of 112 genes using three different algorithms: geNorm, NormFinder and RefFinder in spleen samples from BALB/c mice under different experimental conditions (control and *Leishmania infantum*-infected mice).

3 Results

Our results show that most of the genes traditionally used as RG in this model (i.e. *B2m*(*Beta-2-Microglobulin*)*Polr2a* (*RNA polymerase II subunit A*)and *Tbp*(*TATA-binding protein*)) were constantly ranked in our experiments among the less stably expressed genes; therefore, and should not be used as normalization controls in RT-qPCR analysis, neither for control nor for *Leishmania*-infected BALB/c mice. In contrast, despite the natural heterogeneity of this model, non-classical genes were always ranked among the best scoring candidate RG for every group of mice and every algorithm used, which is indicative of their robustness as RG in gene expression analysis using a high-throughput platform in this experimental model.Finally, we have demonstrated that using "traditional" vs rationally-selected RG for normalization of gene expression data may lead to loss of statistical significance of gene expression changes when using large-scale platforms, and therefore misinterpretation of results

4 Conclusions

Our results highlight the need for a comprehensive, high-throughput search for the most stable RG in each particular experimental model. The use of "traditional" or non-evaluated RG in gene expression experiments may drive scientists to extract flawed conclusions from their research.

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C0815 INDUCIBLE PROTEIN STABILIZATION SYSTEM IN LEISHMANIA MEXICANA

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1 Background

Leishmania are protozoan parasites causative agents of leishmaniases. They alternate between two developmental stages. The flagellated extracellular promastigotes live in the gut of sandflies, while the intracellular non-flagellated amastigotes reside inside the macrophage phagolysosomes of vertebrates.
Targeted regulation of protein levels is an important tool to investigate the role of proteins essential for cell function and development. In recent years, methods based on the *Escherichia coli* dihydrofolate reductase (ecDHFR) have been established and used in various cell types. ecDHFR destabilizes the fused protein of interest and causes its degradation by proteasomes, unless it is stabilized by a specific ligand. In this work we developed an inducible protein stabilization system in *Leishmania mexicana* in which a target protein is stabilized upon Trimethoprim/Trimethoprim-lactate (TMP/TMP-lac) treatment.

2 Methods

For development of the transgenic *L. mexicana* strains, we used the pLEXSY vector encoding Green Fluorescent Protein (GFP) fused to the ecDHFR on its C-terminus. Transgenic cells with this cassette integrated into their 18S rRNA locus were analyzed by fluorescence microscopy and Western blotting.

3 Results

The wild type ecDHFR-based system which was successfully established in *Trypanosoma cruzi*, turned out to be non-functional in *Leishmania.* This probably reflects intrinsic differences between *Trypanosoma* and *Leishmania* species.In order to use the system in *Leishmania*, we tested a series of point mutations in ecDHFR and identified one which provided almost complete degradation of the target protein before induction and substantial stabilization after the TMP/TMP-lac treatment.

4 Conclusions

The inducible protein stabilization system in *L. mexicana* provides us with an opportunity to study function of various proteins (including toxic ones) in *Leishmania.*

C0819 MOLECULAR CHARACTERIZATION OF AN EMERGING L.DONOVANI POPULATION IN THE INDIAN SUB-CONTINENT: ëYETIí PARASITES IN NEPALESE HIGHLANDS

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1 Background

Leishmania donovani causes visceral leishmaniasis (VL) in the Indian subcontinent (ISC). The main population (P), predominant in the last ISC epidemics, emerged around 1850, spread over the region and is genetically very homogeneous. We discovered a genetically distant group of parasites in Nepalese highlands, where more new foci of transmission were recently reported. The so-called 'Yeti' strains (Y) show different patterns of virulence and drug susceptibility than P-ones and are more abundant nowadays in Nepal. We undertook a deep molecular characterization of the Y-strains, by integrative analysis of their genome and metabolome, in comparison to those of P-strains.

2 Methods

DNA and metabolites were extracted in parallel for 4 P and 5 Y strains. Genomes were sequenced using the Illumina HiSeq to identify SNPs, ploidy variations and local CNVs. After chloroform/methanol/water extraction metabolites were measured on the Orbitrap Exactive platform. Data analysis was performed in R using the XCMS and mzMatch packages.

3 Results

Y- and P-strains strongly differed on both the genomic and metabolic levels. We identified over 45,000 different SNPs between Y- and P-genomes. Gene amplifications specific to P include MAPK1, argininosuccinate synthase (ASS), ABC-transporter MRPA; in contrast some specific amplifications were present in Y, like a number of hsp70, GP63 and amastin genes. In total, 294 metabolites were detected of which 39 showed significantly (FDR adjusted $p<0.05$) higher levels (>2 fold increase) in Y, while 33 showed higher concentrations in P. Major differences were observed in lipid metabolism with 35 differentially regulated glycerophospholipids between Y and P. Additional changes were observed in arginine metabolism: in Y, an 11.1 fold decrease in succinyl-L-glutamate and 3.5 fold decrease in ASS.

4 Conclusions

The Yeti group is genomically and metabolomically totally distinct from the main population of *L. donovani* that was the main driver of the last VL epidemics in the ISC. Among several differentiating characters, arginine metabolism was highlighted by both genomic and metabolomics approaches. Previous work showed a link with virulence and resistance against oxidative stress. These data support the phenotypic differences we encountered so far between Y- and P-strains. Particular attention should be paid to monitor the fate of this emerging population, particularly in a post-elimination context.

C0943 MILTEFOSINE RESISTANT FIELD ISOLATE OF INDIAN KALA-AZAR: ISOLATION, IDENTIFICATION AND CHARACTERIZATION

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1 Background

Visceral Leishmaniasis (VL) or Kala-aar (KA), one of the neglected tropical diseases, is caused by the protozoan parasite, *Leishmania donovani.* Pentavalent antimonial drug was introduced in Indian subcontinent for the treatment of Indian KA approximately nine decades ago. The drug has become ineffective due to emergence of resistance in parasites to the drug. Miltefosine (MIL) , an oral drug, in now in clinical use but within years, reports of unresponsiveness of the patients to the drug have started coming out. In the present study, drug sensitivities of the recent clinical isolates (n=26) to MIL and other drugs have been investigated.

2 Methods

We have collected the clinical isolates (n=26) of Indian KA from the field. Among them, one isolate seemed to be a putative MIL- resistant (MIL-R) *Leishmania donovani* by in vitro amastigote-macrophage model. The expressions of MIL transporter (LdMT) and the β subunit of LdMT (LdRos3) genes were studied for all. We have extended our work in *in vivo* hamster model: one group of animals were infected with MIL-R isolate while other two groups of animals were infected with MIL sensitive isolates respectively. Appropriate dose of MIL was given orally to all infected groups of animals and different biological, immunlogical and molecular biological parameters were checked.

3 Results

One field isolate of Indian KA showed EC50 for MIL of 13.47 +/- 0.87 μM, almost 4 times higher than that of the MIL-S isolates and the isolate was considered as putative MIL-R. The expressions of LdMT and its β subunit in this particular isolate were found downregulated. The in vivo hamster model showed reduction in liver weight only by 17.48% as compared to 35.99% and 44.38% reductions in animals' liver infected with MIL-S isolates and then treated with the MIL. Retention of parasite loads of about 51.4% in spleen and liver of the animals infected with MIL-R isolate and then applied treatment with MIL. The splenocytes of this MIL-R group of animals failed to show antileishmanial T cell proliferation and there was no enhancement of cell mediated immunity.

4 Conclusions

It may be concluded that this putative MIL-R field isolate is resistant to the drug, Miltefosine. This is first such kind of report of isolation, identification and characterization of naturally occurring Miltefosine resistant field isolate of Indian KA.

C0997 ISOLATION OF NOVEL TRYPANOSOMATID, ZELONIA AUSTRALIENSIS SP. NOV. (KINETOPLASTIDA: TRYPANOSOMATIDAE) PROVIDES SUPPORT FOR A GONDWANAN ORIGIN OF DIXENOUS PARASITISM IN THE LEISHMANIINAE

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1 Background

The genus *Leishmania* includes approximately 53 species, 20 of which cause human leishmaniais; a significant albeit neglected tropical disease. Leishmaniasis has afflicted humans for millennia, but how ancient is *Leishmania* and where did it arise? These questions have been hotly debated for decades and several theories have been proposed. One theory suggests *Leishmania* originated in the Palearctic, and dispersed to the New World via the Bering land bridge. Others propose that *Leishmania* evolved in the Neotropics. The Multiple Origins theory suggests that separation of certain Old World and New World species occurred due to the opening of the Atlantic Ocean. Some suggest that the ancestor of the dixenous genera *Leishmania*, *Endotrypanum* and *Porcisia* evolved on Gondwana between 90 and 140 million years ago.

2 Methods

In the present study a detailed molecular and morphological characterisation was performed on a novel Australian trypanosomatid following its isolation in Australia's tropics from the native black fly, *Simulium* (*Morops*) *dycei* Colbo, 1976. Phylogenetic analyses were conducted to confirm the relationship between this novel Australian parasite and other trypanosomatids.

3 Results

Phylogenetic analyses confirmed this parasite as a sibling species to *Zelonia costaricensis*, a close relative of *Leishmania* previously isolated from a reduviid bug in Costa Rica. Consequently, this parasite was assigned the name *Zelonia australiensis* sp. nov. Assuming *Z. costaricensis* and *Z. australiensis* diverged when Australia and South America became completely separated, their divergence occurred between 36 and 41 million years ago at least. Using this vicariance event as a calibration point for a phylogenetic time tree, the common ancestor of the dixenous genera *Leishmania*, *Endotrypanum* and *Porcisia* appeared in Gondwana approximately 91 million years ago.

4 Conclusions

Ultimately, this study contributes to our knowledge of trypanosomatid diversity, and to our understanding of *Leishmania* origins by providing support for a Gondwanan origin of dixenous parasitism in the Leishmaniinae.

C1022 COMPARATIVE GENOMIC ANALYSIS OF CUTANEOUS AND VISCERAL SUB-STRAINS OF LEISHMANIA DONOVANI IN SRI LANKA

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1 Background

Leishmaniasis is a complex infectious disease that affects millions of people around the world. Its clinical manifestation ranges from cutaneous leishmaniasis (CL) characterized by self-curing skin lesions to fatal visceralization in internal organs. *Leishmania donovani* causes potentially fatal visceral leishmaniasis (VL) in endemic parts of Asia and Africa, while an atypical *L. donovani* sub-strain is suggested to cause mainly cutaneous and few visceral forms of the disease in Sri Lanka. However, the genetic basis of these sub-strains causing different pathologies, is not well understood and yet to be confirmed at a wider population level of parasites. Here we describe sequencing of whole genomes of six CL (CL-SL) and two VL (VL-SL) clinical isolates from Sri Lankan patients using Illumina MiSeq technology.

2 Methods

Comparative analysis was done to identify the genetic background of Sri Lankan *L. donovani* isolates and how it differs from *L. donovani* isolates from visceral infections elsewhere. Analysis was initially focused on the detection of single nucleotide polynorphisms (SNPs), insertions and deletions (indels), pseudogenes and copy number variations. Reads were mapped to *L. donovani* BPK282A1 reference genome using BWA-mem. Resulted BAMs were cleaned, sorted, indexed, validated and duplicates were marked using GATK, Picard and Samtools. SNPs and indels were called using GATK's HaplotypeCaller. Hard filtering of raw variants resulted in a final high quality set of SNPs and indels which were annotated using snpEff.

3 Results

We found 1,119,459 SNPs shared by both CL and VL isolates against *L. donovani* BPK282A1 reference, while 545,229 and 317,951 SNPs were unique in CL and VL isolates respectively. A total of 81,380 indels were shared among the CL and VL. Indels unique in CL were 66,849 while 56,896 indels were unique in VL. 540,303 SNPs in CL and 313,989 SNPs in VL were detected in protein coding regions. Altogether 584 SNPs in CL and 258 in VL were predicted with high putative impact/deleteriousness.

4 Conclusions

The SNP/indel differences among CL-SL and VL-SL isolates suggest that they may represent two different sub-strains of *L. donovani* and suggest the possibility that these differences may contribute to different phenotypes associated with them. Further analysis including pseudogenes, gene and chromosomal copy number variations is needed to assess if these differences are conserved at a wider population level.

C1030 POPULATION STRUCTURE OF LEISHMANIA (VIANNIA) BRAZILIENSIS ACROSS THE AMAZONIAN RAINFOREST OF PERU

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1 Background

L. (V.) braziliensis is the main causative agent of cutaneous leishmaniasis in the Peruvian Amazon basin where the disease is endemic and there is lack of appropiate control measures. Herein, we evaluated the potential of microsatellite markers to study the population genetics and molecular epidemiology of these parasites between high and low jungle regions of Peru in order to understand the contribution of human activities to the parasite population dynamics.

2 Methods

We analyzed 124 *L. (V.) braziliensis* strains from highland jungle (Junín, Cusco, and Ayacucho) and lowland jungle (Loreto, Ucayali, and Madre de Dios). *Leishmania* DNA extracted from culture was used for microsatellite analysis of 10 neutral markers (AC52, B3H, Ibh3, E11, 7GN, EMI, LBA, ARP, G09 and LRC) that were pseudo-multiplexed for capillary electrophoresis and analyzed on GeneMapper v4.0. Genetic variability and Hardy-Weinberg Equilibrium (HWE) were estimated using the software Cervus v3.0, Genepop v4.0 and GenAlEx. Factorial Correspondence Analysis and Structure v2.2 were used for Bayesian clustering analysis whereas Migrate-n v3.6.11 and Bayes factor were used to estimate the migration model and rates.

3 Results

Our highly informative microsatellite markers (polymorphic information content PIC=0.790) prove to be useful to study the population structure of *Leishmania*. These markers indicate that there is a high genetic diversity in *L. (V.) braziliensis* (14.9 alleles per locus), with mean observed (Ho) and expected (He) heterozigosity of 0.6694 and 0.8161, respectively. In addition they showed that the lowland jungle was constituted by a single cluster with uniformity of alleles, whereas the highland jungle presented alleles from the lowland region mixed with an additional pre-established cluster as a possible event of gene flow.

4 Conclusions

Our study proved the usefulness of microsatellites for assessing the molecular epidemiology of *L. (V.) braziliensis* and indicated that there is an important genetic variability in the parasite population across Peruvian Amazonian rainforest regions. The differences between geographic regions and distribution of parasite clusters underline the importance of human activities on parasite population dynamics and underscore the need to design better surveillance and control strategies.

C1048 PROTEOMIC ANALYSIS OF LEISHMANIA MAJOR PROMASTIGOTE AND AMASTIGOTE

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1 Background

Leishmania (Leishmania) major is etiological agent of cutaneous leishmaniasis. This parasite exhibits two different forms during the life cycle: the extracellular promastigote present in sand fly and *in vitro* culture and the intracellular amastigote. These two parasite forms present morphological and metabolic differences. The aim of this study was to analyze qualitative and quantitative differences between *L. major* promastigote and amastigote using a global proteomic approach.

2 Methods

L. major promastigotes were grown in standard culture and the amastigotes were isolated from infected macrophages. Promastigotes and amastigotes were lysed with a FASP protocol and total protein was obtained, quantified and fractionated. The fractionated protein was digested with tripsin and lysinC to generate peptides that were analyzed in a Thermo LTQ-orbitrap. Data analysis was performed using software package from Orbitrap Thermo software and the proteins were identified by Maxquant software using uniprot database.

3 Results

We have identified 5073 different proteins in promastigote and 80 different proteins in amastigote. Only 11 proteins were expressed exclusively in amastigote and they seemingly are related to genetic information processing and cellular processes. Four of these 11 proteins are hypothetical proteins that suggested to be related to ubiquitination. In addition, four isoforms present in amastigote are not present in promastigotes and further these present in amastigote are shorter than those seen in promastigote. One protein showing different sizes in promastigote and amastigote is suggested to participate in the interaction with the cell. From those 69 proteins found in both forms, 56 are significantly more expressed in amastigotes. They are likely involved in different functions such as Protein turn-over immunomodulation protein synthesis, Glycolitic pathway, Contractile vacuole, Cell redox homeostasis, Vesicle traffic and Signal Transduction .

4 Conclusions

Different proteins or isoforms are expressed in specific form during life cycle of *Leishmania* that may exert specific functions that would be further addressed.

C1192 DE NOVO GENOME ASSEMBLY OF LEISHMANIA (VIANNIA) GUYANENSIS AND LEISHMANIA (VIANNIA) SHAWI

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1 Background

The protozoa of *Leishmania* genus are responsible for different clinical manifestation of leishmaniosis, zoonosis worldwide distributed. *Leishmania* species pathogenic to humans are classified into two subgenera: *Leishmania*, that occur in the Old and New World, and *Viannia*, present only in the Americas. We aimed at sequencing, assembling and annotating the genomes of *Leishmania* (*Viannia*) *guyanensis* and *Leishmania* (*Viannia*) *shawi* in order to get more information and contribute for further studies of these species.

2 Methods

Paired-end DNA sequencing of *Leishmania* (*V.*) *guyanensis* (MHOM/BR/1975/M4147) and *Leishmania* (*V.*) *shawi* (MHOM/BR/1967/PH8) was done on the Illumina MiSeq® platform, with eighty-fold coverage. The genome assemblies were performed using SPAdes as the assembler, after the preassembled scaffolds had been extended with the SSPACE software. Protein annotation was performed by Exonerate software, using other species of annotated *Leishmania* to find the proteins, and in Augustus. For similarity analysis, the BLASTp algorithm and the proteins of *Leishmania* (*V.*) *braziliensis*, *Leishmania* (*V.*) *panamensis*, *Leishmania* (*Leishmania*) *major* and *Leishmania* (*L.*) *donovani* were used. Domain analysis also used these *Leishmania* species and the RPS-BLAST algorithm in the Conserved Domain Database (CDD).

3 Results

We obtained 3,999 scaffolds for *Leishmania* (*V.*) *guyanensis* and 5,032 scaffolds for *Leishmania* (*V.*) *shawi*, with 8,459 and 8,900 noted proteins, respectively. Above 95% protein sequences similarity was observed among *Leishmania* (*V.*) *guyanensis*, *Leishmania* (*V.*) *shawi*, *Leishmania* (*V.*) *braziliensis* and *Leishmania* (*V.*) *panamensis*. When we compared *Leishmania* (*L.*) *major* and *Leishmania* (*L.*) *donovani* proteins it revealed similarity of 75%, showing a higher identity within the same subgenus. In the protein domains analysis we found 10,061 domains shared between L. (V.) guyanensis, L. (V.) shawi and L. (L.) major, while L. (V.) shawi, L. (V.) guyanensis, L. (V.) braziliensis and L. (V.) panamensis shared 10,374 domains. L. (V.) braziliensis was the holder of the highest number of singularities with 120 domains while L. (V.) guyanensis, the lowest presenting 23 unique domains.

4 Conclusions

The assembly of the genomes of these *Leishmania* species amplifies the knowledge of similarity between them supporting even more its classification in distinct subgenera. The information generated can contribute for further studies of these species.

C1236 GENOMIC INSIGHTS INTO THE UNUSUAL POPULATION GENETICS OF LEISHMANIA

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1 Background

Genomic data is starting to provide unprecedented resolution of the genetics of *Leishmania* populations. We now have sufficient data from different populations and species of *Leishmania* to compare their population genetics directly, without the complication that different molecular markers are used in different species. The high density of markers from genome sequencing lets us understand patterns and processes of recombination and selection.

2 Methods

In an extensive series of collaborations, we have now generated whole-genome sequence data for over 900 *Leishmania* isolates from a number of different species.

3 Results

These data confirm our understanding of the evolution of the genus and the genome varaition within Leishmania. In particular, these data have clarified that natural populations of Leishmania are having sex, but that the importance of sexual reproduction occurs seems to vary between different populations: this might correlated with the ecology of the parasites involved. We have some evidence that even the mechanisms of sex might vary - the pattern of recombination in some cases looks like standard meiosis, but in others resembles what we expect from a parasexual process. This complexity is compounded by the presence of extensive aneuploidy, where chromosome copy number varies rapidly between isolates and even within isolates. This impacts the interpretation of population genetic patterns in *Leishmania*: for example, we show that the observed deficit of heterozygosity can be explained by aneuploidy rather than frequent inbreeding.

4 Conclusions

We now have extensive data on genome variation in many Leishmania species. While this gives us a good starting point to understand these populations, these data have all been generated from existing isolates in culture. It is not clear to what extent these collections represent the Leishmania populations circulating in patients and files in the field. To generate more clinically and epidemiologically relevant data, the next step is to be able to sequence directly from clinical samples in well-characterised cohorts of patients. This work was only possible due to an extensive network of collaborations: too many people have contributed to the work I will describe to list all of them as co-authors, but I will acknowledge these critical contributions during the presentation.

C1257 IDENTIFICATION OF DIFFERENTIALLY EXPRESSED PROTEINS BETWEEN ISOLATES OF LEISHMANIA MEXICANA CAUSING LCL AND DCL BY LABEL-FREE PROTEOMICS

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1 Background

Leishmania mexicana can cause two forms of clinical diseases: a benign localized cutaneous leishmaniasis (LCL) characterized by ulcers at sites of parasite inoculation, and the highly destructive and invasive form, diffuse cutaneous leishmaniasis (DCL) characterized by intensely parasitized macrophages within nodules that spread uncontrollably throughout the skin.
In order to understand the differences in virulence and growth rate that LCL and DCL parasites present in vivo and in vitro, we performed a proteomics study (label-free bottom-up).

2 Methods

10 Isolates of Leishmania mexicana were directly obtained from cutaneous lesions of patients with LCL (5 samples) and DCL (5 samples). Total protein was extracted from promastigotes in stationary phase.
For each sample, 2 ug of protein (reduced, alkylated, and trypsin-digested) were injected to the nano LC-MS/MS: Easy-nLC 1000 - Q-Exactive plus mass spectrometer.
Sequest and X! Tandem were searched against Leishmania mexicana database:

- UniProt-Jul-9-2016[8044 proteins]
- Non-redundant sequences from Genome- Transcriptome of L. mexicana project (internal project, still under revision) [880 proteins].
- 155 common contaminants
- 9079 Reverse Decoys.

Scaffold was used to validate MS/MS based peptide and protein identifications:

- Peptide identifications >95.0%
- Protein identifications > 90.0% and at least 2 identified peptides.

NSAF (Normalized Spectral Abundance Factor) was used for normalization. t-Student test was performed between DCL and LCL groups.

3 Results

39 non-annotated proteins from L mexicana Genome-RNASeq project were confirmed by proteomics.
779 proteins were consistently identified in both groups. 10 and 3 proteins were exclusive to LCL and DCL, respectively.
58 Differentially expressed proteins (DCL-LCL), log2FoldChange > 1 and pValue<.05

4 Conclusions

Promastigotes in the stationary phase of DCL and LCL have a differential protein profile, especially in energetic metabolism .
Functional validation must be performed in order to assess the results. Proteins found in this study that could help to explain phenotypic behavior in DCL and LCL isolates:

- Cysteine Peptidases (Clan CA, family C19; clan MF, family M17)
- FKBP-type peptidyl-prolyl cis-trans isomerase putative
- Putative ATP-dependent Clp protease subunit, heat shock protein 100
- High mobility group protein homolog tdp−1(E9ALZ2_LEIMU)
- p-glycoprotein (E9B4S1_LEIMU)

The work was funded by INMEGEN 144-2013-06-26, FINNOVA-CONACyT 242368 and CVU 467639.

C1258 COMPARATIVE GENOMICS OF TUNISIAN LEISHMANIA MAJOR ISOLATES CAUSING HUMAN CUTANEOUS LEISHMANIASIS WITH CONTRASTING CLINICAL SEVERITY

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1 Background

Zoonotic cutaneous leishmaniasis caused by Leishmania (L.) major parasites affects urban and suburban areas in the center and south of Tunisia where the disease is endemo-epidemic. Several cases were reported in human patients for which infection due to L. major induced lesions with a broad range of severity. However, very little is known about the mechanisms underlying this diversity. Our hypothesis is that parasite genomic variability could, in addition to the host immunological background, contribute to the intra-species clinical variability ob- served in patients and explain the lesion size differences observed in the experimental model.

2 Methods

Based on several epidemiological, in vivo and in vitro experiments, we focused on two clinical isolates showing contrasted severity in patients and BALB/c experimental mice model. We used DNA-seq as a high-throughput technology to facilitate the identification of genetic variants with discriminating potential between both isolates.

3 Results

Our results demonstrate that various levels of heterogeneity could be found between both L. major isolates in terms of chromosome or gene copy number variation (CNV), and that the intra-species divergence could surprisingly be related to sin- gle nucleotide polymorphisms (SNPs) and Insertion/Deletion (InDels) events. Interestingly, we particularly fo- cused here on genes affected by both types of variants and correlated them with the observed gene CNV. Whether these differences are sufficient to explain the severity in patients is obviously still open to debate, but we do believe that additional layers of omic information is needed to complement the genomic screen in order to draw a more complete map of severity determinants.

4 Conclusions

With its extraordinary genome plasticity, Leishmania is able to face and adapt to drastic environmental changes, and this behavior is spe- cies-, isolate-, or population-specific. This study relates a sum of geneticdifferences between parasites isolated from patients with ZCL living in an endemic country and causing differential and contrasted clinical outcomes, but also contrasted pathogenicity in BALB/c experimental mode. The reported differences point out to their possible relationship to parasite pathogenicity. However, applying additional high-throughput approaches would help draw a systems-level view complementing the present whole-genome sequencing results.

C1269 DETERMINATION OF GENETIC DIVERSITY OF LEISHMANA TROPICA BY USING MULTILOCUS MICROSATELLITE TYPING (MLMT) IN SANLIURFA, TURKEY

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1 Background

Leishmaniasis, which has been ranked second after malaria as the most important tropical disease in the list published by the World Health Organization, is a disease that is caused by the parasites of Leishmania species, transmitted by the bite of the Philabotomines. It affects 350 million people in over 88 countries. In Turkey, especially cutenous leishmaniasis (CL) is endemic and Sanliurfa has the highest endemicity rating in Turkey in terms of CL; the yearly case number is around 2000. Especially, CL caused by *Leishmania* (*L.*) *tropica* is a major public health threat in Sanliurfa. Despite the measures taken to keep the infection under control, incidence of the disease has not decreased, and even predited to increase in the future due to the arrival of Syrians to the region. In 2013, 2,393 (1635 indigenous + 758 Syrians) cases and in 2014, 1583 (1033 indigenous + 550 Syrians) cases were reported from Sanliurfa. In Sanliurfa, the only causative agent identified to date has been Leishmania tropica which is known to be transmitted antroponotically. However, it is thought that *L.tropica* species that circulate around the region are showing intra-species differences. This study was done to see if indigenous strains of *L. tropica* isolated from human cases of CL are genetically identical to or different from Syrian strains of *L. tropica* isolated from human cases of CL and to see if any genetic differences found in population of L.tropica circulating in Sanliurfa.

2 Methods

To obtain isolates, clinical specimens taken from CL patients were inoculated in NNN. Then, diagnosis and species identification was done by ITS1-PCR-RFLP. Multilocus microsatellite typing (MLMT), employing 12 independent genetic markers specific to L. tropica, was used to determination of genetic polymorphisms and population structures of fifty strains of L. tropica isolated from 38 indigenous and 12 Syrian CL patients in Sanliurfa. The products were carried out on PAGE (Poliakrilamid gel elektrophorez). Tape lengths and differences between them on the imaging device were determined.

3 Results

All genotypes were detected among the 50 strains of *L. tropica*analysed were identical genotypes. Most of them were shared by more than one strain among indigenous and Syrian populations of L.tropica.

4 Conclusions

The propagation of L. tropica appears to be predominantly clonal. Immigration of population may contribute to the development of overlapping, genetically distinct populations of L. tropica in this region

C1306 ASSOCIATION OF VITAMIN D RECEPTOR GENETIC POLYMORPHISM WITH VISCERAL LEISHMANIASIS IN INDIAN POPULATION

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1 Background

Vitamin D receptor polymorphism has been studied in various inflammatory and infectious diseases. Since, Indian population have distinct evolutionary history, the aim of the present study is to explore the association between vitamin D receptor (VDR) gene polymorphisms and Visceral Leishmaniasis (VL).

2 Methods

A total of 176 cases and 202 control samples were included for current study from endemic region of India. We performed Sanger sequencing of the respective region of VDR containing SNP marker FokI (rs2228570), BsmI (rs1544410), TaqI (rs731236) and ApaI (rs7975232).

3 Results

In the controls, all the 4 variations, except rs7975232, were observed in Hardy-Weinberg equilibrium; (1) rs2228570 (P-value = 0.1178), (2) rs1544410 (P-value = 0.6454), (3) rs731236 (P-value = 1) and (4) rs7975232 (P-value = 0.01177). P-value in allele based model were not associated with VL; rs2228570 (P-value = 0.3675), rs1544410 (P-value = 0.5968), rs731236 (P-value = 0.6345) and rs7975232 (P-value = 0.8177). Further, we did not observe any variation associated with VL in both single variation and haplotype based association analysis. .We consider the P-value <0.0125 (Bonferroni corrected P-value = 0.05/4) as significant P-value in statistical analysis.

4 Conclusions

Our analysis revealed that none of 4 variations in VDR were associated with VL in Indian population. To validate the association between VDR gene variation and Leishmaniasis, we are Sanger sequencing the regulatory, UTR and exons regions and will be presented during conference.

C1314 EFECT OF IRON DEPLETION ON LEISHMANIA (VIANNIA) BRAZILIENSIS: ULTRASTRUCTURAL AND PROTEOMICS ANALYSES

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1 Background

Although phagolysosomes are a hostile environment, *Leishmania* amastigotes have developed mechanisms that allow them to resist acid pH and capture host nutrients essential for their survival. One such nutrient is iron which can be purchased as inorganic iron or coupled to porphyrins such as heme and hemoglobin. Iron is essential for the production of energy through the electron transport chain and the oxidative phosphorylation. In addition, *Leishmania* parasites need iron to generate infective forms, colonize host cells and develop skin lesions in mice. This study aimed to analyze the effect of iron depletion on the cell growth, ultrastructure, and protein abundance of *L. (V.) braziliensis*, the main etiological agent of American Tegumentary Leishmaniasis.

2 Methods

Promastigotes were treated with 2,2-dipyridyl and cellular density and iron uptake were determined. Parasites' ultrastructure was analyzed by transmission electron microscopy after treatment with the iron chelator. In addition, flow cytometry and fluorescence microscopy of TMRE, PI and TUNEL labeled parasites were performed in parasites treated or not with the iron chelator. Gene expression of iron transporters was analyzed by qPCR and global protein abundance was determined by a label-free quantitative mass spectrometry-based proteomics approach.

3 Results

Our results showed that iron depletion generates a multifactorial response that initiates by the interruption of cell proliferation and culminates in a marked mitochondrial impairment. This impairment is characterized by the decreased activity of the electron transport chain complex IV which leads to both the reduction of oxygen consumption and the depolarization of mitochondrial membrane potential. However, while some parasites die due to mitochondrial dysfunction, others can resist nutritional stress and survive. It was observed that promastigotes increased mRNA and protein levels of iron (LIT1) and heme (LHR1) transporters in iron depleted medium.

4 Conclusions

The increased expression of iron transporters and antioxidant defenses suggest that parasites can enhance or maintain the intracellular concentrations of the metal allowing part of the population to resist the nutritional stress, survive and resume proliferation. In addition, our results suggest a resilient and plastic role of the parasites' mitochondrion.

C1328 WHOLE GENOME SEQUENCING OF BRAZILIAN LEISHMANIA INFANTUM PROMASTIGOTES. A SNP MAP AND VARIATION ANALYSIS ASSOCIATED WITH CLINICAL OUTCOMES.

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1 Background

Leishmania infantum is the etiological agent of visceral leishmaniasis (VL) in Brazil. Transmitted to humans by the sand fly bite of the species *Lutzomyia longipalpis*, this protozoan causes a clinical spectrum of VL in Brazil that varies from asymptomatic cases to fatal outcomes. VL is currently present in almost all States of the Brazil except for Acre, Amapá, Amazonas and Rondônia in the North and Santa Catarina in the South and presents a growing lethality of 7.8%. Infectious complications and hemorrhages are the main risk factors for death in VL. The role of the parasite in these processes is uncertain, but it is believed that virulence factors and mechanisms of gene regulation and expression may contribute to the maintenance and/or aggravation of the disease. The objective of this work was to the whole genome sequencing of L.infantum promastigotes obtained from culture of biopsyof patients with fatal VL to compare with isolates from patients with non-severe Kala-azar in addition to mapping polymorphisms of those strains.

2 Methods

The strains were obtained from bone marrow biopsy of patients. The strains were grown in NNN (Novy-MacNeal-Nicolle) solid medium supplemented with Schneider's liquid medium with 10% FBS and 2% urine. All collection procedures were approved by the Research Ethics Committee of the Federal University of Piauí under the number 0116.0.045.203-05. Sequencing by Illumina Hiseq2500 101bp paired-end lane (~60Gb/lane), TruSeq DNA PCR-Free library kit. Bioinformatics analyzes were done with in-house scripts and open source tools. Alignment with the reference genome *L. infantum* clone JPCM5 (MCAN/ES/98/LLM-877) (TritrypDB ver 9.0) was done to generate the map of polymorphisms. The sequencing was done at the company Macrogen Inc (Korea).

3 Results

An average of 99% of Mapped Sites with reference (> = 1x) and 98.32% of Mapped Reads, deep 60. Of the 43 samples analyzed, we found an average of 2010.9 SNPs, 697.4 Insertions and 860.8 deletions. The SNP map was generated for all samples.

4 Conclusions

The map of assembled polymorphisms may reveal significant changes in association with severe VL outcomes. We found deletions associated with drug resistance in fatal cases and variations in the number of copies in chromosomes only in samples from fatal cases. New analyzes are under way and we believe that new findings will bring light to the study of severe visceral leishmaniasis.

C1408 WHOLE GENOME SEQUENCING TO STUDY GENOMIC VARIABILITY OF LEISHMANIA INFANTUM PARASITES IN TUNISIA AND ALGERIA IN CORRELATION TO CLINICAL PLEOMORPHISM

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1 Background

Leishmania infantum (*L.i*) species is endemic in Tunisia and Algeria and is responsible of visceral (VL), and sporadic cutaneous (CL) leishmaniasis. This clinical pleomorphism results from a complex interplay where parasite genetic determinants play a crucial role. Whole genomes of several *Leishmania* reference species are now available. Although they constitute a referential source of data on chromosome and gene organization, specific mechanisms in relation to different epidemiological or clinical traits could not be addressed. Our aim is to investigate *L.i* intra-specific genomic diversity with reference to clinical form: VL vs. CL, using NGS.

2 Methods

L.i strains (N=25) isolated from VL (N=14) or CL (N=11) patients, in Tunisia (N=17) and Algeria (N=8) were *in vitro* cultivated and DNAs extracted. Indexed paired-end libraries were prepared using the Illumina Nextera™ kit and DNA sequenced with the HiSeq Illumina technology. Different bioinformatics scripts, programs and pipelines were used or home- made to analyze ploidy, copy number variations (CNV), mutations and phylogenies.

3 Results

Upon mapping of reads on *L.i* JPCM5 reference genome, normalized chromosome read depth was calculated to infer ploïdy, showing a mosaic structure with largely diploïd chromosomes and specific tri, tetra or pentasomic ones. Heatmaps clustered the strains according to disease form independently of geographical origin.
CNVs were analyzed using read depth for CL vs. VL comparison. Statistical analysis using a specific modeling strategy (event wise testing) generated lists of under or over represented genes found in each of the 25 *L.i* genomes.
A homemade pipeline (involving GATK, SNPeff, SNPphylo and other scripts) was developed to identify mutations and allelic variations at the genomes level, and to establish genome phylogenies. The parasites again clustered according to clinical presentation independently of their geographical origin. Mosaïcism was confirmed at the allelic level. Pair wise VL and CL strains comparisons of SNPs are ongoing.
A selection of strains will be further studied for validation and transcriptome analyses.

4 Conclusions

This study brings insights into *L.i* genomic variability and its mechanisms, and resources for biomarkers identification. Ultimately genomic signatures associated to clinical or phenotypical traits will be identified, that could further translate into control strategies including diagnostics.

C1432 EVALUATION OF THE GENOTYPIC PROFILE OF LEISHMANIA LEISHMANIA INFANTUM IN BRAZIL

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2 Background

In Brazil visceral leishmaniasis (VL) is caused by *Leishmania (L.) infantum*, however different genotypes could be involved the transmission cycle of parasite or regarding clinical manifestaions. *Leishmania* has high gene plasticity, being able to adapt to its host to develop better. Different genotypes of *L. infantum*, related mainly to the transmission area and the reservoir, are described. Analyzing different strains of *L. infantum* by means of PCR - RFLP (Polimerase Chain Reaction - Restriction Fragment Lenght Polimorphirsm) of the minicircle of kDNA was observed the presence of different genotypes coming from different reservoirs of geographic areas of Brazil. In this project, the genotypic profile of *Leishmania (L.) infantum* present in bone marrow samples from patients from Rio Grande do Norte - Natal were analyzed. Our main objective is to find different genotypes of *L. infantum* and to analyze if there is any relation with the geographical area of origin or with the clinical outcome of the patient.

3 Methods

For standardization, *L. infantum* (MHOM/BR/1972/LD46) culture promastigotes were used to use as a positive standard. Patients samples came from Natal - RN, with diagnosis of VL, without HIV infection. The project was submitted to the Research Ethics Committee of all participant research centers, the use of the Informed Consent Form was dispensed, because the material used, was previously collected for the diagnosis. The extracted DNA was amplified using primers LINR4 and LIN19. After an amplification, positive samples were digested with RsaI (10U / ul) and HpaII (10U / ul) enzymes and after was made capillary electrophoresis.

4 Results

The patterns of bands obtained in previous studies were convergent with the patterns found when PCR - RFLP of samples of culture promastigotes. Patients samples presented some bands that were conserved when compared to the promastigote sample. However, they presented additional bands of varying sizes, maintaining homogeneity among patient samples, of wich 50% of the analyzed samples had the same pattern of bands, 17% presented a single pattern and 33% presented another uniform pattern.

5 Conclusions

Preliminary data show that within the same geographical region, it is possible that there are different *L. infantum* genotypes, considering that all individuals acquired the disease in Rio Grande do Norte, however, more samples will be analyzed to confirm this data, using samples from other regions from Brazil.
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C1434 FUNCTIONAL GENOME WIDE ASSOCIATION SCAN OF IL-10 RESPONSE OF DOGS NATURALLY INFECTED WITH LEISHMANIA INFANTUM

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1 Background

High IL-10 levels suppress phagocytes and have been associated to enhancement of parasitism in natural infection of dogs by *Leishmania infantum*. The aim of this study was to identify relevant chromosomal regions for IL-10 response in *ex vivo* culture of canine peripheral blood mononuclear cells (PBMC) stimulated with *L. infantum* antigen.

2 Methods

We conducted a genome wide association study (GWAS) of 110,165 SNPs in 127 dogs [20 IL-10 (+) and 107 IL-10 (-)], using variance component model (EMMAX) corrected for significant covariate origin.

3 Results

IL-10 level was positively correlated to parasite load in lymph node ($r = 0.416$, $p < 0.01$). Control of stratification effects due to sample structure was evidenced by the inflation factor approximately 1.0. Three markers were significant ($p < 10^{-5}$) on chromosome 17. Jointly, these markers explained 11% of phenotipic variance. Top SNP BICF2G630213974, $p = 1.04 \times 10^{-6}$ with an estimated odds ratio of 5.27 ± 4.42, frequency of 40% in IL-10 (+) dogs and 11% in IL-10 (-) dogs. This marker mapped on position CFA17:26778592 to approximately 255 kb near from the gene *LTBP1*, whichis involved in targeting of TGF-b to extracelular matrix where it is stored or activated, and to 209 Kb from the gene *RASGRP3* that is involved in down-regulation of proinflammatory cytokines, especially IL-6, induced by toll-like receptors in macrophages.

4 Conclusions

Genomic findings of IL-10 response point to relevant regions associated with T regulatory response and parasite spread.

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C1443 GENOMIC ANALYSIS OF LEISHMANIA (VIANNIA) BRAZILEINSIS ISOLATED IN PERNAMBUCO, NORTHEASTERN BRAZIL.

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1 Background

American Tegumentary Leishmaniasis (ATL) presents a wide spectrum of clinical manifestations depending on the species of Leishmania involved, the relationship with the host, the genetic and immunological constitution of the patient, vectors and epidemiological characteristics. *Leishmania* (*v.*) *braziliensis* is a species that has a great genetic variability, pointed as the main causative agent of american cutaneous leishmaniasis in Brazil. Several studies have shown that aneuploidy has been related to evolutionary strategies of these parasites for adaptation to environmental modifications and drug resistance.

2 Methods

In the present study, the genomes of ten strains of *Leishmania (viannia) braziliensis*, classified previously in 10 zymodemas (Z26, Z45, Z72, Z73, Z74, Z75, Z78, Z105, Z106), were sequenced and annotated. The isolates came from municipalities of Pernambuco, northeastern Brazil. Sequencing were performed in the Miseq/Illumina platform and the reads were mapped against the reference genome (*L. braziliensis* 2904) using the BWA tool. SNP variant calls were performed through GATK package. The variant annotation process was performed using the snpEFF v3.6 program and the number of chromosomal copies were calculated using the program R.

3 Results

The highest rate of variation was found in the Z75 zymodema strain with 1 variation in each 253 bases in a total of 126,616 SNPs. The least variant strain was Z73 with a total of 83,693 SNPs. The aneuploidy was observed in all studied strains ranging from disomy to tetrasomy. The chromosomes with the highest number of copies recorded were 18, 22, 29, 31, 33 and 34 with an intermediate pattern between tri-tetrasomy for Z27 strain. The chromosome 31 presented more than three copies in all strains and tetrasomy for strain Z73. The chromosomal copy numbers calculated from readings showed intermediate values (e.g., between 2 and 3) which may be a consequence of a mixed cell.

4 Conclusions

The results of this study corroborate with the literature that has already reported aneuploidy in the *Leishmania* genus since the end of the 1980s, and it has's seen as a relatively frequent process in eukaryotic pathogens. The discovery of aneuploidy in natural populations refutes the hypothesis that changes in chromosome numbers are merely a long-term maintenance artifact of the parasite in culture and shows that tolerance to aneuploidy makes *Leishmania* a model for studies of genomic instability and ploidy alteration.

C1459 WHOLE GENOME DIVERSITY IN CLINICAL ISOLATES FROM GLOBALLY DISTRIBUTED SAMPLES OF THE LEISHMANIA DONOVANI COMPLEX

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1 Background

The *Leishmania donovani* complex comprises the species, *L. donovani* and *L. infantum*, distributed across the Old and New World. Parasites within the complex are mainly associated with visceral leishmaniasis but are also known to cause asymptomatic and cutaneous leishmaniasis in certain regions. Here, we investigate the whole-genome diversity in a globally distributed sample of 151 clinical isolates from the *L. donovani* complex.

2 Methods

Parasite samples were extracted from clinical isolates sampled between 1968 – 2011 and regrown in culture prior to whole genome next generation sequencing and mapping to the *L. infantum* reference genome. As *Leishmania* species show extensive aneuploidy, sample ploidies were calculated based on chromosomal sequencing coverage prior to SNP calling. Monophyletic groups of isolates were identified and analysed with respect to multiple population genetic parameters including nucleotide diversity (π) and linkage disequilibrium (LD).

3 Results

Phylogenetic analysis of the whole genome polymorphism data identified 2 main clades representing both species. *L. donovani* separated into 4 diverse subclades roughly coinciding with geographical origin, i.e. India, Saudi Arabia, Sudan and Kenya, each including neighbouring countries. In contrast, little diversity was observed in the *L. infantum* clade, leaving a major group comprising European, Middle Eastern and Chinese samples with relatively low levels of diversity. *L. infantum* with the lowest π also showed the lowest levels and fastest decay of LD within 200bp. This is in strong contrast to the *L. donovani* group from India, with the next lowest π but highest and slowest decay of LD with linkage over ~18-38kb. *L. infantum* compared to other groups showed an enrichment of non-synonymous changes that are enriched for low population frequencies. No major differences in patterns of chromosome ploidy were found between groups but there is high variability within each group except for chromosome 31, which has stable tetrasomy. Ploidy patterns across chromosomes were not related to π or LD levels, while SNP density is increased in chromosomes with particularly high ploidies.

4 Conclusions

Genome-wide polymorphism data from globally distributed samples from the *L. donovani* complex confirm differences between both species. While *L. donovani* is more diverse and has geographic sub-structure, little diversity and low LD of *L. infantum* samples suggest mixing between local populations and higher amounts of recombination.

C1477 ISOENZIMACTIC CHARACTERIZATION OF LEISHMANIA SPP. ISOLATED FROM PATIENTS AND SAND FLIES OF FRONTIER AREA OF BRAZIL

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1 Background

Cutaneous Leishmaniasis(CL) is endemic in the State of Amazonas, where it presents a wide spectrum of clinical variability due to the diversity of species that circulate in the region. São Gabriel da Cachoeira (SGC) is a Brazilian municipality in the interior of the state of Amazonas, North Region of the country. Located in the extreme northwest of Brazil, it is one of the border municipalities of the country, bordering two South American countries (Colombia and Venezuela). The aim of this study was to characterize by biochemical method trypanosomatids isolated from patients and sand flies and from this region.

2 Methods

The study was carried out in the municipality of SGC, along the BR 307 (Cucuí road), between coordinates (00 ° 06'21S, 067 ° 01'37''W and 00 ° 35'30 "N, 66 ° 46'26 "W) and on the Miuá Usina highway, in the stretch that includes the coordinates (00 ° 04'18S and 068 ° 00'07" W). Phlebotomines were collected using 16 CDC-type light traps and aspiration collections were also made on the basis of trees with the same trap (modified for tree-base aspiration). The samples of these patients were collected at the Hospital de Garrison (HGU / SGC) by the doctors who provided care to the Laboratory of the IFAM / SGC, lesions biopsy of suspected patients for Leishmaniasis.In this study, eight reference strains, recommended by the World Health Organization (WHO) and two of the genus *Endotrypanum*, were used as standards in the comparison of the profiles with the isolated samples.

3 Results

Three enzymatic loci (GPI, G6PDH and PGM) were used for the analysis of 17 samples: 16 isolates of sandfly (*Lutzomyia dendrophyla*) and one human. The samples presented distinct profiles of *Leishmania* reference strains circulating in Brazil and Colombia. In the electrophoresis of isoenzymes, the enzymes G6PDH and ME are considered as diagnostic for *L.* (*V.*) *guyanensis* and *L.* (*V.*) *panamensis*. In this study there were no alleles similarities with the reference strains used.

4 Conclusions

This work was a pioneer in the epidemiological evaluation of CL in the municipality, as well as the knowledge of the *Leishmania* species that circulate in the municipality of SGC, other techniques should be used to characterize the isolates that were not identified.

C1511 GSK-3 INHIBITORS EMERGE IN VITRO AND IN VIVO AS NEW POTENTIAL ANTITRYPANOSOMATID THERAPY

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1 Background

The trypanosomatid protozoans of the genus *Leishmania* and*Trypanosoma brucei*are the aetiological agent of leishmaniases and Human African trypanosomiasis, respectively. These parasitic diseases are a significant cause of morbidity and mortality in developing countries while over 14 million people are infected and 420 million people are at risk of infection worldwide. Moreover, leishmaniasis is an important public health and veterinary concern in Mediterranean countries while due to the environmental changes, the movement of populations and finally the impoverishment of the population, human cases are alarmingly increasing. Despite these alarming calls, no human vaccines are approved and the current chemotherapy is generally ineffective, mainly due to the emerging drug-resistance and severe toxic side effects. Consequently, there is an urgent need to discover new therapies.

2 Methods

The Alamar blue assay was applied in order to determine the antileishmanial activity of indirubin analogues *in vitro.* Murine model of visceral leishmaniasis was used to test the most potent *in vitro* GSK-3s inhibitors for their *in vivo* efficacy against the acute and the chronic phase of leishmaniasis.

3 Results

Indirubins, a class of bis-indole derivatives are promising candidates for targeted therapy because they are validated as inhibitors of essential parasitic kinases (GSK-3 and CRK3). Herein, a library of 69 indirubins was evaluated for their antitrypanosomal activity. Our results showed that **6** and **29** indirubins had potent antiparasitic activity *in vitro* against *L. donovani* and *T. brucei* parasites respectively. Interestingly, the most potent indirubin inhibitors are displaying activity against the parasitic kinases *Ld*GSK-3 and *Tb*GSK-3. The most potent GSK-3 inhibitors were tested*in vivo* on a murine model of acute and chronic visceral leishmaniasis and displayed substantial reduction of the parasitic burden.

4 Conclusions

In summary, the above results show promising antitrypanosomatidefficacy of GSK-3 inhibitors and are anticipated to lead to drug candidates that are urgently needed for treating parasitic diseases, an arising public health concern.

C1531 CUTANEOUS LEISHMANIASIS IN THE CENTRAL PROVINCES OF HAMA AND EDLIB IN SYRIA: VECTOR IDENTIFICATION AND PARASITE TYPING

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1 Background

Cutaneous leishmaniasis is a disease transmitted by sand fly bites. This disease is highly prevalent in Syria where *Leishmania major* and *Leishmania tropica* are the known etiological agents. In 2011, more than 58,000 cases were reported in the country by the Ministry of Health. The central region of the country harbors 20 % of the reported cases. However, the epidemiology of the disease in this area is not well understood. An epidemiological survey was conducted in 2010 to identity the circulating parasite and the sand fly vector in the central provinces of Edlib and Hama

2 Methods

Sand fly specimens were collected using CDC light traps and identified morphologically. Total DNA was extracted from the abdomens of female specimens and from Giemsa-stained skin lesion smears of 80 patients. *Leishmania* parasites were first identified by sequencing the ITS1 gene amplicons. Then polymorphism analysis was performed using the RFLP technique

3 Results

A total of 2142 sand flies were collected. They belonged to eight species, among which *Phlebotomus sergenti* and *Phlebotomus papatasi* were the most predominant.
L. tropica ITS1 gene was amplified from two pools of *P. sergenti* specimens and from skin smears of cutaneous leishmaniasis patients. This suggests that *P. sergenti* is the potential vector species in the study area. The digestion profiles of the obtained amplicons by TaqI restriction enzyme were identical for all analyzed *L. tropica* parasites. Moreover, *L. infantum* ITS1 gene was amplified from two pools of *Phlebotomus tobbi* in the relatively humid zone of Edlib.

4 Conclusions

L. tropica is confirmed to be the etiological agent of cutaneous leishmaniasis cases in the central provinces. RFLP technique failed to show any genetic heterogeneity in the ITS1 gene among the tested parasites. The molecular detection of this parasite in human skin smears and in *P. sergenti* supports the vector status of this species in the study area. The detection of *L. infantum* in *P. tobbi* specimens indicates a potential circulation of this parasite in the humid zone of Edlib. Further epidemiological studies are needed to evaluate the burden of this visceral parasite in the study region.

C1549 METABOLOMIC APPROACHES TO CHARACTERIZE LEISHMANIA MEXICANA GROWN IN DEFINED MEDIUM

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1 Background

Leishmania Simplified Medium (LSM) is a chemically defined medium developed in our laboratory to cultivate *Leishmania mexicana* promastigotes. The medium offers an enhanced means of discovering more about nutritional requirements for the parasites. The medium is the cost-effective and easy to use offering a routine means to *L. mexicana*. The application of metabolomics technology to evaluate the intra- and extracellular metabolites of parasites grown in defined media allows better understanding of the biochemistry of Leishmania and also to understand how drugs kill these cells.

2 Methods

Promastigote cells were grown in the formulated defined media without serum and standard media with serum, HOMEM. Untargeted metabolomic analysis of the parasites' intracellular environment and spent media using LC-MS was used to determine differences between metabolic profiles in both media. Cells were tested for susceptibility to different anti-leishmanial drugs and also response to oxidative stress.

3 Results

Results showed that LSM is equivalent to HOMEM in allowing growth of promastigotes *in vitro*. Metabolomics analysis indicated several key changes between the metabolic capability of parasites grown in HOMEM or defined medium. Interestingly, the cells in defined media were significantly more sensitive to miltefosine than when grown in HOMEM and metabolomics was used to identify differences in response to the drug on the two media.

4 Conclusions

The use of LSM provides the advantages on metabolomic studies and drug discovery and decrease the dependence of cell to animal serum, a growth supplement with the undefined and variable composition.

C1627 LEISHMANIA BIOMARKERS FOR DIFFERENTIATION OF VISCERAL LEISHMANIASIS PARASITES IN EAST AFRICA

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1 Background

The study exploited polymorphic RAPD markers
selected in a previous study using geographically diverse L. donovani & L. infantum strains complex to develop simple and specific PCR assays for the identification and characterization of VL causing Leishmania parasites.

2 Methods

2 *L.archibaldi* and 2 *L.donovani* differentially amplified RAPD markers obtained from East African *Leishmania* strains, were used to design primers that were screened for the specific/ selective DNA amplification of viscerotropic *Leishmania* parasites. Six primer pairs were retained and tested on 80 *Leishmania* strains representative of the different *Leishmania* species encountered in the Old World and having different geographical and host origins to validate the reactivity of the PCR assays. Sensitivity of detection of the assays was determined on serial dilutions of DNAs from different strains. The assays were applied to 79 DNAs of parasites newly isolated from CL, VL or PKDL patients. Southern blot analysis confirmed the specificity of the PCR assays. Phenetic analysis was conducted to cluster the parasites according to their PCR reactivity. We used the ribosomal DNA internal transcribed spacers ITS1&2 as reference markers for taxonomy DNAs of 48 African strains of this African panel was investigated for ITS1&2 sequences and phylogenetic analyses.

3 Results

AY8 primers consistently amplified DNAs of all Old World Leishmania species or complexes tested. A1Y8, DY14 and
LY8 specifically amplified DNAs of parasites belonging to L. donovani and L. infantum. AY9 and A2Y8 amplified only Sudanese or Ethiopian L. donovani parasites. Sensitivities of detection ranged from 125-1250 parasites. These
assays identified effectively newly isolated parasites in Sudan and Tunisia. They constitute a first step in taxonomic
assignment and genetic differentiation of parasites according to their geographical origins and likely transmission
cycles. At least 3 parasite clusters were identified in Sudan. ITS1&2 sequence analyses showed congruent classification of these clusters into subgroups of one of the clades previously described for the etiological agents of VL in Africa.

4 Conclusions

The study delivers a set of 5 simple & rapid PCR assays for identification and genetic differentiation of VL causing parasites in East Africa.The interest of our tools was confirmed by ITS1& 2 sequence analyses. Our tools will be highly useful for epidemiological
investigations, for diagnosis and in travel medicine.

C1793 DIPEPTIDYL PEPTIDASE III: A NOVEL MARKER TO INVESTIGATE LEISHMANIA SPECIES EVOLUTION AND TAXONOMY

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1 Background

Dipeptidyl peptidase III (DPPIII) member of M49 peptidase family is a zinc-dependent metallopeptidase that cleaves dipeptides sequentially from the N-terminus of its substrates. It is absent in trypanosomes, however, in *Leishmania,* it was reported that the DPPIII with other peptidases, could be key to the parasites' growth and survival. In a previous study, we used a coding sequence annotated as DPPIII to develop a PCR assay that is specific to dermotropic Old World (OW) *Leishmania* species. Thus, our objective was to further assess use of this gene to identify *Leishmania* species or to study their evolution.

2 Methods

Orthologous DDPIII genes were searched in all *Leishmania* genomes and aligned to design primers and to search restriction enzymes. A PCR-RFLP scheme was designed and tested on 51 OW typed *Leishmania* strains belonging to 10 *Leishmania* species (*L. major (L.m), L. infantum (L.i), L. tropica (L.t), L. donovani (L.d), L. aethiopica (L.aet), L. arabica (L.ar), L. turanica (L.tu), L. chagasi (L.ch), L. archibaldi (L.arch)* and *L. tarentolae (L.tar)*), 7 strains isolated from CL patients, and 50 biological samples taken for diagnosis of suspected Tunisian CL patients. Seventy-one PCR products were further studied by sequence analysis using MEGA and DNAsp softwares to infer evolution and phylogenetic relationship of studied species and strains.

3 Results

DPPIII gene is present in all genomes. Inter- and intra-specific genetic diversity was used to develop universal amplification of a 666bp fragment followed by 2 digestions with HaeIII and KpnI. Overall, this PCR-RFLP yielded distinct profiles for each of the species *L.m*, *L.t*, *L.aet*, and *L.tar. L.d, L.arch and L.i* shared their profile with *L.ara* and *L.tur.* Sequence analysis showed 145/589 variable sites (116 being parsimony informative), high haplotype diversity (H=0.81; SD=0.026), and low nucleotide diversity (π=0.04; SD=0.005). Test of natural selection (dN/dS=4.7, Fu and Li's D test (2.79; p<0.02)) inferred a positive selection confirming importance of DPPIII for parasite adaptation to its environment. Phylogenetic relationships confirmed occurrence of 6 clusters congruent to *L.m, L.t, L.aet, L.ar, L.tu, L.tar* species, and one to the *L.i, L.d* and *L.arch* group of species.

4 Conclusions

DPPIII gene is suitable to investigate *Leishmania* epidemiology and evolution and can contribute to timely disease control.

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C1817 GWAS ON CUTANEOUS AND MUCOSAL LEISHMANIASIS: SEARCHING FOR PUZZLES PARTS.

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1 Background

Genome wide association studies (GWAS) have been applied on several human disease scenarios. So far, on Leishmaniases literature we have a lack of studies applying this approach to understand susceptibility patterns or the clinical spectrum of the disease (e.g. Visceral, Cutaneous and Mucosal forms). As a poorly studied subject on Leishmaniases, strong association signals could be found among phenotype/genotype even when looking at small sampling.

2 Methods

With this, we implemented a microarray platform in the North Region of Brazil and analyzed more than 250 thousands of SNPs in 60 male patients form Rondônia State (Brazil), infected by *Leishmania braziliensis*. Those patients were grouped by clinical form and paired by age: 30 of them presenting the cutaneous form (control group) and 30 with mucosal form (case group). The association tests between SNPs and case/control phenotypes were performed using the PLINK package and the results were presented in a Manhattan Plot.

3 Results

First analyses have shown no significant associations when using the Bonferroni correction, but considering its admittedly conservative meaning, we go further on the description of the 20 SNPs with highest association with the phenotypes. Four possible candidate genes related to Tegumentary Leishmaniasis evolution: GRM8, PCLO, NAV3 and RAB20. On chromosome 4, two SNPs were identified in very close positions and, in an adjacent region, four genes related to the immune response in infections by *Leishmania* parasites were identified, making these regions suggestive for future research.

4 Conclusions

These preliminary results compose the first GWAS using microarray to investigate the role of human genetic background on the evolution of Cutaneous to Mucosal forms on Tegumentary Leishmaniasis. Furthermore, we generated a reliable SNP list with disease data associated that is been further investigated and will be soon available on public databases.

C1830 THE GENOME OF LEISHMANIA ADLERI FROM A MAMMALIAN HOST HIGHLIGHTS CHROMOSOME FISSION IN SAUROLEISHMANIA

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1 Background

Control of pathogens arising from human, domestic and sylvatic sources can be enhanced by genome-based investigation. Phylogenetically classifying and optimal construction of these genomes using short sequence reads are key to this. We examined the mammal-infecting unicellular parasite *Leishmania adleri* belonging to the lizard-infecting *Sauroleishmania* subgenus. *L. adleri* has been associated with cutaneous disease in humans, but can be asymptomatic in wild animals.

2 Methods

We assembled and investigated the *L. adleri* genome isolated from an asymptomatic Ethiopian rodent (MARV/ET/75/HO174) and verified it as *L. adleri* by comparison with other *Sauroleishmania* species. Chromosome-level scaffolding was achieved by combining reference-guided with *de novo* assembly followed by extensive improvement steps to produce a final draft genome with contiguity comparable with other references. *L. tarentolae* and *L. major* annotation was transferred and the gene models were manually verified and improved.

3 Results

The first high-quality draft *Leishmania adleri* reference genome is also the first *Sauroleishmania* genome from a non-reptilian host. Comparison of the *L. adleri* HO174 genome with those of *L. tarentolae* Parrot-TarIIand lizard-infecting *L. adleri* RLAT/KE/1957/SKINK-7 showed extensive gene amplifications, pervasive aneuploidy, and fission of chromosomes 30 and 36.

4 Conclusions

There were few genetic differentiation between *L. adleri* extracted from mammals and reptiles, highlighting challenges for leishmaniasis surveillance.

C1863 ANALYZING NATURALLY PROCESSED LEISHMANIA ANTIGENS PRESENT ON DENDRITIC CELLS : IN CONTEXT OF HLA DRB1 BACKGROUND

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1 Background

Leishmaniasis, a neglected tropical disease, prevalent in developing countries with 90% of them in Asia (Bangladesh, India, and Nepal), Sudan, Ethiopia and Brazil. The technical challenges and the complexity in the immunity against the parasites clearly contribute to the absence of vaccines. A major challenge in human vaccine design is to overcome variation in immune response in a genetically heterogeneous population. This is largely determined by genetic heterogeneity in processing and presentation of Ag to T cells, the outcome of which is dependent on binding of T cell epitopes to HLA class I and class II molecules that drive CD8 and CD4 T cell responses, respectively.

In silico screening for putative epitopes binding to DRB1 molecules can identify multiple epitopes per single parasite antigen. Our quest here is to determine what actually occurs during the course of a complex infection *in vivo*. Hence, a combinatorial approach using in silico epitope predictions and an in-vitro study determining scenario of natural antigen processing and presentation could be an initial but important step towards vaccine candidate hunting in Visceral Leishmaniasis.

2 Methods

Here we describe an integrated protocol that combines proteomics methods (immunoaffinity chromatography, mass spectrometry analysis) and epitope mapping to identify the naturally processed peptides that are bound to HLA?DRB1molecules on DCs.

3 Results

we captured the naturally processed Leishmanial peptides from the most potent APC, Dendritic cells (DCs) carrying the DRB1*1301(risk) and DRB1*1501(protective) alleles. The Eluted peptide sequence data revealed specific peptides bound to risk and protective alleles and one common peptide was obtained between both the alleles. *In silico* epitope mapping data revealed greater number and complexity of amino acid residues forming the 9-mer core of risk allele as compared to protective allele suggesting a greater promiscuity in epitopes binding to risk as compared to protective alleles.

4 Conclusions

The major focus of this study was to examine the role of genetic heterogeneity in antigen presentation by risk/protective HLA?DRB1 molecules, which can influence the fate of the subsequent host immune responses. Knowledge derived from these findings can help us develop a strategy for subverting the immune responses towards a protective response against the background of HLA?DRB1?driven genetic susceptibility to clinical VL

C1918 PROTEOME AND TRANSCRIPTOME OF PROMASTIGOTE LEISHMANIA INFANTUM STRAIN ISOLATED OF A PATIENT WITH FATAL VISCERAL LEISHMANIASIS.

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1 Background

Leishmania infantum is the protozoan responsible for visceral leishmaniasis (VL) in the New World. This zoonotic disease which is transmitted by insect vectors family Psychodidae the genus *Lutzomyia* is present in 12 countries in Latin America, with 90% of cases occur in Brazil. The disease evolves in fever, anemia and hepatosplenomegaly, and is fatal if not treated properly. In recent decades the number of fatal VL has grown considerably due to coinfection and other factors that may lead to risk of death. However, the pathogenesis of the complications arising from infection with *L. infantum* are not well understood. It is believed that the severity of illness may be associated with susceptibility and the immune response of the host or the parasite expression of proteins that act as virulence factors.

2 Methods

We developed a study where we compared quantitatively and qualitatively the total protein extract of infective promastigotes of *L. infantum* obtained of a culture of isolated from bone marrow of a patient with fatal VL. The control used was an isolated strain of a patient with classic VL, without complications and with good treatment response.Proteomic analisys were made with a independent data label free mass sspectrometry (label-free nanoLC-MS^E) and confirmed with Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS/MS). Transcriptome was made in illumina hiseq 2500 platform, paired-end libraries with 100 bp, with approximately 12.5 million reads per library. Bioinformatic analisys was made using a open source tools. Some results of genes and proteins find in high quantity were confirmed with real time PCR with Taqman probes.

3 Results

Expression analysis and quantification using label-free MS^E showed increase in quantity in some protein and genes when compared to control. Hypothetical virulence factors as the eukaryotic initiation factor (EIF) 4A, heat shock protein (HSP) 70 and 83-1, elongation factor 1α, kinesin K39, kinetoplastideo membrane protein 11 (KMP-11), cytosolic tryparedoxin (LiTXN1) (LinJ.15.1140) and cysteine ??peptidase were found in fatal outcome.

4 Conclusions

Joining analysis strategies to identify molecules and genes involved with clinical conditions of fatal visceral leishmaniasis can help unravel the role of the parasite in severe disease. Unfortunately for us, these strategies still have high cost.

C1920 PREDICTION OF T-CELL EPITOPES BY USING HYBRID SOFT COMPUTING MODEL TO DESIGN PEPTIDE-BASED VACCINE AGAINST LEISHMANIASIS

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1 Background

Leishmaniasis is a parasitic disease caused by different Leishmania species. Depending upon Leishmania species and the host immune response, exposure to Leishmania might be asymptomatic, or with clinical manifestations ranging from a simple self-healing cutaneous lesion to a lethal form of visceral leishmaniasis. Treatment is based on chemotherapy which is expensive, needs multiple injections, accompanies various side effects, relapse and resistance are reported. Development of an effective vaccine is the most effective tool to control the disease but currently regardless of tremendous efforts no vaccine is available against any form of Leishmaniasis. Immunoinformatics methods such as Bioinformatics tools and computational methods are widely used for modeling, prediction and to define the function of immune system, the information is used to further predict immunogenic peptides to develop peptide-based vaccines. In the current study T cell epitopes for Leishmania parasite are identified using hybrid soft computing model (HSCM).

2 Methods

Data retrieved from the Immune Epitope Database (IEDB, www.iedb.org) and used As data source for the current study. The collected data is divided into two data sets, 70% of the data source were used for training (train data set), and remaining is used for testing and validation purposes (test data set). Ten-fold cross validation was used for optimizing performance of the model. Hybrid soft computing models developed by using combining 3 different classifiers.

3 Results

Hybrid soft computing model was tested against different Leishmania proteins. The results showed that this model highly improves prediction accuracy over the single model (AUC=0.913). Then, the five predicted are defined as candidate peptides for development of an effective vaccine against leishmaniasis.

4 Conclusions

The results indicated that Hybrid soft computing models is an appropriate in silico approach to predict candidate peptides for further in vitro and in vivo evaluation.

Host-parasite cellular and molecular interactions

C0098 SUCCESSFUL TREATMENT OF HUMAN VISCERAL LEISHMANIASIS RESTORES ANTIGEN-SPECIFIC IFN-?, BUT NOT IL-10 PRODUCTION

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1 Background

One of the key immunological characteristics of active visceral leishmaniasis (VL) is a profound immunosuppression and impaired production of Interferon-γ (IFN-γ). However, recent studies from Bihar in India showed using a whole blood assay, that whole blood cells have maintained the capacity to produce IFN-γ. Here we tested the hypothesis that a population of low-density granulocytes (LDG) might contribute to T cell responses hyporesponsiveness via the release of arginase.

2 Methods

A cross-sectional study was conducted on patients with confirmed VL from December 2014 to May 2015 at the University of Gondar Hospital, Ethiopia. Blood was collected in EDTA and heparin tubes from all study participants before and after treatment and from healthy controls to assess the following:
- The effect of anticoagulants on the frequency of low-density granulocytes (LDGs), a population of activated neutrophils with immunosuppressive properties, using flow cytometry;
- The levels of arginase activity using an enzymatic assay;
- The production of IFN-γ and IL-10 by whole blood cells in a whole blood assay using ELISA.

Data were evaluated using GraphPad Prism 6 and differences were considered statistically significant at $p < 0.05$. FACS data were analyzed using Summit v4.3 software.

3 Results

Our results show that the frequency of LDGs is affected by the anticoagulant used to collect blood: it is significantly lower when the blood is collected with heparin as compared to EDTA; however, the anticoagulant does not impact on the levels of arginase released. Next, we assessed the capacity of whole blood cells from patients with active VL to produce IFN-γ and IL-10 in response to antigen-specific and polyclonal activation. Our results show that whole blood cells produce low or levels below detection limit of IFN-γ and IL-10, however, after successful treatment of VL patients, these cells gradually regain their capacity to produce IFN-γ, but not IL-10, in response to activation.

4 Conclusions

These results suggest that in contrast to VL patients from Bihar, India, whole blood cells from VL patients from Gondar, Ethiopia, are hyporesponsive; they have lost their ability to produce IFN-γ during active VL and that active disease is not associated with sustained levels of IL-10 production following stimulation.

C0151 POLYUNSATURATED FATTY ACID (PUFA) METABOLITES: BIOSYNTHESIS IN LEISHMANIA AND ROLE IN PARASITE/HOST INTERACTION

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1 Background

Inside the human host *Leishmania* infection starts with phagocytosis of metacyclic promastigotes mainly by macrophages which are key players of innate immunity. Macrophages could be polarized towards two opposite states, the M1 or classical, and the M2 or alternative activation; parasites such as *Leishmania* induced a M2 activation via unknown mechanisms.In this context, we assessed the production of polyunsaturated fatty acid (PUFA) metabolites by infective and non-infective form of *Leishmania* and explored the role of parasite PUFA metabolites in macrophages polarization.

2 Methods

PUFA metabolites have been quantified by liquid chromatography tandem mass spectrometry in procyclic promastigotes (non-infective form) and metacyclic promastigotes (infective form) obtained *in vitro*. Activity of putative enzymes implicated in the PUFA metabolism of *Leishmania* was studied in transfected CHO cells treated with PUFA and ulterior quantification of PUFA metabolites. Finally, lipids from the parasite were extracted and used to assess their effect on the polarization of mouse bone-marrow derived macrophages.

3 Results

On the 32 PUFA metabolites assessed, eleven were quantified in *Leishmania infantum* and their concentrations were dependent on the life cycle stage. Concentrations of docosahexaenoic acid (DHA) metabolites 14-HDoHE and 17-HDoHE, precursors of pro-resolving lipid mediators: maresins and resolvins D-series respectively, were increased in the infective form of the parasite compared to the non-infective form. Synthesis of these metabolites was impaired by cytochrome P450 inhibitors. Three cytochrome P450-like proteins coded by *LinJ.27.0090* (*CYP1*), *LinJ.30.3610* (*CYP2*), *LinJ.34.3110* (*CYP3*) genes were implicated in DHA metabolism into 14-HDoHE and 17-HDoHE. The treatment of macrophages by lipids extracted from the infective forms polarized macrophages into a M2 phenotype. This phenotype was characterized by an increase in the production of pro-resolving bioactive lipids: resolving D2, maresin 1 and in the expression of several markers of pro-resolving phenotype as IL6 or Arginase1.

4 Conclusions

In the present study we have identified in *Leishmania* new enzymes named CYP1, CYP2 and CYP3 responsible for the production of precursors of pro-resolving bioactive lipids by metacyclic promastigotes. These PUFA metabolites were implicated in parasite/host interactions by promoting polarization of macrophages into a pro-resolving phenotype M2.

C0158 ANTI-LEISHMANIA DONOVANI ANTIBODIES ENHANCE PROMASTIGOTES INTERNALIZATION INTO HOST MACROPHAGE

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1 Background

Leishmania spp. promastigotes preferentially infect host macrophages, where parasite internalization is facilitated by several host and parasite surface molecules. The internalization of the promastigote form has been shown to be mediated by the mannose-fucose receptor, the fibronectin receptor, and the complement receptors type 1 and 3 (CR1 and CR3) on the surface of host macrophages.
This study aimed to demonstrate the role of humoral immunity in *Leishmania* parasite internalization into host macrophages.

2 Methods

First, informed consent sera were obtained from 67 parasitologically confirmed visceral leishmaniasis patients reporting to our field treatment centre, Eastern Sudan. Then following titre determination, sera that had a titre of >102,400 were selected for parasite coating. An *in vitro* parasite internalization system was developed to enhance the *Leishmania*/ macrophage interactions.

3 Results

The mean parasite number per monocytes was 626 ± 91 for antibody-coated *Leishmania donovani*, compared to 412 ± 70 uncoated isolates (p= 0.01). On the other hand, the percentage of infected cells was significantly higher for all antibody-coated isolates (100%) compared to uncoated ones (40%). This evidence of high infectivity probably points to the fact that anti-*Leishmania* antibodies facilitated the parasite uptake by host macrophages and monocytes-derived macrophages (MDM).

4 Conclusions

Leishmania spp. promastigotes preferentially infect host macrophages, where parasite internalization is facilitated by several host and parasite surface molecules. Moreover, the rate of parasite uptake by MDM was significantly higher compared to monocytes. This could be explained by the fact that the functional capabilities of fully differentiated macrophages differ from monocytes. In conclusion, host humoral immunity probably plays a pivotal role in *Leishmania* parasites internalization into host macrophages.

C0185 THE LEISHMANIA VIRULENCE FACTORS GP63 AND LPG TRAFFIC IN VESICLES VIA A SEC22B-MEDIATED PATHWAY

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1 Background

Leishmania parasites conquer their mammalian hosts by using phagocytosis to survive and evade immunity. Phagosomes become microbicidal via membrane exchanges, mediated in great part by soluble NSF attachment protein receptors (SNAREs), with organelles such as the ER-Golgi intermediate compartment (ERGIC). *Leishmania* employs virulence factors such as the GP63 metalloprotease and lipophosphoglycan (LPG) to cleave host proteins involved in immunity, and retard phagosome maturation, respectively. *Leishmania* has no known secretion system; how GP63 and LPG traffic from the phagosome to the cytoplasm is unknown. We hypothesized that these molecules are redistributed throughout the cytoplasm of infected cells in vesicles whose trafficking is mediated by host organelles.

2 Methods

Primary macrophages, dendritic cells or neutrophils were infected with opsonized stationary-phase *Leishmania* promastigotes. Phagocytes were visualized via confocal microscopy after various times of infection. Lysates from infected macrophages were fractionated using discontinuous sucrose gradients followed by ultracentrifugation. Fractions were imaged via electron microscopy and their protein content assayed by Western blot. The role of Sec22b was studied by using JAWSII dendritic-like cells transduced with shRNA to Sec22b.

3 Results

We showed that GP63 and LPG were found in vesicles that dispersed in the cytoplasm of infected phagocytes in a time-dependent manner. This was dependent on contact and independent of GP63 activity. Flotation assays showed that GP63 and LPG were present in low-density fractions containing vesicles and in higher density fractions enriched in ER/ERGIC proteins. Immunofluorescence revealed that GP63 and LPG colocalized with ER/ERGIC$^+$ vesicles. Notably, Brefeldin A-mediated disruption of ER-Golgi transport hindered the redistribution of GP63 and LPG, as well as the degradation of GP63 substrates. This prompted us to study the role of the SNARE Sec22b, a key regulator of ER-Golgi transport, on the redistribution of these parasite molecules. Importantly, Sec22b knockdown also hampered the trafficking of GP63 and LPG and the degradation of GP63 targets.

4 Conclusions

Our findings revealed a novel mechanism whereby intracellular pathogens hijack the host's ER and ERGIC, and resident vesicle fusion proteins, to promote the transport of virulence factors. These data will improve current understanding of how *Leishmania* hijacks the host's membrane fusion machinery to sabotage phagocyte biology.

C0211 DELTA-AMINOLEVULINATE-INDUCIBLE UROPORPHYRINOGENSIS IN TRANSGENIC LEISHMANIA BRAZILIENSIS; A TOOL FOR PHOTODYNAMIC VACCINATION

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1 Background

PDT inactivation of *Leishmania* parasites involves the use of photosensitizers (PS) and light of specific wavelengths; this leads to the production of reactive oxygen species (ROS) that inactivate the parasite. In present study, we generated a transgenic *Leishmania brazileiensis* expressing two enzymes of the heme biosynthetic pathway: porphobilinogen deaminase (PBGD) and aminolevulinate dehydratase (ALAD) and evaluated its potential for photo inactivation through ALA exposure.

2 Methods

L. braziliensis promastigotes were subjected to two rounds of transfection with circular constructs of mammalian genes encoding aminolevulinate dehydratase (pX-ALAD) and porphobilinogen deaminase (pXG-PBGD). Double mutants (DTs) were rescued in presence of antibiotics and population diversity of ALA-induced uroporphyric double transfectantes promastigotes and their viabilityatdifferent time interval (24h, 48h, 72h)was determined by FACs analysis. Viability of cells after white light exposure was assessed by MTT assays

3 Results

A total of twelve double transfectant (DT) clones were obtained and displayed adaptation to high drug selection pressures. Once exposed to externally supplied ALA, all DT clones generated uroporphyrin as evidenced by the presence of fluorescence at UV exposure. FACs analysis showed clonal variability in the accumulation of Uroporphyrin, with the maximum production at 42 h post exposure to ALA. MTT assay confirmed parasite growth inhibition following exposure of DTs to ALA and white light. Two clones were selected based on the highest efficiency for producing uroporphyrinogenic (Lbr_DT11 and Lbr_DT4).

4 Conclusions

Doubletransfection of *L. braziliensis* with ALAD and PBGD induces parasite photoinactivation upon exposure to ALA. The generated DTs are being currently tested with regards to macrophage infectivity and antigen presentation characteristics.

Source of funding: CNPq and FIOCRUZ.

C0240 MACROPHAGE M1/M2 POLARIZATION INDUCED BY LIPIDS FROM LEISHMANIA BRAZILIENSIS PROMASTIGOTES AND AMASTIGOTES

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1 Background

Infections control depends on innate immunity activation and there is evidence of the immunomodulatory role that pathogens´ lipids display in this process where macrophages play a key role, being classified into M1 (classical) and M2 (alternative) activationphenotypes. Control of Leishmania spp infection requires a pro-inflammatory profile with M1 macrophages; though the parasite has developed strategies to evade this response. In *L. braziliensis*, agent of cutaneous Leishmaniasis, we found quantitative differences in the lipid profiles of promastigotes (**PRO**) and amastigotes (**AMA**), as well as after parasite disruption and incubation that generates bioactive lipids which may contribute to inflammation. We here studied the effect of total lipids from PRO and AMA (intact and post-incubation) in lipid bodies' induction, ciclooxigenase-2 (**COX-2**) expression and macrophage M1/M2 polarization.

2 Methods

L. braziliensis MHOM/BR/75M2904, PRO or AMA (1-3x10^8/ml) were lysed by 3 cycles of N_2/37°C + protease inhibitors. Lipids were extracted following Bligh & Dyer, dried under N_2 to constant weight and suspended in ethanol. Parasites were also incubated for 20 h at 37°C to allow lipids hydrolysis and then extracted and processed as mentioned (**PROinc and AMAinc**).Murine peritoneal macrophages (1x10^6/well) were stimulated with 50 µg/ml of the different lipids for 24-48 h at 37ºC and 5% CO_2 to determine: Lipid bodies formation (Gimenez et al, 2010), COX-2, inducible nitric oxide synthase (**iNOS**) and Arginase-1 expression by Immunoblot, Nitric oxide (**NO**) using Griess reaction (Shoda et al, 2000) and Arginase activity (Al-Mutairi MS et al, 2010).

3 Results

We determined that PRO induced the highest Arginase-1 expression/activity, lower iNOS expression and NO production, suggesting an anti-inflammatory response with M2 macrophages. In contrast, AMAinc induced the highest iNOS expression and NO productionand lowerArginase-1 expression/activity, suggesting a pro-inflammatory response, related to M1 macrophages. As concerns lipid bodies, all the lipids analyzed induced their generation, whereas the higher levels of COX-2 expression were obtained with AMAinc.

4 Conclusions

The results suggest that PRO, PROinc, AMA and AMAinc may differentially contribute to the modulation of the immune response; this could be related to their different lipid profiles, thus bioactive lipids from *L. braziliensis* could be good candidates for immunoprophylactic strategies. Supported by CONICET/UBA

C0264 RELATIONSHIP BETWEEN REGULATORY T CELLS AND DENDRITIC CELLS IN ORGANS OF DOGS WITH VISCERAL LEISHMANIASIS

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1 Background

Dendritic cells (DCs) are antigen-presenting cells that migrate to peripheral tissues non-lymphoid to act as sentinels, detecting invading microorganisms, initiating the primary immune response and stimulating T lymphocytes. The functional efficiency of these cells is directly related to their maturation, when they are capable to express MHC molecules. The objective of the present study, was to analyze the skin (nasal surface and ear regions), lymph nodes (popliteal and prescapular), spleen and liver of dogs with visceral leishmaniasis (VL), in order to investigate the relationship between the parasite load measured as DNA copy number of *Leishmania infantum* by quantitative PCR and the immunodetection of regulatory T cells, and mature and immature dendritic cells by immunohistochemistry.

2 Methods

Were used 29 naturally infected dogs from an endemic area for VL and a control group (n = 6) of non-endemic area.

3 Results

The spleen presented the highest number of *Leishmania* DNA copies and the liver a low parasitism. Regulatory T cells (Treg) predominated in infected dogs, but there was a significant difference between groups only in the areas of the nasal surface and prescapular lymph node. The mature DCs (CD83) did not differ between the infected and control groups, already the immature (CD11c) showed differences between the groups only in the liver and nasal surface, with predominance in the infected group.

4 Conclusions

It was concluded that the predominance of immature DCs in the infected group could justify the persistence of the parasite in the tissues, because in this immature stage, the DCs are incapable of presenting antigens to the T cells. Treg cells predominated in the skin regions, followed by the prescapular lymph node of infected dogs, areas of inflammation and parasitized macrophages greater intensity. Possibly this subpopulation of lymphocytes creates an immunosuppressive environment, that can interfere in the efficient activation of DCs, which do not pass to the mature stage and get not activate T cells to initiate efficient cellular immunity against the parasite, consequently the persistence of the infection. Financial support (FAPESP-2013/00763-4).

C0282 L. INFANTUM / L. MAJOR HYBRID CLONES- PHENOTYPIC PROFILES AND DIFFERENTIATION BY MALDI-TOF

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1 Background

Reports on different *Leishmania* hybrid species highlight recombination events of these parasites, which seem to happen within the vector. Different hybrids have already been studied showing more resilience than their putative parents, presenting higher plasticity, phenotypic diversity and pathogenicity such as *L. braziliensis/L. peruviana* strains. Phenotipic characterization and virulence determinants are poorly understood on these emerging *Leishmania* hybrid species, mainly the Old World *L. infantum /L. major* hybrids.
In this work, we present *in vitro* phenotypic profiles of several clones of *L. infantum/L. major* hybrid strains along with their putative parental ones. Moreover, we used Matrix Assisted Laser Desorption Ionisation – Time Of Flight (MALDI-TOF) mass spectrometry for spectral analysis of proteins to differentiate these *Leishmania* strains.

2 Methods

14 clones of *L. infantum/ L. major* hybrids and putative parental species were evaluated in terms of *in vitro* survival capacity at different stages of their life cycle by comparing growth dynamics, oxidative stress tolerance to H_2O_2 (reactive oxygen species) and SNAP (nitric oxide donor) and infection index in the macrophage cell line (J774). MALDI-TOF characterization was performed and spectra were acquired in the mass range of 3000-20000 Da in positive ion mode.

3 Results

Results have shown higher parasite densities in all IMT208 *L. infantum/ L. major* hybrid clones, with ones being similar to *L. infantum* (IMT151). Some of the IMT211 hybrid clones were more similar to *L. major* (LV561). Concerning resistance to the oxidative stresses, hybrid clones presented different responses within each other with significant differences to the parental ones. Infection indexes did not reveal significant differences. MALDI-TOF analysis was able to show micro-diversity.

4 Conclusions

These results showed heterogeneous phenotypes with ones being more similar to *L. infantum* and others to *L. major*. The existence of hybrid strains having diverse or enhanced ?tness and selective advantages may have an impact on virulence and pathogenesis. More studies are in course in terms of proteomic and genomic profiles.
Work supported by funding of the Marie Curie COFUND/BRAIN project (PCOFUND-GA-2013-609440) and Fundação para a Ciência e a Tecnologia for funds to GHTM– UID/Multi/04413/2013.

C0306 LEISHMANIA DONOVANI SILENCE MACROPHAGE MIRNAS BY INHIBITING THEIR TRANSCRIPTION AS A POTENTIAL, NOVEL VIRULENCE STRATEGY

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1 Background

Micro RNAs (miRNAs) have recently emerged as essential regulators of gene expression. Several pathogens have been reported to specifically exploit miRNA expression as a virulence strategy to manipulate host cellular processes to their advantage. Here, we studied the effects of leishmania infection on host miRNA expression, and their potential role in pathogenesis.

2 Methods

Healthy human monocytes-derived macrophages were infected *in vitro* with *Leishmania donovani*. The expression of 800 miRNAs in infected and non-infected human macrophages was assessed using Nanostring nCounter assay, and a subset of these were validated by qPCR. The stability of mature miRNAs was compared by treating infected and non-infected cells with actinomycin, a transcription inhibitor. Western blot was used to determine the expression of several key players of the miRNA maturation pathway, and the location of some of them was assessed by confocal microscopy. Primary and precursor miRNAs were measured by qPCR.

3 Results

Strikingly, our results showed that *L. donovani* infection induced an overall down-regulation of miRNA expression in macrophages, at 24 and 48h post-infection. We then aimed at determining which step or component of miRNA synthesis, maturation or stability is affected during leishmania infection.
First, the stability of mature miRNAs in infected cells was similar to uninfected cells, showing that the downregulation of miRNAs is not due to leishmania inducing a degradation of miRNA, or interfering with miRNA stability in any other way.
Second, we determined that leishmania infection did not alter any protein involved in the miRNA biogenesis pathway: Drosha, DGCR8, Dicer, TRBP and Ago2 were not downregulated by infection. Moreover, Drosha and DGCR8, the two proteins responsible for the nuclear processing of primary into precursor miRNA, were correctly localized in the nucleus, both in infected and uninfected cells.
Finally, we found that similarly to mature miRNAs, both primary miRNAs (product of transcription) and precursor miRNAs (intermediate product) were downregulated in infected cells. This suggests that leishmania infection acts at the level of miRNA transcription.

4 Conclusions

Our results suggest that leishmania inhibits the transcription of miRNAs in host cells, leading to a global downregulation of miRNAs in infected macrophages. This inhibition of miRNA expression may be a novel mechanism of pathogenesis used by leishmania to control macrophage phenotype and promote chronic infection.

C0307 IN VITRO PERMISSIVENESS OF BOVINE NEUTROPHILS AND MONOCYTE DERIVED MACROPHAGES TO LEISHMANIA DONOVANI OF ETHIOPIAN ISOLATE

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1 Background

Epidemiological studies in Ethiopia have documented that the risk of visceral leishmaniasis (VL, Kala-azar) is higher among people living with domestic animals. The recent report on isolation of *Leishmania donovani* complex DNA and the detected high prevalence of anti-leishmanial antibodies in the blood of domestic animals further strengthen the potential role of domestic animals in the epidemiology of VL in Ethiopia. In mammalian hosts polymorphonuclear cells (PMN) and macrophages are the key immune cells influencing susceptibility or control of *Leishmania* infection. Thus to substantiate the possible role of cattle in VL transmission we investigate the permissiveness of bovine PMN and monocyte derived macrophages (MDM) for *Leishmania (L.) donovani* infection.

2 Methods

Whole blood was collected from pure Zebu (Boss indicus) and their cross with Holstein Friesian cattle. *L. donovani* (MHOM/ET/67/HU3) wild and episomal green fluorescent protein (eGFP) labelled stationary stage promastigotes were co-incubated with whole blood and MDM to determine infection of these cells. Engulfment of promastigotes by the cells and their transformation to amastigote forms in MDM was studied with direct microscopy. Microscopy and flow cytometry were used to measure the infection rate while PCR-RLFP was used to confirm the infecting parasite.

3 Results

L. donovani infected bovine whole blood PMN in the presence of plasma factors and all cellular elements. Morphological examinations of stained cytospin smears revealed that PMN engulfed promastigotes. Similarly, we were able to show that bovine MDM can be infected by L. donovani, which transformed to amastigote forms in the cells.

4 Conclusions

The in vitro infection of bovine PMN and MDM by *L. donovani* further strengthens the possibility that cattle might serve as source of *L. donovani* infection for humans.

C0334 THYMUS ALGERIENSIS BOISS. & REUT. AND RHUS TRIPARTITUM (UCRIA) EXTRACTS: FUTURE PROSPECTIVE TO COMBAT CUTANEOUS LEISHMANIASIS

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1 Background

Leishmaniasis is a parasitic neglected disease causing rigorous morbidity and fatality. Unfortunately, no developed vaccine has been available yet, despite advanced research. The majorities of accessible chemotherapeutic compounds have numerous drawbacks. There is an urgent need to explore natural sources for effective and less toxic compounds. We screened the antileishmanial activity of 10 Tunisian medicinal plants on the basis of ethnobotanical features and wide use in folk medicine for the treatment of numerous infections, as well as, their widespread in the leishmaniasis endemic region of collect. The most potent among screened plants were; *Thymus algeriensis* Boiss. & Reut. and *Rhus tripartitum* (Ucria), members of *Lamiaceae* and *Anacardiaceae* families, respectively and, are commonly known as medicinal plants par excellence. We assessed the *in vivo* development of *L. major* infection in BALB/c mice, also the *in vitro* antileishmanial and cytotoxic activities of plant extracts against promastigotes and BALB/c peritoneal infected macrophages.

2 Methods

Infections of BALB/c mice were performed with Ficoll gradient-purified metacyclic *L. major* and, infectious parasites were used for the *in vitro* assays. Antileishmanial activity of natural extracts against promastigotes and amastigotes in BALB/c peritoneal infected macrophages was evaluated with parasites counting. The cytotoxicity of extracts against promastigotes and peritoneal macrophages was assessed with LDH Cytotoxicity Detection Kit. Generated macrophages from mice were determined by flow cytometry.

3 Results

In this research, we highlighted a significant *in vitro* antileishmanial activity, which is time and dose-dependent response, resulting in 50% inhibitory concentration (IC_{50}) of 92.5 µg mL^{-1} and, 240 µg mL^{-1} with ethanolic extracts of *Thymus algeriensis* against *Leishmania major* promastigotes and amastigotes, respectively, after 72h. For *Rhus tripartitum*, IC_{50} ranged between 113.5 µg mL^{-1} and, 85 µg mL^{-1}, and this for promastigotes and amastigotes, respectively. All extracts exhibited no cytotoxic effect.

4 Conclusions

In summary, the screened extracts showed (i) a potent anti-promastigote activity, (ii) an important anti-amastigote ability, by decreasing percentage of infected macrophages and parasite burden and, (iii) no cytotoxic impact of tested concentrations. Further investigations are needed, especially, the deconvolution of active compounds which can be drug prototypes for leishmaniasis treatment.

C0343 EXPRESSION OF A2 PROTEINS DURING THE ESTABLISHMENT OF AN INTRACELLULAR INFECTION BY L. DONOVANI

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1 Background

The family of A2 proteins, first identified in *Leishmania donovani*, are thought to promote visceralization by protecting parasites from the stress brought on by host defenses such as fever and the oxidative response. The stimuli that promote A2 expression, however, have been almost exclusively studied in axenic amastigote and promastigote cultures. In this study, we investigated the expression of A2 proteins in *L. donovani*-infected cells of the monocyte/macrophage lineage, particularly in the initial stages of infection by either the promastigote or the amastigote form.

2 Methods

Peritoneal macrophages (PEM) and splenic amastigotes were isolated from hamsters infected for over 60 days with *L. donovani* LV9 parasites. Murine bone marrow-derived macrophages (BMDM) were infected with stationary-phase promastigotes or with splenic amastigotes for 2h to 96h. A2 expression was monitored by Western blotting throughout the course of infection. In parallel, parasites cultures were incubated at 26°C or 37°C, in neutral (pH 7.4) or acidic (pH 5.5) medium. Phagosome acidification was visualized using Lysotracker.

3 Results

As previously described, *L. donovani* amastigotes obtained from the spleen of infected hamsters did not express A2 proteins. Although PEM obtained from these hamsters were highly infected, A2 proteins were also undetectable in these cells. On the other hand, infection of BMDM with promastigotes promoted A2 expression, albeit significantly less than when the same parasites were cultured in conditions simulating the phagolysosomal environment (pH 5.5/37°C/5%CO_2). This was not due to the fact that lipophosphoglycan delays phagosomal acidification, since a shift in temperature alone was sufficient to induce A2 expression in promastigote cultures.

4 Conclusions

Our data confirm that A2 proteins are not specific to the amastigote stage and suggest that, while they may play a role in visceralization, these proteins cease to be expressed once the infection is well established inside the host. This study also provided evidence that A2 proteins are expressed by promastigotes during the initial stages of infection in cells of the monocyte/macrophage lineage, most likely in response to heat stress. Whether additional intracellular stimuli play a role in triggering A2 expression remains to be investigated.

C0355 LEISHMANIAñMACROPHAGE MULTIFACETED INTERACTIONS: MOLECULAR DOCKING APPROACH OF SURFACE PROTEINS.

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1 Background

Leishmania parasites are protozoans which infect human hosts and are causative agents of a series of diseases known under the name of leishmaniasis. Because Leishmania parasites are obligatory intracellular pathogens, macrophages are indispensable for parasite survival, replication, and differentiation. Although a lot of progress has been made in Leishmania research in the past few years, much remains to be explored in the area of Leishmania–macrophage multifaceted interactions. Hence, new approaches as computational methods would be very helpful to predict protein-protein interactions and validate the obtained experimental results. For instance, In this study, the molecular docking was carried out with the aim of identifying different protein partners from two sets of established proteins, in order to detect protein pairs that may form at the attachment of the parasite *L. major* and human macrophage.

2 Methods

In this work, 3D structure of 3 surface proteins (HASPA, HASPB, and KMP11) of the parasite *L. major* has been determined by homology modeling starting from their peptide sequences. These proteins along others with published 3D structures have been used in a molecular docking study in order to identify the different protein partners involved.

3 Results

Thereby, an interaction network between the different surface proteins of the parasite and those of the host cell (macrophage) has been established. HASPA, HASPB, and gp63 have shown significant interaction (lowest binding energy) with CD181, and CD35.

4 Conclusions

In this work, we studied the protein-protein interactions between the Leishmania major parasite and its human host cell by an *in silico* approach modeling and molecular docking. We succeeded in the first place, to determine three-dimensional structures of three Leishmania major surface proteins, which provided more structural information related to the virulence of the parasite. The exploitation of these structures has allowed us to generate a network of protein-protein interactionand binding energies estimated for different protein complexes formed. These data will be very useful for the detection of pharmaceutical targets and the design of new therapeutic molecules acting at this stage of the disease process and thereby avoiding the proliferation of the amastigote by infecting other neighboring macrophages.

C0391 BRAIN TROPISM OF LEISHMANIA DONOVANI IN IMMUNODEFICIENT MICE

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1 Background

Visceral leishmaniasis (VL) is a systemic disease caused by the *Leishmania donovani* complex. Usually VL affects organs such as spleen, liver and bone marrow, but it can also affect the central nervous system (CNS). However, there are differing opinions in the literature concerning the potential of *Leishmania* to enter the CNS, with the majority of studies using analysis of kDNA as a surrogate for parasite tropism.

2 Methods

The aim of this study was to investigate the brain tropism of *L. donovani* in immunodeficient mice using fluorescent image techniques. C57BL/6 *Rag1*$^{-/-}$ mice (herein called RAG mice) were infected intravenously with 8×10^7 transgenic *L. donovani* (LV9) amastigotes expressing tandem Tomato fluorescent protein (tdTom.*L. donovani).*

3 Results

Brain tissue samples were positive for parasite kDNA by qPCR, in keeping with previous literature. More importantly, using both a non-invasive whole body imaging (IVIS) and stereomicroscopy analysis, we detected the presence of tdTom.*L.donovani* in the intact brain of RAG mice infected for either 80 or 180 days, with no difference among these groups. To further define the localization of amastigotes in the brain, RAG mice were injected with 4mg of 500kDa FITC-dextran in PBS prior to sacrifice. Brain tissues (cerebellum, frontal lobe, diencephalon and occipital lobe) were then fixed, and embedded for cryosectioning and confocal microscopy. Amastigotes were detected in each of the four brain areas and were restricted to the parenchyma rather than being within the blood circulation.

4 Conclusions

Therefore, this study directly confirmed *Leishmania* brain tropism in immunodeficient mice. Moreover, it indicates that the entrance of *L. donovani* into the brain parenchyma of these mice can occur through mechanisms independent of adaptive immunity. The implications of these findings for CNS infection in canine VL and in and humans with immunosuppression due to HIV will be discussed.

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C0409 FIRST REPORT OF SPIROPLASMA INFECTION IN PHLEBOTOMUS PAPATASI SANDFLIES

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1 Background

Spiroplasma is a wall-less and small Gram-positive bacteria where live in their host in symbiotic (mostly commensal, rarely mutualistic) or pathogenic interaction. In insects, spiroplasmas are typically commensals whereas a few numbers of species are pathogens. Blood-sucker insects such as tabanids and mosquitoes are particularly rich sources of *Spiroplasma*. The ability of spiroplasmas to propagate in rodents was experimentally proven, and *Spiroplasma* infections have been reported recently in humans causing transmissible spongiform encephalopathies (TSEs). Sand flies possess a wide spectrum of hosts including rodents, dogs and human, and able to transfer various pathogens including *Leishmania* spp, *Bartonella baciliformis* (infectious agent of the Oroya fever), sandfly (Pappataci) fever virus, however, there is no report on *Spiroplasma* infection in sand flies.

2 Methods

In this study, the symbiont bacteria were assessed in *Phlebotomus papatasi* specimens collected in the central region of Iran. A fragment (a. 1500 bp) of the bacterial 16S rRNA gene was amplified and sequenced.

3 Results

All bacteria positive PCR products were sequenced and analyzed in BLAST queries. We found *Wolbachia* in the majority of specimens, however, *Spiroplasma* were detected by PCR in one sample. We found high similarities to 99% identity with *Spiroplasma melliferum* bacteria found in insect species like honey bees,that has pathological effect in several tissues, and shortening adult longevity.

4 Conclusions

It is the first report of sandfly *Spiroplasma* infection in science, however, further investigation needs to clarify whether the bacterium has pathology and possibly reduce life span of *P.papatasi* and if it can be considered as a biological agent for control of leishmaniasis. Furthermore, due to *Spiroplasma* infection of sand flies inhabiting in rodent burrows adjacent to human setting, the possibility for transferring *Spiroplasma* to humans and other vertebrates in natural conditions should be considered. In the light of the data obtained in the current study, it is recommended investigating such possibilities.

C0424 CANINE NEUTROPHILS ENSURE THE SURVIVAL OF VIRULENT LEISHMANIA INFANTUM PARASITES

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1 Background

Canine leishmaniosis (CanL) caused by *Leishmania infantum* is a severe and zoonotic disease. Although macrophages (MØ) are the definitive host cells, neutrophils are the first cells to encounter the parasite soon after its inoculation into the dermis by the phlebotomine vector. This study aims to evaluate the interaction between dog neutrophils (PMN) and *L. infantum* promastigotes, clarifying PMN contribution to parasite early control or on the contrary to infection establishment.

2 Methods

Blood PMN isolated from healthy dogs were exposed to *L. infantum* promastigotes and parasite-cell contact were evaluated by flow cytometry and by optical and scanning electron microscopy. Intracellular superoxide production, elastase exocytosis and neutrophil extracellular traps (NET) were used to evaluate PMN activation. PMN migration was assessed by a chemotaxis assay and *Leishmania* killing was investigated by transmission electron microscopy. Viability and replication ability of PMN-internalized parasites were also determined.

3 Results

In the majority of the dogs, *L. infantum* was efficiently phagocytized by PMN. Oxidative and non-oxidative intracellular mechanisms were activated in infected PMN, but the release of NET was minimized, indicating modulation by extracellular parasites. Although promastigotes and parasite culture supernatants induced PMN migration, a prior contact with *Leishmania* inhibited chemotaxis, which can ensure PMN retention at the inoculation site. Although PMN-*L. infantum* interaction decreased the parasite viability, some intracellular parasites survived and retained their replicative capacity.

4 Conclusions

Dog PMN are competent effector cells able to control the early phase of *L. infantum* infection. Retention of parasitized PMN at the infection site may favor parasite internalization by recruited MØ. Furthermore, some parasites, probably the most virulent/infective evade intracellular and extracellular PMN effector mechanisms. These findings indicate that both intracellular and extracellular parasites can modulate effector PMN mechanisms, ensuring their survival until be internalized by the definitive host cell. Therefore, PMN action seems to select the fittest parasites able to establish infection and CanL development.

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C0458 EXOSOME SECRETION BY LEISHMANIA INFANTUM: MODULATE INNATE AND ADAPTIVE IMMUNE RESPONSES AND CREATE AN ENVIRONMENT PERMISSIVE FOR EARLY INFECTION

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1 Background

In recent years, several studies demonstrated the role of exosomes in intercellular communications. Specialized secretion systems are used by numerous bacterial pathogens to export virulence factors into host target cells. Leishmania deliver effectors proteins into host cells; however, the mechanisms involved have remained elusive. The aim of this study was to isolate, characterize *Leishmania infantum* exosomes and to investigate the biological activity of exosomes in macrophage cultures.

2 Methods

Human cell lines used were U937 cells and macrophages derived from U937 after treatment with 25 ng/ml PMA.Western blot assay used antibodies to HSP70, HSP83/90. Exosomes were collected by *L. infantum* -conditioned medium by ultracentrifugation.IL10, IFNγ, IL1αIL1β, IL12, IL4 and IL18 secretion were evaluated by ELISA kit.

3 Results

We first purified extracellular vesicles shed by *L. Infantum* promastigotes and amastigotes on a sucrose gradient and later we characterized these extracellular vesicles for HSP70, HSP83/90 and acetylcholinesterase. Furthermore we performed a NanoSight nanoparticle tracking analysis revealed an average of the mode value of 76 ± 5 nm for promastigotes and 94± 5 nm for amastigotes exosomes. All these data demonstrated that *L. Infantum* released exosomes. The treatment of U937 cells with 10 μg/ml of promastigote and amastigote exosomes showed an increase in motility of these cells that can facilitate the progression of infection. We showed also an overproduction of IL10 by macrophages after treatment with exosomes that support parasite persistence and disease establishment, while exosomes limited the production of IFN-γ and IL12 that block the parasite killing and host protection, as well as the production of IL1α, IL1β and IL4. Regarding IL18 production,that is involved in the Th1-type immune responses,L. exosomes determined a reduction in the production of IL18 in U937 monocyte culturescompared to control. Value that does not vary instead macrophages cultures.

4 Conclusions

We demonstrated that exosomes can regulate the immune system and exacerbate disease outcome, adding to the repertoire of virulence factors involved in vector-transmitted infection. The exosomes are able to contribute in the disease estabilishment and may be an appropriate candidate for a vaccine therapy in prophylaxis and treatment.

C0503 MACROMOLECULAR BIOSYNTHETIC PARAMETERS AND METABOLIC PROFILE IN DIFFERENT LIFE STAGES OF LEISHMANIA BRAZILIENSIS: AMASTIGOTES AS A FUNCTIONALLY LESS ACTIVE STAGE

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1 Background

Pathogens are able to change their physiology from a highly proliferative stage when the environment and nutrients are favorable, to a stage with a slow or absent proliferation (semi-quiescence or quiescence) when they face immunological stress or shortage of nutrients. This major adaptive skill allows pathogens to persist in their host for long periods of time, escape chemotherapy without developing resistance and reactivate disease years after the primary infection. These are features shared by *Leishmania*, but reports on its potential for quiescence are scarce. We present the first study focused on amastigotes of *L. braziliensis*, the most aggressive and prevalent species of the New World, difficult to treat and prone to mutilating relapses.

2 Methods

We used the strain MHOM/BR/75/M2904 for the generation of logarithmic promastigotes (Pro^{log}), stationary promastigotes (Pro^{sta}), axenic amastigotes (Ama^{axe}) and intracellular amastigotes (Ama^{int}). We evaluated *in vitro* the kinetic of proliferation together with the expression of indicators of biosynthesis such us the levels of ATP, kDNA, expression of rRNAs (18S and 28S α) and ribosomal proteins (RPL19 and RPS15). For further characterization of the metabolic stage of amastigotes we performed untargeted metabolomics.

3 Results

In contrast to the proliferative Pro^{log}, the Ama^{axe} and Ama^{int} showed limited growth and decreased levels of ATP and kDNA mini-circle. The molecules of rRNA 18S and 28S α decreased in amastigotes with the Ama^{int} showing the deepest drop (0.24% of the amount in Pro^{log}). Similarly, Ama^{axe} showed decreased levels of RPL19 and RPS15. On metabolic level, the Principal Component Analysis of 186 metabolites showed that the first (44.58 %) and second (39.56%) principal component clearly separated the Pro^{log}, Pro^{sta} and Ama^{axe}. The Ama^{axe} showed a down regulation of biosynthetic metabolites such as amino acids and polyamines together with strong changes in the levels of glycerophopholipids and fatty acids. Moreover, $Amas^{axe}$ had increased levels of the pathway of synthesis of endogenous sterols and extremely elevated levels of the exogenous cholesterol.

4 Conclusions

Our results demonstrate that *L. braziliensis* amastigotes are significantly less active than proliferative promastigotes and as such show the features of a quiescent or semi-quiescent stage. Our findings would have a major impact for the understanding of the (sub)clinical presentations associated with that species.

C0506 PHENOTYPIC AND FUNCTIONAL GENETIC ANALYSIS OF VIRULENCE ATTENUATION IN LEISHMANIA DONOVANI

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1 Background

Leishmania donovani cycles between insect and mammalian hosts and has evolved strategies to survive inside phagocytic cells by subverting host antimicrobial activities through expression of virulence factors. Mechanisms that govern intracellular *Leishmania* survival remain widely unknown and only few parasite virulence factors have been identified to date. Our previous results revealed a progressive loss of *L. donovani* infectivity during culture adaptation due to fitness trade off. Here we combine *in vitro* virulence attenuation with functional genetic screening based on CosSeq technology to identify novel *Leishmania* virulence determinants.

2 Methods

In order to assess mechanisms underlying loss of infectivity, virulent promastigotes derived from hamster splenic amastigotes (culture passage P2/ 20 generations) and attenuated long-term cultured parasites (culture passage P20/ 190 generations) were characterized *in vitro* and *in vivo.* A cosmid library was generated using P2 genomic DNA for a functional genetic screen (CosSeq). P20 parasites were transfected with this homologous library and injected into hamsters for *in vivo* selection of parasites carrying a cosmid with genes that enhance infectivity allowing the identification of novel virulence factors. Parasites were extracted from hamster spleens and livers 10 or 20 weeks post-infection. Cosmids were recovered and subjected to HTseq analysis.

3 Results

The phenotypic characterizationof virulent promastigotes P2 and attenuated parasites P20 allowed to correlate virulence attenuation to growth, stress resistance and infectivity.
Hamsters injected with P20 promastigotes transfected with the cosmid library showed clinical signs of illness suggesting increased virulence. High-throughput sequencing of the recovered cosmids revealed highly selected cosmids, some of which carried genes already described as essential for amastigotes survival, thus validating the *in vivo* selection. Our functional genetic screen also uncovered enrichment of organ-specific cosmids, suggesting that virulence may be regulated in a tissue-specific manner. After mapping of virulence genes encoded on the cosmid inserts by transposon mutagenesis, genes will be individually validated *in vivo* and *in vitro* for their capacity to increase parasite infectivity and virulence.

4 Conclusions

Our *in vivo* functional genetic screen sheds new light on amastigote-specific virulence mechanisms and tropism with potential relevance for vaccinology and drug discovery.

C0514 LEISHMANIA DONOVANI INHIBITS TRANSLATION OF IRON EXPORTER FERROPORTIN FOR ITS GROWTH WITHIN HOST MACROPHAGES

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1 Background

The protozoan parasite *Leishmania donovani* (LD) resides within and competes with host macrophage for availing iron. Iron release from macrophage is regulated by hepcidin mediated degradation of iron exporter ferroportin (Fpn) during extra- and intracellular infections and plays a crucial role in host-parasite interaction. The aim of the current study is to understand the fate of iron exporter Fpn during LD infection.

2 Methods

In vivo study was performed on splenocytes isolated from LD infected and uninfected Balb/c mice whereas mouse macrophage cell lines J774A.1 and RAW 264.1 were used for in vitro studies. The protein expression was studied using Western Blot, Immunofluorescence, Immunohistochemistry assay, and mRNA expression by real time PCR. Flag and GFP tagged recombinant Fpn was expressed in macrophage cell lines. S^{35}-metionine labelling was used for *de novo* Fpn synthesis. Iron response element (IRE) - Iron regulatory protein (IRP) interactions were verified by RNA-Electrophoretic Mobility Shift Assay (EMSA), and Luciferase assay. siRNAs were used to silence the protein expression. LD growth was detected by counting intracellular LD.

3 Results

Fpn expression in LD-infected macrophages was down-regulated despite no change in hepcidin expression *in vitro* and *in vivo* infection model of LD. Unaffected Fpn-FLAG expression in LD infected J774 macrophages further suggested that Fpn down-regulation was not due its degradation. Despite increase in Fpn mRNA, protein expression was down-regulated in LD-infected host macrophages implying blocking of Fpn translation that was confirmed by S^{35}-methionine labelling assay. Involvement of IRE present in Fpn 5'UTR was confirmed using RNA-EMSA and luciferase assay. Supershift analysis provided evidence of involvement of IRP2 in IRE-mediated inhibition of Fpn translation in vivo. Significant increase in IRP2 with simultaneous decrease in its protein stability regulator FBXL5 in splenocytes of LD-infected mice suggested the role of IRP2. Higher intracellular LD growth was detected when Fpn expression was decreased by its specific siRNA.

4 Conclusions

This study reveals a complete novel strategy of a pathogen to inhibit iron release from host cell by manipulating IRP2-FBXL5 axis for its survival benefit.

C0520 EXOSOMAL CATHEPSIN B CYSTEINE PROTEASE OF L. DONOVANI MODULATES TGF-B1 AND ARGINASE ACTIVITIES IN MACROPHAGES

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1 Background

Cathepsin B cysteine protease (catB) is a virulence factor in *Leishmania* infection involved in the activation of human Transforming Growth Factor (TGF)-β1. Active TGF-β1 is suggested to play a role in *Leishmania donovani* survival by modulating arginase activity and nitric oxide (NO) production in macrophages. The disruption of catB gene was shown to induce proteome remodeling in *L. donovani*, affecting proteins actively secreted into exosomes, vesicles involved in intercellular communication. In this study, we aimed to investigate the role of *L. donovani* exosomes in modulating TGF-β1 and arginase activities in macrophages and the effect of catB on the expression of exosomal proteins.

2 Methods

To conduct this study, we used *L. donovani* catB wild type (wt), catB null mutants (ko) and episomally complemented catB ko (cm) parasites. Purified exosomes and parasites were analyzed by electron microscopy and immunoblotting to confirm the isolation of exosomes and to validate the expression of the selected virulence factors: Elongation Factor (EF)-1α, Peroxidoxin (Pxn)-4 and catB. To assess the proteolytic activity of exosome-derived catB, we incubated J774.1 and PMA-activated U937 macrophages with exosomes and measured the levels of active TGF-β1 and NO in the supernatants 24h and 48h post-incubation. Also, we measured the activities of inducible nitric oxide synthase (iNOs) and arginase in the exosome-treated macrophages.

3 Results

Pxn4, EF-1α and catB were secreted into exosomes and the expression of Pxn4 and EF-1α were downregulated in exosomes from ko parasites, which is consistent with the decreased levels of these proteins in the lysates of ko parasites. Exosomes from wt parasites were able to induce high level of active TGF-β1 in J774.1 and U937 cells, indicating the active state of exosome-derived catB. The arginase activity in J774.1 cells was enhanced 48h post-incubation with exosomes from wt parasites, despite the fact that no differences in NO production or iNOS activity were observed during the assays.

4 Conclusions

CatB is secreted into exosomes and modulates the expression of exosomal proteins that are associated with *L. donovani* survival and pathogenesis. The high level of active TGF-β1 detected in macrophages incubated with exosomes from wt parasites strongly indicates the immunomodulatory role of exosome-derived catB in TGF-β1 activation and arginase activity, mirroring the properties of parasites in infected macrophages.

C0543 DYSFUNCTION OF BLOOD NEUTROPHILS AND MONOCYTES IN CLINICAL VISCERAL LEISHMANIASIS

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1 Background

Neutrophils and monocytes are rapidly recruited from the circulation to the site of *Leishmania* . The aim of this study was to investigate the response of blood neutrophils and monocytes to *Leishmania (L). donovani* and to activating stimuli as well as their phagocytic activity in VL patients.

2 Methods

Using a whole blood-based assay, the response of neutrophils and monocytes of VL patients and endemic healthy controls (EHC) to *L. donovani* and to the TLR agonists LPS, MALP-2 and poly (I: C) was investigated by assessing the cell surface expression of CD62L and CD66b, and phagocytosis. *Staphylococcus aureus* (*S. aureus)* bio-particles *were* applied to assess phagocytic capacity in a whole blood assay *in vitro*. Cell activation markers and phagocytic capacity were measured by flow cytometery.

3 Results

Neutrophils of VL patients showed reduced response to *L. donovani* and MALP-2, regarding shedding of CD62L. Similarly, *L. donovani* induced degranulation was significantly lower in neutrophils from VL patient than in EHC. The phagocytic activity of neutrophils and monocytes in response to stimuli was significantly compromised in VL.

4 Conclusions

The experimental findings indicate a dysfunction of neutrophils and monocytes in VL, which may contribute to development of life threatening disseminated disease in VL.

C0544 ANALYSIS OF HEPARAN SULPHATE IN THE INFECTION OF HOST MACROPHAGES BY LEISHMANIA PARASITES

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1 Background

With resistant strains emerging to the few drugs available, more detailed information is needed on the interactions of *Leishmania* parasites with host cells to drive development of new therapeutics. The roles of host carbohydrates in *Leishmania* infection remain largely unexplored. The glycosaminoglycan, heparan sulphate (HS), is a linear polysaccharide comprised of a repeating backbone structure of glucuronic acid and N-acetylglucosamine disaccharides. During synthesis, the backbone is modified by a multitude of enzymes, resulting in the fine chemical patterning of the chain. This primary structure is believed to bind target biomolecules and mediate their function. We have analysed the expression and composition of HS from human macrophages infected with *L. donovani* or *L. mexicana* to investigate changes in its structure and explore the involvement of this glycosaminoglycan in leishmaniasis.

2 Methods

Human macrophages were infected with *Leishmania* stationary phase promastigotes and the HS was isolated and purified by anion exchange chromatography. HS chains were degraded with heparinases and labelled with a fluorophore, BODIPY-Fl hydrazide. Separation and analysis of the HS disaccharide composition was achieved using ion-paired reverse-phase high performance liquid chromatography. Flow cytometry was also utilised to analyse changes in HS epitopes present at the cell surface of *Leishmania*-infected human macrophages compared with uninfected macrophages.

3 Results

Production and modification of HS is altered in human macrophages infected with *Leishmania* parasites. HS disaccharide compositional analysis showed an increased abundance of highly sulphated species concurrent with a decrease in unmodified disaccharide residues, suggesting that the biosynthesis and expression of HS is influenced by the *Leishmania* parasite. Flow cytometry data demonstrated that *Leishmania*-infected macrophages display HS with epitopes rich in N-sulphation, suggesting that the domain structure of HS is also distinct from uninfected macrophages.

4 Conclusions

Together, these data show that the biochemistry of HS in *Leishmania*-infected human macrophages differs from uninfected macrophage cells, suggesting that HS may be important for virulence. The observed increase in sulphation of the HS chain will alter the binding properties of target biomolecules and therefore affect the biological role HS plays during *Leishmania* infection. Work is ongoing to further elucidate the relationship between macrophage HS and *Leishmania* parasites.

C0593 ANTIOXIDANT ENZYMES: SAVIOR FOR LEISHMANIA AGAINST MACROPHAGE ARSENALS.

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1 Background

Oxidative burst (ROS) mediated DNA damage is one severe form of threat that intramacrophage pathogens experience immediately after their internalization. However, *Leishmania donovani* infection successfully neutralizes ROS generation to establish the infection. In the present study, we have identified the role of Heme oxygenase1 (HO1) in neutralizing ROS mediated macrophage defence. Our study also investigates the role of Carbon monoxide (CO), a product formed by HO-1 in deactivating TLR dependent macrophage immune activation.

2 Methods

Various biochemical, molecular biology, immunological techniques were applied to demonstrate our findings. All experiments were done in raw 264.7 macrophages and validated in bone marrow derived macrophages isolated from Balb/c mice and also statistically validated.

3 Results

Screening of major macrophage antioxidant enzymes revealed increased level of heme degrading enzyme HO-1 during early point of infection and silencing of HO1 by specific siRNA in infected macrophages documented increased superoxide production and decreased parasite survival. HO-1 expression was found to be inversely related to NAD(P)H oxidase activity and increased NAD(P)H oxidase activity in HO-1 silenced infected macrophages showed the role of NAD(P)H oxidase in HO-1 mediated ROS neutralization.HO-1 induction decreased heme availability essential for the maturation of gp91*phox* subunit of NAD(P)H oxidase and replenishment of heme during infection restored functional gp91*phox* and NAD(P)H oxidase. Persistent HO-1 expression at late hour of infection without any apparent ROS production raised queries about its effect on other host defence parameters and inhibition study revealed a reciprocal relationship of HO-1 with host pro-inflammatory response.CO, a byproduct of heme catabolism by HO-1 documented potent anti inflammatory effects. Quenching of CO decreased the negative modulation of IL-12 and TNF-α production and found that CO inhibits activation of TLR signalling by inhibiting the interaction of TLR4 with adaptor molecules MyD88 and TRIF thereby decreasing proinflammatory cytokine levels. Inhibition of HO1 by tin porphyrin in mice model of visceral leishmaniasis displayed decreased spleen parasite burden and enhanced the production of IL-12 and TNF-α.

4 Conclusions

Our findings thus identified the dual role of infection induced HO-1 during early and late phase to differentially modulate macrophage defense machinery for propagating infection.

C0603 REGULATION OF CELLULAR SIGNALING PATHWAYS IN HUMAN MACROPHAGE DURING LEISHMANIA SPREADING

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1 Background

Leishmania parasites are the causative agent of a wide range of human diseases known as leishmaniasis. The progression of disease pathogenesis is dependent on the ability of intracellular parasites to disseminate between human macrophages. It is assumed that parasites could exit macrophages following cell lysis. However, there is still a major gap in knowledge in understanding the exact mechanism pertaining such critical step. This has been partially caused by the lack of reliable experimental models to study the late stage of host-parasite interaction.

2 Methods

L. aethiopica and *L. mexicana* infected cells at 72 hours from infection were used to infect differentiated THP-1 macrophages and validated as a model for infection spreading. Amastigotes spreading was detected within 12 hours of co-culture as confirmed by flow cytometry and live cell imaging techniques. To address whether parasite spreading is linked to apoptotic induction, this model was used to detect expression of several apoptotic markers. Phosphatidyl serine (PS) externalization and active caspase-3 expression were detected via flow cytometry, expression of AKT, PKC-δ, IκBα, BAD and cytochrom C via western blot analysis.

3 Results

The results showed that *L. aethiopica* (but not *L. mexicana*) dissemination was correlated with a significant increase ($p<0.05$) in phosphatidyl serine (PS) externalization and active caspase-3 expression. Prior to infection spreading a significant reduction in phosphorylation of AKT within *L. aethiopica* infected co-culture cells was detected. Although this caused a decrease in phosphorylated BAD,no effect on cytochrome C release was detected. Western blot analysis of cytosolic extract revealed an increased level of cytochrome C when compared to uninfected co-culture cells. Similarly, the NF-κB signaling was subverted before and during spreading. The infected co-culture cells showed reduced expression of Iκ-Bα and p65 subunit, suggesting that *L. aethiopica* amastigote induced protein degradation. Finally, downregulation of PKC-δ protein was observed within 1 hours of co-culture. Interestingly, this negative effect was consistent throughout the spreading of L8G infection with no clear evidence of PKC-δ CF accumulation.

4 Conclusions

L. aethiopica spreading between macrophaghes is associated with induction of host cell apoptosis via deactivation of AKT, release of cytochrome C and consequent activation of caspase 3 and PS exhibition independently from PKC-δ.

C0608 ANALYSIS OF MIF PROMOTER, VNTR AND NON CODING RNA GENE (LOC284889) IN INDIAN VISCERAL LEISHMANIASIS

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1 Background

Visceral Leishmaniasis (VL) is endemic in Bihar state of India. Macrophage Migration Inhibitory Factor (MIF) is an important host antimicrobial cytokine which is a key regulator of innate immune system.MIF is secreted by lymphocytes, macrophages, dendritic cells, neutrophils, and pituitary cells during infection. MIF is coded by human MIF gene, whose genetic variants were found to be associated with several other inflammatory and infectious diseases. Although VL patients in India display high level of MIF in blood serum, no genetic studies has been conducted to assess the VL susceptibility / resistance. Therefore, we aimed to study the important MIF gene variants in Indian VL.

2 Methods

Study subjects comprised of 214 cases and 220 controls, collected from VL endemic region in India. We sequenced the three important MIF gene variants -794 CATT (5-8) microsatellite repeats (rs145871794; 5'UTR variant), *-173 G>C* (rs755622; promoter variant)and non-coding RNA gene (LOC284889; rs34383331) in an ethnically and geographically matched case and controls.

3 Results

The distribution frequencies for -794 CATT repeats ($\chi2$ = 0.86; p=0.35; OR=85; 95%CI=0.61-1.19), -173 G>C polymorphism ($\chi2$ =1.11; p=0.29; OR=0.83; 95%CI=0.59-1.16) and LOC284889 ($\chi2$ =0.78; p=0.37; OR=0.86; 95%CI=0.61-1.20) were almost similar in both case and control groups. Our study revealed that statistically there was no significant difference in allele, genotype, haplotype, diplotype frequencies for reported MIF variants. All the studied variants were hardy weinberg equilibrium for control subjects. Linkage disequilibrium (LD) pattern showed strong LD pattern between rs755622 and rs34383331 polymorphism.

4 Conclusions

Host *MIF* gene variants were not significantly associated with Indian VL. Our data postulate that other vital genes involved in the MIF pathway may provide genetic clues to elucidate of MIF regulation during VL. Present study provides a better understanding for pathogenesis of Indian VL.

C0610 ARGINASE ACTIVITY IN THE HEALING AND NON-HEALING INFECTED MICE BY PATHOGENIC IRANIAN STRAINS OF LEISHMANIA VERSUS NON-PATHOGENIC L. TARENTOLAE

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1 Background

Leishmania (L) is an intracellular organism requiring a suitable milieu for their growth and invasion within the host macrophages. Arginase (ARG) activity is crucial for survival and proliferation of *Leishmania* in the host cells. In the present study, we characterized the ARG activity of Iran pathogenic strains of *Leishmania* (*L. major* and *L. tropica*) and non-pathogenic *L. tarentolae*. Moreover, we characterized and compared the ARG activity in healing and non-healing mice.

2 Methods

After confirmation and identification of *Leishmania arg* gene by PCR, the *arg* gene was cloned and sequenced. Furthermore, the ARG expressions levels were assayed by RT-PCR. We tested ARG enzymatic activity in promastigotes of parasites in both pathogenic and non-pathogenic strains. Then at the site of infection, the ARG activity was measured in both non-healing BALB/c and healing C57BL/6 mice.

3 Results

After confirmation of *arg* gene in *Leishmania* and its high expression in promastigotes of pathogenic *Leishmania* over non-pathogenic *L. tarentolae*, we found that arginase activity, a signature of disease, was increased in the infected BALB/c mice by *L.major* over *L.tropica* and *L. tarentolae* in the site of infection. Interestingly, in healing C57BL/6 mice, arginase decreased significantly over non-healing mice; further our results showed that high parasite burden in *L. major* infected mice was significantly higher than *L.tropica* and not detectable in *L. tarentolae* infected mice.

4 Conclusions

The outcome of cutaneous leishmaniasis in BALB/c mice has been a controversial issue. Our results showed that *Leishmania* parasites have their own ARG activity that differs from mammalian ARGs. Thus, we conclude that differences in the ARG activity of the different *Leishmania* isolate may act as one of the factors that influence the statue of healing and non-healing in mice. Furthermore, high ARG activity is observable at the site of infection.

C0615 COMPLEX INTERPALAY BETWEEN PKA AND AKAPS: CRUCIAL FOR INTRACELLULAR SURVIVAL OF LEISHMANIA

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1 Background

Leishmania donovani infection led toPGE_2-induced cAMP-dependent PKA activation, which is vital for modulating host inflammatory responses in favour of the parasite. This present study was aimed to gain detailed mechanistic understanding of the molecular events downstream of PKA in *Leishmania* infected macrophages.

2 Methods

PKA activity was measured following *Leishmania* infection. PKA-R subunits,RI and RII, was selectively inhibited by pre-treatment of macrophages with Rp-Cl-cAMPs and Rp-8-pCPT-cAMPS respectively. Disruption of association of RI and RII with scaffold proteins AKAP (A kinase-anchoring protein) was done by inhibitor peptides (Ht31 and RIAD), cytokine levels were measured both *in vitro* and *in vivo* along with spleen and liver parasite burden. siRNA mediated gene silencing was employed to silence specific AKAPs, cytokine and parasite survival was then studied. Co-immunoprecipitation and Immunoblot studies were used to decipher nuclear translocation and association of NF-kB with other proteins.

3 Results

Selective inhibition of RII subunit, significantly reversed the inhibitory effect of *Leishmania* on TNF-α production. On the contrary, specific inhibition of RI markedly abolished *Leishmania* mediated increase in IL-10 production. Of the various AKAPs expressed in macrophages, *L.donovani* infection preferentially upregulated the expression of AKAP8 and AKAP10. siRNA mediated gene silencing revealed that *Leishmania*-induced TNF-α suppression was mediated by AKAP8-anchored RII whereas upregulation of IL-10 was mediated by AKAP10-anchored RI. Coimmunoprecipitation studies documented that PKA-AKAP association leads to formation of a cytosolic complex with NF-κB inhibitor p105, thereby inhibiting both the degradation of p105 and nuclear translocation of NF-κB, leading into decreased TNF-α production. Administration of Ht31 and RIAD in infected BALB/c mice resulted in decreased liver and spleen parasite burden and shifted disease propagating cytokine response in favour of host.

4 Conclusions

Our results demonstrate that *Leishmania* exploits PKA regulatory subunits and their association with specific AKAPs to dampen host immune activation essential for propagating infection. No previous reports have directly shown a role for PKA in *Leishmania* anti-inflammatory activity. Thus, PKA/AKAP represents a versatile new target of intracellular parasite to regulate generation of pro-inflammatory response.

C0634 LEISHMANIA EUCARYOTIC INITIATION FACTOR (LEIF) ANTIGEN AS AN IMMUNOTHERAPEUTICAL MOLECULE

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1 Background

Leishmania, agents of leishmaniases, widespread tropical diseases, are obligate intracellular parasites. They disrupt a multitude of cellular signals and protective mechanisms to infect and survive within macrophages. Understanding these host-pathogen interactions brings knowledge to develop novel strategies to control these neglected diseases. ***Leishmania*** LeIF antigen is known to be a natural Th1 type natural adjuvant that has the ability to stimulate IL12 cytokine expression. However, the exact effect of LeIF on human macrophages has not yet been elucidated. We showed LeIF induces resistance of J774 mice cells to ***L. donovani*** infection. We extend here the study to investigate effect of LeIF on infection of a human macrophage cell line by ***L. infantum*** parasites.

2 Methods

PMA-differentiated THP-1 cells were used in ***in vitro*** infections by ***L. infantum***. Infection conditions (MOI, strain, day of parasite harvest) were set to obtain optimal readout (% infected cells; mean number of amastigotes/cell), done by light microscopy of Giemsa stained slides. Optimal LeIF amounts that are not cytotoxic to the cells were determined. We also established the conditions of an MTT assay to measure the parasite viability in infected cells. Cells were treated before the infection with recombinant LeIF and/ or IFN-γ. Their effect on infection was measured on Giemsa stained slides. Viability was evaluated by MTT assay. Cytokines were quantified using ELISA. Griess reagent was used to measure Nitric Oxide (NO) generation. Immunoblotting was used to analyze LeIF treatment effect on key cellular effectors.

3 Results

In the model we established,the infection rate reached 70% with a mean of 6 intracellular parasites. With LeiF +/- INFγ pre-treatment, infectivity of the PMA-activated THP-1 macrophages decreased to 39% and parasite load to a mean 2/cell, thus inferring that LeIF conferred to these cells resistance to ***L.infantum*** infection (80% parasitic index inhibition).). A specific resistance to ***L.infantum*** infection was checked using a phagocytic assay with latex beads. It also induced secretion of TNF-α a pro inflammatory cytokine. Activation of cells by LPS/IFNγ failed to change the supernatant level of nitrites (NO), these results are in line with some reports. Preliminary analysis highlighted activation of AKT on Ser 473 (but not Thr 308) and ERK1/2 kinase in exposed cells before the infection.

4 Conclusions

The study supports LeIF as a potential therapeutical molecule.

C0641 INHIBITION OF MICRORNA LET-7A INDUCES APOPTOSIS AND NECROSIS IN MACROPHAGES INFECTED WITH LEISHMANIA MAJOR

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1 Background

Protozoan parasites of the genus *Leishmania* are etiologic agents of leishmaniasis which are intracellular pathogens of vertebrates and replicate inside the infected macrophages. *Leishmania* have developed complex strategies to reverse host immune responses in favor of it. One of the major species that causes cutaneous involvements is *Leishmania major*. MicroRNAs (miRNA) are non-coding small RNAs encoding 22-nucleotide (nt) long RNAs. miRNAs influence in diverse biological processes, including the cell cycle, proliferation, differentiation, growth and development, metabolism, aging, apoptosis, gene expression and immune regulation . This study aimed to evaluate apoptosis and necrosis after transfection locked nucleic acid (LNA) inhibitor of let-7a in the human macrophages miRNAs upon infectionwith *L. major*.

2 Methods

We performed inhibition of let-7a in macrophages which were derived originally from the human monocytes (MDM), with locked nucleic acid (LNA) antagomir. The total cellular RNA was extracted 24 and 48 hours post transfection. To measure the expression levels of let-7a, quantitative real-time PCR was done with specific related primers using the SYBR® Green master mix Kit™. Annexin-V/Propidium Iodide staining method was performed to detect apoptosis and necrosis in the MDM cells. Resuls were analyzed using the Kruskal-Wallis and Mann-Whitney tests.

3 Results

Let-7a inhibition decreased apoptosis and necrosis of the MDM cells using flow cytometry method.

4 Conclusions

The results suggest that inhibition of let-7a could be a new approach to treat leishmaniasis.

C0648 EXPRESSION OF HSA LET-7A MICRORNA OF MACROPHAGES INFECTED BY LEISHMANIA MAJOR

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1 Background

Leishmaniasis is a vector-born disease caused by species of the genus Leishmania and is transmitted from host to host through the bite of an infected sandfly. MicroRNAs (miRNAs) are non-coding small RNAs with 22-nucleotide length. They are involved in some biological and cellular processes. We aimed to evaluate the expression of let-7a in human macrophages miRNA when are infected by Leishmania major. We also evaluated the impact of Leishmania major infection on the expression of let-7a at two different times, 24 and 48 hours, after infection.

2 Methods

Blood samples were collected from ten healthy volunteers with no history of leishmaniasis. Development of macrophages from peripheral monocytes and infection with stationary phase of Leishmania major promastigotes were done through serial cultures under 5% CO_2 environment and 37iC.To measure the expression levels of let-7a real-time PCR was performed with specific related primers using the SYBR® Green master mix Kit™.

3 Results

The real-time PCR showed let-7a was expressed in cells infected with parasites after 24 and 48h post-infection. Comparison of let-7a miRNA expression after 24 and 48 h revealed that let-7a miRNAs were down-regulated at 48 h post-infection more than 24h after infection.

4 Conclusions

The results of this study suggest that according to the main function of miRNA in repression of mRNA translation it could be possible to manipulate host cells in order to alter miRNA levels and regulate macrophage functions after establishment of intracellular parasites such as Leishmania.

C0654 CASPASE-1 INHIBITION BY LEISHMANIA (L.) AMAZONENSIS AMASTIGOTES: A NEW EVASION MECHANISM?

Thais Boccia da Costa, Cortez Mauro Javier Veliz

Institute of Biomedical Sciences, University of Sao Paulo Brazil

1 Background

Leishmaniasis is an infectious disease that affects people worldwide under cutaneous, mucocutaneous and visceral forms. In macrophages the infection with *L. (L.) amazonensis* induces the expression of CD200, a molecule involved in the inhibition of iNOS expression and NO production, being an evasion mechanism used by the parasite to escape the imune response. Also during the infection, promastigotes from *L. (L.) amazonensis* are able to induce the activation of inflammasomes, also related to parasites and bacteria control.

2 Methods

Bone marrow-derived macrophages were primed with LPS (25ng/mL) for 3h and then infected with *L. (L.) amazonensis* promastigotes or amastigotes for 6h. Treatments with recombinant CD200 and neutralizing antibodies were also performed during infection. Cells were prepared for western blotting detection of caspase-1, and supernatants were collected for IL-1ß detection.

3 Results

Here, we show that infection with *L. (L.) amazonensis* promastigotes induces activation of inflammasomes, shown by caspase-1 processing and IL-ß production, while infection with amastigotes is able to inhibit caspase-1 activation in macrophages primed with LPS. Furthermore, activation of inflammasomes has been related to be controlled by CD200 signaling, and our results show a dose dependent caspase-1 inhibition by CD200Fc treated macrophages. Interestingly, neutralizing CD200 during infection of macrophages with *L. (L.) amazonensis* amastigotes partially restores caspase-1 activation and IL-ß production, suggesting that CD200 signaling might play a role also in controlling inflammasome activation.

4 Conclusions

Our results suggest a role for CD200 signaling during infection with *L. (L.) amazonensis* amastigotes, controlling caspase-1 activation and IL-ß production, being a possible new evasion mechanism for parasite survival.

C0659 EXPLORING THE PATHWAYS TO INDUCE CD200 IN MACROPHAGES BY LEISHMANIA

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1 Background

Leishmania (L.) amazonensis, a causative agent of cutaneous leishmaniasis, avoids macrophage response through the induction of CD200 in the host cell. When in contact with its receptor CD200R, an inhibitory signal is generated preventing local macrophage activation. Macrophages have on their membrane surface Toll-like receptors (TLRs), one of the first recognition systems against invasive microorganisms. TLR-ligand interaction triggers activation of signaling pathways dependent of MyD88 or TRIF, resulting in immune response gene activation. In this context, the aim of this work is to investigate the role of TLRs in the CD200 induction in macrophages by *L. (L.) amazonensis*.

2 Methods

Bone marrow macrophages (BMMs) from wild type (WT), MyD88$^{-/-}$, TRIF$^{-/-}$, TLR2$^{-/-}$, TLR3$^{-/-}$, TLR4$^{-/-}$ and TLR9$^{-/-}$ mice were infected with lesion amastigotes of *L. (L.) amazonensis* and CD200 levels were analyzed by immunoprecipitation. TLR agonists were also used to evaluate CD200 induction. The importance of the entry and parasite viability for CD200 induction were evaluated concomitantly.

3 Results

We observed that the parasite induces an increase in CD200 protein levels in WT macrophages after 1hr of infection, as well as in TLR2$^{-/-}$, TLR3$^{-/-}$ and TLR4$^{-/-}$ macrophages. However, in MyD88$^{-/-}$, TRIF$^{-/-}$ and TLR9$^{-/-}$ macrophages was not observed any signal of CD200 induction. When treated with TLR agonists for 1hr, we observed a slight increase in CD200 levels when TLR2 was activated and a strong increase in TLR9 activation. Furthermore, we also observed that the amastigotes need to be alive and be internalized to induce the protein.

4 Conclusions

These results suggest that signaling mediated by TLR is required to induce CD200 in macrophages infected by *L. (L.) amazonensis*. Further experiments will be performed to confirm in more detail our results.

C0666 DIFFERENCES IN CD200 EXPRESSION PROFILE IN LEISHMANIA ISOLATES ASSOCIATED TO HUMAN DIFFUSE CUTANEOUS LEISHMANIASIS

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1 Background

Diffuse cutaneous leishmaniasis (DCL) is a rare clinical manifestation of cutaneous leishmaniasis (CL) caused by *Leishmania* parasites of the mexicana complex in the new world. It differs from the localized cutaneous leishmaniasis (LCL) by presenting multiple non-ulcerated, nodule-like lesions throughout the skin and the host mediated immune responses. The gravity of a DCL form relays on the poor response to treatments and the high frequency of relapse on infected patients. Previous studies revealed molecular differences between LCL and DCL infected-macrophages that are crucial for the outcome of infection. Moreover, it was shown that a virulent *Leishmania (Leishmania) amazonensis* isolate is capable of inducing the expression of CD200, an immunoregulatory molecule responsible to inhibit macrophage activation. In this study, we examined the differences in CD200 expression levels by macrophages infected with *L. (L.) amazonensis* isolates from human cases of DCL.

2 Methods

Bone marrow macrophages from C57BL/6 mice were infected with of different DCL isolates amastigotes for 1h to confirm capacity and levels of CD200 expression. Additionally, intracellular multiplication of these isolates was tested to confirm their capacity of infection in a more resistant model. Finally, our *in vitro* findings on host-parasite interaction were analyzed by *in vivo* model using C57BL/6 mice.

3 Results

All DCL isolates amastigotes were capable of inducing CD200 1h after infection as previously reported for a *L. (L.) amazonensis* virulent strain. However, it was evident that the levels of expression of this immunoregulatory protein differed between these parasites. Meanwhile the levels of the signaling receptor (CD200R) remained unchanged. Moreover, we observed differences in infection capacity *in vivo* and *in vitro* within DCL isolates using C57BL/6 as an experimental model.Interestingly, highly infective *Leishmania* isolates induced increased levels of CD200 on infected macrophages *in vitro*.

4 Conclusions

As previously reported, CD200 is important to maintain and enhance *Leishmania* replication inside the parasitophorous vacuole. Thus, we showed that regardless all the isolates tested in this study were associated to DCL in humans, they presented differences in their capacity to induce CD200, resulting in divergent infectivity rates during the infection in mice models.

C0671 CASPASE-1 INHIBITION BY LEISHMANIA (L.) AMAZONENSIS AMASTIGOTES: A NEW EVASION MECHANISM?

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Institute of Biomedical Sciences, University of Sao Paulo Brazil

1 Background

Leishmaniasis is an infectious disease that affects people worldwide under cutaneous, mucocutaneous and visceral forms. In macrophages the infection with *L. (L.) amazonensis* induces the expression of CD200, a molecule involved in the inhibition of iNOS expression and NO production, being an evasion mechanism used by the parasite to escape the imune response. Also during the infection, promastigotes from *L. (L.) amazonensis* are able to induce the activation of inflammasomes, also related to parasites and bacteria control.

2 Methods

Bone marrow-derived macrophages were primed with LPS (25ng/mL) for 3h and then infected with *L. (L.) amazonensis* promastigotes or amastigotes for 6h. Treatments with recombinant CD200 and neutralizing antibodies were also performed during infection. Cells were prepared for western blotting detection of caspase-1, and supernatants were collected for IL-1ß detection.

3 Results

Here, we show that infection with *L. (L.) amazonensis* promastigotes induces activation of inflammasomes, shown by caspase-1 processing and IL-ß production, while infection with amastigotes is able to inhibit caspase-1 activation in macrophages primed with LPS. Furthermore, activation of inflammasomes has been related to be controlled by CD200 signaling, and our results show a dose dependent caspase-1 inhibition by CD200Fc treated macrophages. Interestingly, neutralizing CD200 during infection of macrophages with *L. (L.) amazonensis* amastigotes partially restores caspase-1 activation and IL-ß production, suggesting that CD200 signaling might play a role also in controlling inflammasome activation.

4 Conclusions

Our results suggest a role for CD200 signaling during infection with *L. (L.) amazonensis* amastigotes, controlling caspase-1 activation and IL-ß production, being a possible new evasion mechanism for parasite survival.

C0675 DIFFERENCES IN CD200 EXPRESSION PROFILE IN LEISHMANIA ISOLATES ASSOCIATED TO HUMAN DIFFUSE CUTANEOUS LEISHMANIASIS

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1 Background

Diffuse cutaneous leishmaniasis (DCL) is a rare clinical manifestation of cutaneous leishmaniasis (CL) caused by *Leishmania* parasites of the mexicana complex in the new world. It differs from the localized cutaneous leishmaniasis (LCL) by presenting multiple non-ulcerated, nodule-like lesions throughout the skin and the host mediated immune responses. The gravity of a DCL form relays on the poor response to treatments and the high frequency of relapse on infected patients. Previous studies revealed molecular differences between LCL and DCL infected-macrophages that are crucial for the outcome of infection. Moreover, it was shown that a virulent *Leishmania (Leishmania) amazonensis* isolate is capable of inducing the expression of CD200, an immunoregulatory molecule responsible to inhibit macrophage activation. In this study, we examined the differences in CD200 expression levels by macrophages infected with *L. (L.) amazonensis* isolates from human cases of DCL.

2 Methods

Bone marrow macrophages from C57BL/6 mice were infected with of different DCL isolates amastigotes for 1h to confirm capacity and levels of CD200 expression. Additionally, intracellular multiplication of these isolates was tested to confirm their capacity of infection in a more resistant model. Finally, our *in vitro* findings on host-parasite interaction were analyzed by *in vivo* model using C57BL/6 mice.

3 Results

All DCL isolates amastigotes were capable of inducing CD200 1h after infection as previously reported for a *L. (L.) amazonensis* virulent strain. However, it was evident that the levels of expression of this immunoregulatory protein differed between these parasites. Meanwhile the levels of the signaling receptor (CD200R) remained unchanged. Moreover, we observed differences in infection capacity *in vivo* and *in vitro* within DCL isolates using C57BL/6 as an experimental model.Interestingly, highly infective *Leishmania* isolates induced increased levels of CD200 on infected macrophages *in vitro*.

4 Conclusions

As previously reported, CD200 is important to maintain and enhance *Leishmania* replication inside the parasitophorous vacuole. Thus, we showed that regardless all the isolates tested in this study were associated to DCL in humans, they presented differences in their capacity to induce CD200, resulting in divergent infectivity rates during the infection in mice models.

C0688 TRANSCRIPTOME ANALYSIS OF LEISHMANIA MEXICANA AND INFECTED MACROPHAGES: DIFFERENTIAL BEHAVIOR IN PARASITE ISOLATES AND INFECTION.

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1 Background

Leishmania mexicana can cause a benign localized cutaneous leishmaniasis (LCL) characterized by ulcers at sites of parasite inoculation, and cause the highly destructive and invasive form, diffuse cutaneous leishmaniasis (DCL) characterized by intensely parasitized macrophages within nodules that spread uncontrolledly throughout the skin. Previous work in our lab showed that human NK cells of DCL patients had down-regulation in TLR2, IRAK-3, NF-kB1, NF-kB2, IFN-gR2, IL-12Rb2, STAT-1 and TNF-a genes. An approach considered by our group was to analyze whether the cause of disease progression in DCL patients was related to differences in parasite virulence.

2 Methods

Now, using RNA-seq was analyze the transcriptome of *Leishmania mexicana* isolates from LCL and DCL lesions. Expression and miRNAs microarrays were using to analyze the transcriptome time-course in infected human macrophages with LCL and DCL isolates.

3 Results

Preliminary results showed over expression in genes involved in dynein complex, phosphatidylserine biosynthetic process and microtubule motor activity, in DCL isolates. Additionally, we observed different pattern in human macrophages phagocytosis between LCL vs DCL isolates and differential activation of metabolic pathways, endocytosis, cytokine receptors, apoptosis, PI3K-Akt, MAPK and TLR signaling pathways.

4 Conclusions

The cause of the differential gene expression in DCL patients remains under study. However, it is tempting to speculate that DNA methylation or epigenetic modulation may play a role. It has been shown that the response of immune cells to invading pathogens can lead to genomic instability and DNA damage and that intracellular pathogens can alter the epigenome integrity of the host. Future work will address whether these alterations happen through DNA methylation or microRNA regulation. The work was funded by INMEGEN 144-2013-06-26, CONACyT 221405 and FINNOVA 242368.

C0698 TCTP DECIDING FACTOR FOR MCL-1 STABILITY: ENSURES LEISHMANIA DONOVANI SURVIVAL WITHIN HOST MACROPHAGES

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1 Background

Myeloid Cell Leukemia-1 (MCL-1), an anti-apoptotic protein helps *Leishmania donovani* to survive within macrophages by inhibiting host apoptosis. Interestingly, MCL-1 is extremely labile and *Leishmania* requires a minimum of 72 h to stay within macrophage for propagating infection. The present study therefore aims to find out whether infection leads to sustained intracellular MCL-1 level and if so, what is the underlying mechanism behind this.

2 Methods

To elucidate the underlying mechanism for MCL-1 stability in *Leishmania* infection various biochemical analysis and immunological techniques were utilized. All our experiments were done in triplicates in RAW 264.7 cells and also validated in Bone marrow derived macrophages (BMDM) cells isolated from BALB/c mice.

3 Results

The transcript level of MCL-1 showed a sharp decline at 36 h post-*Leishmania* infection, but translational level of MCL-1 was found to be maintained at a steady level upto 48 h post-infection. Upon Actinomycin D treatment, MCL-1 silenced infected cells demonstrated enhanced caspase activity and increased apoptosis compared to wild type infected cells. When IL-6 or GM-CSF, strong inducers of MCL-1, was withdrawn from macrophages, the protein level expression of MCL-1 was significantly reduced but again got restored upon infection with *Leishmania.* We next studied the ubiquitination pattern of MCL-1. Decreased MCL-1 ubiquitination was found upon *Leishmania* infection. However, MCL-1 specific E3 ligase MULE, responsible for ligating ubiquitin moieties with MCL-1, was strongly associated with MCL-1 during infection. Since the only other possible explanation for reduced ubiquitination of MCL-1 would be the blockade of ubiquitin binding site on MCL-1 by some other proteins, we screened different well known binding partners of MCL-1. During infection, amongst different binding partners of MCL-1, TCTP (translationally controlled tumour protein) was found to be strongly associated with MCL-1. TCTP was found to be phosphorylated by the mitotic polo-like kinase (Plk) and silencing of TCTP showed decreased MCL-1 level, increased apoptosis and decreased macrophage parasite burden.

4 Conclusions

Our observations indicate that during *Leishmania* infection, ubiquitin moieties attachment site on MCL-1 is masked by TCTP, thus preventing rapid proteasomal degradation of MCL-1, inhibiting mitochondria dependent apoptosis and prolonging its survival within host macrophages.

C0712 DIFFERENTIAL CODON USAGE PATTERN IN THE EXPRESSION OF SUSCEPTIBLE AND NON-SUSCEPTIBLE GENES OF HUMAN, AFFECTING VISCERAL LEISHMANIASIS INFECTION

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1 Background

Codon usage pattern of a gene determines the functionality of its product. Differential pattern of codon usage can affect the sequence of amino acids being added to the newly synthesized protein. This study represents the preferential utilization of codon, for coding a specific amino acid among susceptible and non-susceptible genes of human host during visceral leishmaniasis (VL) infection.

2 Methods

A literature survey was carried out to find out the genes with susceptible and non-susceptible behavior during human VL. Total 35 susceptible and 37 non susceptible genes were obtained along with their respective sequence. These sequences were utilized to calculate the GC percentage and nucleotide composition. The codon usage pattern of the susceptible and non-susceptible genes were also calculated using the codon usage calculator.

3 Results

This comparative study reveals the presence of susceptible and non-susceptible genes on human chromosome with similar GC content. The codon usage pattern was found to be similar for most of the amino acids in both gene types. However, a significant differential codon utilization pattern was obtained for the susceptible and non-susceptible genes. Major codon biases (≥10%) were identified for alanine, proline, threonine, glutamine, histidine and Arginine while remaining amino acids have shown minor codon usage biases (≤10%) .

4 Conclusions

This study highlights the role of codon utilizing machinery in promoting the VL infection.

C0717 TOWARDS A HIGHER LEVEL OF COMPLEXITY IN LEISHMANIA-HOST RELATIONSHIP. LEISHMANICIDAL ACTIVITY OF PHENOL ñSOLUBLE MODULINS, MICROBICIDAL PEPTIDES FROM SKIN STAPHYLOCOCCUS SP.

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1 Background

Leishmania has been traditionally studied as an isolated organism or in relation with the macrophage. However, in nature *Leishmania* shares its ecological niche with microbiomes either from the sandfly gut or from the skin, the latter extended in time on the ulcers of cutaneous leishmaniasis. Antimicrobial peptides (AMPs) were consistently used as tools to strive for survival among microorganisms at the same anatomical location. *Staphylococcus epidermidis*, a common skin commensal, and *S. aureus,* a pathogen frequently found in infected cutaneous ulcers, secrete a set of microbicidal peptides known as phenol-soluble modulins (PSM), endowed with a broad spectrum of activity and effectors for immune cells. The leishmanicidal effect of PSMs was approached.

2 Methods

PSMα1 and PSMα2, their N-terminal dipeptide truncated PSMα1Δ1-2 and PSMα2Δ1-2 from *Staphylococcus aureus*, and δ- and γ-toxins from *S. epidermidis* were synthesized by Fmoc chemistry, and assayed on *L. major* promastigotes, and on axenic- and intracellular *L. pifanoi* amastigotes. Membrane permeation was studied by in vivo variation of intracellular ATP, plasma membrane depolarization, entrance of SYTOX green and transmission electron microscopy

3 Results

IC_{50}s (μM) for promastigotes were: PSMα2Δ1-2 (7.7) <PSMα1 (11.0) = PSMα1Δ1-2 (12.4) ≤ PSMα2 (15.5) << δ-toxin (<30) = γ-toxin (<30), with additive effect in combination. In axenic amastigotes PSMα2 showed the highest activity (IC_{50}=3.3 μM) and the selectivity index (3.0). Under IC_{50} PSMα2 appeared inside *Leishmania*, endorsing intracellular targets, although at higher concentrations, it permeated the plasma membrane under an all-or-none pattern This peptide decreased by 30% intracellular infection at subIC_{50} values. In combination with LL-37 and dermcidin, both human skin AMPs, PSMα2 showed antagonistic or nil effects, respectively.

4 Conclusions

In all, modulins, as components from the skin microbiome may modulate *Leishmania* infection, leading to a higher complexity of the parasite-host interactions. Due to their short length, linear structure, and absence of modified amino acids they may constitute new leishmanicidal hits.

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C0730 THE ROLE OF TREM-1 IN THE SEVERITY OF HUMAN CUTANEOUS LEISHMANAISIS (CL) THROUGH A TRANSLATIONAL STUDY.

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1 Background

Leishmaniasis remains as one of the most seriously neglected tropical diseases in the world. The host immune response against Leishmania has a critical role in promoting parasite killing, but it also accounts for inflammation and disease severity. In this context, the Triggering Receptor Expressed on Myeloid Cells (TREM) was recently identified as an amplifier of the immune response, which acts synergistically with Toll-like receptors (TLRs) in the production of proinflammatory mediators. TREM- is expressed strictly in cells of myeloid lineage, mainly neutrophils. Its signaling pathway depends on the adapter protein DAP12, which results in the activation of NFkB and expression of inflammatory genes, as well as degranulation and ROS production. Based on that, our study investigate the role of TREM-1 during Leishmania infection.

2 Methods

First, publicly available transcriptome data from lesions of patients infected with Leishmania braziliensis compared to uninfected skin were obtained from GEODataSets (www.ncbi.nlm.nih.gov/geo/). After, the results from these analyzes were validated by Real-Time PCR (qRT-PCR) with new samples. Finally, we investigated the mechanisms underlying TREM activation during the infection of human neutrophils in vitro.

3 Results

The analysis of publicly transcriptome data revealed an increase in the expression of TREM-1 pathway, including all signaling factors involved in this path. Afterwards, we confirmed, by qRT-PCR, the increased expression of DAP12 and TLR4 mRNA in the lesions of L. braziliensis-infected patients. On the other hand, the expression of these mRNAs is not significantly altered in the peripheral blood mononuclear cells (PBMC) from these patients. However, a down regulation in the expression of TREM-1, DAP12, TLR2 and TLR4 mRNA is observed in circulating neutrophils from LCL patients. On in vitro assays, we observed that the exposure to L. braziliensis increases the expression of TREM-1 in the surface of human neutrophils, leading to the release of Matrix Metalloproteinase 9 (MMP9). Further assays are being conducted to define the importance of TREM-1 in parasite killing.

4 Conclusions

These preliminary results suggest that TREM-1 and TLRs may have an important role in the balance between tissue damage and parasite killing in CL.

C0762 IDENTIFICATION OF PHOSPHOLIPID EXPOSED BY LEISHMANIA TO ESCAPE FROM THE INFLAMMATORY RESPONSE OF THE VERTEBRATE HOST.

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1 Background

The parasites of the genus *Leishmania* use some mechanisms to escape from the immune response of the vertebrate host. One of the mechanisms, called apoptotic mimicry, induces the macrophage phagocytosis, resulting in the internalization of the parasite into the macrophage without the killing of the parasite. Data in literature indicate that phosphatidylserine (PS) is the major phospholipid recognized by the macrophage in this process. However, *Leishmania* species lack this phospholipid. So, the aim of this communication is to identify the phospholipid present in the external plasma membrane layer of *Leishmania* that is recognized by the macrophage.

2 Methods

As a strategy, we performed a competition between liposomes synthesized with a single phospholipid class, at concentrations of 0.2 µM, 20 µM or 2 mM, and promastigotes of *L. (L.) amazonensis* in a macrophage (MOI 5:1) infection assay. The amount of amastigotes/macrophage and the rate of infected macrophage, by counting 100 macrophages, were determined after 4 and 24 hours of infection. The infectivity rate was then calculated by the multiplying both parameters.

3 Results

The assay performed with phosphatidylcholine (PC) liposomes, resulted in a dose dependent decrease in the rate of infected macrophage and in the infectivity rate, in both 4h and 24h of infection – 40% of decrease in the infectivity rate at 2 mM in 4h of infection. However, the decrease was not observed in the presence of liposomes constructed with PS. We did not observe a change in the number of amastigotes/macrophage in any of the experiments. Assays with cardiolipin (CL) showed to be not viable because CL induced apoptosis in cells. To perform the assay with phosphatidylethanolamine (PE), we had to synthesize the liposome with PC and PE (1:1) to make sure the stability of the liposome and the result did not present the same decrease dose dependent when compared with the PC assay.

4 Conclusions

The results suggest that PC liposomes are the preferential phospholipid recognized by the macrophage, possibly blocking macrophages receptor and thus inhibiting the entry of the parasites. As the number of amastigotes inside the infected macrophage was not altered, we can say that the *Leishmania* that is phagocytosed by macrophages replicates normally.

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C0773 LEISHMANIA INFANTUM PROMASTIGOTE FRACTIONS AND KMP-11 RECOMBINANT PROTEIN INDUCE AN INFLAMMATORY CYTOKINE RESPONSE IN AN IN VITRO CANINE MACROPHAGE MODEL

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1 Background

Leishmanial ligands which induce production of Th1 cytokines are not fully identified. It is essential to identify those antigens/immunogens that induce the production of Th1 inflammatory cytokines, which may further be employed to develop vaccine candidates, adjuvants or immunotherapeutic drugs for humans and dogs. Kinetoplastid Membrane Protein 11 (KMP-11) is a major conserved surface protein expressed in both promastigotes and amastigotes. *In vitro* and *in vivo* studies have identified KMP-11 as a multifunctional immunogenic factor required for protection of vertebrate hosts from infection. However, the majority of studies have been carried out in rodent models or humans. The aim of this study was to examine the effect of *L. infantum* promastigote fractions and KMP-11 recombinant protein in inducing inflammatory cytokine responses in canine macrophages.

2 Methods

Cells of the DH82 canine macrophage line were incubated for 24 hours and stimulated with medium alone, lipopolysaccharide (LPS, 20µg/mL), several eluted *L. infantum* fractions (>100 KDa, 50-100 KDa and 50-10 KDa at 1, 2, 5, 10 and 20 µg/mL), *L. infantum* soluble antigen (LSA) at 2 and 5 µg/mL; or KMP-11 protein at 1. 2, 5, 10 and 20 µg/mL. Culture supernatants were harvested for measurement of canine TNF-α and IL-6 cytokines by ELISA

3 Results

LPS induced a significant production of both cytokines while LSA did not produce IL-6 neither TNF-α at any concentration studied. Interestingly, *L. infantum* fractions of >100 KDa induced production of both cytokines at 1, 2 and 5 µg/mL while no production was detected at higher concentrations (10 and 20 µg/mL). Similar findings were observed for the other *L. infantum* fractions at 1 and 2 µg/mL. KMP-11 at all concentrations showed a significant increase of TNF-α and IL-6 concentrations.

4 Conclusions

This study illustrates an *in vitro* macrophage activation assay that appears to be useful for screening potential *Leishmania* antigens as future prophylactic or immunotherapeutic candidates for canine leishmaniosis. *Leishmania infantum* fractions appear to induce a strong immune response. However, further studies are required to identify the antigens that promote an inflammatory response in these *L. infantum* promastigote fractions. Moreover, KMP-11 is a potent immunogenic protein as previously demonstrated in other species

C0852 EFFECT OF SUPERNATANT OF PERIPHERAL BLOOD MONONUCLEAR CELLS INFECTED WITH LEISHMANIA MAJOR ON HUMAN FIBROBLAST CELLS

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1 Background

Leishmaniasis is a disease caused by obligate and intracellular parasites belonging to the genus ***Leishmania. Cutaneous Leishmaniasis (CL)*** caused by Inoculation of parasite Leishmania by sand flies to humans. The severity of the disease depends on the species of parasite and inducing host immune responses. ***Leishmania*** Mechanisms for generating and wound healing is unclear.In other words, the behavior of the dermis and epidermis cells in response to ***Leishmania*** infection is unknown yet.Even there is no complete information in the case of loss of cells (apoptosis, necrosis or reduce proliferation). The aim of this study was to determined effect of supernatant of peripheral blood mononuclear cells infected with ***Leishmania major*** on human fibroblast cells**.**

2 Methods

L. major promastigotes (MRHO / IR / 75 / ER) entered into the culture medium (RPMI 1640 + 10% FCS + 1% L -glutamine) and after amplification, are harvested in stationary phase.PBMC cells were washed three times after isolation and a suspension with a concentration of 5 × 106 cells ml in RPMI1640 medium (Bio-idea, Iran) were prepared.Macrophages after being infected with the parasite Leishmania major at a ratio of 5 to 1 in the early stages of stationary phase were incubated at 37 ° C for 4 hours. The plates in order to remove extracellular parasites were washed three times with PBS.Next, the cell supernatant at 4, 24, 48, 72 and 96 hours were collected.

3 Results

Survival fraction after incubation with 72and 96 h-supernatant decreased so that this reduction was more considerable for 96hour over the whole range of supernatant concentrations (***p***-value <0.05). These results indicate that, 96h-supernatant have remarkable cytotoxicity on fibroblast cells, therefore it was selected as an effective supernatant.Significant increase in the apoptosis of cells that incubated with 96h-supernatant compared to control cells was observed (p-value <0.05).

4 Conclusions

Information obtained from this study showed that the main cause of scarring by cutaneous Leishmaniasis infection occurred by interactions between parasite and immune cells and the subsequent effects on fibroblasts. Identify key factors and efforts to suppression are appears to be necessary in future studies.

C0853 LEISHMANIA DONOVANI HAMPERS INFLAMMASOMES FOR SUCCESSFUL INTRA-CELLULAR SURVIVAL

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1 Background

Leishmania donovani as a survival strategy in macrophages subverts the production of pro-inflammatory cytokines. IL-1β is a crucial pro-inflammatory cytokine produced by macrophages which is necessary for parasite clearance. However, during infection there is a reduction in the level of biologically active IL-1β. Inflammasomes have been implicated with IL-1β maturation in a caspase-1 dependent manner. Hence, we tried to investigate the role of inflammasomes and the key regulators associated with the reduction of IL-1β during infection.

2 Methods

All in vitro experiments were performed in RAW 264.7 cell line or BMDM. For in vivo studies Balb/c mice model and *Leishmania donovani* AG83 strains were used. IL-1β, pro-IL-1β, pro-caspase-1, caspase-1, NLRP3, ASC, A20, p65, UCP2 levels in uninfected/ infected cells were checked by immunoblotting. The *in vivo* levels of IL-1β were confirmed by ELISA. Caspase-1 activity was also checked using caspase-1 activity assay. mRNA levels of pro-IL-1β were analysed by RT-PCR. Silencing of caspase-1, NLRP3, A20, UCP2 was mediated by siRNA/ shRNA. Intracellular ROS levels were checked by FACS using DCFDA staining.

3 Results

Our results suggest that *Leishmania donovani* infection reduced stimuli-induced levels of NLRP3. Reduction in NLRP3 levels in turn reduces activation of caspase-1, thereby abrogating mature IL-1b levels. Reduced expression of NLRP3 by the parasite can be attributed to the decrease in NF-kB activity by the infection-induced up-regulation of the deubiquiting enzyme A20, a negative regulator of NF-kB signalling. Silencing A20 in infected cells did not increase the matured IL-1β production although it increased NLRP3 and pro-IL-1β expression, suggesting the presence of a second signal in order to activate inflammasome complex. ROS generation which has been associated with the oligomerisation of NLRP3 inflammasome complex were found to be impaired during infection. This can be attributed to the infection-induced up-regulation of UCP2, an inner mitochondrial protein. Silencing UCP2 in infected cells increased the stimuli-induced levels of matured IL-1β. Silencing of A20 and UCP2 in infected mice by shRNA supported in vitro findings and resulted in a significant decrease in liver and spleen parasite burden.

4 Conclusions

Leishmania donovani hampers inflammasome expression and activation by exploiting host A20 and UCP2, thereby reducing levels of IL-1β as a survival strategy.

C0868 LEISHMANIA DONOVANI EXPLOITS A SINGLE TRANSMEMBRANE EXPORTER ATP BINDING CASSETTE TRANSPORTER A1 TO DECREASE BOTH CELLULAR CHOLESTEROL AND INFLAMMATION - CRITICAL FOR ITS INTRAMACROPHAGE SURVIVAL

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1 Background

Leishmania donovani infection decreases intracellular cholesterol content of the host, thus facilitating its intramacrophage survival. One of the possible mechanisms by which the this is attained is cholesterol export from the cell. Macrophage has 2 major cholesterol exporter proteins ABCA1 and ABCG1. Other than cholesterol export, ABCA1 has infection propagating anti-inflammatory properties. The present study is aimed to gain a comprehensive understanding about roles of ABCA1 and ABCG1 in pathogenesis of visceral leishmaniasis.

2 Methods

We checked ABCA1 and ABCG1 expression in RAW cells by RT-PCR and Immunoblot. By using ABCA1 agonist TO901317 and ABCA1 siRNA we checked the parasite survival by confocal microscopy and various cytokine level (TNF-α, IL-12, IL-10, TGF-β) by ELISA. We checked nuclear translocation of NF-κB subunits by immunoblot and surface TLR4 level by cellular ELISA. Lipid Raft organization was checked by microscopy with anti-GM1 antibody. CREB phosphorylation was checked which contributes to enhanced secretion of anti-inflammatory cytokines. Upstream mediators cAMP and PKA activation were checked by assay kit. STAT3 phosphorylation which works downstream to IL-10 , was checked in presence of IL-10 neutralizing antibody. Co-IP studies were carried out for JAK-2 and ABCA1. Finally we treated female BALB/c mice with ABCA1 agonist and ABCA1 shRNA and checked the parasite load in spleen and liver by giemsa staining. We also checked cytokine levels at 2, 4 and 6 weeks of infection.

3 Results

ABCA1 level significantly increased at 3 and 6 hours P.I, but not ABCG1. Cells treated with ABCA1 agonist showed higher parasite count in macrophages and increased secretion of IL-10 and TGF-β as well as reduced secretion of TNF-α and IL-12. ABCA1 disrupt the lipid raft organization resulting in decreased surface expression of TLR4 and hence diminished nuclear translocation of NF-κB. ABCA1 induced by *L.d* causes a hike in intracellular cAMP level resulting in the activation of PKA in ABCA1 agonist treated infected macrophages. We also found that, even in the presence of IL-10 neutralizing antibody JAK2-STAT3 phosphorylation occurs as a result of JAK2 and ABCA1 association. In-invo experiments validated our observations of in-vitro experiments further.

4 Conclusions

Together our study advocates the occurrence of *Leishmania* mediated exploitation of ABCA1 for immune-suppression in infected macrophage to aid its own survival and propagation.

C0882 SIMULTANEOUS TRANSCRIPTOMIC PROFILING OF THE HOST MACROPHAGE AND LEISHMANIA PARASITE THROUGH RNA SEQ: MECHANISTIC INSIGHT DEPICTING THE CAUSE OF DISEASE AND CURE

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1 Background

Visceral leishmaniasis is an immunosuppressive disease. Central to its pathogenesis lies on the ability of the *L. donovani* parasites to infect the host macrophages. The tug of war between the parasite and the host cell culminates in either disease or cure. Parasite derived virulence factors modulate the host signaling network in this process. But how this happens is not completely understood.

2 Methods

To understand this, our approach was to infect murine peritoneal macrophages with the virulent and the non-virulent parasites for 12 h and determine subsequent changes in the host and parasite transcriptomic profile. To this end, the infected macrophages were processed for RNA isolation using Trizol method. These RNAs were used for library preparation before next generation sequencing analysis. Using bioinformatics tools raw data of the RNA-seq were further processed to get the gene expression profile (transcriptome) of the host as well as the parasites which was further validated by real time PCR analysis.

3 Results

Analysis of the host response using KEGG pathway revealed that it varied enormously between the infecting parasites. The virulent parasites induce a biased anti-inflammatory response in the host macrophage. Moreover, they upregulated genes predominantly involved in cytokine-cytokine receptor interactions. In contrast, these parasites downregulated many of the genes involved in inflammation and macrophage activation. However, the macrophage infected with the non-virulent parasites appears to be activated, acquiring inflammatory phenotype. This was reflected from significant number of up regulated genes involved in diverse pathways including immune response and signaling like cytokine signaling in immune system, signaling by GPCR, Toll-like receptor signaling and NF-kappa B signaling. Gene ontology analysis of the parasite genes depicted that the genes for virulent factors and those involved in parasite survival were significantly up regulated in the intracellular amastigotes of the virulent parasites compared to the non-virulent ones.

4 Conclusions

The study provides global gene expression pattern of both the host and the parasite depicting a broad and clear picture of how the changes occurring in both the host and the parasite are crucial in determining either disease or cure. Moreover, it unravels many unknown molecules that are associated with disease susceptibility and resistant model.

C0935 EFFECT OF PLASMA LIPOPROTEINS ON THE PARASITISM OF THP-1 CELLS INFECTED WITH LEISHMANIA (LEISHMANIA) INFANTUM

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1 Background

In active phase of visceral leishmaniasis infection, both in man and in animal models, alterations of lipid profile are detected with a reduction in high density lipoprotein (HDL) level and increase in total cholesterol and very low density lipoprotein (VLDL) levels. Some studies have shown that the progression of the disease may be related to changes in lipids and lipoprotein metabolism. Macrophages are the host cells of Leishmania that have receptors for natural and/or modified lipoproteins, being possible targets for immunomodulatory activities exerted by lipoprotein fractions. Since changes in lipoprotein levels during the active phase of VL appear to modulate Leishmania infection, we propose to evaluate the direct effect of lipoprotein fractions on the parasitism of THP-1 monocytic cells by *Leishmania (L.) infantum*.

2 Methods

PMA differentiated THP-1 cells were infected with stationary phase *L. infantum* promastigotes with a multiplicity of infection of 8, in presence or absence of the VLDL and HDL fractions (fractionated with ultracentrifugation) separately or in combination. Cell culture medium was enriched with infranatant of ultracentrifugation lacking lipoproteins. After 6h, the cells were washed for the removal of non-internalized parasites and were incubated for 24h and 48h for determination of parasitism by ligh microscopy and arginase activity by urea production.

3 Results

The cells infected in the presence of the VLDL and HDL fractions separately showed two times higher parasitism when compared to the infected cells in the absence of the fractions after 24h of incubation. However, when the fractions VLDL and HDL were combined, the parasitism was similar to or less than the parasitism presented by the cells infected without the lipoproteins. When analyzing arginase activity, we observed that the cells infected in the presence of the HDL or combined fractions presented greater activity, 1.375 and 2.94 mU/10^6 cells, respectively, compared to control (0.849 mU/10^6 cells) mainly after 48h of incubation.

4 Conclusions

The data suggest that lipoproteins present immunomodulatory activity on the infected macrophages, since the cells presented greater parasitism when infected in the presence of certain lipoprotein fractions. However, analysis of arginase activity revealed an unexpected result, since its activity was increased while the parasitism was decreased in cells infected with the combination of VLDL and HDL fractions.

C0948 COMPARISION OF THE INVOLVEMENT OF AUTOPHAGY IN LEISHMANIA AMAZONENSIS AND LEISHMANIA MAJOR INFECTION IN CBA MOUSE MACROPHAGES IN VITRO

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1 Background

CBA macrophages (MΦ) control *Leishmania major* infection yet are permissive to *Leishmania amazonensis*. Few studies have been conducted to assess the role autophagy plays in *Leishmania* infection. Herein, we took advantage of the CBA model to compare the role autophagy plays in *L. amazonensis* and *L. major* infection *in vitro*.

2 Methods

Initially, we compared by PCR array the expression of autophagy genes of *L. amazonensis*- and *L. major*-infected MΦ. Then, we evaluated by western-blot whether *Leishmania* infection interferes with the autophagic activity, determined by LC3-II/LC3-I ratio. We also analyzed the autophagic flow, comparing LC3-II accumulation in untreated cells and cells treated with chloroquine, a drug that inhibits the fusion of autophagosomes with lysosomes resulting in autophagic flow inhibition and consequent accumulation of LC3-II. After that, we characterized *L. amazonensis*- and *L. major*-induced parasitophorous vacuoles (PVs) regarding the presence of LysoTracker or DQ-BSA, acidic degradative markers, and LC3 an autophagy marker. Next, we evaluated the effect of autophagy modulation on infected CBA MΦ.

3 Results

First, we demonstrated by PCR array that *L amazonensis* and *L major* infection differentially altered the expression of autophagy genes. Additionally, we found that *L. amazonensis*- or *L. major*-infected MΦ expressed at least 1.5 times more LC3-II than uninfected control MΦ, suggesting that *L. amazonensis* and *L. major* induces autophagy in CBA MΦ. Chloroquine treatment promoted a higher accumulation of LC3-II compared to untreated cells demonstrating that *L. amazonensis* and *L. major* did not block the autophagic flow in CBA MΦ. We also found that, in comparison with *L. amazonensis*, *L. major* colocalized in a greater extent with the markers of acidic degradative compartments, LysoTracker and DQ-BSA. On the other hand, LC3 colocalized more with *L. amazonensis* inside PVs than with *L major*. Finally, we demonstrated that autophagy induction by starvation or inhibition by wortmannin, ammonium chloride, and VPS34-IN1 did not alter the infection rate or parasite load in infected MΦ.

4 Conclusions

These findings demonstrate that the intracellular *L. amazonensis* PVs in comparison to those from *L. major* interact in a greater extent with the autophagic pathway. Additionally, autophagy induction or inhibition did not alter *L. amazonensis* and *L. major* infection. Further studies are underway to clarify the actual role autophagy plays in *Leishmania* infection.

C1012 ANTILEISHMANIAL PROPERTIES OF GALACTOFURANOSYL EXPLORED BY FLUORESCENT DYES

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1 Background

Lipophosphoglycan (LPG), a major component of *Leishmania* cell wall, contains a galactofuranosyl unit, β-D galactofuranose (Gal*f*) which is completely lacking in mammalian cell membrane. This is therefore a promising therapeutic target. Anti-parasitic effect of chemically synthesized octyl-β-D-galactofuranose (Gal*f*-oct) against promastigotes and amastigotes of *Leishmania donovani* (*L. d.*) was already shown (Suleman *et al*. AAC2014), but its possible mechanism of action remains to be understood.
This work aims at synthesizing Galf-oct conjugates with fluorescent dyes to characterize the mechanism of action of Gal*f*-oct on amastigotes in a human macrophage cell model.

2 Methods

Human macrophages from peripheral blood mononuclear cells were seeded and differentiated on culture slides then infected with *L. d.* (DOI 10:1). Infected macrophages were treated during 6 to 48h with Gal*f*-oct-rhodamine 6G or Gal*f*-oct-fluorescein or unconjugated Rhodamine, fluorescein or Gal*f*-oct. Nuclei were labeled with Hoechst then pictures of each condition were taken using epifluorescence microscopy or colored with May Grünwald Giemsa stain to determine anti-parasitic effect by optical microscopy.

3 Results

Gal*f*-oct conjugates entered in all macrophages whatever the using dyes. Intracytoplasmic distribution was uniform throughout the cytoplasm and no co-labelling with Hoechst was observed in nuclei, nor accumulation dyes around amastigote nuclei. Regarding anti-parasitic effect, Gal*f*-oct remained effective when conjugated with rhodamine but fluorescein inhibited its effect. Surprisingly, there was a significantly higher efficacy of Rhodamine conjugated Gal*f*-oct compared to Gal*f*-Oct ($p<0.05$), a tendency of Rhodamine 6G to decrease infection ($p=0.11$) was observed and could explain the better anti-parasitic effect of this conjugate.

4 Conclusions

Fluorescent dyes conjugated to known molecules may change their properties. Biological activities need to take into account chemical and physical properties of the used dye. In this work, rhodamine 6G was interesting in two ways:

i) Synergistic effect with Gal*f*-oct and
ii) Its possible use as in-vivo dyes on small animals.

C1021 EVALUATION OF IMMUNOMODULATORY PROPERTIES OF LEISHMANIA PROMASTIGOTES EXTRACELLULAR ANTIGENS

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1 Background

The use of secretion pathways for effector molecule delivery is an essential part of the millennia struggle between pathogens and their host immune system. *Leishmania* protozoan are no exception. Recent emphasis has been given to the parasite released extracellular vesicles (EVs) as important agents in this process. Still, these EVs are not alone as players and their significance in the context of other *Leishmania* secreted antigens is still to be observed. To address this we evaluated the immunological potential of the promastigote exoproteome (EXO) and its associated components EVs and vesicle depleted exoproteome (VDE).

2 Methods

Secreted molecules were recovered after 4 days of promastigote culture using a combination of ultracentrifugation/ultrafiltration. Local cell recruitment was evaluated using an air pouch model with different doses of EVs, VDE, EXO or parasites. Six and 24h after inoculation, animals were euthanized, the pouch exudate collected, and the presence of different cell populations studied by flow cytometry. *In vitro* studies with bone marrow-derived dendritic cells and macrophages were performed evaluating by flow cytometry the dose dependent effects of the secreted molecules and also the state of responsiveness of the cells upon re-stimulation with LPS or Poly I:C.. *In vivo* infections with *L. infantum* with or without increasing doses of EVs, VDE and EXO were also performed. Parasite burden in spleen and liver was determined by limiting dilution.

3 Results

In the air pouch model a dose-dependent recruitment of immune cells was observed for all the exoproteome components. Interestingly, EVs and VDE induced a different recruitment compared with parasites, attracting significantly more dendritic cells, which remain non-activated at the site of inoculation, and less neutrophils. Interestingly *in vitro*, EVs, VDE and EXO, as well as the parasites, were able to diminish the response capacity of DCs and macrophages to TLR ligands suggesting that these components interfere with functional aspects of these cells. Overall the secreted antigens conferred a real biological advantage as they increases the parasite burden in a dose-dependent manner.

4 Conclusions

Overall these results suggest that *L. infantum* extracellular molecules by themselves might contribute to the infection process in the early stages. Therefore, presence of these secreted components in the initial infectious inoculum should be confirmed and addressed in more complex infection models.

C1036 INSULIN-LIKE GROWTH FACTOR-I MRNA EXPRESSION UPON INFECTION OF MACROPHAGES BY DIFFERENT SPECIES OF LEISHMANIA

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1 Background

Different *Leishmania* species, genetic and immunological constitution of the host define different clinical manifestations that include cutaneous, mucosal and visceral forms. In *Leishmania* infection besides specific immune elements non-specific growth factors influence the infection fate and some are responsible for promoting the proliferation of parasites inside the host such as insulin-like growth factor-I (IGF-I). In our previous studies we demonstrated that extrinsic IGF-I favors the parasite proliferation and the infection development interfering with arginase activity and nitric oxide production. However, IGF-I is constitutively present in macrophages contributing also to parasite growth but the influence of *Leishmania* infection on its expression is not known. Thus here we aimed to evaluate the IGF-I expression on macrophages upon infection by different species of *Leishmania.*

2 Methods

Cutaneous strains, *L.* (*L.*) *major* and *L.* (*L.*) *amazonensis*, and visceral strain, *L.* (*L.*) *infantum chagasi*, were used to infect BALB/c mice derived-macrophages and the latter species also to infect the human monocyte cell line THP-1. $5x10^5$ cells were infected with stationary phase promastigotes (8 parasites/cell) and maintained for 24 and 48 hours. The parasitism was evaluated by light microscopy, the IGF-I mRNA expression by RealTime PCR relative quantification and Nitric oxide (NO) production by Griess reaction.

3 Results

In 24h of infection, we observed a decrease in the IGF-I mRNA expression accompanied by an increase in the NO production in all *Leishmania* species-infected macrophages compared with the control group except in *L. amazonensis*-infected cells where opposite results were seen. At 48h, the IGF-I mRNA expression was decreased and the NO production slightly increased in *L. major*– and *L. amazonensis*-infected macrophages compared with the control. In contrast, *L. infantum* infected macrophages revealed an increased IGF-I expression and NO production. Similar results were obtained using mouse or human cells. No significant difference was observed in parasitism comparing at 24 and 48 hours of infection.

4 Conclusions

IGF-I mRNA expression on macrophages shows differences among different species of *Leishmania* that may result in different development of infection. Depending on the *Leishmania* species, host cells up or down regulate the expression of IGF-I favouring or deterring the parasite growth.

C1049 MACROPHAGE CELL MEDIATED IMMUNE RESPONSES IN CUTANEOUS LEISHMANIASIS AGAINST LEISHMANIA DONOVANI

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1 Background

In Sri Lanka, localised cutaneous leishmaniasis (LCL) is caused by *Leishmania donovani*; a visceralizing species. We hypothesized that the distinct alterations in the early immune response determines the outcome of infection in Sri Lanka. Aim of this study was to characterize the immune response in LCL by macrophages; which is central to both replication and elimination of the parasite.

2 Methods

We studied the peripheral blood mononuclear cell (PBMC) derived macrophages from LCL patients (n=20) and healthy non endemic (n=10) and endemic controls (n=10). Production of cytokines and expression of cell surface markers were assayed at predetermined intervals after stimulation with *Leishmania* antigen and compared between the groups using nonparametric tests.

3 Results

Patient derived macrophages were characterized by a higher production of IL-10 at 72 hours, TNF at 48 & 72 hours with TGFβ remaining high throughout in comparison to both endemic and non endemic controls. The presence of $CD14^+CD68^+CD80^+$ macrophages were significantly higher in LCL patients when compared to healthy individuals at 24 hours while the proportion of $CD14^+CD40^+$ macrophages were significantly higher at 72 hours.

4 Conclusions

A mixed immune response with both pro and anti inflammatory cytokines and early M1 polarization (classical activation) appear to play a role in determining disease outcome in LCL due to *L. donovani*.

C1054 IMPACT OF THE INFECTION BY LESIHMANIA MEXICANA ON THE INTEGRITY OF THE GOLGI APPARATUS OF THE MACROPHAGES

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1 Background

During the infection of the macrophages by *Leishmania mexicana,* the parasite form a spacious vacuole from the phagosome in which he can survive and multiply. Those vacuoles grow in size during the infection by recruiting membranes from multiple sources in the cell like the endosomes. We already know that the Golgi apparatus is the principal organelle implicated in the protein and membrane transport, so **we suppose that he could be implicated in the formation of the vacuole and could be modified by the infection**, in its integrity or in the expression level of his proteins. Given that the metalloprotease leishmanolysin (GP63) is one of the principal virulence factor of the parasite, and is under the control of the Cysteine protease B (CPB), the inhibition of this CPB could induce an alternate response in the cells. Using our four mutants of CPB, we observed the integrity of the Golgi by looking at three proteins, P115, GM-130 and Golgin-84. These three proteins are implicated in the formation and the structure of the Golgi. The first two work in a complex on the cis-face and are also used in the vesicle transport from the endoplasmic reticulum. The third one is located in the trans-face.

2 Methods

To verify our hypothesis, we infected bone-marrow derive macrophages (BMM) with our four CPB mutants of *Leishmania mexicana,* being the wild type (WT), CPB knock out (KO), CPB rescue and CPB KO add-back GP63, the metalloprotease is continually induce in the absence of CPB. The infection lasted for 72h but we stopped it at 2-24-48-72h. At these times, we divided the samples in two groups. One was used for the Western blot and the second one for the confocal microscopy.

3 Results

The two methods didn't gave exactly the same results. In the Western blot, we saw a modification for both Golgin-84 and GM-130, a smaller band was present in addition to the normal size protein. However, when we looked by microscopy, we only saw significant modification of the expression of GM-130. No effects were observed for P115 in both methods for all the mutants. The WT and KO mutants had the biggest effect for GM-130 and Golgin-84, the other two had lesser effect on the proteins.

4 Conclusions

With our results, we can conclude that the Golgi apparatus is modified following the infection of the macrophages by *Leishmania mexicana,* but is not disrupt. These modifications are not dependant of the GP63 neither of the CPB, but the RE and the add-back are not as efficient in the degradation of the protein of the Golgi.

C1057 A MOLECULAR STUDY INVOLVING NITRIC OXIDE SYNTHASE AND ARGINASE ENZYMES FROM DIFFERENT LEISHMANIA INFANTUM STRAINS

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1 Background

Leishmania infantum is one of the species that cause the visceral form of the disease and is present throughout the Mediterranean basin, but there are also found in the Middle East and South Asia. *L. chagasi* with strains circulating in the New World, is responsible for visceral leishmaniasis (VL) in Latin America and is considered identical to *L. infantum*, so it refers to it as *L. infantum* (syn *L. chagasi*). Our group has been studying the enzymes nitric oxide synthase (NOS) and arginase in different species of *Leishmania*. We characterized constitutive NOS from these species and associated the enzyme activity to the parasites infectivity. Also, we presented some data concerning arginase in *L. amazonensis*. It has been described in the literature that *L. infantum* of the Old World and the one from the New World are identical from the molecular point of view. As we observed in previous studies, there are significant biological differences between these strains, we decided to study it under a molecular approach.

2 Methods

In this work, a comparative study of arginase and NOS from both strains was carried out. The NOS activity was measured, in an indirect way, by nitric oxide production using DAF marker to detect this radical intracellularly through fluorimetry. Concerning arginase activity, it was initially measured the urea production and later on by gene sequencing and real time PCR. Besides, a western blot analysis is being performed. It is important to notice that both strains were maintained in vivo in two mice lineage (Swiss and BALB/c) to evaluate the influence of it in the enzymes activities.

3 Results

The fluorimetric assay using the DAF-2DA marker was able to detect the intracellular NO production of the promastigotes. The parasites of the New World presented a higher content of NO independent of the mice. The highest arginase activity was seen on the third day of growth in both strains, but significantly prominent in the Old World strains. Arginase expression is different among parasite strains, indicating no relation to distinct hosts on this expression.A comparative analysis was also performed between the DNA sequences of the arginase gene; however, no difference was observed and the strains were identical with each other and with sequences deposited in the TriTrypDB database.

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C1085 IMMUNOPATHOLOGICAL STUDY OF DISTRIBUTION OF LAMININ, FIBRONECTIN AND TCD3+ CELLS IN THE LIVER OF NATURALLY INFECTED DOGS WITH LEISHMANIA INFANTUM.

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1 Background

Canine visceral leishmaniasis is a life-threatening vector-borne zoonotic disease caused by *Leishmania infantum*. In general, an intense chronic inflammatory reaction is observed in liver. Chronic inflammation, a common consequence of parasitic infections, is a potent promoter of extracellular matrix (ECM) formation. Laminin (LN) and fibronectin (FN) are ECM components that are important in the development and maintenance of cellular organization. Studies with *Leishmania* spp. have provided strong evidence that the parasite uses host FN and LN to bridge their association with host monocytes and macrophages. The aim of this work was to study the alterations in distribution of LN, FN and $TCD3^+$ cells in liver from naturally infected dogs and correlate with symptomatology and parasitological findings.

2 Methods

Liver fragments were collected for the parasitic load quantification by qPCR. Frozen sections were stained by immunohistochemitry for FN (n=38), LN (n=35) and $TCD3^+$ (n=11). The percentual area occupied by the stained LN and FN was measured using ImageJ. $TCD3^+$ cells were counted in granulomas, portal space and hepatic parenchyma. This research uses tissues fragments after compulsory euthanasia (recommended by the Brazilian Ministry of Health) without any manipulation, so ethical approval are not required according to CEUA-Fiocruz and Brazilian's law 11794/08.

3 Results

FN deposition was significantly greater in the polisymptomatic (n=15) than asymptomatic (n=13) animals (p=0.048) No significantly correlation between LN and symptomatology or parasite load was observed. There was a positive correlation between the parasite load and FN deposition (p=0.053 r=0.330). Six dogs with low parasite load had number $TCD3^+$/granuloma (Median=22.7%) greater than the dogs with high parasite load (Median=9.4%). Inverse correlation between parasite load and $TCD3^+$/granuloma (p= 0.018 and r^2= -0.842) was observed.

4 Conclusions

Our work corroborates the studies that report canine visceral leishmaniasis is a cause of fibrogenesis in liver. High levels FN expression associated with parasite load in symptomatic animal reflects the inflammatory and degenerative processes. The number of granuloma TCD3+ cells was inversely correlated with parasite load which may suggest a role of TCD3+ cells in parasite burden control, possibly activating macrophages and contributing to granuloma formation.

C1098 LEISHMANIA MEXICANA: MECHANISMS EMPLOYED FOR THE INHIBITION OF APOPTOSIS OF DENDRITIC CELLS

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1 Background

Leishmania mexicana is the causal agent of cutaneous leishmaniasis in Mexico. This intracellular parasite alternates between the flagellated promastigote transmitted by the vector and the aflagellated amastigote found in host cells. The principal host cells for *Leishmania* are macrophages and dendritic cells. Intracellular microorganisms inhibit host cell apoptosis as a strategy to ensure their survival in infected cells. It has been shown that *Leishmania* inhibits apoptosis of macrophages, neutrophils and dendritic cells, but the mechanisms involved in the inhibition of apoptosis have not been fully deciphered. Mitogen-activated protein kinases (MAPK), phosphoinositide 3-kinase (PI3K) and protein kinase B (Akt) participate in the process of apoptosis and are modulated by different species of *Leishmania*. In this work we analyzed the effect of *L. mexicana* promastigotes and amastigotes in the activation of diverse signaling pathways that participate in apoptosis such as MAPK, PI3K/Akt and Bcl-xL.

2 Methods

Monocyte-derived dendritic cells (moDC) were differentiated from $CD14^+$ human monocytes with GM-CSF and IL-4 during 7 days and infected with *L. mexicana* promastigotes or amastigotes for different times. Apoptosis was induced with the topoisomerase-1 inhibitor, camptothecin. Stimulated moDC were lysed and the activation of the kinases and other proteins that participate in apoptosis was determined by Western blot. Also, MAPK p38 and JNK were inhibited with SB203580 and SP600125, respectively, and DNA fragmentation was measured by the TUNEL assay.

3 Results

As shown in this study, the infection of moDC with *L. mexicana* promastigotes and amastigotes diminished significantly the phosphorylation of the MAPK p38 and JNK, which are signaling pathways that lead to apoptosis. The inhibition of both kinases diminished DNA fragmentation in moDC stimulated with camptothecin, but in a major extent was the reduction of DNA fragmentation when JNK was inhibited. On the other hand, *L. mexicana* amastigotes activated anti-apoptotic mechanisms such as PI3K/Akt and Bcl-xL.

4 Conclusions

The capacity of *L. mexicana* to diminish MAPK activation and induce anti-apoptotic mechanisms is probably one of the strategies employed to delay apoptosis induction in the infected moDC and may have implications for *Leishmania* pathogenesis by favoring the invasion of its host and the persistence of the parasite in the infected cells.

This work was supported by grant no. IN225116 from PAPIIT, DGAPA, UNAM.

C1098 LEISHMANIA MEXICANA: MECHANISMS EMPLOYED FOR THE INHIBITION OF APOPTOSIS OF DENDRITIC CELLS

Laila Gutiérrez Kobeh[1], Jorge Rodríguez-González Gutierrez Kobeh[2], Rosalino Vázquez-López[3], Oscar Olvera-Salas[4], Jesús Argueta-Donohué[5], Alma Escalona-Montaño[6], Magdalena Aguirre-García[6], Arturo Wilkins-Rodríguez[6]

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1 Background

Leishmania mexicana is the causal agent of cutaneous leishmaniasis in Mexico. This intracellular parasite alternates between the flagellated promastigote transmitted by the vector and the aflagellated amastigote found in host cells. The principal host cells for *Leishmania* are macrophages and dendritic cells. Intracellular microorganisms inhibit host cell apoptosis as a strategy to ensure their survival in infected cells. It has been shown that *Leishmania* inhibits apoptosis of macrophages, neutrophils and dendritic cells, but the mechanisms involved in the inhibition of apoptosis have not been fully deciphered. Mitogen-activated protein kinases (MAPK), phosphoinositide 3-kinase (PI3K) and protein kinase B (Akt) participate in the process of apoptosis and are modulated by different species of *Leishmania*. In this work we analyzed the effect of *L. mexicana* promastigotes and amastigotes in the activation of diverse signaling pathways that participate in apoptosis such as MAPK, PI3K/Akt and Bcl-xL.

2 Methods

Monocyte-derived dendritic cells (moDC) were differentiated from $CD14^+$ human monocytes with GM-CSF and IL-4 during 7 days and infected with *L. mexicana* promastigotes or amastigotes for different times. Apoptosis was induced with the topoisomerase-1 inhibitor, camptothecin. Stimulated moDC were lysed and the activation of the kinases and other proteins that participate in apoptosis was determined by Western blot. Also, MAPK p38 and JNK were inhibited with SB203580 and SP600125, respectively, and DNA fragmentation was measured by the TUNEL assay.

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4 Conclusions

The capacity of *L. mexicana* to diminish MAPK activation and induce anti-apoptotic mechanisms is probably one of the strategies employed to delay apoptosis induction in the infected moDC and may have implications for *Leishmania* pathogenesis by favoring the invasion of its host and the persistence of the parasite in the infected cells.

This work was supported by grant no. IN225116 from PAPIIT, DGAPA, UNAM.

C1101 MOLECULAR PATHWAYS TRIGGERED BY LEISHMANIA INFANTUM INFECTION IN IN VITRO DIFFERENTIATED MACROPHAGES

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1 Background

Leishmaniases are parasitic diseases caused by protozoa of the *Leishmania* genus transmitted by a sandfly vector, affecting both humans and other vertebrates. The alterations of many host cell gene expression/signaling pathways have been shown to be among the strategies that *Leishmania* has evolved to allow its own survival and replication. For example, *Leishmania* is able to confer resistance to apoptosis in the early phase of the infection by activation of host PI3K/Akt pathway and inhibition of caspase-3 activation. Many intracellular pathogens hijack organelles such as ER, to facilitate survival and replication, activating ER stress and modulating the unfolded protein response (UPR) in the host cell. The aim of this study was to investigate the molecular pathways –focusing in particular on UPR- in *Leishmania*-infected macrophages to gain insights about the pathogenic mechanisms in parasitized cells.

2 Methods

Macrophages differentiated from human monocytic cell lines (U937 and THP-1) and murine primary macrophages were infected with *L. infantum* MHOM/TN/80/IPT1 (1:10). Several ER stress/autophagy expression markers and survival/apoptosis markers (phospho-Akt and cleaved caspase-3) were evaluated by qPCR and/or by western blotting. As ER stress positive control, cells were treated with tunicamycin (TUN) or dithiothreitol (DTT). To deeply investigate the molecular mechanisms involved, RNAseq analyses were performed in both host macrophages (Tru-seq RNA-seq) and *L. infantum* (Spliced leader RNA-seq) RNA samples.

3 Results

The gene expression analysis showed a significant induction of the ER stress/autophagy markers following infection. The western blot analysis revealed that *Leishmania* infection induced Akt phosphorylation and significantly inhibited the induction of caspase-3 cleavage, eIF2α phosphorylation and *DDIT3/CHOP* expression in TUN and DTT treated cells. RNAseq analyses are ongoing to evaluate the gene expression in parasites and host macrophages.

4 Conclusions

The significant increase in ER stress expression markers and the delay/attenuation of the effects of ER stress inducers in infected cells support the hypothesis that *L. infantum* could promote survival of host cells by inducing a mild ER stress response. This mechanism could explain many previous findings regarding the subversion of host cellular pathways and could represent not only a common pathogenic mechanism among *Leishamania* species but also a target for development of new drugs.

C1106 IMMUNOPATHOLOGICAL STUDY OF SPLEEN OF NATURALLY INFECTED DOGS WITH LEISHMANIA INFANTUM

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1 Background

One of the main affected organs in canine visceral leishmaniasis (CVL) is spleen. Disorganization of splenic white pulp (SWP) has been associated with immunosuppression and disease progression This study aims to asses structural and cellular changes in the splenic extracellular matrix of dogs with CVL correlating them with the parasite load and symptoms.

2 Methods

41 animals were grouped according to SWP organization into 1- Organized to slightly disorganized (OR-SD, n = 11); 2- Moderate to intense disorganization (MD-ID, n = 30). Splenic fragments were collected for the parasitic load quantification through qPCR, histopathological analysis and immunohistochemistry. $CD3^+$, $CD4^+$, $CD8^+$ T, $CD21^+$ B cells, IFN-γ^+ and IL-10^+ expressing cells, MMP-9, ADAM-10, as well as, laminin and fibronectin expression were evaluated.

3 Results

The disorganization was accompanied by reduction in the quantity of lymphoid follicles (p> 0.0001).There were no correlation between SWP organization and parasite load or clinical groups. MD-ID animals showed increased of clinical signs (p=0.021), increased deposition of laminin (p=0.045) and collagen (p=0.036) and reduction in $CD4^+$ T (p=0.027). ID animals presented increasing in MMP-9 expression (p=0.025) and in animals with moderate disorganization of SWP the ADAM-10 expression was lower than the other animals (p=0.05). Positive correlations between CD8 and parasite load (p=0.042, r=0.320), CD8 and CD4 (p=0.028 r=0.343), IFN-? and IL-10 (p=0.001 r=0.497), laminin and fibronectin expression (p= 0.043 and r^2 =0,393) were observed. Inverse correlations between laminin and $TCD3^+$ cells (p= 0.019 and r^2= -0637), ADAM-10 and follicles/mm^2 (p=0.022 r=-0.486) were observed.

4 Conclusions

The data suggested that the disorganization of the SWP could be a standard alteration in CVL and partially could be caused by a higher laminin, collagen deposition and ADAM-10 and MMP-9 expression due to remodeling of the MEC that is required initial stage of infection. This disorganization could lead to reduction of $CD3^+$ and $CD4^+$ cells, but not $CD8^+$ cells, and consequently to immunological failure and disease progression. Laminin, fibronectin and collagen deposition together with extra-cellular matrix remodeling by metallopeptidase activity, might lead to reduction of spleen T cell subpopulations, by a mechanism not yet understood, that impairs cell-mediate immune response contributing to immunosuppression and disease progression.

C1110 INTERACTION OF INSULIN-LIKE GROWTH FACTOR-I AND IL-4 ON ARGINASE ACTIVATION IN LEISHMANIA MAJOR-INFECTED MACROPHAGE

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1 Background

In *Leishmania* infection we showed previously that extrinsic and intrinsic intracellular IGF-I favor the parasite growth inducing an increase in arginase expression and activity in infected macrophages. In addition it is known that arginase is activated by IL-4. Thus here we aimed to study the role of intrinsic IGF-I in conjunction with IL-4 in the macrophage activation focusing the arginase expression and activation both on the parasite and macrophage in *Leishmania major*-infected cells in vitro through silencing IGF-I mRNA expression.

2 Methods

L. major promastigote -infected RAW 264.7 cells were transfected with 150µM IGF-I-siRNA and analyzed after 48 hours. In some experiments IL-4 (2ng/ml) were added. IGF-I mRNA expression was quantified by Real Time (qPCR), and nitric oxide (NOS2) by qPCR and Griess method and, arginase (Arg1) by qPCR and arginase activation by urea production, and the parasitism by microscopy.

3 Results

siRNA treatment of cells resulted in 70% reduction of IGF-I mRNA expression. In all groups treated with siRNA we observed a decrease in the arginase mRNA expression and arginase activity in macrophage and parasite when compared with control group without IGF-I siRNA. In parallel IGF-I siRNA treatment decreased the parasitism from 149 (median) parasites per 100 cells in control without siRNA treatment to 93 ($p<0.05$). Using IL-4 stimulus IGF-I mRNA expression was increased as well as the parasitism that reached 171. However in siRNA-treated cells IL-4 stimulus did not induce any increase in IGF-I mRNA expression and the parasitism reduced to 87. To ascertain the role of IGF-I, experiments were carried out to restore IGF-I activity after knockdown. Fifty ng/ml recombinant IGF-I (rIGF-I) were added in the cells treated with siRNA when an increase in the parasite arginase mRNA expression was seen but not in macrophages and no difference in the nitric oxide production. In parallel, restoring with rIGF-I the parasite number was increased to the level similar to the controls. In those experiments with cytokine, the effect of IL-4 stimulus was restored when rIGF-I was added.

4 Conclusions

These results suggest that IGF-I is directly related to expression and activation of arginase mainly on *Leishmania* in infected macrophages leading to increase in the parasitism. The data also suggest the essential role of IGF-I on IL-4 effect on arginase activation during *Leishmania*-macrophage interaction.

C1119 MICRORNA EXPRESSION IN LEISHMANIA (LEISHMANIA) INFANTUM-INFECTED HUMAN MACROPHAGE CELL LINE

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1 Background

Visceral leishmaniasis is caused by *Leishmania (Leishmania) infantum* that may evolve to lethality even under treatment. Infection development and disease progression may be related, in part, to certain changes in the control of gene expression related to immune and inflammatory responses. Our group has been studying insulin-like growth factor I (IGF-I) in *Leishmania* infection showing its role inducing proliferation of the parasite and accelerating lesion development. Studying involvement of IGF-I in disease, we observed high mRNA levels of IGF-I in the liver in *L. infantum*-naturally infected dogs but a decreased serum levels of this factor suggesting a post-transcriptional regulation by microRNAs (miRNAs). Thus the aim of this study was to analyze changes of miRNAs expression in *L. (L.) infantum*-infected cells to value the biological significance of these changes.

2 Methods

Monocyte cell line THP-1 ($5x10^5$) were infected with stationary phase *L. (L.) infantum* promastigotes (8 parasites/cell) and maintained for 6 and 24 hours. Then the cells were harvested for total RNA isolation using mirVANA kit. After RNA isolation, the total RNA was reverse transcribed and used for assess miRNAs levels using qPCR kit Inflammatory Response and Autoimmunity miScript miRNA PCR Array. qPCR relative quantification was evaluated using the web suite *miScript miRNA* PCR *Array* Data *Analysis*. The computational prediction of targets was performed with the DIANA-miRpath 3.0 server (TargetScan algorithm).

3 Results

We have identified 11 differentially expressed miRNAs when compared with non-infected cells at 6 and 24 hours. For 6 hours of incubation, 5 miRNAs was down regulated and 6 was upregulated. For 24 hours of incubation, 8 miRNAs was upregulated and 3 was downregulated. All these miRNAs have 51 different targets predicted in the TGF-β pathway, suggesting modulation of the TGF-β pathway by *Leishmania* infection. Other validation experiments have to be done to validate our hypothesis.

4 Conclusions

These results suggest that different miRNAs are expressed or dynamically regulated by *Leishmania infantum* infection.

C1120 LEISHMANIA MEXICANA INHIBITS RECRUITMENT OF MACROPHAGE SPHINGOSINE KINASE 2 TO THE PERIPHERY OF NASCENT PHAGOSOMES

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1 Background

Leishmaniases are a major cause of infectious morbidity in endemic countries. The pathogenesis of these parasitoses involves inhibition of phagosome maturation, leading to survival of the parasite within human macrophages. As *Leishmania*, *Mycobacterium tuberculosis* (Mtb) survives and proliferates in vacuoles inside macrophages. The inhibition of traslocation of the macrophage enzyme sphingosine kinase (SK) 1 to the periphery of phagosomes, which activity normally induces Ca2+ signaling and phagosome maturation, has been identified as a key determinant of Mtb survival inside this immune cell. Given the similarity between *Leishmania* and Mtb in terms of survival inside the same target cell, we decided to explore whether internalization of *Leishmania mexicana* (Lmex) by macrophages induces changes in the localization of SKs during the formation of the phagosome and after establishment of the parasitophorous vacuole.

2 Methods

Changes in the subcellular localization of J774 murine macrophages SK1 and SK2 enzymes were evaluated by confocal microscopy after 5, 10, 20 and 40 min of internalization of live or fixed Lmex promastigotes, or latex beads, and after the establishment of the infection (48 hours), using polyclonal antibodies directed against the enzymes SK1 and 2, and secondary antibodies conjugated to fluorescent probes.

3 Results

During the internalization process, Lmex live promastigotes inhibited the translocation of SK2 to the periphery of the phagosome, a progressive and transient phenomenon observed during the phagocytosis of fixed promastigotes and latex beads. Once the infection was established, SK2 accumulated in the nucleus in infected macrophages compared to a location consistent with the endoplasmic reticulum in uninfected cells. No major changes were observed in macrophage SK1 localization during internalization of fixed promastigotes, latex beads, or live promastigotes, nor after establishment of infection.

4 Conclusions

The results showed represent a contribution to the understanding of the survival mechanisms shared by microorganisms that succeed in living in vacuoles inside macrophages. Further studies will be necessary to determine the impact of the changes induced by *Leishmania* on macrophage SK2 localization on its survival. These findings open a new field for the search of potential drug targets against leishmaniases.

C1128 FUNCTIONAL ANALYSIS AND B-CELL EPITOPE PREDICTION OF HYPOTHETICAL PROTEINS IN LEISHMANIA DONOVANI: A BIOINFORMATICS BASED APPROACH

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1 Background

The global control of visceral leishmaniasis remains a challenge from the standpoint of diagnosis and treatment. Major serodiagnostic limitations include cross-reactivity and low specificity in detecting active infection, while resistance to drug should be of concern. Hence, there is a need to look into newer targets. The proteome information available suggests that among the 7960 proteins in *Leishmania donovani*, a staggering 65% remains classified as a hypothetical uncharacterized set. In this study, we developed a bioinformatics based approach to analyze a sub-set of hypothetical proteins from this uncharacterized set and predicted structural and functional properties as well as antigenecity of proteins with available tools.

2 Methods

An entire set of 570 proteins, termed as 'hypothetical protein, unknown function' was primarily screened for such proteins having conserved domains with high confidence using a combination of the protein family databases. In the second step, 57 of such potential target proteins were extensively analyzed by means of physicochemical characteristics , protein-protein interaction, sub-cellular localization, structural similarity and functional classification with gene-ontology term association. In addition, we made an approach to find out probable antigenic proteins from the entire set and to identify linear and conformational B-cell epitopes of these antigenic proteins.

3 Results

We predicted these hypothetical members belong to various classes of proteins such as enzymes, transporters, receptors, structural proteins, transcription regulators and other proteins. However, the structural similarity prediction of the annotated proteins substantiated the functional classification of 50 out of those 57 proteins. Further, based on higher antigenicity score and sub-cellular localization, we choose three of the antigenic proteins to exemplify B-cell epitope prediction approach. We found 19 linear epitopes in total for the three hypothetical proteins and 14 of the conformational epitopes located partially or fully in the linear epitope region. Two known proteins- heat shock protein and kinesin protein were used to evaluate this prediction approach.

4 Conclusions

The in silico methodology used in this study for the particular hypothetical protein group of *L. donovani* may be followed for all other proteins in the group, which may produce data that can be useful in facilitating swift experimental identification of potential serodiagnostic and drug targets.

C1159 LEISHMANIA BRAZILIENSIS INDUCES INCREASED LEVELS OF INFLAMMATORY MEDIATORS IN MURINE NEUTROPHILS

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1 Background

Neutrophils are the major cells during the first hours after *Leishmania* infection and therefore, can these cells contribute to a protective immune response against this parasite. In our study, we evaluated *in vitro* response of neutrophils infected with different *Leishmania* species.

2 Methods

Neutrophils were harvested from bone marrow of C57BL/6 mice and cultured in the presence of *Leishmania amazonensis*, *Leishmania braziliensis* or *Leishmania major*.

3 Results

Our date demonstrated that regardless of *Leishmania* species, the percentage of viable cells did not change. *L. braziliensis* induced highest percentage of neutrophils phagocytosis in addition to an increased intracellular expression of both nitric oxide (NO) and reactive oxygen species (ROS). Moreover, neutrophils infected with the three analyzed *Leishmania* species displayed a decreased in CD62L expression and an increased in TLR2 and TLR9 expression. Interestingly, neutrophils infected with *L. braziliensis* displayed uppermost TLR2 expression. Finally, the intracellular cytokine expression showed increased TNF-alpha levels and no difference in TGF-beta, in all species of the parasite after infection in neutrophils.

4 Conclusions

Taken together, our data demonstrated that *L. braziliensis* was more effective in inducing an activation profile in neutrophils in addition to expression of inflammatory mediators when compared to *L. amazonensis* or *L. major* infections.

C1169 THE C-TYPE LECTIN BOTHROPS LEUCURUS SNAKE VENOM BLL INCREASES IL-1B PRODUCTION IN MACROPHAGES INFECTED WITH LEISHMANIA BRAZILIENSIS: THE PUTATIVE ROLE OF INFLAMMASOME ACTIVATION

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1 Background

Leishmania brasiliensis, is the main etiological agent of cutaneous leishmaniasis in Brasil. This illness leads to severe and disfiguring lesions. During the early stages of infection in macrophages, the parasites are recognized by a variety of pathogen-associated molecular pattern receptors that include TLR family member and the intracellular Nod-like receptor (NLR) family. A subset of NLRs, the NLRP3 inflammasome, forms a multiprotein complex that lead to the maturation IL-1β in response to different stimulus and plays an important role in innate immune response against intracellular pathogens. C-type lectins have been recognized as important pattern-recognition proteins, essential for the innate immunity of vertebrates and invertebrates. In this regard, the aim of this study was to investigate the effects of a C-type lectin, from *Bothrops leucurus* venom (BLL), on the immune response against *L. braziliensis.*

2 Methods

Peritoneal macrophages are allowed to adhere in 24-well culture plate and infected with promastigotes forms of *L. braziliensis.* Next, the cultures were treated with BLL for 24 hours, stained with Giemsa and analyzed by light microspcopy for estimation of the survival index of amastigotes and the percentage of macrophage infection. The culture supernatants were incubated with Griess reagent for NO quantification and the cytokine profile was analyzed using BD CBA Mouse Th1/Th2/Th17 cytokine Kit. The infected cells were also submitted to transmission electron microscopy for evaluation of morphological alterations.

3 Results

BLL significantly inhibited the survival of intracellular amastigote (86%±7.8) and the percentage of infected macrophages (92.1 %), compared with non-treated infected cells. The lectin also induced the enhancement of NO and the release of proinflammatory, but not of Th2 cytokines. In particular, we observed the increase in IL-1β, indicative of NLRP3 inflammassome activation. The incubation of infected macrophages with BLL previously bonded to galactose, was able to revert the inhibitory effect of BLL on macrophage infection. Our ultrastructural analysis showed that BLL induced morphological alterations on both host cells and the parasites.

4 Conclusions

Our results suggest that the specific interaction of lectin with macrophage membrane receptors may activate NLRP3 inflammasome and increase the IL-1β, which ultimately lead to an increased NO production and the inhibition of amastigote survival inside the macrophages.

C1185 A NEW FUNCTIONAL FLOW CYTOMETRIC ASSAY USING SIMULTANEOUSLY MEASURE TO ANALYZE LEISHMANICIDAL ACTIVITY, CYTOTOXIC AND IMMUNOMODULATORY ACTIVITIES OF GLUCANTIME AND AMPHOTERICIN B

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1 Background

Our group described the standardization of a new quantitative functional methodology as a predictive assay to confirm leishmanicidal potential of new chemical entities in drug discovery programs. This method allowed a simultaneous confirmation of the leishmanial and cytotoxic/immunotoxic activities of Glucantime® and Amphotericin B, using a multiparametric analysis by flow cytometry.

2 Methods

The new methodology is done through the quantification of internalization rate of labeled-*L. braziliensis* for monocytes and neutrophils by human peripheral blood with a concurrently assessment of cytotoxicity (late apoptosis or necrosis) by flow cytometry. In this work, we described for the first time the application of this methodology to predict the immunomodulatory effect of two drugs used in the leishmaniasis treatment. We evaluated the immunomodulatory effect of Glucantime and Amphotericin B through measurement of intracellular cytokines by monocytes and granulocytes using this new multiparametric assay by flow cytometry. Human whole blood culture were incubated or not in the presence of live promastigote forms of *L. braziliensis* and treated with Glucantime® and Amphotericin B; cell viability was measured by 7AAD label; intracellular cytokines (IL-10, TNF-α and IL-12) were evaluated in monocyte/granulocytes populations.

3 Results

Data demonstrated a significantly reduction in internalization of *L. braziliensis* by phagocytes, without significant cytotoxicity, after treatment with both drugs when compared with the non-treated cultures. The analysis of the intracellular cytokines profile showed an increase of IL-10, IL-12 and TNF-a in the treated cultures with Glucantime® and Amphotericin B in the presence of live promastigote forms of *L. braziliensis*, enabling the access of different important parameters for drug discovery. The methodology was able to detect the impact of Glucantime® and Amphotericin B on intracellular cytokines (IL-10, TNF-α, IL-12) production from mononocytes and neutrophils, that are directly involved in the progression of *Leishmania* disease.

4 Conclusions

The new assay presents itself as a new alternative in the area of drug discovery, allowing accurate prediction the leishmanicidal and immunomodulatory activities of substances in the preclinical phases of study. Moreover, it can be considered an alternative to the decrease of the use of models with animals in this stage.

C1205 MACROPHAGE REPROGRAMMING AND ANTIGEN PRESENTATION IN INDIAN POST KALA-AZAR DERMAL LEISHMANIASIS

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1 Background

The *Leishmania* parasite is the causative organism for Post-Kala-azar Dermal Leishmaniasis (PKDL), a chronic dermal sequel to Visceral Leishmaniasis. It deviously manipulates host immune responses for ensuring its survival. Information regarding host immune responses in Leishmaniasis is primarily derived from animal models, but growing evidence is emphasizing the inadequacy of direct inter-species translation. This study aimed to define the status of antigen presenting cells (dendritic cells and monocytes/macrophages) and their impact on $CD8^+$ T-cells in PKDL.

2 Methods

In dermal lesions of patients with PKDL, the expression of CD1a, MHC-II and Langerin along with markers of alternative activation (*arg1, mrc-1, vdr, cyp27b1, ll-37)* were evaluated.Additionally, the status ofPerforin, Granzyme, PD-1 and CCR4 was evaluated both in dermal lesions and peripheral blood, along with plasma levels of CCL17, CCL22, IL-4, IL-13 and IL-10.

3 Results

In patients with PKDL, a decrease in CD1a, MHC-II and Langerin indicated a decrease in the presence of lesional dendritic cells. The raised plasma levels of IL-4/IL-13 and IL-10 confirmed the presence of a microenvironment conducive for alternative activation of monocytes/macrophages. The elevated mRNA expression of *il-10*, *ifn-γ*, *mrc-1, arg1, vitamin D signaling pathway (vdr, cyp27b1, ll-37)* at lesional sitescorroborated the presence of alternatively activated macrophages. Alongside, markers of classical activation showed a consistent decline in terms of generation of reactive oxygen intermediates, CD282/284and MHC-II. Although there was an absence of $CD4^+$ T-cells, there was an enhanced infiltration of $CD8^+$ T-cells, which demonstrated an absence of perforin, granzyme and Zap-70, concomitant with an enhanced expression of PD-1 and the skin-homing chemokine CCL17. Furthermore, a raised $CCR4^+CD8^+$ population was accompanied by an increased lesional expression of IL-10 and IL-5. In peripheral blood, the enhanced $CD8^+/CCR4^+$ T-cell population and raised CCL17/22 was associated with an increased frequency of PD-1 and decreased CD127.

4 Conclusions

The enhanced presence of alternatively activated monocytes/macrophages along with a decrease in CD1a dendritic cells, led to an impairment in antigen presentation. This translated into an enhanced presence of anergic/exhausted $CD8^+$ T-cellsthat accounted for an overall immunosuppression that supported disease progression in PKDL.

C1218 LYMPHOCYTE ACTIVATION GENE 3, (LAG-3) AS IMMUNE CHECK POINT IN INDIAN VISCERAL LEISHMANIAISIS.

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1 Background

Visceral Leishmaniasis , a chronic infection caused by protozoan parasite *Leishmania donovani* complex and suppression of T-cell response (exhausation of T-cells) is thought to be involved in pathogenesis of it. Different inhibitory markers play role in the negative regulation of T-cell activation. LAG-3 is one of them who behave as immune checkpoint. The proposed study aims to see Lymphocyte activation gene, i.e, LAG-3 involvment *Leishmania* infection both in vitro and vivo .

2 Methods

This study was approved by RMRIMS Ethics committee. Blood specimens for this study was obtained from RMRIMS.

RNA isolation and cDNA Ppreparation from PBMC was done by RNAeasy Qiagen kit and ABI cDNA preparation kit as per manufacture's instructions. Gene expression study was done by REAL-TIME PCR . Experiment was performed on 11 samples of each active VL and Healthy individuals. Parasite load estimation was done by quantiataive polymerase chain reaction using kDNA specific absolute quantification method. DNA was extracted from 200 ul of blood collected in citrate tubes. DNA extraction was carried out using a QIAamp DNA mini kit. LAG-3 ELISA was performed using plasma by human specific LAG-3 ELISA kit (Thermoscientific, Life technology)

Jurkat T cell line and THP1 cell line (monocyte) were chosen for stimulation experiment.Cells were cultured in RPMI media and promastigote infection given to them for stimulation. Experiment repeated 3 times . PBMC of 3 different individuals were taken for this study . Time interval taken for study was 5hr, 24 hr. and 48 hr. gene expression and ELISA study was done. There is significant difference in gene as well as protein level in T cell line and stimulated PBMC .

3 Results

We found there is significant difference in expression pattern of LAG-3 gene both at the RNA level and protein level in Active VL compare to treated or Healthy individuals. Although, there is no any correlation exists between parasite burden in peripheral lood of VL patients with LAG-3 gene. We also found expression pattern of LAG-3 in terms of time kinetics and found that after 24 hr there is more difference in LAG-3 expression in Leishmania stimulated samples (T cells and PBMC)

4 Conclusions

Thus this study is helpful in finding out involvment of functional existence of LAG-3 in immune response during VL and to find out new ways of T cells based immune modulaton during Leishmania infection and further new therapeutic options to treat VL through regulation of immune activation

C1260 HEMATOLOGY AND SERIC ELETROPHORESIS PROTEINOGRAM IN POSITIVE LEISHMANIASIS DOGS

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1 Background

Visceral leishimaniasis (VL) is a chronic disease existent in all world. It can attack humans, dogs and wild animals. Most of hematologic alteration in attacked dogs are hyperproteinemia, hyperglobulinemia, hypoalbuminemia, anemia, thrombocytopenia, proteinuria and azotemia. The aim of this study was to determine changes in serum total protein concentration and fractions by electrophoresis method in dogs attacked by VL.

2 Methods

15 natural infected dogs from LV endemic area were studied, the diagnosis was performed by popliteal liph node biopsy by needle puncture and aspiration, and serology by ELISA. Jugular vein was punctured after local antisepsis, and the blood was processed to obtain serum, it was employed for the electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels (SDS-PAGE) to obtain complete proteinogram.

3 Results

The most important blood changes were anemia 40% (6 out of 15 dogs), thrombocytopenia 80% (12 out of 15 dogs), leukocytosis 13,33% (2 out of 15 dogs), leukopenia 13,33% (2 out of 15 dogs), proteinuria 60% (9 out of 15 dogs), hyperproteinemia 66,66% (10 out of 15 dogs), hypoalbuminemia 100% (all dogs), hyperglobulinemia 93,33% (14 out of 15 dogs) e decrease of albumin: globulin ratio 93,33% (14 out of 15 dogs). In globulin fraction, it was observed increase in gamma globulins, what is called polyclonal gammopathy.

4 Conclusions

The gamma globulins increase in VL infected dogs is related to polyclonal antibodies productions, what promotes a humoral answer and contributes to albumin: globulin ration decrease. These results confirm that most importante findings in chronic infected dogs are related to hypergammaglobulinemia.

C1263 L-ARGININE TRANSPORT CRITICALLY REGULATES SURVIVAL OF LEISHMANIA DONOVANI INSIDE MACROPHAGE: INVOLVEMENT OF HOST CATIONIC AMINO ACID TRANSPORTER-2 (CAT-2)

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1 Background

Leishmania donovani, the causative agent of Indian visceral leishmaniasis (VL), is an obligate intracellular parasite that possesses an excellent survival strategy to escape the host defence. Macrophages are the major target cells for *Leishmania* replication and their activation status is the principal determinant for the outcome of infection. It has been previously reported that L-arginine plays an important role for activation of macrophages. However, the role of L-arginine transport in disease manifestation of VL still remains elusive.

2 Methods

A human monocytic macrophage like cell line, THP-1 was infected with *Leishmania donovani*. Argininine uptake was measured by scintillation counter. The expression of CAT-1, CAT-2, Arg-1, Arg-2, iNOS were measured at the transcript level by semi-quantitive RT PCR and protein level by western blot. Polyamines were measured by HPLC. siRNA mediated transfection were performed to downregulate the expression of different genes. Released cytokines were measured by sandwich ELISA.

3 Results

Infection of macrophage with *Leishmania donovani* resulted in upregulation of L-arginine transport. While investigating the involvement of the L-arginine transporters, we observed that CAT-2 is the major isoform associated with L-arginine transport in macrophages. Simultaneously, *Leishmania* survival was greatly impaired when the transporters were blocked. L-arginine availability and its transport was also found to regulate host arginase and iNOS balance in *L. donovani* infection in *ex-vivo* condition. Host arginase and iNOS expression were found to be reciprocally regulated. Interestingly, induction of iNOS expression and significant increase in extracellular nitric oxide production were observed in case of inhibition of arginase in infected macrophages resulting in decreased parasite survival. Simultaneously, inhibition of L-arginine transport as well as arginase resulted in decreased polyamine production limiting parasite survival inside macrophages. Upregulation of L-arginine transport and induction of host arginase in case of *Leishmania* pathogenesis were found to be correlated with increased level of Th2 and decreased level of Th1 cytokines.

4 Conclusions

Our study is the first report describing the importance of L-arginine availability and its transport by CAT-2 in regulation of *Leishmania donovani* survival inside macrophages. Our findings provide evidence for targeting L-arginine metabolism as an adjunct therapy for the control of *Leishmania* infection.

C1271 INTEGRATED ANALYSIS OF THE TRANSCRIPTOME OF LEISHMANIA DONOVANI AND MACROPHAGE HOST CELL UPON INFECTION WITH ANTIMONY-RESISTANT AND ANTIMONY-SENSITIVE STRAINS

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1 Background

Antimonials like sodium stibogluconate (SSG) have been widely used to treat leishmaniasis worldwide, but the emergence of drug resistance has limited their use in the Indian subcontinent (ISC). It has previously been demonstrated that antimony-resistant (SSG-R) *Leishmania donovani* from ISC show a higher infectivity and a general better fitness than sensitive strains. This has been linked, among others, to the occurrence of specific glycans on the surface of SSG-R parasites that activate a cascade of signaling events ultimately leading to increased levels of IL-10 and decreased production of reactive oxygen and nitrogen intermediates. In order to understand the influence of the SSG susceptibility background on both the parasite's survival skills and the host cell response, we have initiated the first integrated study of gene expression patterns of parasites and macrophages infected with SSG-R or SSG-S *L.donovani*.

2 Methods

Macrophage and parasites transcriptomes were analysed in parallel, by RNAseq of mouse peritoneal macrophages *in vitro* infected with two sets of SSG-R and SSG-S strains. A specific protocol was applied for the amplification of parasite transcriptomes from infected host cells, based on the detection of mini-exon spliced transcripts. These experiments were carried out in the absence of drug pressure.

3 Results

First experiments confirmed upregulation of IL-10 in SSG-R infected cells, while type I interferon (IFN) signaling and IFN regulatory factors were downregulated, as compared to SSG-S infected cells. Remarkably, the same transcriptional pattern was observed after infection with two different SSG-R strains. Analysis of parasite transcriptomes showed upregulation in SSG-R parasites of, among others, amastins, GP63 and galactosyltransferase, the enzyme responsible for addition of the SSG-R-specific galactose residue. Integrated transcriptome analyses in presence of SSG are currently being carried out.

4 Conclusions

These data suggest that SSG-R parasites acquired different abilities than their SSG-S counterparts to avoid the SSG-induced immune activation, and that these abilities remain in the absence of drug pressure. SSG-R parasites differentially modulate the host response ultimately leading to a better infection.

C1283 REACTIVE OXYGEN AND NITROGEN SPECIES LEVELS PRODUCED BY LEISHMANIA MAJOR-INFECTED PHAGOCYTES AS HOST SURROGATE PARASITE VIRULENCE MARKERS

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1 Background

Zoonotic cutaneous leishmaniasis (ZCL) caused by *Leishmania (L.) major* shows various clinical manifestations, ranging from asymptomatic infection without apparent lesion, to extensive lesions that might cause severe disfiguring scars. This clinical expression is due to the host immune status, to the parasite intrinsic virulence or to both. Ability to predict clinical outcome is crucial when prescribing treatment or determining possible control measures in epidemiological studies.

During its life cycle, Leishmania encounters various hostile conditions such the two highly toxic radicals for *Leishmania*, reactive oxygen and nitrogen species (ROS and RNS) generated during phagocytosis by host cells

In this work, we sought to identify host cellular surrogate markers for *Leishmania* virulence that can discern between high and low virulent isolates i.e., the cellular levels of nitric oxide (NO) and reactive oxygen species production.

2 Methods

Using a selected couple of *L. major* isolates according to their contrasting virulence and three cell types i.e., human activated THP1 cells, human polymorphonuclear neutrophils (PMN) and murine macrophagic Raw 264.7 cells, we monitored NO and ROS levels in each cell type upon *in vitro* infection.

3 Results

Results showed that virulent parasites are inducing higher NO and ROS amounts than non-virulent ones, contrasting with their corresponding parasite load.

4 Conclusions

This study shows that *L. major* virulence seems to be correlated with cellular NO and ROS levels. A collection of well-characterized isolates will be used to infect cells, to monitor NO and ROS produced amounts upon infection and to correlate them with lesion severity and pathogenicity in experimental animal model.

C1290 THE COMPOSITION OF EXTRACELLULAR VESICLES DERIVED FROM LEISHMANIA DONOVANI INFECTED MACROPHAGES PROVIDES PERSPECTIVES INTO THEIR BIOGENESIS AND CONTRIBUTIONS TO PARASITE PATHOGENESIS

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1 Background

Extracellular vesicles (EVs) have emerged as important mediators of cell-to-cell communication and have been shown to contribute to the pathogenesis of infectious microorganisms. In *Leishmania* infections, it has been proposed that infected cells release EVs loaded with *Leishmania* derived molecules that promote *Leishmania* pathogenesis.

2 Methods

To better understand the properties of EVs produced by *Leishmania*-infected macrophages, we analyzed the proteome of EVs derived from RAW 264.7 mouse macrophages infected with *L. donovani*. EV preparations obtained by established protocols were analyzed by liquid chromatography and tandem-mass spectrometry (LC MS/MS), then protein composition was validated by Western blotting. Immunofluorescence assays were used to visualize the distribution of EV proteins in infected cells and to track the biogenesis of EVs within infected macrophages. In light of the observations that parasites lacking the centrin gene (LdCen-/-) do not thrive within macrophages, the proteome of EVs from cells infected with LdCen-/- was determined to further validate the parasite derived proteins in EVs from infected cells.

3 Results

In addition to host derived molecules such as CD63, HSP70, and CD9 that are characteristic of mammalian exosomes, EVs from infected cells contained additional host derived proteins including annexins A1, A2, A5 and cathepsin B. In regards to parasite derived proteins in EVs, a subset of proteins that were previously identified in exosomes released by axenic parasites were also found in EVs from *Leishmania*-infected cells, including gp63, Elongation factors 1 and 2 and HSP70. A significant number of parasite-derived proteins were exclusively identified in EVs from established *Leishmania* infections, including proteophosphoglycan 3, putative apical membrane antigen 1, sulfate transporter-like protein, and putative serine/threonine protein kinase. EV preparations derived from LdCen-/- lacked this later subset of parasite proteins, which was interpreted to imply that parasite proteins in EVs were derived only from metabolically active intracellular parasites. Immunofluorescence assays with antisera reactive to parasite derived proteins in EVs hint at a novel pathway of EV biogenesis involving a complex interplay between parasite and host.

4 Conclusions

Taken together, a comprehensive molecular profile of the molecules released in extracellular vesicles from *L. donovani*-infected macrophages and insight into the mechanism of their biogenesis will be presented.

C1292 A NEW EX VIVO MODEL: INFECTING GLIAL CELLS WITH ANTIMONY-RESISTANT L.TROPICA

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1 Background

Leishmaniasis as a vector-borne zoonotic disease has different clinical features such as cutaneous, visseral, mucocutaneous forms. People living in tropical and subtropical regions of the world are under risk for infection. Treatment protocols for leishmaniasis are toxic and has many limitations during the applications. There is also a need for the description of new therapy options especially for the resistant patients. In addition to in vitro and in vivo studies, ex vivo models using in house developed primary cell cultures could be a good source for evaluation of new drug options in patients with antimony resistance. Here we described an ex vivo culture model to evaluate the possible treatment alternatives for the patients with resistant cutaneous leishmaniasis.

2 Methods

Leishmania tropica promastigotes previously isolated from 9 years old female CL case were used for experimental infection model. After two unresponsive courses of intralesional meglumine antimoniate (Glucantime ® injectable, Sanofi-Aventis, France)application, finally parenteral sodium stibogluconate (Pentostam ® injection,
GlaxoSmithKline, UK)was used. Due to unsuccessful pentavalent antimony treatment, patient had been defined as "antimony-resistant case". Primary astroglial cell cultures were prepared from new born rat (d1-d2) forebrain cortex according to the methods for Mc Carthy and de Vellis with minor modification.

3 Results

The cultures were kept in DMEM (%10 FCS) at 37oC in 5% CO2 humidified air and passaged to 6 well plates (5x104 cells/well) after 11-12 days in vitro for further experiments. Following the incubation at 72 hours at 37oC in 5% CO2 humidified air, astroglial cell cultures infected with antimony-resistant *Leishmania* promastigots were stained with Giemsa. Infected glial cells were shown by microscopy.

4 Conclusions

We expect that this new ex vivo model will provide additional information to in vitro and in vivo models especially to evaluate new drug candidates. Surprisingly, to be able to infect glial cells using antimony-resistant *Leishmania* promatigotes could also be an evidence for the presence of *Leishmania* parasites in hidden areas of nervous system escaping from immune response. Long term maintenance of amastigotes in nervous system might also provide unexpected explanations for drug resistant cases.

C1307 THE LEISHMANIA VIROME AND ITS ROLE IN DISEASE SEVERITY

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Leishmania in South America often bear the dsRNA *Leishmaniavirus* (LRV1). Like most Totiviruses, LRV1 is neither shed nor infectious, and thus may be viewed as a persistent endobiont. Perspectives on the importance of protozoal viruses changed upon discovery that *L. guyanensis* LRV1 is associated with hypervirulence and increased metastasis in animal models, the latter being a hallmark of the more severe forms of leishmaniasis (Ives *et al. Science* 2011). We have been pursuing this observation intensively as a new paradigm of protozoal virulence. For *Leishmania* we developed RNA interference tools for reproducibly generating isogenic lines lacking LRV1s (Brettmann *et al PNAS* 2016). This has allowed extension of findings with *L.guyanensis* to *L. braziliensis*, the predominant agent of mucocutaneous leishmaniasis (MCL). An important question is the contribution of LRV1 with *Leishmania* pathogenicity in human infections, where disease manifestations differ greatly from those seen in murine models, which is complicated by several factors. Recently we showed that the presence of LRV1 was associated with increased relapse and/or treatment failures in human *L.braziliensis*-infected patients treated with pentavalent antimonials in Peru and Bolivia, as well as in *L.guyanensis* infections treated with pentamidine (Adauai *et al* & Bourreau *et al. J. Inf. Dis* 2016). The association of LRV1 with clinical drug treatment failure could serve to guide more effective treatment of tegumentary disease caused by *Leishmania* sp.
We have embarked on a systematic survey of known and new viruses in Leishmania as well as their monoxenous insect trypanosomatid relatives, using a wide range of methods including next-gen RNA sequencing. The properties and roles of these in various aspects of parasite biology will be the focus of future studies.

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C1336 LEISHMANIA MAJOR-HUMAN INFECTED MACROPHAGES : A SYSTEMS-LEVEL PROFILING

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1 Background

Leishmania (*L.*) parasites are able to survive and replicate in the hostile phagolysosomal environment of infected macrophages in human host. To establish a successful infection and ensure their own survival, these parasites have developed sophisticated strategies to subvert the host macrophage responses. Identifying factors that participate to this complex interplay is an important step towards better understanding of the clinical outcomes of leishmaniasis.
Despite huge community efforts, only a small number of host and parasite molecules have been identified to date for their crucial role during infectious process. The aim of our study was to identify mRNA targets of deregulated microRNAs(miRNAs) following infection and vice versa.

2 Methods

We established the miRNA and mRNA transcriptional profile of monocyte-derived human macrophages from healthy donors infected with *L. major* metacyclic promastigotes. This was realized using microfluidic cards and microarray set including 364 miRNAs and 17,838 mRNAs respectively. An integrated analysis using the 64 deregulated miRNAs and the 67 differentially expressed mRNAs was then applied to identify key pathways and molecules involved in macrophage-*Leishmania* interaction.

3 Results

GO enrichment and KEGG pathway analysis of differentially expressed mRNA and miRNA allowed us to identify among the most highlighted pathways and top functions deregulated during the infection process, chemokines signaling pathways, MAPK signaling pathways, as well as Toll-like receptor signaling and apoptotic pathways.

4 Conclusions

Our data indicate that combining the two complementary mRNA and miRNA transcriptomic approaches may lead to better understand the involvement of key genes in these pathways. This comprehensive approach is likely to allow the identification of new molecules and functional pathways that could be targeted to fight this parasitic infection.

C1337 PHENOTYPIC CHANGES IN LEISHMANIA DONOVANI SUBJECTED AS INTRACELLULAR AMASTIGOTE TO SUCCESSIVE CYCLES OF MILTEFOSINE PRESSURE

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1 Background

Experimental studies with miltefosine (MIL) in *Leishmania infantum* have shown that deficiency in the expression of the MT/ROS3 transporter complex confers full drug resistance coinciding with the inability of MIL to accumulate inside the parasite (Mondelaers *et al. PLOS one* 2016). Although increasing relapse rates are being reported, treatment failure is generally not been associated with the acquisition of a prominent MIL resistance phenotype based on standard *in vitro* susceptibility assays. Due to the scarcity of naturally resistant clinical isolates, this study aimed at understanding treatment failure beyond the scope of drug susceptibility by focusing on some (epi-) phenotypic changes of the parasite.

2 Methods

Resistance was selected *in vitro* through eight sequential cycles of increasing MIL exposure on intracellular amastigotes of *L. donovani*. Uptake and efflux of BODIPY-labeled MIL was evaluated by flow cytometry and correlated with the level of transcription of the *MT*-gene by quantitative RT-PCR for the parent, intermediate (cycle 5) and final (cycle 8) selected line. The fitness of the parent versus the cycle 8 selected line was evaluated by determination of intracellular amastigote multiplication and total burdens upon *in vitro* infection of primary mouse macrophages.

3 Results

The lines recovered from the sequential selection cycles displayed unaltered MIL-susceptibility of amastigotes but a decreased susceptibility of back-transformed promastigotes. Relative to a fully (natural) resistant MT/ROS3 deficient *L. infantum* strain, parasites isolated after successive MIL exposureonly displayed a limited reduction in BODIPY-MIL uptake that was also associated with a moderate decrease in *MT* transcription. Efflux rates were not significantly altered suggesting that the activity of outward drug transporters may not be primarily involved in the observed decreased susceptibility. However, the fitness studies revealed that parasites under repeated MIL pressure showed a more efficient entry into the host cell leading to higher intracellular burdens.

4 Conclusions

Repeated *In vitro* exposure of *L. donovani* intracellular amastigotes to MIL did not reveal prominent changes in intra-parasitic MIL accumulation but was rather associated with an increased infectivity for the macrophage host cell.

C1338 CHANGES IN STEROL AND PHOSPHOLIPID COMPOSITION DURING THE INTRAMACROPHAGIC TRANSFORMATION OF THE LEISHMANIA DONOVANI COMPLEX PROMASTIGOTES INTO AMASTIGOTES

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1 Background

Leishmania donovani and *L. infantum*, the causative agents of visceral leishmaniasis (VL) in humans and of canine leishmaniasis in dogs are intracellular pathogens, whose establishment depends on their successful internalization and multiplication inside macrophages, their mammalian host cell. In the purpose to analyze the variations in the different lipid categories needed by the parasite at the time of its transformation from promastigote to amastigote, cholesterol, ergosterol and different phospholipids were quantified.

2 Methods

Promastigotes were cultivated axenically and obtained massively for the different analyses. The amastigotes were extracted from J774 infected macrophages after cell disruption and were purified by a series of centrifugations and filtrations. Their integrity was monitored by optical and electron microscopy that showed that they were in overall good condition.
Lipid Extraction was achieved using the method of Bligh and Dyer, followed by thin-layer chromatography. The samples are subsequently analyzed by GC-MS.

3 Results

The transformation of the promastigote into the amastigote stage was correlated with an enrichment in phospholipids and cholesterol.

4 Conclusions

We suggest that parasites metabolize cholesterol into ergosterol and use it for infection and multiplication in the host.

C1342 LIPIDS ANALYSIS AND STUDY OF THE ROLE OF FATTY ACIDS IN THE INFECTIVITY OF LEISHMANIA INFANTUM, L. TROPICA AND L. MAJOR ISOLATED IN TUNISIA

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1 Background

To date, studies have essentially focused on the role of surface glycosylated residues and proteins in host-parasite interactions. Recent interest has grown for the role of lipids in Trypanosomatids and especially in *Trypanosoma cruzi*, *T. brucei* and *Leishmania* spp. Lipid metabolism is of paramount importance for parasites, and especially for intracellular parasites, which rely on a complex system of uptake and synthesis mechanisms to satisfy their lipid needs.

2 Methods

Nine human strains and one canine strain originating from Tunisia, where leishmaniasis in endemic, were isolated in order to analyze the variations in lipid composition from *Leishmania infantum, L. major* and *L. tropica*. Total lipids and cholesterol were quantified in the promastigote forms cultivated axenically and obtained in great amounts for analysis. Lipid extraction was performed using the method of Bligh and Dyer. The samples are analyzed by gas chromatography or GC-MS. The study of the role of docosahexaenoic acid, arachidonic acid and oleic acid on the infectivity of *Leishmania* has also been carried out.

3 Results

The total lipids composition was the same in the *L. infantum* and *L. tropica*, while the *L. major* strains exhibited some variations. No major difference in the saturated fatty acids of the tested parasite strains was evidenced, as well as in their mono-unsaturated fatty acids. The fatty acid precursors of the family n-6 (18:2 n-6) and n-3 (18:3 n-3) were present in a significant proportion.

4 Conclusions

Our results suggest that the elongation and desaturation reactions have a fairly low rate in the parasites. In addition, n-3 were in greater amounts than n-6 fatty acids, as well as 22:6 n-3 were far more present than 20:4 n-6 fatty acids, a fact that seems to differentiate *Leishmania* promastigotes from most eukaryotic cells. The results show that the 3 fatty acids studied alter the virulence of the parasite.

C1367 ADIPOCYTES INFECTED BY LEISHMANIA: A MODEL FOR UNDERSTANDING PARASITE PERSISTENCE AND LIPID METABOLISM

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1 Background

Leishmania amastigotes live in phagolysosomal compartments of phagocytic mononuclear cells. However, there are studies reporting that *Leishmania* is also capable of infecting different cell types including fibroblasts, melanocytes, neuronal and muscle cells. It has been recently proposed that adipose tissue-derived mesenchymal stem cells are permissive to old World *Leishmania* species infection, although the cellular aspects that orchestrate this process have not yet been elucidated. The aim of this work is to evaluate the susceptibility of adipocyte-derived 3T3.L1 fibroblasts to *L. (L.) amazonensis* and correlate infection rates and parasite maintenance throughout the infective process.

2 Methods

Briefly, 3T3.L1 fibroblasts were differentiated into adipocytes after incubation in DMEM medium with appropriate concentrations of dexamethasone, isobutyl-1-methylzanthine, and insulin for 4 days. Later, cells were cultured in insulin-containing DMEM for 3 days. After 12 days, fully transformed adipocytes presenting lipid droplets were infected with GFP-expressing *L. (L.) amazonensis* axenic amastigotes. Adipocytes were infected for 1, 24, 48, 72, and 144h (MOI=5) and parasite number was determined by fluorescence microscopy.

3 Results

Our results show that adipocytes are infected by amastigotes that are able to replicate in a time-dependent manner up to 48h. However, growth is not sustained after later time points, even though GFP-parasites can be found in adipocytes 144h post-infection. Electron microscopy studies are being carried out to analyze the ultrastructure of the infected adipocyte and will provide evidence of *Leishmania* amastigotes persistence in non-phagocytic cells.

4 Conclusions

Our data suggest that the adipose tissue is a potential site for maintenance of *Leishmania*. This hypothesis is under investigation in our laboratory in an attempt to explore the role of adipocytes as host cells in latent leishmaniasis and to understand basic aspects of lipid metabolism during *Leishmania* infection. Supported by: FAPESP (FUNDAÇÃO DE AMPARO À PESQUISA DO ESTADO DE SÃO PAULO; 14/21129-4; 15/17902-2; 15/26408-1).

C1371 RNA-BINDING PROTEINS AS MAJOR REGULATORS OF THE LEISHMANIA RESPONSE TO INTRACELLULAR STRESS AND AMASTIGOTE DIFFERENTIATION

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1 Background

RNA-binding proteins (RBPs) are key regulators of post-transcriptional control. Not only regulate mRNA processing, stability, and translational efficiency but they also play major roles in the cellular response to environmental changes, cell differentiation, and cell death. In *Leishmania*, we have recently identified a DEAD-box RNA helicase of the DDX3 subfamily that together with its interacting partners monitor the parasite response to intracellular stress. DDX3 is a highly conserved ATP-dependent DEAD-box RNA helicase with multiple roles in all steps of RNA metabolism and cellular signaling.

2 Results

We showed that *L. major* and *L. infantum* DDX3$^{(-/-)}$ knockout parasites become highly susceptible to various stress stimuli encountered in macrophages and are thus unable to undergo amastigote differentiation, to cope with oxidative burst, and to promote disease in an experimental mouse model. Genetic inactivation of DDX3 leads to increased accumulation of reactive oxygen species (ROS), which upon stress results in mitochondrial membrane potential loss, mitochondrial fragmentation, and cell death. Furthermore, in the absence of DDX3 the unfolded protein response (UPS) is activated and poly-ubiquitinated proteins accumulate both in the cytosol and the mitochondrion, as an indicator of protein damage. Increased proteotoxicity in DDX3$^{(-/-)}$ parasites leads to translation inhibition due to a defect in the elongation process. Complementation studies using DDX3 mutants lacking conserved functional motifs highlighted the essential role of the DDX3 ATPase activity in this phenotype. Consistent with these findings, co-immunoprecipitation and mass-spectrometry studies revealed potential interactions of DDX3 with key components of the cellular stress response, particularly the antioxidant response, the UPS, and essential components of the protein quality control. These studies also revealed additional interactions of DDX3 with other RBPs that seem to negatively regulate the stress response.

3 Conclusions

Taken together, these findings provide for the first time insights into the central role DDX3 RNA helicase and its interacting partners play in monitoring the *Leishmania* response to stress stimuli inside macrophages by preventing ROS-mediated protein damage. A comprehensive understanding of how DDX3 regulates protein quality control upon stress would possibly open new avenues for the development of therapeutic strategies not only against parasitic but also other human diseases.

C1398 ROLE OF CD200 IN MACROPHAGE INFECTION BY LEISHMANIA (LEISHMANIA) INFANTUM CHAGASI

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1 Background

To guarantee the successes of the infection, *Leishmania* has to manipulate the immunological response of the host. Many studies have been published in the last years, but little is still known about how Leishmania can manipulate the activation of macrophages for their own benefit. It was previously shown that *L. (L.) amazonensis*, which produces the cutaneous form of the disease, induce the expression of CD200 in macrophages, molecule involved in the inhibition of macrophage response mediated by iNOS. In this study, we examined the involvement of CD200 macrophage infection by *L. (L.) chagasi*, *Leishmania* associated to the visceral form of the disease

2 Methods

Bone marrow macrophages (BMM) from C57BL/6 mice were infected with amastigotes or promastigotes of *Leishmania (L.) infantum chagasi*. Intracellular parasite proliferation and CD200 levels were determined during different time points after infection by immunofluorescence and immunoprecipitation /qPCR, respectively. In another set of experiments, macrophage were infected with amastigotes and incubated in the presence of CD200-Fc or iNOS inhibitor, LNMMA. In vivo infections were performed injecting stationary phase promastigotes in the footpad of mice untreated or treated with CD200Fc. Lesion progression was followed by weekly measurements with a caliper. After 5 weeks, animals were euthanized to quantify parasite load from the footpad and spleen by limiting dilution assay

3 Results

Parasites were unable to proliferate inside of BMM, but parasites are not eliminated by macrophages. Quantification of CD200 in different time points of infection showed that *Leishmania (L.)* chagasi modulate the levels starting at 72 until 120h. Treatment of BMM with LNNMA and CD200-Fc slightly rescued their ability to sustain the intracellular replication. Next, we examined the progression of cutaneous lesions following the inoculation of *Leishmania* into mouse footpads, from mice untreated or treated with CD200Fc. Interestingly, quantification of the parasite load in infected footpads showed a small quantity of parasites in untreated mice and no parasite in the footpad from mice treated with CD200-Fc. When parasite load were quantify from the spleen, more parasites were found in CD200Fc treated mice, compared to a small quantity of parasites in untreated mice.

4 Conclusions

Our results suggest that L. (L.) chagasi induces CD200 in later time points of infection and may be important for the migration of Leishmania from the local infection to the spleen

C1399 GENE EXPRESSION PROFILE IN SYMPTOMATIC VL AND RECOVERED VL AFTER SLA STIMULATION

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1 Background

Visceral leishmaniasis (VL) is a parasitic disease caused by *Leishmania infantum* in Latin America. The main characteristic of the immune response in VL is anergy, which Leishmania antigen-specific, observed during disease, but reversed after successful treatment. A Th1 immune response is required to effective control the parasite. This study aimed to assess the global gene expression of peripheral blood cells of people with symptomatic (sVL) and recovered VL (rVL).

2 Methods

Whole blood cells from sVL (n=7) or rVL (n=4) were stimulated with Soluble Leishmania Antigen (SLA). RNA was obtained from whole blood either with or without SLA stimulation. Total RNA was extracted and hybridized against ~ 48,000 probes using a microarray platform (HumanHT-12 BeadChip). Data were analyzed in a paired-way by linear models allowing for interaction.

3 Results

The rVL group had 604 differentially expressed (DE) genes whereas sVL group had only 128 genes. Furthermore, sVL and rVL differed in the gene expression pattern for a total of 306 genes (interaction term). We evaluated the top 10 up- and down-regulated genes in sVL and rVL, after SLA stimulation. Besides characteristic anergy in sVL, there was an increased expression of genes (fold change>4) related to cell recruitment (CCL8, CCL7, CXCL9, CXCL10) as well as genes coding for pro-inflammatory cytokines such as, IFNγ, IL1A and IL1B. On other hand, genes such as CD1D (fold change>3), involved in antigen presentation, and genes involved in platelet activation, signaling and aggregation (AMICA1, NFE2) were down regulated. Whereas, in rVL, genes related to cell recruitment (CCL8, CCL7, CCL20) and pro-inflammatory cytokines such as, IFNγ and IL1A were up-regulated. IL8RB and CD1D (both fold change>4) were decreased in rVL. In rVL, the 604 DE genes were related to different pathways including cell recruitment (CXCL1, CXCL2, CXCL6, CXCL5, CXCL9, CXCL10, CXCL11, IL8), immunoproteasome (IFNγ, TNF, PSMA6) and inflammasome (IL1A, IL1B; IL1R1, GBP5). In sVL, the 128 DE genes were related to cell chemotaxis and inflammatory response pathways, among others. The 306 significantly DE genes for the interaction term were related to pathways including TNF signaling pathway, NOD-like receptor signaling, and Cytokine-cytokine receptor interaction (KEGG Pathways).

4 Conclusions

During acute VL, pro-inflammatory pathways were activated. These were not effective in controlling disease and likely are involved in promoting disease symptoms.

C1404 LEISHMANIA AMAZONENSIS AND LEISHMANIA BRAZILIENSIS-INFECTED NEUTROPHILS DIFFERENTLY MODULATE DENDRITIC CELLS FUNCTION AND INFECTION: IMPACT OF ADENOSINE RECEPTORS.

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1 Background

Neutrophils are essential for the innate immune response against a variety of pathogens. They are able to modulate immune response by cytokine and chemokine production, release of granules and their direct interaction with other cells at the infection site. Dendritic cells are recruited in response to cytokines and chemokines produced by neutrophils. The interaction between cells of the innate immune system is essential for targeting the adaptive immune response, which is responsible for eliminating microorganisms and the maintenance of immunological memory.
Objective: To evaluate the effect of the interaction between human DCs and neutrophils infected by Leishmania braziliensis or Leishmania amazonensis.

2 Methods

Methods: Neutrophils purified from peripheral blood of healthy donors were infected or not with L. braziliensis or L. amazonensis and co-cultured with DCs generated in vitro with IL-4/GM-CSF. Afterwards, neutrophil enzymes were evaluated in the supernatants and cells harvested for phenotype and function determination by the expression of Mac-1 (CD18 and CD11b), CD16, CD39, CD73 and ROS production. Analyzing DCs in these co-cultures we assessed their infection rate, expression of DC-SIGN, CD80, CD86, CD39 and CD73 surface molecules and whole cytokine production. Parasite load in these cells was evaluated after inhibition of CD39 and CD73 expressed in neutrophils.

3 Results

Dendritic cells co-cultured with L. amazonensis-infected neutrophils showed a significant increase in parasite load, reduction in the expression of surface molecules and production of pro-inflammatory cytokines, as well as increase in anti-inflammatory cytokines production. Opposite results were obtained in the co-cultures of DCs and L. braziliensis-infected neutrophils. Blocking CD39 but not CD73 in neutrophils induces a decrease in parasite load in DCs co-cultured with L. amazonensis-infected neutrophils. Additionally, inhibition of A2b receptor leads to a decrease of the parasite load after DCs co-culture with L. amazonensis-infected neutrophils. This same treatment did not alter the parasite load in DCs co-cultivated with neutrophils infected by L. braziliensis.

4 Conclusions

Conclusions: Neutrophils could be an important cell in mediating the activation or inhibition of DCs and different species of Leishmania present differential behaviors in the interactions between neutrophils and dendritic cells.

C1422 THE ROLE OF EPIGENETICS IN MACROPHAGE INFECTION BY LEISHMANIA

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1 Background

Background: The downregulation of host macrophage innate immune responses by *Leishmania* is key to successful infection, parasite survival and disease progression. While, many of the signalling pathways involved in this downregulation have been dissected, the underlying mechanisms remain largely unknown. We have examined the role that epigenetics changes via DNA methylation play in the macrophage response to *Leishmania donovani* infection.

2 Methods

Methods: Using an Illumina Human DNA Methylation 450K arrays genomic DNA from THP1 cells infected with *L. donovani,* 24 h post-infection was compared to controls.

3 Results

Results: We quantified genome-wide changes in >485,000 cytosine methylation sites at the single nucleotide level, revealing significant changes in DNA methylation patterns. Interestingly, one of the genes found to have a decrease in methylation that correlated with increased mRNA expression was a class IIa histone deacetylase (HDAC4). HDAC4 belongs to a family of epigenetic mediators involved in controlling chromatin structure, DNA accessibility and gene expression. Using specific HDAC inhibitors we are investigating the role that HDACs play in promoting macrophage infection by *Leishmania*.

4 Conclusions

Conclusion: Epigenetic modifications may be a major contributing factor controlling host responses to *Leishmania.* Since macrophages are terminally differentiated, changes in methylation could lead to permanent downregulation of host defense responses that promotes parasite survival and disease progression.

C1435 GRANULOCYTIC CELLS (NEUTROPHILS, EOSINOPHILS AND MAST CELLS) IN THE GUT WALL OF DOGS NATURALLY INFECTED WITH LEISHMANIA INFANTUM

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1 Background

Canine visceral leishmaniasis (CVL) is a chronic disease caused by the protozoa *Leishmania infantum*, an intracellular parasite that infects macrophages residing in several organs and lymphoid tissues, including the gastrointestinal tract of dogs. The aim of this study was to quantify and correlate the profile of granulocytic cells (neutrophils, eosinophils and mast cells) of dogs naturally infected with *L. infantum* in the presence or absence of this parasite in the intestinal tract.

2 Methods

For this study, 17 dogs naturally infected with CVL were submitted to euthanasia and 03 dogs were uninfected and had accidental death. The dogs were divided into three groups according to the following characteristics: Group 1 (G1, n = 9) were CVL positive dogs with *L. infantum* amastigote in the intestine; Group 2 (G2, n = 9) were CVL positive dogs, but without intestinal amastigotes; and group 3 (G3, n = 3) were CVL negative control dogs.Coproparasitological exams were also done in order to detect some other intestinal parasite co-infection. The identification of the parasite in the intestinal amastigotes and granulocytic cells was by histochemical and immunohistochemical methods. The cell quantification was done in 10 villus/crypt units (VCUs), submucosa and muscular layers of duodenum, jejunum, ileum and colon of each animal.

3 Results

Granulocytic cells (neutrophils, eosinophils and mast cells) were significantly ($P \leq 0.05$) increased in animals from group G2, followed by group G1 in relation to the control group (G3). These cells were present in the mucosa (VCU), submucosa and muscular layers, but significantly higher in the mucosa. Neutrophils and eosinophils were significantly higher in the small intestine than in the large intestine of infected groups (G1 and G2), but mast cells were found in similar numbers in both intestines (small and large) in each group. The intestinal tissues of the most infected dogs with amastigotes (G1 group) had fewer neutrophils, eosinophils and mast cells than those animals with lower burden of intestinal amastigote parasites or in the absence of them.

4 Conclusions

CVL infected dogs had a granulocytic cellular hyperplasia (neutrophils, eosinophils and mast cells) in the intestinal wall, but it was not correlated with the burden of the intestinal amastigote parasites.

C1465 ROLE OF LEISHMANIA CALCINEURIN FROM DIFFERENT INFECTIVE FORMS IN MACROPHAGE INFECTION

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1 Background

L. (L.) amazonensis from the Leishmania Mexicana complex grows in a unique and big parasitophorous vacuole inside of macrophages. The generation of this structure has to be tightly controlled by the parasite to survive and proliferate in such harsh environment. Calcineurin is a Ca2+ dependent phosphatase involved in different cellular process related to thermotolerance and adaptation to oxidative stress. In this study, we examined the involvement of calcineurin of *Leishmania* from two different infective forms during the process of macrophage infection.

2 Methods

Promastigote and amastigote forms were treated with calcineurin specific inhibitors (CsA, FK506) and parasite viability was measured by different methods such as PI/Annexin staining and MTT assay. Moreover, bone marrow-derived macrophages (BMM) were infected with the different forms of *L. (L.) amazonensis*, pretreated or not with calcineurin inhibitors, to measure intracellular parasite proliferation and parasitophorous vacuole size, overtime. Furthermore, 96h after infection RNA samples and supernatant of each treatment were used to quantify macrophage transcripts (Lyst and CD200) and cytokines / Nitric oxide levels, respectively, as host cellular response against *Leishmania*.

3 Results

L. (L.) amazonensis promastigote and amastigote present different susceptibility to calcineurin inhibitors. In fact, when the different forms were treated with 80 µM of FK506, more than 90% of the different infective forms are viable. Moreover, when amastigotes were treated with CsA almost 80% are still viable (80 µM of treatment). Interesting, promastigotes are more susceptible to CsA, diminishing parasite viability to 60% (40 µM) and to 35% at 80 µM. When promastigotes are pretreated with 25 µM of CsA or FK506 (concentration when the drugs are not toxic to the parasite), the intracellular parasite proliferation is affected, compared to parasites untreated. However, when amastigotes of *Leishmania* are treated with the drugs, only treatments with CsA impair parasite growth, affecting also the PV size in infected macrophages.

4 Conclusions

These results demonstrate a different role of calcineurins in the infective forms of *L (L.) amazonensis*. Further experiments will be prepared to deeply understand the specific role the calcineurins during the infection of macrophages.

C1501 MODULATION OF THE HOST IRON METABOLISM UPON LEISHMANIA INFANTUM INFECTION DISCLOSES IRON AS AN IMPORTANT FACTOR FOR PARASITE CONTAINMENT

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1 Background

Iron is crucial for the survival of both parasites and their hosts, yet it can be highly toxic if in excess. As a means to control infections, hosts often take advantage of the dual properties of iron engaging a diversity of microbicidal mechanisms such as deprivation of this essential metal or intoxication of the microorganisms with this micronutrient. In this work our ultimate goal is to understand the importance of iron for the development of *in vivo* infections by *Leishmania infantum*, a causative agent of canine and human visceral leishmaniasis. Thus, as a starting point, we investigated whether ferroportin-1 (FPN-1), the sole iron exporter of mammalian cells, and hepcidin, the main FPN-1 regulator, where altered in the liver of *L. infantum*-infected mice.

2 Methods

For that, we performed a set of *in vivo* infections and, at different time-points, the liver of both *L. infantum*-infected and control mice were collected. Immunofluorescence assays were used to assess the expression and localization of FPN-1 and iron deposition was identified through Perls-DAB staining. Iron chelation was also employed as a means to understand the importance of this metal as microbicidal element.

3 Results

We show that i) in *L. infantum*-infected kupffer cells FPN-1 expression is altered, this protein being either internalized or highly downregulated. This is probably important for the accumulation of iron that is observed in those cells. ii) Strikingly, FPN-1 alterations in infected macrophages occur through a hepcidin-independent mechanism which likely requires the contact between the parasite and its host cell. Importantly, we iii) have data suggesting that iron deposition is an important factor for the containment of *L. infantum* in mice.

4 Conclusions

Our data clearly shows that *L. infantum* infection impacts on the host iron metabolism. More importantly, we report that parasite elimination is likely achieved through iron intoxication occurring upon FPN-1 downregulation. In the future we will investigate whether iron is indeed used by the host as a microbicidal strategy and we will also dissect the mechanism leading to FPN-1 decrease.

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C1535 LEISHMANICIDAL ACTIVITY OF HUMAN SERA: A COMPARATIVE STUDY.

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1 Background

After inoculation, host serum proteins constitute the first barrier of innate defense against *Leishmania*. The complement system is very effective removing and neutralizing the parasite, while the last subverts complement-mediated lysis and uses opsonization to infect host cells. Certain clinical conditions like immunosupression by either HIV infection or chemotherapy may impair complement function. Here, we evaluated whether a potential complement deficiency condition in these patients renders them more susceptible to *Leishmania infantum* infection.

2 Methods

In the present study, we tested human sera collected from individuals living in endemic areas for visceral leishmaniasis. Particularly, 111 serum samples from clinic healthy donors, HIV infected subjects, patients under biological treatments (BT) and solid organ transplant (SOT) recipients were used.We generate transgenic *L. infantum* (JPC strain, MCAN/ES/98/LLM-722) constitutively expressing GFP. Stationary phase GFP transfectants complement-mediated lysis was assessed after 0, 2.5 and 5 minutes of sera incubation by loss of GFP fluorescence in a flow cytometer.

3 Results

As expected, the number of viable parasites was reduced in the four groups within the first seconds after sera incubation. At this stage, HIV+ and SOT patient's sera showed the highest leishmanicidal activity (≥ 60%). Furthermore, these groups displayed a narrower range of percentage of *Leishmania* killing (23,08±12,84 and 18,27±8,20 respectively) when compared to BT (36,39±22,4). On the other hand, complement-mediated lysis in HIV+ and SOT groups stopped before reaching 2.5 minutes incubation whilst lysis of GFP transfectants continued in BT donor´s sera during the whole assay.

4 Conclusions

Overall, our data supports the pivotal role of complement in limiting initial parasite load and spreading. In addition, HIV+ and SOT complement is unable to continue its lytic activity after a few seconds; as a result, *Leishmania* might easily overturn host innate system and cause disease. Currently, further analysis are being carried out to determine if this phenomenon is caused by a combined deficiency in circulating IgM and Ca^{2+}, essential elements for proper complement classical and mannose binding lectin pathways function.

C1589 LEISHMANIA USES MINCLE TO TARGET AN INHIBITORY ITAM SIGNALING PATHWAY IN DENDRITIC CELLS THAT DAMPENS ADAPTIVE IMMUNITY TO INFECTION

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1 Background

Leishmania can be detected by several Pattern Recognition Receptors, which trigger activating or inhibitory pathways. *L. major* is a poor inducer of dendritic cell (DC) activation. Moreover, this parasite inhibits DC migration to draining lymph nodes (dLN) and consequently, priming of T helper 1 (Th1) cells and macrophage microbicidal activity. The mechanisms underlying this early *L. major*-induced DC suppression remain ill-defined.

Mincle is an FcR gamma-chain-coupled myeloid-inducible C-type lectin receptor (CLR) for endogenous SAP-130 exposed and released by dead cells and for glycolipids on the cell walls of bacteria and fungi. Binding of these ligands to Mincle triggers phosphorylation of immunoreceptor tyrosine-based activation motif (ITAM) tyrosine residues in the FcR gamma-chain, followed by the recruitment and activation of the kinase Syk, which generates an activating signal that boosts immunity to infections and inflammation. We hypothesized that *Leishmania* infection is sensed by Mincle.

2 Methods

We have used Mincle-deficient mice or mice with depletion of SHP1 or Syk in the CD11c$^+$ compartment. Mice were infected intradermally into the ear pinnae with *L. major.* Next, we evaluated pathology, parasite load and immune responses in the skin and dLN. We also generated bone marrow derived DC cultures from these mice to further characterize DC-parasite interaction.

3 Results

We have found that human and mouse Mincle bind to a soluble ligand released by *Leishmania*. Mincle-deficient mice had milder dermal pathology and a tenth of the parasite burden compared to wild-type mice after *Leishmania major* infection. Mincle deficiency enhanced adaptive immunity against the parasite, correlating with increased activation, migration and priming of a Th1 cell response to parasite antigens by Mincle-deficient dendritic cells (DCs). *Leishmania* triggered a Mincle-dependent inhibitory axis characterized by SHP1 coupling to the FcR gamma-chain. Selective loss of SHP1 in CD11c+ cells phenocopies enhanced adaptive immunity to *Leishmania* observed in the absence of mincle.

4 Conclusions

Leishmania shifts Mincle to an inhibitory ITAM (ITAMi) configuration that impairs DC activation. Thus, ITAMi can be exploited for immune evasion by a pathogen to delay sterilizing immunity in vertebrate hosts. and may represent a paradigm for ITAM-coupled receptors sensing self and non-self. The low effectiveness of vaccines based on whole *Leishmania extracts* could partially rely on the presence of a ligand for Mincle.

C1656 DIFFERENTIAL MICRORNA EXPRESSION IN PERIPHERAL BLOOD MONONUCLEAR CELLS FROM DOGS WITH VISCERAL LEISHMANIASIS

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1 Background

Leishmania (L.) infantum is the etiologic agent of visceral leishmaniasis (VL), in Brazil represents a serious public health problem. Studies have shown that regulation of immune response appears to depend on miRNAs. In canine VL due to cell immune suppression being determinant of disease progression, knowledge of miRNAs may be important for the pattern of change in response immune.The objective of this study was the characterization of the miRNAs in PBMC from dogs naturally infected by *L. infantum.*

2 Methods

This investigation was performed in a endemic region for canine VL in Brazil. A group of 3 healthy dogs were included in the study as negative controls. A total of 5 dogs with diagnosis of VL determined through ELISA and PCR. The blood was obtained by venipuncture. DNA was extracted from PBMC using DNeasy Kit (QUIAGEN™) in accordance with the manufacturer's recommendations. The *L.* species were identified by means of PCR-restriction fragment length polymorphism. The samples were subjected to PCR using primers that amplify ITS1 and amplified DNA was digested using the restriction enzyme HaeIII. A restriction profile identical to *L. infantum* was observed. miRNA was extracted from PBMC using Mirvana Kit (Invitrogen™) in accordance with the manufacturer's recommendations. The miRNA was quantified using a fluorometer (Qubit 3.0, Invitrogen™), the degree of purity was performed by capillary electrophoresis (Bioanalyser, Agilent™) then stored at -80°C. The miRNA was performed to microarray using FlashTag Biotin HSR RNA Labeling Kit (Affymetrix™). The microarray was performed using Affymetrix™ miRNA 4.1 Strip in accordance with the manufacturer's recommendations. The gene chip was washed, stained and scanned using Affymetrix™ GeneAtlas instrument. The analysis was performed Expression Console™, Transcriptome Analysis Console (Affymetrix™), miRWalk 2.0.

3 Results

We identify 116 miRNAs that were associated with *L. infantum* infection in dogs. Of these, 5 miRNAs were identificated in *Canis familiaris*: miR-185, miR-192, miR-21, miR-370, miR-451. The expression level of these miRNAs were higher than observed in controls. The miR-185 target T cell receptor signaling pathway and miR-21target MAPK signaling pathway

4 Conclusions

The miRNAs identification that regulation the immune response in canine VL can created immunotherapeutic strategies that should be investigated further.

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C1662 METABOLIC ENZYMES FROM LEISHMANIA MEXICANA AND THEIR INVOLVEMENT IN CARBON METABOLISM, STRESS RESISTANCE AND PARASITE SURVIVAL.

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1 Background

The geneal objective of the research is to understand how phospho*enol*pyruvate carboxykinase (PEPCK), pyruvate phosphate dikinase (PPDK) and glycerol kinase (GK) from *Leishmania mexicana* could support parasite survival and growth in mammals. PEPCK, PPDK and GK are localized in the glycosomes, peroxisome-like organelles involved in the energy and intermediary metabolism. In trypanosomatids, these enzymes have been involved in carbon metabolism and energy balance inside glycosomes. By using individual Δ*pepck*, Δ*ppdk* and Δ*gk* null mutants generated by homologous recombination, I have previously demonstrated the specific roles of PEPCK, PPDK and GK in gluconeogenesis (Rodriguez-Contreras and Hamilton, 2014).

2 Methods

Glucose uptake assays, growth curves at several temperatures and *in vitro* infectivity assays, using murine bone marrow macrophages (MΦ), were carried out to increase our knowledge about PEPCK, PPDK and GK roles in metabolism, stress resistance and parasite viability.

3 Results

I) Rate of glucose uptake in Δ*pepck* was moderate but significantly reduced by 41.7% in early/logaritmic phase of growth (uptake rate: 90.9 ±20.2 versus 156.0 ±13.5 pmol/mg protein/min in Δ*pepck* and wild type parasites, respectively), supporting PEPCK involvement in glucose catabolism, although also showed its no essentiality for maintaining glycosomal homeostasis in promastigotes. II) Promastigotes growth curves carried out in the presence or not of 10 mM glucose at the increasing temperature of 32.5 °C showed that Δ*gk* promastigotes were unable to grow under glucose starvation when exposed to temperature stress. III) Finally, when wild type and null mutant promastigotes were used to infect primary macrophages at 1:5 MΦ/parasite ratio, a significantly attenuated Δ*pepck* survival was observed at day 6 post-infection, while in Δ*ppdk* the reduced survival phenotype was observed after 24 h of infection, comparing to wild type parasites. Despite the reduced infectivity phenotypes, amastigotes from each of three null mutants were observed at day 6 post-infection.

4 Conclusions

The studied null mutant parasites have different capacities to resist stresses, and to transform and survive within macrophages; however, none of the studied enzymes is essential, by itself, for survival in the mammalian hosts. Double and triple null mutants will clarify redundant roles in metabolism, energy balance inside glycosomes and parasite viability.

C1663 MODULATION OF THE ACTIVITY OF INFLAMMASOME IN MACROPHAGES BY LEISHMANIA MEXICANA

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1 Background

Leishmania mexicana (*L. mexicana*) can cause both localized (LCL) and diffuse (DCL) cutaneous leishmaniasis in Mexico. Patients affected with this disease have an increased amount of IL-1β. Its been shown that the levels of this cytokine are associated with the activation of the NLRP3. There are studies that show that *Leishmania major*, *Leishmania braziliensis,* and *Leishmania amazonensis* activate the inflammasome and stimulate the production of IL-1β. Phosphatases are enzymes that participate in the phosphorylation and desphosphorylation mechanisms to regulate several functions of the host cell; they are considered a pathogenic mechanism in other parasites. Little is known about the interaction of molecules from the parasites as phosphatases with the NLRP3 inflammasome. Recently, we reported that the effect of secreted protein phosphatases (SPP) by *L. mexicana* correlates with cytokines production in macrophages. The goal of this work is to analyze the participation of the parasite and SPP from *L. mexicana* in the activation of NLRP3 inflammasome.

2 Methods

RAW 264.7 macrophages were used in this work. *L. mexicana* parasites were obtained from LCL and DCL patients. The cells were primed with LPS and then stimulated with: a) different infection ratios of promastigotes and amastigotes of *L. mexicana* and b) SPP from *L. mexicana*. Afterwards, macrophages were lysed and Western Blot was done for NLRP-3, caspase-1 and IL-1β proteins. IL-1β was quantified by ELISA.

3 Results

We observed that *L. mexicana* of both clinical forms induce the sinthesis of NLRP3 and caspase-1 in macrophages in a dose dependent manner. This induction was reflected in an increase in IL-1β production by macrophages. Also SPP modulated the activity of the NLRP-3 inflammasome, the expresion of caspase-1, and the production of mature IL-1β.

4 Conclusions

These results show that *L. mexicana* parasites and the SSP modulated the activity of the NLRP3 inflammasome.
This work supported by grant from DGAPA-PAPIIT IN220816

C1682 INSERTS ON COMPARATIVE SCANNING ELECTRON MICROSCOPE STUDY OF LEISHMANIA EXOSOMES

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1 Background

Leishmania parasites are a group of kinetoplastid pathogens that cause a variety of clinical forms. Emerging technologies have been adapted for the studies of *Leishmania*-host-cell interactions to enable broad scale analysis of parasite extracellular vesicles, collectively termed exosomes. Although *Leishmania* exosomes has increasingly gained in importancemuch is still unexplained, including bio availability and function in the complex molecular mechanisms of pathogenesis. Considering the importance of the exosomes in the parasite-host interaction and in the parasite-parasite relationships, the current study aims to characterize the morphology and production of these vesicles by cultured promastigotes and amastigotes of *Leishmania* spp.

2 Methods

Promastigotes of visceral (*L. infantum*) and cutaneous *Leishmania* species (*L. amazonensis*, *L. guyanensis* and *L. shawi*) were cultured in Schneider's Drosophila medium and axenic amastigotes were differentiated. Parasites were then evaluated by scanning electron microscope and acquired images were subject to normalization of the color histogram and converted to 8 bits in the GNU Image Manipulation Program for Windows. Exosomes associated with the parasite cell membrane were assessed by pixel density. Shape, size and frequency of exosomes were estimated in both parasitic morphological forms. Mann–Whitney's *test* was used for statistical analysis.

3 Results

All parasites analyzed presented exosome secretion on membrane surface, independently of their morphological form or species. Morphologically, the nano vesicles showed spherical shape and irregular topography. The highest density of exosomes was observed in the body of the cell when compared with the surface of the flagellum, indicating the predominance of nano vesicles throughout the cell body. *L. amazonensis* amastigotes exhibited greater exosomes secretion and *L. guyanensis* evidenced the lower density of nano vesicles.

4 Conclusions

To the best of our knowledge, this is the first morphological and morphometric study of nano vesicles associated with the cellular membrane of both morphological forms of *L. infantum, L. amazonensis, L. shawi* and *L. guyanensis*. The pattern of exosome distribution seems to differ throughout the parasite life cycle and between different regions of promastigote. However, there seems to be no relationship between vesicle secretion and parasite virulence.

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C1691 IDENTIFYING OF SALIVARY GLAND PROTEIN PROFILE IN IRANIAN PHELOBOTOMUS PAPATASI, PROVEN VECTOR OF ZOONOTIC CUTANEOUS LEISHMANIASIS, FROM BOJNORD ENDEMIC FOCUS (NORTH EAST OF IRAN)

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1 Background

leishmaniasis is a neglected tropical disease and is fatal if untreated. Because of lacking vaccine against *leishmania*, salivary gland proteins of sand flies considered as a candidate vaccine and protection for leishmaniasis. Each region of sand flies has local criteria for this reason Iranian *Phlebotomus papatasi,* proven vector of ZCL, isolated salivary gland proteins was choosen.
Sand fly saliva contains a series of bioactive molecules which are necessary for the successful uptake of blood meals and for establishment of *Leishmania* in vertebrate hosts.

2 Methods

The specimens were collected by CDC trap and put in empty tubes without any buffer on ice then transferred immediately to laboratory. Sand flies identified morphologically and dissected. Five salivary glands of each species cut and put in Lysis buffer in a tube. Extraction process was including Centrifuging (30 min and 14000 rpm), filteration (3KDa) and adding acetone to precipitate proteins.Proteins in PBS, loaded on 15% SDS-PAGE electrophoresis.

3 Results

We identified salivary gland protein profile from wild crowd *Phlebotomus papatasi.* Out of 144 collected samples 76 specimens were female and after distinguishing of morphological objectives, 17 individuals were *papatasi*.
It was revealed that there are 12 separable bands in *papatasi* profile.

4 Conclusions

There were vivid differences between our results and previous published profiles of *papatasi* (from 4 to10 bands) in other foci of world. It confirmed that longitudinal and geographical conditions affected strongly on gene expression and protein secretion in salivary glands of sand flies. This study is pending to further investigation regarding protein purification in order to vaccine production.

C1692 HUMAN MACROPHAGES ARE SUPERIOR TO DENDRITIC CELLS AS HOST CELL FOR LEISHMANIA MAJOR PARASITES

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1 Background

An appropriate T-cell response to *Leishmania* infection is critical for an effective immune response. Human macrophages (hMDMs) as well as dendritic cells (DCs) can internalize *Leishmania* parasites and serve as antigen presenting cells, leading to T cell activation.

2 Methods

In this study we compare hMDMs and DCs for their suitability as host cell for *Leishmania major* (*L.major*) by analyzing infection rate, parasite transformation and their ability to induce T cell proliferation, having an effect on parasite survival.

3 Results

L.major parasites were able to infect both pro-inflammatory hMDM1 ($CD14^+$ $CD206^+$ $CD209^+$ $CD163^-$ $MHCII^+$) and anti-inflammatory hMDM2 ($CD14^+$ $CD206^+$ $CD209^-$ $CD163^+$ $MHCII^+$) as well as DCs ($CD14^-$ $CD206^+$ $CD1a^+$ $CD209^+$ $MCHII^+$ $CD163^-$). After 24 hours, hMDM2 ((45.5 % ± 5.9) shows to be most susceptible for *L.major* infection, followed by hMDM1 (32.5 % ± 2.5) and DCs (12.4% ± 1.8). Remarkably, over a period of 7 days parasite developed is the strongest in DCs. The intracellular promastigotes (ABC^{high} and $GP63^{low}$) transformed into the multiplying amastigote form (ABC^{low} and $GP63^{high}$), analyzed by RT-PCR[1]. Upon coculturing infected cells with autologous CFSE labeled PBMCs, proliferation, detected as $CFSE^{low}$ cells, was induced the strongest by DCs (49 % ± 2.4) followed by hMDM1 (44.1 % ± 2.8) and hMDM2 (36.6 % ± 4.2). As a consequence of proliferation, *L.major* infection rates were dramatically reduced about 20% in hMDM1, 40% in hMDM2 and almost 60% in DCs. Interestingly, preliminary data showed the presence of nitric oxide to be increased in DCs, which may contribute to the strong parasite elimination. When hMDMs encounter virulent *L.major* promastigotes, the autophagy machinery got activated, leading to a significantly reduction of T cell proliferation[2]. In contrast, induction of the autophagy machinery upon *L.major* infection in DCs is absent.

4 Conclusions

So far, we could demonstrate that both human macrophage phenotypes as well as dendritic cells can be infected with *L.major* promastigotes, which develop, in all 3 cell types, into amastigotes. Moreover, apoptotic *L.major* misuse the autophagy machinery in macrophages – but not in dendritic cells - to lower T cell proliferation, securing intracellular survival, making human macrophages superior to dendritic cells as host cell for *L.major*.

1. Wenzel UA et al. *FASEB journal* 2012;26(1):29-39.
2. Crauwels P et al. *Autophagy*. 2015;11(2):285-297.

C1721 INTERACTION OF LEISHMANIA WITH MAST CELLS LEADS TO EFFECTOR RESPONSES FOR EXTRACELLULAR PATHOGEN CLEARANCE

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1 Background

Mast cells (MCs) are one of the first immune cells encountered by invading pathogens and play an important role in innate and adaptive defenses. As MCs are found particularly in locations that are in close contact with the external environment, such as skin, airways, and intestines, they may have an important role in Leishmaniasis caused by the protozoa *Leishmania* that are inoculated by infected sandfly vector on the skin surface. It is still not clear if their contributions occur directly through active pathogen killing or antigen uptake. Our objectives were to use *Leishmania donovoni* causative agent of visceral Leishmaniasis and *L. tropica* causative agent of cutaneous Leishmaniasisfor studying the interactions between *Leishmania* and MCs to clarify if MCs are directly involved in active antigen uptake, and clearance of *Leishmania*.

2 Methods

CFSE labelled promastigotes of *L. tropica* and *L. donovoni* co-cultured with rodent MCs RBL-2H3 were incubated for specific time and analyzed by flowcytometry. Generation of Reactive Oxygen Species (ROS) was assessed through CMH_2DCFDA stain which was measured through flowcytometer.To assess killing of promastigotes on co-culture with rodent MCs trypan bue staining of promastigotes was done and apoptosis was confirmed by Annexin V staining by flowcytometry. To visualize Mast Cell Extracellular Traps, DNA was stained by DAPI and anti-tryptase and anti-histone antibodies and visualized by confocal microscope.

3 Results

We found that co-culture of *Leishmania* withMCsled to significant killing of *L. tropica* and to a lesser extent of *L. donovoni.* Also, while there was significant uptake of *L. tropica* by MCs, *L. donovoni* was not phagocytosed by MCs. Uptake increased with time of exposure in case of *L. tropica.* We also found significant generation of (ROS) by MCs on co-culture with these species of *Leishmania,* which may contribute to their clearance. However interactions of MCs with *Leishmania* led to generation of MC extracellular traps comprising of DNA, histones and tryptase probably to ensnare these pathogens.

4 Conclusions

These results clearly establish that MCs may contribute to host defences to *Leishmania* in a differential manner by actively taking up these pathogens and also by mounting effector responses for their direct clearance by extracellular and non-phagocytic means. This gives us an understanding that MCs can also be used as a target for therapeutic approaches.

C1781 CELL DIVISION SPECIFIC VIRULENT GENES IN LEISHMANIA AS POTENTIAL TARGETS FOR LEISHMANIASIS ELIMINATION

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1 Background

Global elimination of *Leishmania*, a protozoan parasite, responsible for several clinical form of diseases, would save million of lives. *L. donovani* causes fatal visceral leishmaniasis in India and Africa. Null mutations in the cell cycle involved genes in *Leishmania* would develop live attenuated vaccine candidates as has been observed with the deletion of basal body associated centrin1 gene by us in *L. donovani*. Centrin1 knockout parasites were attenuated specifically at the intracellular stage *ex vivo* and *in vivo* after arrest in cell cytokinesis and as a vaccine candidate protected the animals from virulent challenge with protective immune responses. This presentation describes analysis of additional cell cycle functioning genes important for the virulence of the parasite.

2 Methods

Recombinant '30b' and 'Centrin4' proteins were expressed in *E. coli* and purified by affinity chromatography. The polyclonal antibodies raised against the recombinant proteins in mice were used for detection of proteins in the parasite lysate by Western blot analysis as well in protein localization studies. Episomal expressions of 30b and Centrin4 fusing with HA-tag were attempted in the parasites to look for a dominant negative effect in its function followed by infection study in the macrophages *ex vivo.*

3 Results

Amastigote specifically transcribing 30b (hypothetical protein) and Centrin4 (putative centrin) unique to the parasites have never been studied before. We found that 30b was localized to isolated glycosome vesicles and existed as multimer (pentamer). Upon episomal expression of 30b the growth of the parasite was completely arrested *in vitro* with accumulation of cells at G0 cell cycle phase. We found that Centrin4, similar to Centrin1, is a calcium binding protein associated with basal bodies of the parasite. Upon episomal expression of C' terminal deleted Centrin4, that has lost EF hand 4 calcium binding site, the parasite displayed reduced growth. The recombinant parasites of either type did not survive in the newly differentiated macrophages from human PBMC. Complete gene knockouts for these genes via homologous recombination are underway.

4 Conclusions

The recombinant parasites displaying dominant negative effect observed with either genes' episomal expression suggest the importance of these genes for the virulence of the parasite in the host cells. This study recommends targeting cell cycle functioning proteins for live attenuated vaccine development.

C1807 RIPK1-RIPK3-MLKL-ASSOCIATED NECROPTOSIS DRIVES LEISHMANIA INFANTUM KILLING ON NEUTROPHILS

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1 Background

Even though macrophages are the preferential host cell for *Leishmania* establishment and persistence, neutrophils have been also implicated in the immunopathogenesis of Leishmaniasis. These cells are rapidly recruited to the infection site and its role in the magnitude of disease is controversial. Necroptosis has been described as a proinflammatory cell death, which happens in the absence of caspase-8 allowing activation of the axis RIP1-RIP3-MLKL. Recently, necroptosis has emerged as a key component of resistance against pathogens including *Leishmania*-infected macrophages. We tested the hypothesis whether blockage of caspase-8 contributes to kill *Leishmania infantum* parasites by inducing necroptosis in infected neutrophils.

2 Methods

Mouse neutrophils were pre-treated with zVAD-fmk (pan caspase inhibitor) and zIETD-fmk (caspase-8 inhibitor) or with appropriated controls and were infected with *L. infantum* promastigotes. In order to evaluate the involvement of necrosome on Leishmania viability we used inhibitors of necroptosis cell death pathway (Nec-1, GSK'872 and NSA). Brazilian Committee on the Ethics of Animal Experiments (CPqGM; Permit Number: 004/2014).

3 Results

Murine neutrophils pretreated with caspases inhibitors presented a significant decrease of *L. infantum* viability and presented higher levels of ROS. Interestingly, Nec-1 and GSK'872 (RIPK1 and RIPK3 inhibitors, respectively) abrogated the low parasite viability observed when caspase 8 is blocked. Electron Microscopy revealed that *L. infantum* infected-neutrophils pretreated with RIPK1 and RIPK3 inhibitors do not have alterations in membrane integrity, preserved intracellular content and presented viable *Leishmania* within parasitophorous vacuoles; in opposition, groups pre-treated with zVAD-fmk only exhibited morphological aspects of necroptosis. Finally, we investigated MLKL (a molecule downstream necroptosis pathway) role in parasite survival using human neutrophils. Human neutrophils treated with NSA inhibitor showed high parasite burden when compared with controls only pretreated with caspase inhibitors.

4 Conclusions

The sophisticated interplay between Leishmania and the innate immune response allow us to see diverse finish related to the intricate cell death scenario ranging from protection to susceptibility. Our data suggest that intervention of neutrophil caspases contributes to eliminate *L. infantum* parasites, probably by stimulating an inflammatory response associated with necroptosis cell death.

C1859 PHENOTYPIC AND FUNCTIONAL CHARACTERIZATION OF SC ANTIGEN- SPECIFIC CD8 + T CELLS IN CUTANEOUS LEISHMANIASIS PATIENTS FROM ENDEMIC REGION.

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1 Background

Immunological host response is a key factor which determines the pathogenesis of cutaneous leishmaniasis. Thus, a strong Th1 cell response is correlated to the infection control and a Th2-biased response with parasite persistence. Therefore, antigen-specific memory T cells are critical in developing a quick and strong protective cellular response.

2 Methods

The study is focused on leishmaniosis patients from endemic areas of Colombia (N=18). Patients who suffered cutaneous lesihmaniasis but were cured after quimiotherapeutic treatment (i) and those with an active leishmaniasis process caused by *L. panamensis* (ii), together to healthy donors positive (iii) or negative (iv) to Montenegro test were selected. Peripheral blood was collected and mononuclear cells (PBMC) isolated from all patients. PBMC were stimulated with *L. panamensis* total soluble antigens (SLA) and with two different parasite-specific recombinant proteins (Ag1 and Ag4). A multiparameter flow cytometry panel was designed in order to phenotypically characterize the $CD8^+$ T cell populations and to study the expression of inhibitory receptors of immune response (CD160, CD244, CTLA-4, PD-1 and TIM-3) and expression and co-expression of cytokines (IFN-g, TNF-α and IL-2) and cytotoxic molecules (Granzyme B and perforin) by these cells. A statistical analysis was performed using the Mann-Whitney *U* test for two groups or Wilcoxon signed-rank test.

3 Results

A higher frequency of antigen-specific CD8+ T_{EM} and T_{naive} cells was found in cured patients than that with an active leishmaniasis process. Furthermore, these cells showed to have an improved multifunctional response, with a significant increase in the frequency of cells expressing INF-γ and cytotoxic molecules. A slight higher frequency of CD8+ T cells coexpressing three or four inhibitory receptors was observed in cured patients versus those with an active leishmaniosis process. However, the cured patients had a significant higher frequency of CD8+ T cells expressing CD160. On the other hand, the T CD8+ cells from healthy donors with a Montenegro positive test showed a lower multifunctionality capability than those from healthy donors with a negative Montenegro test.

4 Conclusions

The antigen-specific $CD8^+$ T cells probably play a relevant role in the control of cutaneous leishmaniasis.

C1903 THE ROLE OF ANNEXIN A1 IN THE CONTEXT OF LEISHMANIA AMAZONENSIS INFECTION

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1 Background

Annexin A1 (AnxA1, a 37-kDa calcium-dependent phospholipid-binding protein) has important roles in the modulation of inflammatory responses. However, there are no investigations on its role in *Leishmania amazonensis* in infections, which may provide new insights for the development of effective therapies against leishmaniasis. This study has evaluated the differences in the inflammatory responses' profiles of BALB/c wild type (WT) and AnxA1 Knockout (KO) mice infected by *L. amazonensis*.

2 Methods

Animals were infected with parasites in the stationary promastigote phase and the course of infection was evaluated weekly. Cytokine levels (IL-4 and IL-10) were assessed in lymph node cells and tissue damage was evaluated by histological analysis. Parasite loads were evaluated by a limiting dilution assay. The WT and KO mice cell infection were analyzed by *in vitro* assays utilizing bone marrow-derived macrophages (BMDM), and the rate of metacyclogenesis was evaluated by Ficoll density gradient

3 Results

. The results showed that after eight weeks of infection the footpad lesions were significantly larger in the KO group and during the first five weeks of infection, IL-4 and IL-10 cytokine levels were higher in the KO animals and histological analysis demonstrated an increased damage to the epidermis of $Anx^{-/-}$ animals, with the presence of a mixed inflammatory cell infiltrate, even though the parasite load was the same for both groups. During the first 24 hours of infection, the *in vitro* assays showed an increased number of infected BMDM in KO mice cells, as well as a higher number of parasites per cell, when compared to WT group

4 Conclusions

These preliminary results indicate that *in vitro* $Anx^{-/-}$ BMDM are more susceptible to infection. Already *In vivo*, the lack of AnxA1 results in more intense inflammatory responses, which are not controlled by increasing IL-4 and IL-10 levels. Thus, AnxA1 seems to contribute for the control of inflammatory responses during infection with *L. amazonensis.*

C1910 ROUTE OF INFECTION AFFECTS PATHOGENICITY AND VISCERAL GROWTH OF LEISHMANIA MAJOR IN BALB/C MICE

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1 Background

Leishmaniasis is a group of diseases caused by *Leishmania* parasite. Experimental models can be used for development of new methods of prevention and treatment for these diseases. Route of infection is one of the variables that have been reported to influence the immune responses as well as the disease outcome in experimental models of leishmaniasis. Aim of this research is to study the effect of infection route on the virulence of *Leishmania.(L.)* parasite

2 Methods

Low (10^3 parasites / mouse) or high (10^6 parasites / mouse) doses of *L. major* was injected subcutaneously into foot pad or intradermally into ear dermis of BALB/c mice. Parasite load was estimated by quantitative limiting dilution assay. Lesion diameter was assessed by measuring the thickness of ear or footpad. One-tailed two-sample equal variance t test was used to compare lesion diameter and parasite load results between experimental groups.

3 Results

Results showed that subcutaneous infection route has substantial differences with intradermal infection route which results in higher pathogenicity of *L. major* in BALB/c mice as assessed by lesion diameter, parasite load in the draining lymph node, and dissemination of the parasite to spleen.

4 Conclusions

Our findings suggest that subcutaneous infection route has substantial differences with intradermal infection route. The different pathogenicity of *L. major* in subcutaneous in comparison to intradermal may be due to presence of different immunoregulatory mechanisms in these two infection routes.

C1916 DIVERGENT ROLES FOR LY6C+CCR2+CX3CR1+ INFLAMMATORY MONOCYTES AT PRIMARY OR SECONDARY SITES OF INFECTION IN THE SKIN WITH LEISHMANIA MAJOR

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1 Background

Bone marrow-derived monocytes undergo maturation to populate numerous peripheral cell subsets under different inflammatory or steady state conditions. Monocytes recruitment to sites of inflammation combined with their immature phenotype suggests they may be targets for infection by *Leishmania spp.* Following inoculation, *Leishmania major* parasites are predominantly engulfed by neutrophils, but are not killed. After 1-2 days parasites transition into poorly defined secondary CD11b$^+$ cells. IFN-gamma-producing T helper 1 (Th1) CD4$^+$ T cells mediate protective immunity against *Leishmania* infection and primary infection mediates protective immunity at a site of secondary challenge. Neither the phagocytic cell that mediates parasite killing during secondary challenge, nor the phenotype of the secondary host cell during acute primary infection, have been carefully defined in the skin.

2 Methods

We employed *Leishmania major*-red fluorescent protein (RFP) parasites and multi-color flow cytometry to define and enumerate infected and uninfected inflammatory cells in the skin at primary or secondary sites of infection.

3 Results

During primary infection, infected monocytes had altered maturation and were the initial mononuclear host cell for parasite replication. At secondary sites, this same population rapidly produced inducible nitric oxide synthase (iNOS) in an IFN-gamma-dependent manner and was critical for parasite killing. Maturation to a dendritic cell-like phenotype was not required for monocyte iNOS-production. In contrast, neutrophils were a safe haven for parasite at both primary and secondary sites.

4 Conclusions

These studies reeal divergent roles for inflammatory monocytes at primary or secondary sites of infection and identify a critical early time window during which protective immunity must act to prevent monocyte modulation and parasite expansion.

C2057 DIFFERENTIAL REGULATION OF L-ARGININE METABOLISM THROUGH ARGINASE-1 DURING INFECTION WITH LEISHMANIA MEXICANA ISOLATES OBTAINED FROM PATIENTS WITH LOCALIZED AND DIFFUSE CUTANEOUS LEISHMANIASIS

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1 Background

In Mexico, *Leishmania mexicana* is the main etiological agent responsible of cutaneous leishmaniasis and can cause two different clinical manifestations in patients: localized cutaneous leishmaniasis (LCL) and diffuse cutaneous leishmaniasis (DCL). The factors determining the development of LCL or DCL after infection with *L. mexicana* remain unclear. Host L-arginine metabolism via arginase-1 and iNOS has emerged as a pivotal regulatory mechanism that allows parasite replication or killing, respectively, during infection with different *Leishmania* species. In the present work, we investigated in an *in vivo* murine model whether a *L. mexicana* isolate obtained from a patient with LCL and a *L. mexicana* isolate obtained from a patient with DCL were able to regulate differentially L-arginine metabolism through arginase-1 and iNOS and affect infection course and severity in the host.

2 Methods

Susceptible BALB/c mice were inoculated with *L. mexicana* isolates and infection progression was assessed both by measuring the size of developed lesions as well as determining the parasite load present in these. Arginase-1 and iNOS protein presence levels in the lesions were analyzed by Western blot and their localization in the infected tissues was detected by immunohistochemistry. Additionally, enzymatic activities of arginase-1 and iNOS in the lesions were determined through the Archibald and Griess reactions, respectively. Due to the fact that *Leishmania* parasites also express an arginase and a nitric oxide synthase, protein presence and activity of these enzymes were also evaluated in parasites purified from lesions of mice infected with both *L. mexicana* isolates.

3 Results

Mice infected with the *L. mexicana* isolate obtained from the DCL patient developed bigger lesions in size and with higher parasite loads than mice infected with the isolate obtained from the LCL patient. Importantly, higher sizes and parasite loads in mouse lesions paralleled with higher levels of arginase-1 protein presence and enzymatic activity. Immunohistochemical analysis revealed the presence of greater parasite numbers-harboring macrophages with an arginase-1 staining more intense and widespread in the lesions of mice infected with the *L. mexicana* isolate obtained from the DCL patient than in the lesions of mice infected with the isolate from the LCL patient. Interestingly, minimal or no iNOS protein presence was detected in the lesions of mice infected with both *L. mexicana* isolates. *Leishmania*-encoded arginase presence was detected only in parasites purified from lesions of mice infected with the isolate obtained from the DCL patient, while *Leishmania*-encoded nitric oxide synthase was not detected in parasites from any of both isolates. Furthermore, detected enzymatic activity values of *Leishmania*-encoded arginase were almost imperceptible when compared to those detected in the mouse lesions.

4 Conclusions

Taken together, our results suggest that *L. mexicana* isolates obtained from patients with LCL and DCL regulate differentially the expression and function of host arginase-1, constituting this parasite regulatory ability a possible factor involved in the development of LCL or DCL after infection with *L. mexicana.*

Molecular and Cell biology

C0077 VACCINATION WITH AN ANTIGEN COCKTAIL PROTECTS BALB/C MICE AGAINST LEISHMANIA MAJOR INFECTION

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1 Background

In previous works, the antigenicity of four recombinant proteins from the *Leishmania (L) major* ribosome (L3, L5, S4 and S6) was observed using sera from *Leishmania*-infected dogs. Furthermore, two of the proteins (L3 and L5) conferred protection in BALB/c mice against *L. major, L. braziliensis, L. amazonensis* and *L. chagasi* infection when immunized individually and/or combined in the presence of a Th1 adjuvant.
In the present work, the protection induced by the combination of the four proteins, as DNA vaccines or as recombinant proteins, towards the infection of BALB/c mice with *L major* was studied.

2 Methods

BALB/c mice were vaccinated in the left footpad with three doses, two weeks apart, of either the mixture of the recombinant proteins, with or without CpG ODN, or the mixture of the four genes as DNA vaccines. Four weeks after the last immunization half of the population were euthanized and the cellular responses post vaccination were analyzed. The remaining mice were challenged in the right footpad with *L. major* promastigotes and footpad lesions were monitored weekly. Seven weeks after challenge, mice were sacrificed and the parasite load in spleen and draining lymph node (DLN) and the cellular responses induced were measured.

3 Results

In spleen cells from mice vaccinated with the combination of recombinant proteins formulated with CpG ODN and stimulated with soluble *Leishmania* proteins (SLA), there was an induction of antigen-specific IFN-γ, indicating a Th1 cellular response.
In the case of vaccinated and challenged mice, lower inflammatory lesions were developed and lower number of parasites in spleen and in DLN were quantified than in control groups. When splenocytes from this mice group were stimulated with SLA or with the individual proteins, the level of antigen-specific IFN-γ was higher than in the remaining groups, confirming the redirection to a Th1 response.
In the case of mice vaccinated with the genes and further challenged with *L. major*, there was a delay in the development of the lesions, a decrease in the parasite load and induction of the Th1 cytokine IFN-γ, but lower than in mice immunized with the proteins in the presence of the adjuvant.

4 Conclusions

The combination of the L3, L5, S4 and S6 recombinant proteins with CpG ODN, induces stronger Th1 protective immune responses in BALB/c than the individual proteins and than the combination of their genes. Therefore, it seems to be a good prophylactic tool against *Leishmania* infection.

C0081 SPECIFICATION OF BLOOD MEALS IMBIBED BY FEMALE SAND FLIES CAUGHT IN PALESTINIAN FOCI AND EXPOSURE AND IDENTIFICATION OF THEIR CONCOMITANT LEISHMANIAL INFECTIONS

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1 Background

Since leishmaniases are zoonotic vector-born diseases with female sand flies transmitting them between hosts when the female sand flies imbibe hosts' blood, identifying the animal blood donors' species together with exposing and identifying concomitant leishmanial infections in female sand flies caught in the field should help to discern animal reservoirs, clarify the epidemiology and facilitate the control of local leishmaniases.

2 Methods

CDC light traps, aspirators and sticky traps were used in four Palestinian areas to collected sand flies, 691 of which were females. Their blood meals were used, to identify the species of the donors.

3 Results

The females were also screened for leishmanial infections, indicated by the presence of leishmanial kDNA and by an ITS1-PCR-RFLP, which gave a positive rate of 18.5% (128/691), and 6.4% (44/691), respectively. Of these, only 1.5% were identified as being caused by either ***L. tropica*** from female ***Phlebotomus sergenti*** or ***L. major*** fromfemale ***Ph. papatasi***. Twenty species were collected: 11 ***Phlebotomus spp.*** and 9 ***Sergentomyia spp***. The most abundant sand fly species were ***Ph. papatasi*** at 33.7% and ***Ph. sergenti*** at 21%. Interestingly, the blood meals of two females of the species ***S. neophlebotomus*** contained DNA from ***L. tropica***.Respectively, 90 and 104 caught engorged female ***Ph. papatasi*** and ***Ph. sergenti*** had fed on a large variety of vertebrate hosts, as determined by Cytochrome b RDB of the DNA from their blood meals. The female ***Ph. sergenti*** had fed on humans, hyraxes, rats, cows, goats and birds. Regarding mixtures of blood in blood meals, the RDB blood meal assay revealed the presence of hyrax and rat blood in 8/104 (7.7%) female ***Ph. sergenti***.

4 Conclusions

Hyrax blood in blood meals lends support to the role of hyraxes serving as an animal reservoir of L. tropica. Rat blood in blood meals could be significant as there have been rare cases of strains of L. tropica and L. infantum having been isolated from rats. Further study must be undertaken before finite conclusions can be drawn.

C0173 EXTRACELLULAR RELEASE OF VIRULENCE FACTOR MAJOR SURFACE PROTEASE VIA EXOSOMES IN LEISHMANIA INFANTUM PROMASTIGOTES

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1 Background

Leishmania spp. protozoa are introduced into humans through sand fly blood meal, depositing the infectious metacyclic promastigote into human skin. Parasites enter and replicate intracellularly in macrophages, leading to chronic leishmaniasis which causes considerable human morbidity in endemic regions. *Leishmania* evade host microbicidal mechanisms using virulence proteins such as the major surface protease (MSP or GP63), which inactivates immune factors in the host environment. MSP is a metalloprotease encoded by a tandem array of genes belonging to 3 *msp* gene classes, that are differentially expressed among different life stages of the parasite. Like other cells, *Leishmania* spp. release small membrane-bound vesicles called exosomes into their environment. The purpose of this study was to detect MSP proteins in exsomal vesicles of *Leishmania* spp. protozoa.

2 Methods

Using mass spectrometry data we determined the profile of MSP class proteins released in *L. infantum* exosomes derived from promastigotes in their avirulent procyclic (logarithmic) stage and virulent stationary and metacyclic stages. MSP protein isoforms belonging to each of the three *msp* gene classes could be identified by unique peptides.

3 Results

Metacyclic promastigote exosomes contained the highest, and logarithmic exosomes had the lowest abundance of total MSP. Among the MSP classes, MSPC class had the greatest variety of isoforms, but was least abundant in all exosomes. Nonetheless, all MSP classes were present at higher levels in exosomes released from stationary or metacyclic promastigotes than logarithmic promastigotes.

4 Conclusions

The data suggest the efficiency of exosome release may be more important than the identity of MSP isoform in determining the MSP content of *Leishmania* spp. exosomes.

C0189 ARTEMISININ MODULATES THE MITOCHONDRIAL RESPIRATORY PATHWAY TO MEDIATE ITS ANTI-LEISHMANIAL ACTIVITY

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1 Background

Toxicity and drug resistance are the major limitations of anti-leishmanial chemotherapy, making it imperative to identify new compounds. Artemisinin, a plant derived sesquiterpene lactone endoperoxide has been shown to exert leishmanicidal effects via generation of reactive oxygen species (ROS). As mitochondria is the major energy for parasites concomitant with generation of ROS, this study aimed to delineate the role of Artemisinin on the respiratory chain and ATP production in *Leishmania donovani* promastigotes.

2 Methods

The effect of Artemisinin (0.5 – 1 mM) on mitochondrial generation of ROS was measured in *L. donovani* promastigotes using MitoSOX™ along with its effect on enzymes of the respiratory chain complexes; ATP production was measured spectrophotometrically.

3 Results

Artemisinin showed a significant increase in MitoSOX™ fluorescence, GMFC being 272.70 ± 10.19 (0.5 mM, $p<0.05$) and 409.30 ± 8.37 (1 mM, $p<0.005$) vs. 209.60 ± 17.01. Artemisinin (0.5 and 1 mM) caused a dose dependent inhibition of the activity of complexes I-III, baseline cytochrome c reduction being 43.81 ± 3.53 and 10.12 ± 2.18 respectively vs. 62.09 ± 5.35 µmoles/min/mg. Similarly, Artemisinin (0.5 mM and 1 mM) caused a 25.36% and 66.38% inhibition respectively of complexes II-III activity. Additionally, Artemisinin also caused a significant inhibition of mitochondrial F_0F_1-ATP synthase activity, which corroborated with a decrease in levels of ATP.

4 Conclusions

Artemisinin mediated enhanced generation of mitochondrial superoxide and depletion of ATP levels in *L. donovani* promastigotes that was mediated following inhibition of respiratory complex III (Cytochrome C reductase) and complex V (F_0F_1-ATPase). Taken together, targeting the mitochondrial bioenergetics is a potential leishmanicidal target, worthy of future consideration.

C0268 GENETIC VARIABILITY OF MOROCCAN LEISHMANIA INFANTUM POPULATION BASED ON PCR-RFLP OF KDNA MINICIRCLES

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1 Background

In Morocco, *Leishmania infantum* is the main etiologic agent of human visceral leishmaniasis (VL) and canine leishmaniasis (CanL). This parasite has been also proven to be an opportunistic agent in HIV+ patients and can also causes sporadic cutaneous leishmaniasis (CL). The study of intraspecific genetic variability among *L. infantum* population collected from different geographical areas and different types of hosts are of great clinical and epidemiological interest. This work aims to evaluate the genetic variability of Moroccan *L. infantum* strains based on PCR-RFLP analysis of the kinetoplastid DNA (kDNA) minicircles.

2 Methods

A total of 75 DNA samples were studied: 32 DNA samples were extracted from positive Giemsa-stained smears and 43 were extracted from cultured *L. infantum* strains, which were previously identified by MLEE as belonging to the zymodeme MON-1. These samples were originated from: VL patients with or without HIV, patients with CL and dogs. The PCR amplified the hypervariable region (447 bp) of kDNA using the MC1 and MC2 primers. The RFLP analysis were performed by using a panel of nine restriction enzymes. The presence or absence of restriction fragments was scored in a binary matrix and the SplitsTree4 software was used for the construction of a Neighbor-Net network. For each genotype identified in this work a representative strain was chosen and its kDNA-PCR product was sequenced.

3 Results

Moroccan population of *L. infantum* showed an important level of variability with the identification of 6 genotypes. The sequences corresponding to kDNA hypervariable region of each genotype were deposit in the GenBank database (KX098509 – KX098514). The predominant profile was the genotype B, which is the most widely distributed. A genotype, named Q was detected in the first time. The Neighbor-Net network segregates Moroccan *L. infantum* population into 3 clusters.

4 Conclusions

The kDNA-PCR-RFLP assay was suitable for use directly with biological samples; it reveals an important degree of genetic variability among Moroccan *L. infantum* strains even those belonging to the same zymodeme.

C0294 EPIDEMIOLOGIC STUDY AND MOLECULAR DETECTION AND GENOTYPING OF LEISHMANIA TROPICA IN A CUTANEOUS LEISHMANIASIS FOCUS IN HIGH ATLAS OF MOROCCO

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1 Background

Cutaneous leishmaniasis due to *Leishmania tropica* has a large distribution in Morocco, and is considered as a public health threat. Although, dogs were found infected with *Leishmania tropica* in some rural areas, the cutaneous leishmaniasis caused by *L. tropica* in Morocco is still considered to be anthroponotic. The involvement of different vertebrate hosts in the transmission of anthroponotic *Leishmania tropica* remains debatable. *Leishmania tropica* is recognized as a very heterogeneous species, in terms of isoenzyme profile variability, Morocco is characterized by the largest number of zymodemes ever described in *Leishmania tropica*.

2 Methods

This survey aimed to study *Leishmania* infection of *Phlebotomus sergenti* naturally infected, to investigate molecular variations within *Leishmania tropica* isolated from patients and vector, and to identify host-blood feeding preferences of *Phlebotomus sergenti.* Sand flies were collected in three rural areas of Azilal province, using CDC miniature light traps. 965 females of *Phlebotomus sergenti* were screened for *Leishmania* infection by ITS1-PCR-RFLP. Nucleotide heterogeneity of PCR-amplified ITS1-5.8S rRNA gene-ITS2 was analyzed in 31 *Leishmania tropica* from vector and 93 sequences from patients.

3 Results

The sequences of *Leishmania tropica* isolated from sand flies showed a high diversity. The source of blood meal of engorged females identified by *Cytb* sequences showed that *P. sergenti* feeds from a variety of vertebrate hosts, including humans, chickens, rodents, rabbit, bat and monkey. Sequence analysis of *L. tropica* isolated from CL patients identified 19 haplotypes in of which one predominates, and is previously found in *P. sergenti*, Furthermore. Significant correlation between intraspecific variants of *Leishmania tropica* and the age of the patients and geographical origin were observed. Finally, the correlation between the identified haplotypes and the age of patients suggests the existence of one haplotype that seems more pathogenic among children, and other haplotypes probably mainly involved in the development of a cumulative and partial immunity over the years.

4 Conclusions

Our findings supported that *Leishmania tropica* is genetically very heterogeneous. Furthermore, our results might be suggestive of a certain level of heterozygosity in *Leishmania tropica* population isolated from vector. This finding, as well as the feeding of the vectors on different animals are of interest for further investigation.

C0321 MICROSATELLITE PANEL DEFINITION TO CHARACTERIZE LEISHMANIA STRAINS ISOLATED FROM HUMAN SAMPLES IN AN ITALIAN ENDEMIC REGION

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1 Background

The leishmaniasys affects people, domestic and wild animals in temperature, subtropical and tropical regions. The natural cycle involves phlebotominae sandfly vectors transmitting the parasite to the vertebrate host. The insects influence the epidemiology of the disease by their geographical distribution in the seasons and the specific vectorial capacity. Human Leishmania infections are increasing every year in Sicily, which represent the region with the highest endemic level of the disease in Italy. Among different approaches employed for the diagnostic the parasites isolation remains the gold standard.

2 Methods

The samples directly taken from the lesions allowed us to obtain a big number of isolated strains that we stored in nitrogen. We used this strains collection to check the genetic distance trough the parasites isolated in various part of Sicily by applying two different molecular approaches. A multiplex PCR were carried out to amplify 11 independent polymorphic microsatellites to cluster the strains in various groups. For other hand, a K26 gene targeted PCR-RFLP amplification analysis was conducted to reveal and confirm the polymorphisms based on the SNPs.

3 Results

This combined analytical method allowed us to distinguish various genotypes of *L. infantum* strains by isoenzyme-independent approach.

4 Conclusions

The isoenzymatic analysis is based on the polymorphisms of the protein structures and it is performed as previously described by Martin-Sanchez et al. in the 1994 using potato amid gel. The technology is time expensive a can result difficulties for some laboratories. Microsatellite approach is a semi automatized analytical method using Structure Software. This software uses multi-locus genotype data to investigate population structures by studying hybrid zones and estimating allele frequencies. The results permitted us to discriminate geographically the parasite infective cases. The methods exhibited a high level of discrimination for closely related strains at epidemiological level and could be used on large-scale investigation.

C0324 METACASPASE BINDING PEPTIDE INHIBITS HEAT SHOCK INDUCED DEATH IN LEISHMANIA (L.) AMAZONENSIS

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1 Background

Leishmania (Leishmania) amazonensis is an important agent of cutaneous leishmaniasis in Brazil. This parasite faces cell death in some situations during transmission to the vertebrate host, and this process seems to be dependent on the activity of metacaspase, an enzyme bearing trypsin-like activity present in protozoans, plants and fungi. In fact, the association between metacaspase (MCA) expression and cell death induced by different stimuli has been demonstrated for several *Leishmania* species, but regulators and natural substrates of metacaspase are poorly known.

2 Methods

We analyzed trypsin-like activity in parasite extracts with or without heat shock at 37º C and employed phage display over recombinant *L. (L.) amazonensis* MCA to identify peptides that could interact with the enzyme and modulate its activity.

3 Results

We have shown for the first time that heat shock at 37º C induces *L. (L.) amazonensis* death and increases MCA activity. We searched for MCA ligands using peptide phage display over the recombinant *L. (L.) amazonensis* enzyme. Among several peptides that bound to MCA one significantly reduced trypsin-like activity and parasite death after heat shock. This data indicates that even a small inhibition of MCA by the peptide has an impact on parasite death induced by stimuli such as heat shock. The similarity of this peptide to *Leishmania* inhibitor of serine peptidase 3 (ISP3) suggests that this protein may regulate endogenous MCA activity and thus parasite death.

4 Conclusions

Our data demonstrates that *L. (L.) amazonensis* MCA activity and promastigote death are induced by heat shock. We showed that peptides that bind to recombinant MCA, selected by phage display, may affect enzyme activity. One of the peptides, similar to *Leishmania* ISP3, reduced parasite trypsin-like activity induced by heat shock and promastigote death after the shock. We suggest for the first time that ISP3, considered a serine peptidase inhibitor, may also inhibit cysteine proteases such as metacaspases.

C0381 POST KALA-AZAR DERMAL LEISHMANIASIS: IS IT A RESULT OF ENDOSYMBIOTIC INFECTION OF LEISHMANIA WITH PARVIBACULUM LAVAMENTIVORANS

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1 Background

Post kala-azar dermal leishmaniasis (PKDL) is a well-known dermal sequel of visceral leishmaniasis (VL) or kala-azar. In about 5–10% of patients this complication manifests as maculo-papular skin lesions several months or years after the cure of VL. It is not known why only 5-10% kala-azar patients develop PKDL. Our group has been of the opinion that PKDL is a result of *in-vivo* generation of quasi-species of *Leishmania donovani* either as *in-vivo* hybridization of various endemically circulating species within the host cells or due to super-infection with other organisms.

2 Methods

Therefore, we sequenced the whole genome of the Leishmania isolated from the skin lesions of a PKDL patients. The whole genome sequencing was performed using 100bp pair-end reads on an Illumina HiSeq 2500 system, which produced 36.3 million reads, corresponding to more than 110 fold sequencing depth.

3 Results

The draft genome of the Leishmania strain consisted of 2803 sequence scaffolds of 200bp with a total length of 32035718 bp (32Mbp). Pairwise alignment showed 6712 variable sites (SNPs & INDELs) in the genome of our PKDL isolate with 744 synonymous, 1068 non-synonymous SNPs and 20 frame-shift INDELs. A total 138 tRNAs were identified in the genome. Sequence mapping revealed that the PKDL isolate was closely related with *L. infantum, L. major, L. donovani* and *L. braziliensis*, but surprisingly only 4-5% reads could be mapped with *L. infantum* (4.43%), *L.major* (4.73%), *L. donovani* (4.35%) and *L. braziliensis* (3.66%). While comparing with the genome of three Indian VL isolates, we found only 31.9%, 57.4% and 50.4% mapping, with *L. donovani* BHU 1095, *L. donovani* Ld2001 and *L. donovani* Ld39, respectively. The genome of our isolate showed maximum coverage (98.7%) with *L. donovani* Ld2001. Most surprisingly, on BLAST analysis we found 893 contigs of a heterotrophic bacterium *Parvibaculum lavamentivorans DS*-1 in the sequencing data, covering its 53.8% genome.

4 Conclusions

These findings indicate a possibility of endosymbiotic infection of the visceralizing species of Leishmania with *Parvibaculum lavamentivorans* leading to PKDL manifestations.

C0389 GENE EXPRESSION OF PATTERN RECOGNITION RECEPTORS AND CYTOKINES IN THE BRAIN OF NATURALLY INFECTED DOGS WITH LEISHMANIA

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1 Background

Visceral leishmaniasis (VL) is an important anthropozoonosis. Infected dogs are the main host of the disease and they may present barely neurological disorders. However, the pathogenesis of the cerebral form remains to be elucidated. Pattern recognition receptors (PRRs) play a role in the innate immune response through the recognition of conserved pathogen associated molecular patterns (PAMPs). Toll-like receptors (TLRs) and nucleotide binding oligomerization domain (NOD)-like receptors are PRRs reported previously by recognize molecules present in the *Leishmania* parasite, as lipophosphoglycan, mediating the production of inflammatory cytokines.

2 Methods

The aim of this study was to determine the gene expression of TLRs, NODs and cytokines along with the histopathological lesions in the brain from fifteen dogs with VL and four uninfected. Samples from brain (pool of thalamus, hippocampus, piriform/temporal cortex and periventricular white matter) were collected in RNAlater and others samples were subjected to routine histological procedures and staining with haematoxylin – eosin. RNA was extracted, followed by reverse transcription procedure to cDNA and submitted to RT-qPCR to quantify the relative gene expression of TLR-2, TLR-9, NOD-1, NOD-2, IL-1β and TNF-α, using specific primers and Taqman probes. G3PDH was used as a reference gene.

3 Results

Meningitis and choroiditis were detected in the infected dogs, ranging from mild to intense. There was upregulation of IL-1β (6.42-fold more; $p<0.001$) and TNF-α (4.35-fold more; $p=0.001$) in the brain of infected dogs, along with trend of upregulation for TLR-2 (2.69-fold more; $p=0.0799$). On the other hand, TLR-9, NOD-1 and NOD-2 presented no differences between groups ($p>0.05$).

4 Conclusions

This indicates that TLR-2 is an immune sensor during VL, and that it can be produced in the brain during the infection along with inflammatory cytokines, contributing to the development and maintenance of the local inflammatory process. In contrast, NOD-1, NOD-2 and TLR-9 seem not to be related to the brain inflammation.

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C0429 ANALYSIS OF INTERACTOME-ASSOCIATED SCD6 AND RASPUTIN PROTEINS IN LEISHMANIA BRAZILIENSIS

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1 Background

Leishmanias are protozoan parasites with a digenetic life cycle in which the parasite alternates between an invertebrate vector and a vertebrate host; during it, the parasite experiences morphological and biochemical changes that allow them to survive and to adapt to the cellular stress that they must face in both hosts. This adaptation depends on the regulation of the expression of a wide variety of genes, which occurs, mainly at the posttranscriptional level. This differential gene expression is achieved by mechanisms based mainly in RNA binding proteins that regulate the translation and/or stability of mRNA targets by interaction with *cis* elements principally located in the untranslated regions (UTR). In consequence, the study of these proteins is of great importance. In recent studies, our group identified two proteins, SCD6 and Rasputin, based on their association with UTRs of the *L. braziliensis* HSP70 transcripts. The aim of this study was to determine the interactome associated to SCD6 and Rasputin proteins in *L. braziliensis* promastigotes, considering that these proteins could potentially be involved in the gene expression regulation of these parasites.

2 Methods

To achieve this objective, co-immunoprecipitation assays were performed by the UV-Cross-linking and immunoprecipitation (CLIP) technique, using the corresponding antibodies coupled to magnetic beads. The eluted proteins were analyzed by mass spectrometry. After identification of the co-immunoprecipitated proteins, an *in silico* functional analysis was performance using the TriTryp database.

3 Results

After data cleaning and comparison of replicates, 67 proteins were found associated with SCD6, whereas 135 proteins were found in the Rasputin interactome. Forty of these proteins were observed as constituents of both interactomes. Notably, SCD6 and Rasputin proteins were found to interact each other. Either directly or indirectly. Interestingly, the SCD6 and Rasputin proteins were associated to many constitutive proteins of different cellular granules like stress granules and processing bodies.

4 Conclusions

The SCD6 and Rasputin proteins interact with constitutive proteins of ribonucleoprotein granules, which are implicated in the expression regulation of different transcripts by accumulation or decay processes.

C0455 DETERMINING THE MODE OF ACTION OF A NOVEL ANTI-LEISHMANIAL COMPOUND SERIES

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1 Background

Visceral leishmaniasis (VL) is caused by the protozoan parasites, *Leishmania donovani* and *L. infantum*, and is fatal if left untreated. Due to a combination of toxicity, drug resistance, high cost and often complicated treatment regimen, there is a major need for new, safer drugs for the treatment of the disease. A promising anti-leishmanial that formed part of GlaxoSmithKline's Kinetobox library (Pena et al. 2015, *Sci. Rep.* 8771), was subject to further medicinal chemistry leading to the development of a compound series with potent *in vitro* activity but with an undefined mechanism of action. Here, we report the result of our studies to determine the mechanism of action of these compounds.

2 Methods

A range of molecular, genetic and biochemical techniques were employed to ascertain the mechanism of action of this compound series. Since these compounds are equally potent against all three TriTryp parasites, in certain instances *Trypanosoma brucei* was used as a model organism in these studies.

3 Results

Screening of compounds from this series against a genome-scale RNAi library (RIT-seq) in *T. brucei* (Alsford et al. 2012, *Nature* 482: 232-236) identified genes encoding putative zinc transporters as playing a role in compound resistance. The addition of exogenous Zn^{+2} to culture medium protected *in vitro* cultures of *T. brucei* and *L. donovani* from the cytotoxic effect of these compounds. Using a cell permeable, fluorescent zinc probe, we established that addition of these compounds results in a marked decrease in the intracellular levels of zinc within *Leishmania* promastigotes and bloodstream trypanosomes. The apparent zinc chelating properties of these compounds were also evident in compound-treated mammalian cells as well as in cell-free competition assays. Finally, *Leishmania* promastigotes treated with compounds from this series, presumably starved of intracellular zinc due to chelation, maintained higher levels the principal established zinc importer (*zip3*; Carvalho et al. 2015, *Mol. Microbiol.* 96: 581-595).

4 Conclusions

Collectively, these data suggests that compounds from this chemical series act as non-specific zinc chelators and thus have been deemed unsuitable for further drug development. However, the compound series may promise as a chemical tool. These studies highlight the value of carrying out mechanism of action studies when developing drugs and prioritising resources.

C0467 IDENTIFICATION OF A NOVEL PROKARYOTIC LIKE TOXIN-ANTITOXIN MACHINERY IN LEISHMANIA DONOVANI: A UNIQUE THERAPEUTIC TARGET

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1 Background

All prokaryotic genomes encode for toxin-antitoxin (TA) systems which have been linked to various cellular functions such as virulence, antibiotic resistance, programmed cell death, etc. Zeta toxin is a member of such TA family encoded "exclusively and throughout" the genome of pathogenic prokaryotes. We, for the first time identified and characterized such an interesting system in a deadly eukaryotic pathogen, *Leishmania donovani*. Zeta toxin is a small molecule kinase converting UDP-N-acetylgalactosamine (UNAG) to uridine diphosphate-N-acetylglucosamine-3'-phosphate (UNAG-3P). UNAG is conjugated to phosphoenolpyruvate by MurA enzyme eventually leading to the biosynthesis to peptidoglycan. UNAG-3P resembles MurA tetrahedral intermediate and could bind to MurA but does not get further processed or released leading to the termination of reaction.

2 Methods

We identified a zeta toxin like protein (ZLP) encoded in the genome of *L. donovani* by homology search. The recombinant zeta toxin domain of ZLP (rZLP) was expressed in *E. coli* and its growth was analyzed. Furthermore, the bacterial cells were tested for cell membrane permeability by Propiodium Iodide staining. FM-64 dye staining was performed to study artifacts in cell wall biogenesis. Kinase activity of ZLP was measured by recording conversion of ATP to ADP. The anti-ZLP serum was used for immunofluorescence assays and Western blots of parasites.

3 Results

E. coli cells expressing rZLP displayed massive cell death as compared to vector control. Further, cells expressing rZLP were PI positive, and were unable to undergo cytokinesis while karyokinesis was complete. We further found that rZLP displayed strong kinase activity in presence of UNAG, while in its absence it also, it exhibited weak kinase activity suggesting autophosphorylation of ZLP. Moreover, formation of UNAG-3P in the assay was identified by HPLC. IFA of promastigotes with anti-rZLP serum indicated strong expression of ZLP in the parasites which further was enhanced when parasites were shifted to lower pH at 37°C, the ambient conditions for amastigotes growth. The inhibition of *L. donovani* ZLP, affects growth of the parasites.

4 Conclusions

ZLP, an important component of TA system having kinase activity, is expressed in *L. donovani* parasites and blocking the activity affects the growth of parasites. Presence of zeta toxin in trypanosomatids and prokaryotes but not in higher eukaryotes makes it an excellent drug target.

C0468 INCREASED LEVELS OF OXIDATIVE AND NITROSATIVE STRESS-RESISTANCE PROTEINS DURING GROWTH AND DIFFERENTIATION OF LEISHMANIA AMAZONENSIS PROMASTIGOTES.

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1 Background

Leishmania amazonensis is one of the major etiological agents of the neglected, stigmatizing disease termed american cutaneous leishmaniasis, is a zoonosis and rodents are the main reservoirs. Most cases are reported in Brazil, Bolivia, Colombia and Peru.

2 Methods

In this study, changes in protein abundance have been detected by 2DE/MALDI-TOF/TOF at the main growth phases of *L. amazonensis* promastigotes in axenic culture.

3 Results

Sixty three out of 339 identified proteins are differentially abundant. The most remarkable finding is the increase of the level of proteins involved in resistance to nitrosative and oxidative stress at the last stages of growth and differentiation. These proteins are the arginase, a light variant of the tryparedoxin peroxidase, the iron superoxide dismutase, the regulatory subunit of the protein kinase A and a light HSP70 variant.

4 Conclusions

The most remarkable finding is the increase of the level of proteins involved in resistance to nitrosative and oxidative stress at the last stages of growth and differentiation. These data taken together with the decrease of the stress-inducible protein 1 levels are additional evidence supporting the previously described pre-adaptative hypothesis, which consists of preparation in advance towards the amastigote stage.

C0469 RESISTANCE TO NITRIC OXIDE IN PROMASTIGOTES OF LEISHMANIA CHAGASI.

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1 Background

Leishmania chagasi is the causative agent of zoonotic visceral leishmaniasis in Brazil. Domestic and stray dogs are the main reservoirs. It is well known that the mammalian phagocyte attempts to deploy a nitric oxide production response to clear amastigotes, which are able to resist against NO to some extent. We have found that promastigotes show ability to survive in the presence of this compound.

2 Methods

In this study, an NO-resistant and an NO-sensitive strain were isolated from infected dogs and differential protein abundance analyzed by 2DE-MALDI-TOF/TOF.

3 Results

This analysis has revealed that the glucose-6-phosphate dehydrogenase increases in the NO-resistant strain and the arginase and the glutathione peroxidase decrease in the NO-sensitive strain. Therefore, they contribute to NO resistance in *L. chagasi* promastigotes. These genes may be related with infectivity and/or resistance of amastigotes within the phagocytic host cell because NO-resistant promastigotes are highly infective *in vitro*, yielding a high rate of infected cells and amastigotes per infected cell of the human myeloid U937 line differentiated with phorbol esters. Changes in the abundance of certain enzymes involved in sugar metabolism (aldolase, enolase, gGAPDH, phosphoglycerate kinase C and triose phosphate isomerase), the pyruvate decarboxylase complex (GVCL-2) and the Krebs cycle (aconitase, isocitrate dehydrogenase and malate dehydrogenase) have been also found between the NO-resistant and the NO-sensitive strain. Other important variations in protein abundance have also been found in the case of proteins involved in gene expression regulation (e.g. EF1α), protein folding (hsp9, hsp60 and hsp83-2) and intracellular signaling (e.g. rPP2A).

4 Conclusions

Given the more infective nature of NO-resistant strain, the proteins mentioned above might be studied as potential drug targets and/or vaccine candidates in the near future.

C0480 THE ROLE OF HSP90 AND ITS INTERACTION WITH STI1 IN LEISHMANIA DONOVANI

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1 Background

HSP90 is a highly abundant, constitutively expressed protein in *Leishmania (L)* spp. Treatment of *L. donovani* with Geldanamycin (GA) or Radicicol (RAD), which are inhibitors of the heat shock protein 90 (HSP90), lead to an amastigote-like morphology and an increased expression of the amastigote-specific A2 protein family (Wiesgigl & Clos,2001). Both GA and RAD bind HSP90 thereby abrogating the assembly of the foldosome and leading to cell growth arrest. Parasites over expressing a RAD-resistant HSP90 (HSP90rr) transgene are able to growth under RAD treatment. Parasites bearing HSP90rr transgenes which lack the putative Sti1 interaction motif (Hsp90rr:Δ695) are significantly impaired in their growth under RAD (Hombach et al.,2013). This illuminates the important role of the HSP90-Sti1 interaction for the cell proliferation. Ribosome profiling is a new technique based on the deep sequencing of ribosome protected mRNA fragments, which allows the direct monitoring of protein synthesis (Ingolia et al.,2009). We applied ribosome profiling on parasites challanged with RAD to understand the role of HSP90 and of its interaction with Sti1 for gene expression and cell signaling.

2 Methods

L. donovani wt cells and cells bearing the transgenes HSP90rr or HSP90rr:Δ695 were challenged for 24h with RAD at an IC_{50} and subjected to either mRNA or monosome purification. Both the ribosome protected mRNA fragments and the chemically fragmented total mRNA were used for a library preparation and a subsequent deep sequencing analysis. The HiSeq-generated (Illumina) reads were then aligned to the *Leishmania* reference genome.

3 Results

Generated reads were mapped to the *Leishmania* spp. reference genome for the gene loci identification. We obtained an average number of 6000 gene IDs per sample. Preliminary data show that the RAD-treatment dramatically change the translational efficiency in the parasites, especially in wt parasites.

4 Conclusions

Further evaluation of the deep sequencing data in the next month will show us which pathways are mainly altered after RAD-treatment and illuminate the impact of HSP90 and the HSP90-Sti1 interaction on the expression of certain genes.

C0507 NEUTROPHIL ELASTASE IS REQUIRED FOR GROWTH OF LEISHMANIA DONOVANI IN THE VISCERA VIA TLR INDUCTION OF INTERFERON BETA

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1 Background

Virulence factors that contribute to the pathogenesis of dermal or visceral leishmaniasis are still elusive. *Leishmania sp.* have three genes similar to bacterial ecotin: *ISP*1, *ISP*2 and *ISP*3. Ecotin is an inhibitor of S1A family proteases, such as neutrophil elastase (NE). In *L. major*, ISP2 inactivates host NE and prevents triggering of TLR4 and TLR2 during parasite phagocytosis, which is important for parasite survival and growth in macrophages. Here, we describe that ISP2 expression is not detected in *L. donovani*.

2 Methods

We performed infections of mouse peritoneal macrophages *in vitro*, or infected C57B6 mice with *L. donovani* or *L. donovani* transgenic lines expressing the ISP2 gene of *L. major* (*L.donovani*:ISP2), and analysed parasite intracellular survival and growth, and parasite load in the viscera.

3 Results

We found that NE activity abrogation by a synthetic inhibitor or infection of macrophages from *ela*-/- (NE knockout), *tlr*4-/- or *tlr*2-/- mice with *L. donovani* prevented the intracellular growth of parasites. Addition of exogenous NE restored the intracellular growth of *L. donovani* in *ela*-/- macrophages. NE and TLR4 co-localized with the parasite in the parasitophorous vacuole. Parasite loads in the liver and spleens of *ela*-/- mice at 7d post-infection were reduced in comparison to C57BL/6 mice, and accompanied by increased nitric oxide (NO) and decreased transforming growth factor β (TGFβ) production. *L. donovani* lines expressing *L. major* ISP2 (*L. donovani:ISP2*) were generated and these transgenic parasites displayed impaired intracellular growth *in vitro* and decreased parasite burden in mice. Inhibition of NO or superoxide generation in macrophages enhanced survival of wild type parasites but failed to recover the growth of *L. donovani:ISP2* at 24h. Macrophages infected with *L. donovani*, but not with *L. donovani:ISP2*, showed increased levels of the transcription factor IRF3 in the nucleus at 6h up to 24h. Intracellular growth of *L. donovani* in *ela*-/- macrophages or of *L. donovani:ISP2* was fully recovered by addition of exogenous IFNβ, but not by IFNα.

4 Conclusions

We propose that *L. donovani* utilizes the host NE-TLR pathway to generate IFNβ and fuel parasite growth.

C0526 MOLECULAR CHARACTERIZATION OF AQUAGLYCEROPORINE: A NOVEL MUTATION IN LMAQP1 FROM LEISHMANIA MAJOR (MRHO/IR/75/ER)

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1 Background

The first line treatment for cutaneous leishmaniasis is pentavalent antimony such as sodium stibogluconate (pentostam) and meglumine antimonite (glucantime). One of the most important way to uptake the drug is by a transmembrane protein, called aquaglyceroporin encoded by *Aquaglyceroprotein1* (*LmAQP1*). So far, there is no report on *LmAQP1* from *L. major* (MRHO/IR/75/ER), therefore, in this study, molecular characterization of *LmAQP1* was reported.

2 Methods

L. major (MRHO/IR/75/ER) promastigotes were cultured, and then DNA extraction and RNA extraction were done and followed by cDNA synthesis. Amplicons resulted from PCR and RT-PCR using specific primers were purified and sequenced. Molecular characterization was done by bioinformatically softwares such as BLST, *ClustalW2*, and RMSD.

3 Results

Amplicons resulted from PCR and RT-PCR showed equal size in length. BLASTn analysis showed a point nucleotide change in *LmAQP1* gene that encoded 282-amino-acid long protein with a mutation at position 154 including replacement of alanine by threonine. The observed mutation in the interested gene was assessed using the above mentioned software. The mentioned gene was submitted at GenBank, NCBI with accession number of KU514052.

4 Conclusions

The functional prediction of the protein encoded from *LmAQP1* showed that the mentioned mutation could not affect the three dimension structure but it may modify the drug uptake potential of this important channel. Based on from *LmAQP1* role, it seems to be an appropriate candidate for drug development. According to search through internet, this is the first report of *LmAQP1* from *L. major* (MRHO/IR/75/ER).

C0529 LEISHMANIA BRAZILIENSIS EXPRESSES A DIPEPTIDYL PEPTIDASE PROTEIN.

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1 Background

Parasites of *Leishmania* genus yield several clinical manifestations known as leishmaniasis. In Colombia, the main pathologies are cutaneous and mucocutaneous leishmaniasis, being *Leishmania braziliensis* one of the main etiological agents. The increase in leishmaniasis worldwide and the existence of strains resistant to treatments, make it necessary searching new therapeutic targets. Proteases are interesting therapeutics targets because they have important functions in the survival and pathogenesis of the *Leishmania* parasites. Dipeptidyl peptidase enzyme 3 (DPP3) is a protease that has been found in organisms such as mammals, insects, yeasts and has been involved in protein recycling and protection against oxidative stress. However, to date, this enzyme has not been studied in *L. braziliensis*. Our objective is to evaluate the expression of DPP3 enzyme in *L. braziliensis*, express the recombinant enzyme functionally active, and compare its structure with its human orthologous.

2 Methods

Promastigotes of *L. braziliensis* strain MHOMBR75M2904 cultured at 26°C in Schneider Medium were submitted to 35°C and 5% of CO_2, and collected to different time periods (0, 4, 8, 20 and 48 hours). Protein extract was obtained to evaluate the expression of LbDPP3 by Western blot. The recombinant protein of *L. braziliensis* (rLbDPP3) into pCold-TF was expressed in *E. coli* BL21-DE3 and purified by Amicon Ultra Centrifugal Filter Units. The enzymatic activity of rLbDPP3 was measured using the fluorogenic DPP3 assay kit (BD Bioscience).
The human and *L. braziliensis* DPP3 amino acid sequences were modeled by Phyre 2. Then, an energy minimization was made by the Yasara server, the structures were validated by Ramachandran plot. Finally, the structures were compared with the Pymol software.

3 Results

The DPP3 was expressed at similar levels in promastigotes during their differentiation to axenic amastigotes. The activity of the rLbDPP3 was lower than that of its orthologous in humans. Most of the amino acid differences between the human and LbDPP3 enzymes, are found on the protein surface, but also differences were noted near the active site and the cleft where the substrate is bonded.

4 Conclusions

The DPP3 protein seems to constitutively expressed during promastigote-to-amastigote differentiation in *L. braziliensis*. The protein was expressed and purified as a functional recombinant enzyme; this warrants future studies dealing the usefulness of this protein as target for treatment of leishmaniasis.

C0532 COMPARISON OF PROTEOMES OF AMASTIGOTES FROM TWO L. AMAZONENSIS STRAINS WITH DIFFERENT VIRULENCE

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1 Background

It is estimated that 14 million people are infected and 350 million are at risk of developing leishmaniasis worldwide. In Brazil cutaneous leishmaniasis is caused by different *Leishmania* species, including *L. amazonensis*. The intracellular amastigote form of *Leishmania* lives in phagocytic cells, mainly macrophages, while the promastigote form is found in the sand fly vector. Molecules called virulence factors are expressed by both parasite forms, and some of them may be secreted. These factors are essential for successful infection and are therefore related to pathogenicity. Proteome analysis is an effective tool to identify parasite virulence factors. The strains PH8 and LV79 of *L. amazonensis* have different virulence in BALB/c mice. We have shown that they also have differences in infectivity of macrophages *in vitro* and in viability after isolation from lesions. Proteome comparison revealed some differences between the two strains that may be associated to virulence.

2 Methods

We compared the infection of BALB/c and C57Bl/6 mice by promastigotes from strains PH8 and LV79 during 12 weeks. Amastigotes derived from BALB/c lesions had their viabilities measured by MTT. They were used to infect macrophages and BALB/c mice, and lesion development was accompanied during 8 weeks. We compared the soluble proteins of amastigotes from the two strains by LTQ – Orbitrap and identified differentially expressed proteins using MaxQuant.

3 Results

We have shown that PH8 is more virulent in both strains of mouse, and that lesion-derived parasites from this strain are more viable and more infective *in vitro*. Amastigote proteome comparison identified GP63 as highly expressed in PH8 strain, and Superoxide Dismutase, Tryparedoxin Peroxidase and Heat Shock Protein 70 as more abundant in LV79 strain.

4 Conclusions

Our data demonstrates that *L. amazonensis* PH8 strain is more virulent than LV79, and that the comparison of their proteomes may help to identify proteins associated to virulence in this parasite.

C0546 MITOCHONDRIAL HETEROGENEITY AND NUCLEAR GENE DIVERSITY HAVE A KEY ROLE IN SAND FLIES' ADAPTATION (DIPTERA: PSYCHODIDAE) TO THE REGIONAL SPECIALIZATION AND ENVIRONMENTAL ASPECTS

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1 Background

Nucleotide variations of the genera Phlebotomus and Sergentomyia always has a prominent effects on the adaptation of sand flies species to environmental and climatic oscillations transmitting Leishmania parasites to their afflicted patients. The EF-1α and Cyt b genotype regions have been applied to describe the sand fly fauna and genetic aspects collected at war-torn sites of the Khuzestan boundary between Iraq and Iran.

2 Methods

The distribution of CL vectors was determined based on the climatic regionalization using the kriging method in ArcGIS model. DNA of sand fly pools were screened via polymerase chain reaction (PCR) using neutrality (Tajima's D) and neutral allele frequency (Fu's F_s) tests to measure the effect of randomly evolving DNA sequence on the genetic diversity of sand fly populations in response to habitat fragmentation and landscape modification. The climatic regionalization was used with the principal component analysis (PCA) and the clustering integration method (CIM) to obtain the appropriate classification.

3 Results

For this investigation, we identified ten species among Phlebotomus and Sergentomyia sand flies next to the Khuzestan boundaries. The non-native species Phlebotomus sergenti was unequivocally found for the first time in the studied regions. Nucleotide substitutions of sand fly sequences varied most in the most disrupted districts (disparity index test: $P < 0.05$). The haplotypes of Cyt b from the subgenus Sergentomyia and P. papatasi revealed more heterogeneity (Tajima's $D > +2$) than P. alexandri ($D > +1$), which suggests widespread heteroplasmic mitochondrial DNA mutations in the same mtDNA gene among different sand fly species. Subgenus Sintonius exhibited greater fitness ($D = 0$) and (neutrality test; $P > 0.05$) no evidence of selection. The sequence of the nuclear gene EF-1α indicated similar nucleotide differences, as observed for the Cyt b gene, in all sand fly species, but lower levels of polymorphisms ($D > +1$) were observed compared with the mitochondrial Cyt b gene ($D > +2$) in the subgenus Sergentomyia.

4 Conclusions

Our findings highlight the importance of haplotype variations for the development of adaptability. The neutrality and molecular analyses provide compelling evidence of randomly evolving DNA haplotypes to support the fitness and survival of wild-caught CL vector population with neutral mutation due to anthropogenic influence.

C0549 POLYMERASE CHAIN REACTION AT DIFFERENT DEPTHS OF CUTANEOUS LESIONS IN PATIENTS SUSPECTED OF HAVING AMERICAN TEGUMENTARY LEISHMANIASIS.

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1 Background

American Tegumentary Leishmaniasis (ATL) is an infectious disease responsible for causing skin and mucosal damage. Molecular biology techniques are promising strategies for the improvement of diagnostic accuracy and adequate sampling methods are crucial for the correct interpretation those tests. The purpose of this study was to test polymerase chain reaction (PCR) aiming to detect *Leishmania* kinetoplast DNA (kDNA) in different layers of the skin (epidermis, upper dermis, lower dermis and hypodermis).

2 Methods

Patients were consecutively recruited at the University Hospital of Brasília-HUB, with suspected ATL. The diagnosis of ATL was performed using culture of lesion aspirates inoculated in Novy-MacNeal-Nicolle medium (NNN), smears from skin biopsies and PCR from skin biopsies. The separation of the epidermis from the dermis was performed using the salt-split skin technique and the remaining layers were separated manually using sterile blades. DNA extraction was performed using the commercial Kit NucleoSpin® Tissue and PCR reactions were performed aiming to detect a 120bp fragment of *Leishmania spp.* kDNA.

3 Results

Seventeen patients were included in the study. The mean age of the selected patients was 48 years, being 10 of the male gender and 7 of the female gender. Eleven patients presented negative results in all tests analyzed. Of the 6 remaining patients, 4 had positive smears, 3 had positive cultures and all 6 had positive PCR on the four previously-separated skin layers. Although accuracy of PCR was similar in all tested skin layers, the exam from epidermis performed better considering bands definition in DNA gel electrophoresis.

4 Conclusions

The present results demonstrated that all the layers of the skin and subcutaneous tissues from a cutaneous ATL lesion present *Leishmania* kDNA. However, the epidermis analysis seems to provide better results in therms of exam interpretation. Further studies using real-time-based PCR are needed in order to detect the number of parasites in each skin layer. We conclude that adequate sampling methods, including biopsy depth, directly influence the results of PCR. In addition, it has been found that superficial sampling methods such as skin shaving and scrapping are sufficient for *Leishmania* DNA detection in ATL.

C0552 MOLECULAR MODELING AND ENZYME ACTIVITY RELATIONSHIPS OF OTULI, A DEUBIQUITINASE FROM LEISHMANIA INFANTUM

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1 Background

Deubiquitinating enzymes (DUBs) play an important role in regulating protein degradation and other non-degradative processes, such as immune response modulation and signaling of DNA double strand break. In this context, exploring the role of deubiquitination in *Leishmania infantum* could be a promising alternative to search new therapeutic targets for leishmaniasis. Here we present the enzymatic characterization of a DUB from *L. infantum*, the otubain(OtuLi), and its localization within parasite.

2 Methods

To explore the role of this deubiquitinase in *Leishmania infantum*, we performed enzymatic assays with the recombinant OtuLi (rOtuLi) and 3 other variants obtained by site-directed mutations. These recombinant enzymes were produced in *Escherichia coli* and purified by affinity chromatography. The OtuLi molecular models were constructed using the human Otubain-1 as template. We also established strains carrying an extra copy of otubain (OtuLi) and validated the integration of the cassettes into the 18S rRNA gene locus by PCR and the expression in promastigotes of OtuLi fused to a 6 histidine tail or to a FLAG peptide were accessed by western blotting and immunofluorescence analysis.

3 Results

The recombinant OtuLi showed improved activity on lysine 48 (K48)-linked over K63-linked tetra-ubiquitin substrate and is inhibited by *N*-ethylmaleimide and ubiquitin-aldehyde. Site-directed mutations on amino acids closed to the catalytic site (F82) or involved in the interaction with the ubiquitin (L265 and F182) caused structural changes as shown by molecular dynamics, resulting in a reduction or loss of enzyme activity, respectively. Furthermore, OtuLi localized in small cytoplasmic vesicles with a strong labelling in the kinetoplast area in promastigotes, confirming the results obtained using antibodies specific to OtuLi.

4 Conclusions

Our findings suggest that OtuLi is a cytoplasmic enzyme with K48-linked substrate specificity and the establishment of these strains overexpressing OtuLi is the first step for future identification of proteins interacting with it aiming better understanding of the importance of the deubiquitination process in *L. infantum* biology.

C0561 ANALYSIS OF LEISHMANIA INFANTUM GENETIC DIVERSITY IN PARAGUAY BY KDNA-PCR-RFLP

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1 Background

Leishmania infantum is the agent for human and canine visceral leishmaniosis in Paraguay. Human visceral leishmaniasis is an important public health problem related to disordered urbanization, poverty, HIV/AIDS, malnutrition and immunocompromised patients. In America, *Canis familiaris* is the main reservoir in the transmission cycle. In Paraguay, the high proportion of infected dogs and the relatively huge number of human cases require molecular tools to evaluate risk factors related to different types of the parasite. The genus *Leishmania,* as all the *Kinetoplastida* order, presents an extrachromosomal DNA localized in the mitochondria, which replicates independently, known as kinetoplastid DNA (kDNA), which can be used to typify these kind of protozoa.

2 Methods

A semi-nested PCR was performed, using LINR4, LIN17 and LIN19 primers. PCR products were digested by RsaI restriction enzyme and the RFLP pattern was observed in a 3% agarose electrophoresis gel. A distance matrix was constructed using *RESTDIST*, subsequently a *UPGMA* dendogram was made using the software *NEIGHBOR,* available in the package *PHYLIP* v3.6. The dendogram was visualized by *FigTree* v1.3.1.

3 Results

The present study included 53 samples, distributed in three collections: Nine human bone marrow aspirates, 24 canine spleen biopsies and 20 culture promastigotes strains isolated from dogs. The samples showed a high genetic polymorphism, with 19 different genotypes, separated into five groups. It was not observed a cluster tendency among the three collections of samples; however, it was detected an arrangement tendency by geographic regions.

4 Conclusions

kDNA-PCR-RFLP is a simple and useful tool, throughout the analysis of biological samples, to monitor relapses, to define outbreaks and to determine the presence of new strains in a geographic area. Genotyping could be important to correlate genetic variation with clinical manifestations, virulence and drug resistance. Identification of these genetic variations may have significant implications to understand the biology of the parasite, leading subsequently to the development of control strategies for the agent.

C0574 THE N-TERMINUS OF LEISHMANIA PROLINE/ALANINE TRANSPORTER (LDAAP24) REGULATES ALANINE TRANSPORT

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1 Background

Leishmania have developed mechanisms of adaptation to the distinct environments of host and vector that favor utilization of proline and alanine. LdAAP24 is the *L. donovani* proline-alanine transporter that influences proline, alanine, glutamate and arginine homeostasis. It is a member of *Leishmania* system A, responsible for neutral amino acid transport. Long N-terminal tails of amino acid transporters are known to act as sensors of the internal pool of amino acids and as positive regulators of substrate flux rate. In this study, we establish that N-termini of amino acid transporters can also determine substrate specificity.

2 Methods

Amino acid transport was determined using the rapid filtration technique of radiolabeled amino acids, developed in our laboratory. All other techniques are well established.

3 Results

We show that due to alternative *trans* splicing, the human pathogen *Leishmania* naturally expresses two variants of the proline/alanine transporter, one 18 amino acid shorter than the other. We demonstrate that the longer variant (LdAAP24) translocates both proline and alanine, whereas the shorter variant (?18LdAAP24) translocates just proline. Remarkably, co-expressing the hydrophilic N-terminal peptide of the long variant with ?18LdAAP24 was found to recover alanine transport. This restoration of alanine transport could be mediated by a truncated N-terminal tail, though truncations exceeding half of the tail length were no longer functional.

4 Conclusions

Taken together, the data indicate that the first 18 amino acids of the negatively charged N-terminal LdAAP24 tail are required for alanine transport and may facilitate the electrostatic interactions of the entire negatively charged N-terminal tail with the positively charged internal loops in the transmembrane domain, as this mechanism has been shown to underlie regulation of substrate flux rate for other transporters.

C0710 STRUCTURAL AND FUNCTIONAL STUDIES OF A LEISHMANIA DONOVANI PHOSPHOTYROSINE AND PHOSPHOINOSITIDE PHOSPHATASE HOMOLOGUE OF BACTERIAL SECRETED VIRULENCE FACTORS

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1 Background

Modulation of the host phosphoinositide (PI) metabolism is an important strategy used by many pathogens to suppress the host cells' defense mechanisms. While PI bacterial phosphatases interfering with PI metabolism in the host are already attractive drug targets, this aspect is poorly explored for intracellular protozoan parasites like *Leishmania*. The Leishmanias' phosphatome contains a subgroup of Atypical Lipid Phosphatases with no human homologues that are homologs of the bacterial virulence factors MptpB and LipA, PI phosphatases from *Mycobacterium tuberculosis* and *Listeria monocytogenes* respectively. A *Leishmania donovani* (*L. donovani*) member of this group is the LDBPK_220120 gene product, highly conserved amongst *Leishmania* species.

2 Methods

The LDBPK_220120 gene ortholog from the *L. donovani* LG13 (MHOM/ET/0000/HUSSEN) strain was cloned into plasmid vectors specific for protein expression in bacteria, *Leishmania* or mammalian cells. Transgenic *L. donovani* strains overexpressing the putative phosphatase and specific antibodies were generated and used to localize it in axenic *L. donovani* promastigotes and amastigotes by confocal and STED microscopy and by biochemical subcellular fractionation. The activity of recombinant putative phosphatase produced in bacteria was assayed using as substrates pNPP, peptides phosphorylated on tyrosine, serine or threonine residues and PI species.

3 Results

The LDBPK_220120 open reading frame is expressed in *L. donovani* cells as a ~30 kDa protein (calculated molecular mass 29,1 kDa) that dephosphorylates phospotyrosine (pTyr) residues and PtdIns3*P* and PtdIns4*P* monophosphorylated PIs. This phosphatase, named by us *Ld*TyrPIP_22, was localized in the promastigote cell body, in vesicle-like or distinct filament-like subcellular structures and next to the kinetoplast, at the base of the flagellar pocket. It was also detected as spots throughout the flagellum length. By STED microscopy, *Ld*TyrPIP_22 was shown to partially colocalize with LeishActin in the cell body and the flagellum of the promastigotes and the cell periphery of the axenic amastigotes. In mammalian cells, ectopically expressed *Ld*TyrPIP_22-GFP localizes on plasma membrane lamelipodia and filopodia where it partially colocalizes with F-actin.

4 Conclusions

This is the first report for the characterization of a pTyr/PI phosphatase homolog of pathogenic bacteria virulence factors in *L. donovani*. The role of *Ld*TyrPIP_22 in the parasite's developmental cycle is under investigation.

C0718 ATYPICAL CUTANEOUS LEISHMANIASIS CASE FROM PARAGUAY DUE TO LEISHMANIA INFANTUM

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1 Background

Leishmaniasis is cluster of heterogeneous diseases caused by various species of *Leishmania* genus. There is a very wide range of clinical forms, from self-healing cutaneous forms to fatal presentations with visceral compromise. In the New World, mainly cutaneous forms are caused by parasites belonging to *Leishmania braziliensis* and *L. mexicana* complexes and the visceral form is assigned to *L. infantum*. Nevertheless, in many countries of Central America, occurs several atypical cutaneous leishmaniasis forms due to *L. infantum,* the only autochthonous specie of the *L. donovani* complex described in America. There is no description of skin lesions owing to *L. infantum* in Paraguay, before this case.

2 Methods

The case was a one-year-old boy, from Mariano Roque Alonso district, near Asunción, Paraguay's capital, who had a cutaneous scabby sore of seven month of evolution in the face, resistant to local antibiotics and antiseptics treatments. A biopsy sample was obtained from the lesion and was processed for histopathology, conventional PRC and RFLP-PCR techniques.

3 Results

Amastigotes of *Leishmania* were observed in the skin samples, the presence of *Leishmania donovani* complex was confirmed by conventional PCR and *L. infantum* by RFLP-PCR. The patient was treated with intravenous pentavalent antimony salt, 20 mg/kg/day for 20 days with a total recovery of the lesion.

4 Conclusions

This is the first case described for an atypical cutaneous leishmaniasis caused by *Leishmania infantum* in Paraguay. As the child never left out of the neighborhood, we can assume that the transmission of the parasite occurred in a typically known visceral leishmaniosis area due to *L. infantum*

C0720 MITOCHONDRIAL ABC TRANSPORTERS FROM LEISHMANIA: NOVEL DRUG TARGETS TO TREAT THE DISEASE?

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1 Background

Mitochondria play essential functions including the synthesis and trafficking of porphyrins and iron/sulfur clusters (ISC), processes that in mammals involve the ATP-Binding Cassette (ABC) transporters ABCB6 (and maybe also ABCB8 and ABCB10) and ABCB7, respectively. *Leishmania* require ISC to live and is auxotrophous for heme.

2 Methods

LmABCB3 and LmABCB1, new *L. major* proteins with significant similarity to human ABCB6/ABCB7 and ABCB8/ABCB10, respectively, were characterized using bioinformatic tools. Fluorescent microscopy, biochemical and molecular genetic techniques and heterologous expression in yeast were used to characterize their functions. Structural analysis of LmABCB3 was performed using molecular modeling software. An *in vivo* mouse model was used to analyze its role in virulence.

3 Results

LmABCB1 and LmABCB3 were localized at the mitochondria of the parasite. LmABCB3 had a unique N-terminal extension, required to target the protein to the mitochondrion, which included a potential metal-binding domain. LmABCB3 plays a main role in the biogenesis of cytosolic ISC as it complemented the severe growth defect shown in yeast lacking ATM1. This protein is an orthologue of human ABCB7 involved in exporting from the mitochondria a gluthatione-containing compound required for the generation of cytosolic ISC. Docking analyzes performed with a LmABCB3 structural model using trypanothione, the main thiol in this parasite, as a ligand showed how both, LmABCB3 and yeast ATM1, contain a similar thiol-binding pocket. Point mutations in this pocket abolished the ability of LmABCB3 to complement ATM1 function in yeast. Additionally, LmABCB3 interacted with porphyrins and was required for the mitochondrial synthesis of heme from a host precursor. On the other hand, we show solid evidence suggesting that LmABCB3 is an essential gene as dominant negative inhibition of LmABCB3 was lethal for the parasite. Moreover, the abrogation of only one allele of the gene did not impede promastigote growth in axenic culture but prevented the replication of intracellular amastigotes and the virulence of the parasites in a mouse model of cutaneous leishmaniasis. Finally, ongoing work concerning LmABCB1 characterization will be also presented.

4 Conclusions

LmABCB3 is an unusual mitochondrial ABC transporter essential for *Leishmania* survival through its role in the generation of heme and cytosolic ISC. Hence, LmABCB3 could represent a novel target to combat leishmaniasis.

C0731 MITOGEN ACTIVATED PROTEIN KINASE 1 OF LEISHMANIA DONOVANI TARGETS HSP90 FOLDOSOME COMPLEX

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1 Background

MAP kinases (MAPK), the furthest downstream kinases in signal transduction cascades, regulate critical cellular activities such as cell proliferation, differentiation, mortality, stress response, and apoptosis. In Leishmania, MAPK1 has been shown to play roles in parasite survival and drug resistance. It negatively regulates the expression of P-glycoprotein type efflux pumps in parasite. The decrease in efflux pump activity with increase in LdMAPK1 expression may result in increased antimony accumulation in the parasite, making it more vulnerable to the drug. In the current study, we have identified potential targets of MAPK1 in *Leishmania donovani*.

2 Methods

Coimmunoprecipitation was performed using anti-LdMAPK1 antibodies and the pulled out complex of associated proteins was separated on one-dimensional gel electrophoresis. The various LdMAPK1 interacting proteins were identified through MS/MS analysis.Western blot analysis was employed to confirm the mass spectrometry identification. The enzyme kinetic assay was performed using purified native recombinant protein rLdMAPK1 and myelin basic protein as standard and identified target protein(s) as the possible kinase substrates.

3 Results

Co-immunoprecipitation studies revealed that LdMAPK1 interacts with the all subunits of HSP90 foldosome complex.The interaction is sensitive to treatment with AMTZD, a competitive ERK inhibitor. MAPK1 also exhibited kinase activity with HSP70 and HSP90 as substrates. By phosphorylating the two HSPs in the foldosome complex, MAPK1 may regulate the stability and activity of the foldosome which in turn plays a pivotal in the life cycle control of *L. donovani*.

4 Conclusions

The study may thus demonstrate a novel role for the MAP kinase1 in the regulation and possible post-translational modification of heat shock proteins.

C0768 FUNCTIONAL ACTIVITIES OF LBRDEVH1, A LEISHMANIA BRAZILIENSIS RNA HELICASE

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1 Background

Leishmania braziliensis LbrDEVH1 protein belongs to the DExD/H-box family of helicases. RNA helicases are essential enzymes that participate in many aspects of the RNA metabolism due to their biological functions as unwindases, RNPases and/or chaperones. It was previously reported that cytoplasmic LbrDEVH1 is accumulated into stress-mRNA granules when the parasite is grown under adverse conditions as lack of nutrients or heat shock. Herewith, functional properties of the recombinant LbrDEVH1 protein are described.

2 Methods

The LbrDEVH1 helicase recombinant protein was purified by Ni-NTA affinity chromatography. Binding, annealing and unwinding abilities were analysed employing increasing amounts of rLbrDEVH1 on ^{32}P-labeled RNA, dsDNA or pre-formed RNA:DNA duplexes by electrophoretic mobility-shift assays (EMSA). The rLbrDEVH1 activity was also tested on a RNA sequence endowed with HDV-like ribozyme activity.

3 Results

Recombinant LbrDEVH1 possesses RNA binding capacity in an ATP-independent manner and also to dsDNA. The rLbrDEVH1 is a functional and bidirectional RNA helicase as it catalyzes the unwinding of RNA:DNA duplexes with both 5´ and 3´-ssRNA overhangs exhibiting ATP dependence. It also has the ability to promote the annealing of complementary nucleic-acid strands. Depending on the protein concentration, LbrDEVH1 shows a switching behavior between unwinding and annealing processes as described for other RNA-binding proteins. Moreover, rLbrDEVH1 accelerates post transcriptionally autocatalytic cleavage of a *Leishmania* HDV-like ribozyme.

4 Conclusions

The obtained results suggest that LbrDEVH1 is a multifunctional helicase protein which catalyzes the rearrangement of secondary RNA configuration of DNA:RNA and RNA:RNA duplexes. These properties are required for remodeling of ribonucleoprotein structures and consequently, for the rapid adaptation to the environmental stresses in which *Leishmania* parasites are exposed during their life cycle.

C0799 THE ROLE OF MYELOID CELLS IN LIPOSOMAL AMPHOTERICIN B DISTRIBUTION DURING EXPERIMENTAL VISCERAL LEISHMANIASIS

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1 Background

During an experimental visceral leishmaniasis infection myeloid cells play many key roles in resolving infection; encompassing hosting parasites, inducing effector mechanisms to reduce parasite burden and trafficking to sites of infection. Some myeloid cells have also been shown to interact with liposomal drugs via uptake/release and the presence/absence of these cells might dramatically affect tissue distribution of liposomal drugs. Liposomal Amphotericin B (L-AmB) is the treatment of visceral leishmaniasis (VL) in humans but the role of myeloid cells on the distribution of L-AmB has not yet been fully characterized. We aim to utilise in silico modelling combined with in vivo experimentation in mice to decipher the key myeloid cells interacting with L-AmB in the liver during infection and to investigate

2 Methods

To determine the distribution of L-AmB in the liver during infection, a physiologically-based pharmacokinetic model (PBPK) is being constructed utilizing published pharmacokinetic data. To optimize the detection of L-AmB, RAW264.7 cells were incubated with DiO stained L-AmB and analyzed by flow cytometry and confocal microscopy. Distribution of labeled L-AmB within myeloid cells in the livers of C57BL/6 mice infected with $3x10^7$ *Leishmania* donovani amastigotes is determined by flow cytometry and confocal microscopy at various times post injection. By combining the *in silico* PBPK with *in vivo* data, simulations will be possible to determine the effect of distinct myeloid cell:L-AmB interactions on liver L-AmB distribution and ultimately parasite clearance.

3 Results

Conditions for optimal the labeling of L-AmB with DiO+ have been developed and DiO-labeled L-AmB have shown to be taken up by RAW264.7 cells *in vitro*. Ongoing preliminary experiments are aimed at establishing the optimal imaging parameters for *in vivo* detection of L-AmB post-administration under different dose regimens. In addition, parameter estimates for a fully developed PBPK model of steady state L-AmB distribution have been generated using a genetic algorithm, and this is being updated to account for physiological changes that occur in infected mice.

4 Conclusions

Our data has shown that it is possible to detect labelled L-AmB in myeloid cells using flow cytometry and confocal microscopy after *in vitro* exposure. We have also shown that L-AmB distribution can be simulated *in silico*. Further analysis using these two interlinked approaches to studying drug-immune cell interactions will be presented.

C0804 PRO-DEATH ACTIVITY OF A BH3 DOMAIN IN AN AQUAPORIN FROM LEISHMANIA

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1 Background

Apoptotic cell death has been deeply studied in metazoans for more than two decades but the presence of apoptosis-like processes in protozoans is still a matter of debate. The almost absence of demonstrated molecular pathways that regulate this process is a major concern that hampers acceptance of the existence of PCD in protozoa. Endonucleases G and metacaspases constitute the only two relevant enzymes in single-celled organisms for which a role in apoptosis-like processes has been demonstrated so far. Ectopic expression of some members of the Bcl-2 family of proteins can inhibit (e.g. Bcl-XL) or promote (e.g. Bax) cell death processes when expressed in yeast or *Leishmania* cells. However, up till now the yeast BH3-only Ybh3p was the unique protein from a single-celled organism that contains a functional BH3 domain with a demonstrated pro-death activity.

2 Methods

The PHI-BLAST algorithm was applied to search for putative BH3-containing sequences in the *Leishmania* genus. The central region (residues 5–12) of the Bcl-2 family BH3 motif signature (Prosite entry PS01259) was used as the query sequence.

3 Results

Because the BH3 domain is the smallest common signature in all the proteins of this family of apoptosis regulators and also because it is essential for molecular interactions between antagonistic members, we looked for sequences with significant similarity to the BH3 motif in the *Leishmania infantum* genome. Among the top scoring ones, we found the MYLALQNLGDEV amino-acid stretch at the C terminus of a previously described aquaporin, now renamed as Li-BH3AQP. This motif is highly conserved in homologous proteins from other species of the *Leishmania* genus. The association of Li-BH3AQP with human Bcl-XL was demonstrated by both co-immunoprecipitation and yeast two-hybrid experiments. Ectopic expression of Li-BH3AQP reduced viability of HeLa cells and this deleterious effect was abrogated by the simultaneous overexpression of Bcl-XL. Also, a prodeath effect could be observed when parasites overexpressing Li-BH3AQP were treated with staurosporine or antimycin A. Surprisingly, these parasites were more resistant, compared with wild-type parasites, to hypotonic stress or nutrient deprivation. The prodeath activity was abolished upon replacement of two highly conserved amino acids in this BH3 domain.

4 Conclusions

Our data support the inclusion of Li-BH3AQP as the first non-enzyme member of the very short list of proteins involved in cell death regulation in trypanosomatids.

C0826 PROTEIN KINASES THAT REGULATE LEISHMANIA DIFFERENTIATION

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1 Background

Leishmania encounters a number of challenges during its life cycle following environmental changes that trigger the transition from an intracellular amastigote that replicates inside a parasitophorous vacuole in the mammalian host to the extracellular insect stages, a replicative procyclic promastigote and an infective metacyclic promastigote. The differentiation process is thought to be regulated in part by phosphorylation resulting in dramatic changes in protein expression, which allows adaptation. Here we aim to identify and characterize protein kinases involved in this process, as well as understand which mechanisms *Leishmania* uses to overcome stress and regulate its life cycle.

2 Methods

We have applied the CRISPR-Cas9 genome editing system to *L. mexicana* in order to investigate 2 protein kinases previously characterized in *Trypanosoma brucei* as regulators of bloodstream to procyclic form differentiation, RDK1 and RDK2 (Repressor of Differentiation Kinase) [1].

3 Results

Our data show that while RDK1 is non-essential for *L. mexicana* promastigotes, its absence impacts on amastigote proliferation and differentiation to promastigotes *in vitro*, but doesn't affect infectivity in mice. It was not possible to generate constitutive over-expression lines for RDK1 in promastigotes so inducible overexpression and endogenous tagging of this gene is being used to better understand its importance on the dynamics of differentiation. RDK2 is located on supernumerary Chromosome 30, making it a challenging gene to study. *In situ* tagging and inducible knock-out strategies will be used to better understand the importance of RDK2 in *Leishmania* differentiation.

4 Conclusions

Progress on the application of CRISPR-Cas9 technology to generate protein kinase null mutants and investigate their involvement in Leishmania life cycle progression will be discussed. [1] Jones et al., 2014. PMID:24453978.

C0835 THE LEISHMANIA DUAL SPECIFICITY TYROSINE REGULATED KINASE 1: THE POSITIVE SIDE OF A NEGATIVE REGULATOR

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1 Background

Regulation of the balance between procyclic and metacyclic *Leishmania* forms is a key factor for parasite intracellular survival**.** Central mediators of environmental sensing and differentiation are protein kinases and amongst these are the dual-specificity tyrosine-regulated kinases (*DYRKs),* which in other organisms are known to act as negative regulators of growth and as positive regulators of cell differentiation. Interestingly, a *Leishmania* DYRK1 homologue (LinJ.15.0180), contains all structural motifs of an active kinase. Our working hypothesis is that *Leishmania* DYRK1 plays a major role in parasite differentiation, controlling the development of the infective stationary phase promastigotes.

2 Methods

We performed genetic manipulation to over-express *Leishmania infantum* (*Lin*) DYRK1 and to generate facilated knockout mutants, as null mutants often result in a lethal phenotype. First we induced the over-expression of *Lin*DYRK1 in *Leishmania* by using a negative selectable plasmid pXNG that also carries a GFP reporter. Then we sequentially knocked out the two endogenous DYRK1 alleles. pXNG-*Lin*DYRK1 persistence after 15 passages under negative selection was used as a readout of essentiality. To evaluate the phenotypes of our mutants we used cell cycle analysis, confocal fluorescence microscopy, scanning and transmission electron microscopy and *in vitro* infectivity assays.

3 Results

*Lin*DYRK1 over-expressing parasites displayed a significant delay in proliferation, associated with a delayed cell-cycle G1/S transition. Facilitated knockouts tolerated the loss of pXNG-*Lin*DYRK1, suggesting that *Lin*DYRK1 is not essential for viability. However, *Lin*DYRK1$^{-/-}$ parasites displayed a defective stress response showing a marked thermosensitivity to a 26→34?C temperature shift. Moreover, *Lin*DYRK1$^{-/-}$ parasites displayed defects in stationary growth phase as they rounded up, and showed an abnormal cell-cycle distribution with a marked increase of G2/M phase parasites. Defects in morphology were also associated with an intense cytoplasmic vacuolization and lipid body accumulation, suggestive of endocytic pathway impairment. Finally, *Lin*DYRK1$^{-/-}$ showed a pronounced reduction in their ability to survive and replicate in mouse macrophages.

4 Conclusions

*Lin*DYRK1 mediates the growth/stress response balance, acting as a negative regulator of growth to promote pro-survival and fitness of infective stationary phase parasites inside the host cell.

C0840 IN VITRO INDUCTION AND EVALUATION OF METACYCLOGENESIS IN LEISHMANIA BRAZILIENSIS

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1 Background

Leishmania parasites present different morphologies throughout its life cycle: amastigotes, inside host cells (macrophages), and two promastigote forms, in the vector (metacyclics and procyclic promastigotes respectively). Metacyclogenesis occurs in the vector midgut and consists in the transformation from poorly infective procyclic promastigotes into highly infective metacyclic promastigotes. Metacyclogenesis is therefore a vital process on the infection mechanism. Nevertheless, in vitro purification and isolation of *L.braziliensis* metacyclic forms is difficult, and for this reason the infections models with *L.braziliensis* are complicated to establish.

2 Methods

Metacyclogenesis in *L. braziliensis* was identified by morphometric analysis of the parasites along the growth curve. Once promastigotes reach the stationary phase, metacyclic promastigotes were isolated by negative selection with PNA (Peanut Agglutinin) lectin. The evaluation of the infectivity of the selected parasites was performed by in vitro macrophage infection (murine J774A cell line) after 24 hours, and detected by Giemsa staining and confocal microscopy.

3 Results

Morphometric analysis revealed statistical significant differences ($p<0.05$) in the size of the parasites from 3rd to 6th days of culture, consistent with the typical changes observed in *Leishmania* metacyclic promastigote forms. Isolated stationary phase promastigotes showed higher infectivity in vitro compared to logarithmic or non-selected stationary phase parasites ($p<0.05$).

4 Conclusions

In the present study, we have induced and evaluated metacyclogenesis in vitro in *L.braziliensis.* We have observed morphological changes along the growth curve, which in turn resulted in higher virulence of the isolated parasites, as reflected by the infectivity rate on murine J774A macrophages. These results open interesting possibilities around *L.braziliensis* infection models.

C0870 COULD HEME AUXOTROPHIC LEISHMANIA PARASITE REQUIRE DE NOVO HEME SYNTHESIS?

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1 Background

Trypanosomatid parasites are auxotrophous for heme and must rescue porphyrins from the host. Unlike other trypanosomatids, *Leishmania* acquired the enzymes for the last three steps of the heme biosynthetic pathway (CPOX, PPOX and FeCH) from proteobacteria by lateral gene transfer. These enzymes could use macrophage heme precursors or repair heme chemically modified in the acidic environment of the parasitophorous vacuole. As the group heme is essential for the parasite, heme trafficking and metabolism is a promising target for new leishmanicidal drugs. Due to the bacterial origin of CPOX, PPOX and FeCH genes in *Leishmania*, these enzymes have low similarity to their human counterparts, which could help to identify specific inhibitors avoiding cytotoxic side effects. The aim of our work is to evaluate the potential of these enzymes as new drug targets.

2 Methods

The activity of the CPOX, PPOX and FeCH of *L. major* was analyzed by functional complementation assays in mutant yeast and bacteria with the orthologous genes deleted. Their subcellular localization was analyzed by confocal microscopy. To determine the essentiality of these proteins in the infection processes, we are generating double knockout parasites for these genes using CRISPR-Cas9 technology.

3 Results

Leishmania PPOX and FeCH rescued the growth of heme auxotrophic bacteria lacking PPOX and FeCH, indicating that these enzymes were functional. Additionally, PPOX was not affected by inhibitors of eukaryotic PPOX, suggesting the possibility of inhibit specifically the protein. On the other hand, CPOX was localized in the cytosol, contrary to the mitochondrial localization of human CPOX, while PPOX and FeCH were localized in the mitochondrion. Finally, we have generated $FeCH^{-/-}$ parasites, which have been confirmed by PCR and by Southern blot. Whereas wild-type promastigotes could growth in the presence of heme-free medium supplemented with the FeCH substrate protoporphyrin IX (PPIX), $FeCH^{-/-}$ parasites were not able to use PPIX to synthesize heme, indicating the absence of FeCH activity.

4 Conclusions

The *de novo* heme synthetic pathway in *Leishmania* is functional and involves the cytosol and the mitochondria. Using the CRISPR-Cas9 technology we have generated $FeCH^{-/-}$ *L. major* parasites that cannot use PPIX as a source of heme. These mutant parasites will allow determining the essentiality of this pathway in mammals and sandflies and its potential to become a novel drug target.

C0871 OVEREXPRESSION OF LEISHMANIA DONOVANI DNA POLYMERASE ? CONFERS SURVIVAL ADVANTAGE AGAINST DNA DAMAGING AGENTS

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1 Background

Leishmania donovani is an intracellular protozoan parasite that causes endemic disease Visceral Leishmaniasis (VL) or Kala-azar. Currently used drugs are experiencing resistance and toxicity issues and therefore identification of novel drug targets is of paramount importance. As this parasite multiplies under highly oxidative environment inside the human macrophage, it must have robust DNA repair machinery to ensure its genomic integrity. Among various DNA repair mechanisms, base excision repair (BER) is the predominant one, but details of BER has not been studied in *L. donovani*. DNA polymerase-β (*Ld*Pol-β) is one of the essential enzymes involved in BER pathway and could be a potential therapeutic target.

2 Methods

Leishmania donovani DNA Pol-β was cloned, expressed and purified from *E. coli* cells. The purified protein was characterized *in-vitro* by using generated substrate pUC19. For the overexpression of *Ld*Pol-β gene was cloned in pXG-GFP plasmid and transfected into *L. donovani* cells by electroporation method. And this overexpressed cells were subjected to different DNA damaging agents (UV, Methotrexate and Hydrogen peroxide) and checked the percentage of viability of the cells by Trypan blue exclusion method and MTT assay.

3 Results

To establish this Leishmania Pol-β gene was cloned in pXG-GFP plasmid and transfected in *L. donovani* promastigotes. The overexpression of Pol-β was confirmed by Western hybridization with anti-GFP antibody, and overexpressing cells were subjected to different DNA damaging agents (UV, Methotrexate and Hydrogen peroxide) and interestingly these cells show more survival as compared to control cells. When *L. donovani* cells was exposed to UV and check the m-RNA and protein expression levels of *Ld*Pol-β by semi-quantitative RT PCR and western blot method respectively and found that Pol-β level 2-3 fold increased in UV exposed cells as compared to UV unexposed cells.

4 Conclusions

DNA polymerase β is the key enzyme of Base Excision Repair pathway, which re-synthesizes DNA during gap filling in eukaryotes and bacteria. *L. donovani* is a protozoan parasite which encounters a highly oxidative environment inside human macrophage has a strong Base Excision repair pathway for DNA repair. Overexpression of DNA polymerase β makes it tolerant to DNA damaging agents. This suggests that DNA polymerase β has a major role in DNA repair of *L. donovani* and could be an attractive target for therapeutic intervention.

C0879 STRUCTURAL ANALYSIS OF LISIR2RP1 RESOLVED AT 2.2 Å REVEALS FEATURES BOTH COMMON AND UNIQUE TO SIRTUIN DEACETYLASES

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1 Background

In recent years,*Leishmania infantum* Silent Information Regulator 2 related protein 1 has emerged as a promising drug target deserving exploitation but, prior to this work, the lack of structural information on this protein has hampered structure-based drug design.

2 Methods

Crystallisation was attempted under multiple conditions for several different recombinant LiSir2rp1 proteins.

3 Results

The crystal structure of LiSir2rp1has been resolved at 2.2 Å in complex with the NAD cofactor and acetyl-lysine substrate. The structure is broadly commensurate with SIRT2 proteins of yeast and human origin, reproducing many of the structural features common to these sirtuin deacetylases, including the characteristic small N-terminal Zn-binding domain (Cys_{152}-$X_{(2)}$-Cys_{155}-$X_{(20)}$-Cys_{176}-$X_{(2)}$-Cys_{179}), and the larger Rossman-fold domain involved in NAD –binding interactions. The two domains are linked via a cofactor binding loop ordered in open conformation. The peptide substrate binds to the SIR2rp1 protein via a cleft formed between the small and large domains, with the acetyl lysine side chain inserting farther into the resultant hydrophobic tunnel. Crystals were obtained only with recombinant LiSIR2rp1 proteins possessing extensive internal deletions of an unstructured and proteolytically-sensitive loop that appears to be unique to the sirtuins of kinetoplastid origin. Thus, deletion of 50 internal amino acids from LiSir2rp1r253-303 did not appear to alter Ac-p53 substrate interactions in deacetylation assays (Km values remained similar to the full length protein) but this deletion was a requisite for the production of ordered crystals. Nonetheless, the removal of the unstructured region, that otherwise extrudes from the classical structural elements that form the Rossman-fold, appears to produce less stable protein, leading to lower specific activities in the recombinant protein and lower accumulation of the protein when expressed from episomal vectors in *Leishmania infantum* promastigotes.

4 Conclusions

A biological role for the unstructured loop in LiSir2rp1 remains elusive. Its presence unique to kinetoplastids might suggest that the corresponding sirtuins have additional cellular functions that are distinct from their human counterparts. These unique properties, along with the resolution of the present LiSir2rp1 structure, present novel opportunities for drug design against a protein target previously established as essential to parasite survival and proliferation.

C0914 THE ROLE OF INFLAMMATORY MONOCYTES IN EXPERIMENTAL VISCERAL LEISHMANIASIS

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1 Background

Ly6C^{hi}inflammatory monocytes develop in the bone marrow and migrate to the site of infection during inflammation. Upon recruitment, Ly6C^{hi} monocytes can differentiate into dendritic cells (Mo-DCs) or macrophages (Mo-Ma). According to the tissue environment they can also acquire different functions. How this recruitment and differentiation of myeloid cells affects the outcome of experimental visceral leishmaniasis is poorly understood.

2 Methods

C57BL/6 wild type (B6) and B6.*Rag2*$^{-/-}$ mice were infected by intravenous injection of 3x10^{7} *Leishmania donovani* amastigotes. At different time point post-infection, the parasite load in the spleen and liver was determined using impression smears, and the phenotype of the myeloid cell infiltrate was analysed by flow cytometry in the bone marrow, the blood and the spleen.

3 Results

Ly6C^{hi} monocytes are recruited in the spleen of B6 mice and at day 28 p.i, the peak of infection, these cells are mainly differentiated into Mo-DCs, expressing high levels of MHCII, CD86 and CD40. In B6.*Rag2*$^{-/-}$ mice, Ly6C^{hi} monocytes maintained an immature phenotype at this time post infection, but at later stages of chronic infection (>3mths) they had become mainly differentiated into macrophages. In B6 mice, Ly6C^{hi} monocytes in the blood and bone marrow displayed a highly activated phenotype, with >90% of monocytes expressing MHCII. In contrast, in B6.*Rag2*$^{-/-}$ mice, only <10% of the monocytes were MHCII^{+} at day 28 p.i. These results suggest that during *L. donovani* infection, Ly6C^{hi} monocytes can be primed in the bone marrow in a process that is likely to be dependant of the presence of lymphocytes.

4 Conclusions

These preliminary data showed that during *L. donovnai* infection, Ly6C^{hi} monocytes are recruited in the spleen and differentiate into Mo-DCs. In addition,Ly6C^{hi} monocytes are most likely primed in the bone marrow. Further studies are in progress to understand the mechanisms involved in the Ly6C^{hi} monocytes priming in the bone marrow and differentiation and how this impacts on *L. donovani* infection.

C0915 INCREASING IN CYSTEINE PROTEINASE B EXPRESSION AND ENZYMATIC ACTIVITY DURING LEISHMANIA (VIANNIA) BRAZILIENSIS IN VITRO TRANSFORMATION: FIRST EVIDENCES FOR MODULATION DURING MORPHOLOGICAL TRANSITION

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1 Background

Leishmania sp. is a digenetic protozoan parasite causing leishmaniasis in humans. It is important understanding of the host–pathogen–vector triangle, and particularly of their intimate interactions at the molecular level. *Leishmania (Viannia) braziliensis* presents adaptive mechanisms dependent of proteinases, as cysteine proteinases B (CPB). The focus of this work is the expression of three cpb gene isoforms and the CPB enzymatic activity during the parasite differentiation.

2 Methods

In vitro differentiation of promastigotes to amastigotes was initiated with log-phase promastigotes in Schneider´s insect medium pH 7.2/ 26ºC. The promastigotes were suspended in Schneider's insect medium pH 5.5 and incubated at 32 °C. We assessed the relative expression levels of the gene LbrM.08.0810 and LbrM.08.0820 and LbrM.08.0830 by quantitative polymerase chain reaction (qPCR), using primers based on respective 3` Untranslated Region (3'UTR). The proteolytic activity was essayed through variance in the relative fluorescence units (RFU) corresponding to enzymatic cleavage of the substrates and monitored with a Molecular Devices SpectraMax spectrophotometer (Gemini XPS).

3 Results

Observed a higher quantity of transcripts of LbrM.08.0810 in logarithmic phase promastigotes than in stationary phase promastigotes (> 1.5 times). The cbp gene trend to decrease during acid pH shock was followed by an increase after temperature (> 1.3 times).The genes LbrM.08.0820 and LbrM.08.0830 showed expression profiles similar to the gene LbrM.08.0810, but with overall lower levels. The proteolytic activity shows a gradual increase during parasite's differentiation process, with low levels in samples of logarithmic phase promastigotes (3.1 ± 0.03 mmol min-1.mg protein-1) to a peak of activity after 72 hours of incubation at 32ºC (4.2 ± 0.03 mmol min-1.mg protein-1), followed by a subsequent decrease of 68% after 96 hours of incubation at 32ºC (2.8 ± 0.03 mmol min-1.mg protein-1). These activities were inhibited by Z-Phe-Phe-fluoromethyl ketoneand and transepoxysuccinyl-L-leucylamido(4-guanidino)butane proving their source as cathepsin-like proteinases.

4 Conclusions

These results indicate for the first time a modulation of cathepsin L-like expression during the *L. (V.) braziliensis* in vitro differentiation induced by acid pH and high temperature.

C0918 MOLECULAR CHARACTERIZATION OF URACIL DNA GLYCOSYLASE OF LEISHMANIA DONOVANI AND ITS POTENTIAL AS NEW ANTI-LEISHMANIAL DRUG TARGET

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1 Background

Currently used anti-leishmanial drugs are experiencing resistance and toxicity concerns and therefore identification of novel drug targets is of seminal importance. *Leishmania donovani* is an intracellular parasite that able to survive and replicate concomitantly under lethal oxidative environment of host macrophages. Between different survival strategies for parasite, active DNA repair machinery must exist to ensure genomic stability. Base excision repair (BER) is the predominant one among various repair pathways. In order to access the role of BER in protecting parasite against DNA damage, we studied Uracil DNA glycosylase (UNG), which is one of the essential enzymes involved in BER pathway and could be a potential therapeutic target.

2 Methods

Initially, partially purified parasite crude extract was checked for the glycosylase activity. The existence of the leishmanial UNG activity prompted the recombinant expression of LdUNG for further characterization. LdUNG was identified, cloned, expressed and purified from *E. coli* as His_6-tag fusion protein. The recombinant UNG was analyzed by SDS PAGE and western blotting. The *in vitro* repair activity of the LdUNG was checked on synthetic 47 base pair oligonucleotide as a substrate. Biochemical properties were also characterized. We have overexpressed LdUNG in *Leishmania donovani* and checked the survival and sensitivity of the parasite against various DNA damaging agents.

3 Results

After cloning of 1185 base pair PCR product, the 47-kDa hexa-histidine tagged LdUNG was expressed and identified. In vitro activity assay indicate that LdUNG specifically excise uracil from both single and double stranded oligonucleotide. UNG overexpressing parasite showed an increased resistance to hydrogen peroxide, a mutagen that produces oxidative stress, UV rays which produces large adducts in genomic DNA, and also to methotrexate (MTX), an inhibitor of thymidylate biosynthesis which causes copious incorporation of dUTP into DNA when compared to control cells.

4 Conclusions

The recombinant LdUNG was characterized for biochemical properties. The over expression of LdUNG increases promastigotes viability when they are exposed to acute ROS/UV/mutagen attack. Our data con?rm that the BER pathway is involved in *Leishmania donovani* resistance to DNA oxidative damage and indicate the role of Uracil DNA glycosylase in parasite survival. Here we presented LdUNG as an attractive target for therapeutic intervention.

C0931 LMFLVCRB IS A HEME IMPORTER REQUIRED FOR THE VIRULENCE OF LEISHMANIA MAJOR

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1 Background

An Achilles' heel of *Leishmania* parasites is their absolute dependence on scavenging the essential compound heme from its human hosts. Proteins involved in this rescue of heme could be new therapeutic targets for drug development. *Leishmania* spp. can fulfill heme requirement by receptor-mediated endocytosis of host hemoglobin or by the uptake of free heme through the membrane. The essential transporter LHR1 plays a role in both mechanisms: at the plasma membrane LHR1 is involved in the internalization of porphyrins whereas at the lysosome, LHR1 transport to the cytosol the heme released after hemoglobin breakdown. In mammals, the transporter FLVCR2 can mediate the uptake of porphyrins through the plasma membrane. We have identified a putative orthologue of this mammal transporter in *Leishmania* (LmFLVCRb) and, in this work, we have studied its role in the trafficking of porphyrins.

2 Methods

L. major has been used as model organism. *LmFLVCR* genes were identified and characterized using bioinformatic tools. Fluorescent microscopy was used to determine their cellular localization. Their level of expression was modulated by molecular genetic techniques. Flow cytometry and HPLC-MS/MS were used to measured porphyrin transport. Functional characterization of LmFLVCRb was carried out in *Leishmania* promastigotes and in *Xenopus laevis* oocytes. A mouse model of leishmaniasis was used to analyze LmFLVCRb role in *L. major* virulence *in vivo*.

3 Results

We have found four *FLVCR* genes in the *L. major* genome, showing *LmFLVCRb* the highest similarity to mammal *FLVCR2*. LmFLVCRb was located at the plasma membrane of the parasite. It was efficiently pulled down with hemin-agarose, and the interaction was competed by free hemin. LmFLVCRb overexpression increased the uptake of fluorescent porphyrins in the parasite up to 40 %. In contrast, the deletion of one allele of *LmFLVCRb* diminished the accumulation of porphyrins by around 60 % with respect to control parasites. In addition, LmFLVCRb heterologously expressed at the plasma membrane of *Xenopus laevis* oocytes promoted the uptake of heme in a temperature, pH and ion-dependent manner. Finally, although we were not able to generate double KO parasites, the abrogation of only one allele of the gene prevented the virulence of the parasites in mice.

4 Conclusions

These results show that LmFLVCRb is a novel plasma membrane heme importer required for the virulence of the parasite.

C0939 COMPARATIVE STUDIES ON BIOLOGICAL BEHAVIOR OF FLUORESCENCE TRANSFECTED PARASITES AND THEIR UTILITY IN BIOLOGICAL ASSAYS

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1 Background

In the search for alternatives for the treatment of leishmaniasis more precise methodologies have been developed and implemented with the use of recombinant parasites for the in vitro screening of antileishmanial activity. However, differences or similarities in biological behavior between transfected species are not yet established.

2 Methods

Green fluorescent protein (GFP) and mCherry transfectantspromastigotes of five *Leishmania*species (*L. panamensis, L. braziliensis*, *L. guyanensis*, *L. mexicana* and *L. amazonensis*) were tested for growth in culture, infectivity to human U937 macrophages and sensitivity to meglumineantimoniate (MA), miltefosine (Milte), pentamidine (Penta) and amphotericine B (AmB). Parasites were cultured in NNN medium and parasites were counted daily during 12 days. The Infective concentration 50 (IC_{50}) was determined after infection of U937 macrophages at different promastigotes:cell ratio and evaluation of percentage of infection by flow cytometry. In turn, the sensitivity of these transfected *Leishmania* strains to serial dilutions of antileishmanial drugs was evaluated also by flow cytometry in intracellular amastigotes and expressed as the effective concentration 50 (EC_{50}).

3 Results

All *Leishmania* strains showed similar growth behavior in culture showing a log phase andstationary phaseof growth at days 2-3 and 5-6, respectively. Both GFP and mCherry transfected *L. brazilienzis* showed higher infective capability to human U937 cells. All *Leishmania* strains were highly sensitive to AmB with not significant differences in the EC_{50} values. At contrary, all transfected *Leishmania*species except mCheery-*L. panamensis*showed a moderate sensitivity to Milte.*L. panamensis*-GFP was the most sensitive to Penta while *L. guyanensis*-GFP and *L. mexicana*-GFP were less sensitive. Al transfected *Leishmania*species exhibited low sensitivity to MA.

4 Conclusions

Transfected parasites are similar in growth and infective capability but different in sensitivity to antileishmanial drugs. Therefore, during screening of drug candidates the use of MA as control drug is not recommended.

C0945 TOCOPHEROL BIOSYNTHESIS IN LEISHMANIA (L.) AMAZONENSIS.

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1 Background

Leishmaniasis, a neglected tropical disease, is caused by a protozoan of the genus *Leishmania* and is transmitted by the bite of sandflies. It affects either the skin or the internal organs and is estimated 2 million of new cases occur annually, with about 12 million people currently infected. Isoprenoid biosynthesis in *Leishmania* spp has not been studied yet and this pathway could be a possible therapeutic target.

2 Methods

Using metabolic labeling with {1-(n)-^{3}H} GGPP or {1(-n)-^{3}H} FPP, two different methods of RP-HPLC purification and scintillation counting we detected in *Leishmania (L.) amazonensis* promastigotes extracts, radiolabeled fractions with a coincident retention time of α and γ-tocopherol standards, which we confirmed the presence of their chemical structures by GCMS in non-radiolabeled parasites extracts.

3 Results

The treatment of usnic acid (UA), an inhibitor of HPPD enzyme that catalyzes the conversion of p-hydroxyphenylpyruvic acid to homogentisic acid, a precursor of tocopherol biosynthesis, inhibited the growth of parasite in a concentration-dependent manner. Parasite growth inhibition by UA was reversed by the addition of α-tocopherol (the viability of parasites was measured by MTT and Sybrgreen DNA incorporation)

4 Conclusions

In conclusion, this study shows for the first time evidences for tocopherols biosynthesis in *Leishmania (L.) amazonensis* promastigotes and antileishmanial activity of UA. This pathway could serve as an antiprotozoan target, once it is absent in mammal. This research was supported by FAPESP and CNPq.

C0958 IDENTIFICATION OF POTENTIAL MOLECULAR TARGETS IN LEISHMANIA SPP. THROUGH IN SILICO RECONSTRUCTION OF THE PI3K/AKT AND APOPTOTIC SIGNALING PATHWAYS INTERPLAY

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1 Background

For the last decades, induction of apoptosis via PI3K-AKT pathway effectors has being exploited as an effective therapeutic strategy in cancer and other human maladies. In order to study if the same approach would also be feasible in *Leishmania*, we reconstructed through a bioinformatics approach the survival, proliferation and apoptotic cross-talk signaling, determining also effector and regulatory molecules pivotal for parasite biology.

2 Methods

Different reference biological databases were used to characterize the proteins; subsequently we performed a similarity analysis with human counterparts based on a best reciprocal hits protocol. Then, we exhaustively searched the literature for those mammalian PI3K-AKT members considered good candidates in anti-cancer therapy known to trigger apoptosis when disrupted. Simultaneously, we reviewed experimental studies to seek whether the same proteins had been previously described in *Leishmania*. Additionally, these were mapped to a protein-protein interaction network previously built for different *Leishmania* species.

3 Results

According to the literature reported, *Leishmania* homologs of the PI3K-AKT pathway, eIF4E, GSK-3, Hsp90, TOR and 14-3-3 are necessary for survival, cell cycle progression or infection. We further identified *in silico* counterparts for NF-κB, C-myb, cyclin E-Cdk2, JAK, AMPK, S6K, PTEN and PHLPP in the parasite. Last, we found PI3K-AKT apoptotic homologs for Apaf-1, CytC, ENDO-G, PARP, ATM, Calpain-1, and XIAP. Moreover, the key signalling nodes: AKT, CDK and GSK3, are highly connected and interconnected within the protein-protein interaction network.

4 Conclusions

Overall, our data supports the presence of mammalian PI3K-AKT homologues in *Leishmania* spp. that when disrupted may trigger apoptosis in the parasite.In addition, the proteins listed constitute a preliminary reconstruction of the molecular signaling connecting cell cycle and death in this kinetoplastid. From a clinical perspective, clearance of apoptotic rather than necrotic cellular debris, alleviate inflammatory response reducing self-amplifying tissue injury as there is no release of damaging cellular components. These findings could be exploited for drug repurposing and the design of multi-target drugs; thus preventing parasite resistance and/or driving treatments sooner, safer and cheaper.

C0980 INFLUENCE OF SUCCESSIVE IN VIVO PROPAGATION ON VIRULENCE OF LEISHMANIA MAJOR

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1 Background

How many passages the parasites have been propagated *in vitro* is of importance for virulence and is partly related to the expression of the known virulence factors, such as LPG and GP63. In this study we wanted to investigate the difference between parasites repeatedly propagated *in vitro* with parasites repeatedly propagated *in vivo* by looking at infectivity in mice, ability to resist complement lysis, expression of known virulence factors, ability to infect known host cells and the differential gene expression in the parasites.

2 Methods

L.major promastigotes were repeatedly propagated *in vitro* (>100 passages), the medium was changed every 7 days. For the *in vivo* propagation of parasites, stationary promastigotes were inoculated into the footpad of BALB/c mice. 57 days post infection, parasites were purified and transferred to RPMI medium and propagated maximum 4 passages *in vitro* before being used to infect new mice. This process was repeated 4 times.
Parasites propagated in mice were compared to parasites that had only been propagated *in vitro*. Parasite load in mice footpads was measured by RT-qPCR using AAP3 as a target. Known virulence factors, such as resistance to complement lysis was measured by flow-cytometry, and expression of GP63 measured by ELISA. To investigate the difference in phagocytosis of promastigotes by neutrophils and monocytes, flow cytometry was used. Phagocytosis by macrophages (RAW 264.7) was measured using light microscopy. To evaluate differential gene expression Illumina HiSeq 125 bp paired-end RNAseq analysis was performed.

3 Results

There was an elevated parasite load in mice footpads infected with *in vivo* propagated parasites. These parasites also had increased expression of GP63 and resistance to complement lysis. There was a significant difference in phagocytosis by macrophages, but not by neutrophils and monocytes. The sequencing data revealed that for the *in vivo* versus *in vitro* passaged parasites, there was 25 upregulated genes involved in 31 pathways, 79 downregulated genes involved in 82 pathways, and 28 pathways were common to the up- and down-regulated genes. The most upregulated gene was GP63.

4 Conclusions

This study confirms earlier studies on the importance of GP63 and resistance to complement lysis for the infectivity of parasites *in vivo*. However, this study could not relate this to functional studies of phagocytosis *in vitro* by neutrophils or monocytes.

C0983 TLR-2 IS PRODUCED IN THE CENTRAL NERVOUS SYSTEM AND PERIPHERAL IMMUNNE SYSTEM DURING CANINE VISCERAL LEISMANIASIS

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1 Background

The innate immune system detects microorganisms including protozooans such as *Leishmania* through pattern recognition receptors (PRRs). Toll-like receptors (TLRs) are PRRs that act in the recognition of microbial structures stimulating a pro-inflammatory immune response. During visceral canine leishmaniasis (CVL) dogs were reported to present barely neurological clinical signs in adition to the systemic alterations, with liberation of inflammatory cytokines and chemokines in the brain. However, there is a limited number of studies related to TLRs during this disease. The aim of this study was to evaluate the presence of TLR-2 in choroid plexi (CP) and lymph nodes (LN) during CVL in animals that presented up-regulation of this gene in these tissues previously.

2 Methods

Slides from nineteen infected dogs were deparaffinised and rehydrated. Immunohistochemistry for detection of TLR-2 in CP and LN was made blocking the endogenous peroxidase with hydrogen peroxide. Antigen retrieval was performed with citrate buffer; slides were treated with 1% Triton X-100 and washed, blocking the non-specific binding sites with fetal bovine serum 2%. The sections were then incubated overnight with goat polyclonal primary antibody (Abcam, cod.ab1655). One slide was incubated without primary antibody and used as a negative control. Biotinylated secondary antibody (polyclonal goat anti-Rabbit; Dako) was applied on each section, followed by an avidin- reagent. Substrate used for detection was DAB. Tissues were counterstained using hematoxylin, and were dehydrated, cleared and mounted. Positive cells for TLR-2 were quantified by counting the total number of immunolabeled cells in five consecutive fields.

3 Results

We detected the presence of TLR-2 in cells in the CP and LN from naturally infected dogs. LN presented a higher proportion ($p<0.05$) of immunostained cells but there was no correlation between the amount of cells in the two tissues ($p>0.05$).

4 Conclusions

Our results indicate that in addition to the increase of gene expression of TLR-2 observed in previous study, inflammatory cells from the CP and lymph node are indeed producing TLR-2 during CVL. Therefore, the data presented here indicate that TLR-2 is at least one of the innate immune sensor activated in the CVL in the peripheral immune system and would be a connection to trigger inflammation in the brain through CP. **Acknowledgments:** Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, grant number 2014/01202-9).

C1018 EVALUATION OF PROINFLAMMATORY CHEMOKINES IN THE CHOROID PLEXUS OF NATURALLY INFECTED DOGS WITH LEISHMANIA

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1 Background

Visceral leishmaniasis (VL) is an anthropozoonosis with a large range of clinical manifestations, including neurological disorders in chronic cases. Nevertheless, the pathogenesis of the cerebral form of VL is still to be elucidated. Brain of dogs with VL presents choroiditis and meningitis, as well glial cells activation and degenerative alterations in neurons. Some previous data support the occurrence of blood-brain barrier and blood cerebrospinal fluid barrier disruption in dogs with spontaneous visceral leishmaniasis and cerebrospinal fluid (CSF) would be an important disseminator of inflammatory stimuli within the central nervous system (CNS). The choroid plexi (CP) are strategically positioned as an interface between the CNS and the peripheral immune system. Additionally to classically known role of CP in generating the CSF, it can enable bidirectional communication between the CNS parenchyma and blood circulation. The aim of this study was to evaluate the gene expression of chemokines (CCL3, CCL4, CCL5 and CXCL10) in the CP of dogs with VL.

2 Methods

For this purpose, fifteen dogs with VL and four uninfected dogs were included in this study. CP were collected from lateral ventricles and fourth ventricle in RNAlater and others samples of brain were collected to be stored in 10% buffered formalin and subjected to routine histological procedures, following by staining with haematoxylin–eosin and histopathological analysis. The RNA was extracted and reverse transcript to cDNA and submitted to RT-qPCR to quantify the gene expression of CCL3, CCL4, CCL5 and CXCL10, using specific primers. G3PDH and RPL32 were used as reference gene.

3 Results

70% of infected dogs presented lymphocytes and plasm cells in the CP. Moreover, we observed that CCL3 was upregulated ($p=0.001$) in the CP of infected dogs. On the other hand, CCL4, CCL5 and CXCL10 presented no differences between groups ($p>0.05$).

4 Conclusions

CP was shown to rapidly respond to the traumatized CNS parenchyma, releasing a wide array of adhesion molecules and chemokines that are essential for leukocyte trafficking. Therefore, our data indicate that CCL3 may be produced by cells of the CP during the canine VL and suggest that it plays a role in the brain inflammation. In contrast, CCL4, CCL5 and CXCL10 seem not to be related to the brain inflammation.
Acknowledgments: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, grant number 2014/01202-9.

C1129 LEISHMANIA MEXICANA DIFFERENTIATION INVOLVES A SELECTIVE PLASMA MEMBRANE AUTOPHAGIC- LIKE PROCESS.

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1 Background

The complex process of *Leishmania* differentiation during its life cycle is accompanied by morphological and metabolic changes, gene expression, organelle modification, surface protein expression and cell size reduction. The importance of autophagy in this process has been highlighted since it leads to changes in the morphology and metabolic capacity of the parasites to adapt to extreme conditions, the removal of redundant or damaged organelles, as well as a survival mechanism under starvation or stress conditions. The aim of the work was to find out, using an in vitro model and different culture media at pH 7.0 and 5.5, how cell size reduction occurs, from an elongated 20 µm motile promastigotes to non motile 5µm rounded amastigotes .

2 Methods

The whole morphological process of differentiation was followed by taking electron micrographs every 24 h until 120 h. Promastigotes grown at 26C pH 5.5 and pH 7.0 and after incubation at 35C pH 5.5 in Liver Infusion tryptose , 199 and RPMI media were washed in cacodylate buffer, fixed in karnosky solution and osmium tetroxide , dehydrated and embedded in Epon resin. Sections were observed under a Jeol transmission electron microscope .

3 Results

Soon after promastigotes were exposed to 35C either pH 7.0 or 5.5 present an ameboidal type form generated by an extensive process of autophagocytosis of the cell membrane- microtubule complex forming deep pockets of different sizes and wideness that close and fusionate inside the cytoplasm leading to the formation of large vesicles surrounded by microtubules , that later depolymerized . This process ended at about 48 h After 72 h cells are quite ovoidal .The differentiation was faster in RPMI than in 199 and slower in Liver Infusion tryptose medium. The flagella was reduced to the flagellar pocket size and large lipid droplets accumulate in the cytoplasm.

4 Conclusions

The cell size reduction is the result of an impressive process of plasma membrane- cytoskeleton complex invagination . We denominate this mechanism as a selective autophagic-like process of the plasma membrane. To our knowledge it is the first time this mechanism has been reported. Differentiation speed was dependent of the culture medium and temperature is the key environmental factor inducing *Leishmania* differentiation. Flagella size reduction is due to the continuos depolimerization of the microtubular assembly. Drugs able to block parasite differentiation could be very important for new leishmaniasis treatment.

C1131 EXPRESSION OF THE ANNEXIN A1 IN CUTANEOUS LEISHMANIASIS PATIENTS

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1 Background

The aim of this study was to quantify annexin-A1 (ANXA1) protein expression in macrophages $CD163^+$ and $CD4^+$ and $CD8^+$ T cells from the skin of patients with cutaneous leishmaniasis (CL) and correlate with histopathological aspects.

2 Methods

This study was performed in 55 patients with symptomatic cutaneous leishmaniasis who were treated at Julio Müller University Hospital [Hospital Universitário Julio Müller (HUJM)], Cuiabá, State of Mato Grosso, Brazil. All patients were infected with *L. brasiliensis*, characterized by ITS1-PCR/RFLP. ANXA1 expression and cell markers were analysed by immunofluorescence.

3 Results

CD163+ macrophages expressed ANXA1 at basal levels in uninfected individuals. However, CD163+ macrophages in infected lesions expressed ANXA1 more abundantly (107.0 ± 2.7AU versus 64.6 ± 3.0AU in uninfected control, $p < 0.0001$). Expression was higher in necrotic lesions (123.5 ± 6.9AU) than in granulomatous (100.0 ± 4.1AU, $p < 0.01$) and cellular lesions (104.6 ± 3.0AU, $p < 0.05$). In all cases, ANXA1 was expressed in the cytosol and cell membrane. ANXA1 was also mainly expressed in the cytoplasm of CD8+ and CD4+ T cells. Expression in CD8+ cells was higher in cellular lesions (121.3 ± 9.0U) than in granulomatous (76.0 ± 11.4AU, $p < 0.05$) and necrotic lesions (77.0 ± 10.4AU, $p < 0.05$). Similarly, expression in CD4+ cells was higher in cellular (123.9 ± 11.6AU) than in granulomatous (87.7 ± 5.2AU, $p < 0.05$) and necrotic lesions (53.7 ± 15.7AU, $p < 0.01$). This difference might be explained by the widespread aspect of lymphohistiocytic and plasmocytic infiltrate.

4 Conclusions

Our study evaluates the differential ANXA1 expression in different histophatological lesions of patients with cutaneous leishmaniasis (CL). The results were very positive; however, all patients were infected with Leishmania brasiliensis. It is possible that other parasite species shows a different pattern of expression. In summary, our data show for the first time that ANXA1 is differentially expressed in macrophages and T cells in lesions due to leishmaniasis, and expression is dependent on the histopathological characteristics of the lesion. We anticipate that future studies will further clarify the regulatory mechanism of cell action of ANXA1 during Leishmania infection.

C1152 CONFIRMATION OF LEISHMANIA (VIANNIA) GUYANENSIS/ LEISHMANIA (VIANNIA) SHAWI SHAWI HYBRIDS CAUSING CUTANEOUS LEISHMANIASIS IN THE AMAZON REGION, PARÁ STATE, BRAZIL

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1 Background

Mechanisms of reproduction and generation of genetic diversity of *Leishmania* organisms has been changed with the demonstration of inbreeding at laboratory and genome sequencing of hybrids isolates. Reports in the literature have showed the occurrence of hybrids in the natural environment, associated with cases of American cutaneous leishmaniasis (ACL).Seven *Leishmania* strains isolated from patients with ACL in the Amazon region of Brazil showed mixed profile between the species *L.* (*V.*) *guyanensis* and *L.* (*V.) shawi shawi* by the phenotypic characterization performed through the use of monoclonal antibodies and the electrophoretic pattern of isoenzymes. From these findings, the present study aimed to confirm the hybrid identity these seven isolates using analysis of polymorphism by cleavage of the polymerase chain reaction product (PCR RFLP) targeting the coding sequence of the heat shock protein (*hsp*70) that enables the distinction between *L.* (*V.*). *shawi* shawi and *L.* (*V.*) *guyanensis*.

2 Methods

Seven isolates (M15983, M15984, M15987, M15988, M19672, M19676 and M19697) were cloned by cultivate in solid medium or limiting dilution. The DNA of clone of each isolate and of reference parental strains was obtained by phenol-chloroform followed by precipitation with sodium acetate and ethanol method. The DNA was quantities and subjected to PC and PCR products were purified and cleaved by the restriction enzyme *Hae*III to detect polymorphisms in this sequence.

3 Results

The results from the cloning of the isolates showed that these were homogeneous populations of parasites.The isolates digestion profile presented two standards, being one heterozygous and another homozygous profile.

4 Conclusions

Our findings confirm that the strains analyzed are true hybrids between *L. (V.) guyanensis* and *L.* (*V.*) *s. shawi* from Amazon region, an area with high occurrence of ACL in Brazil and biodiversity of parasite species, vectors and reservoirs. Further studies will be necessary to describe ecological and epidemiological aspects of these organisms.

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C1166 MOLECULAR CHARACTERIZATION OF LEISHMANIA SPP. ISOLATED FROM PATIENTS WITH AMERICAN CUTANEOUS LEISHMANIASIS FROM AMAZONIAN BRAZIL BY HSP70 PCR-RFLP

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1 Background

The wide variety of *Leishmania* species responsible for human American cutaneous leishmaniasis (ACL) combined with the mechanisms of the host immune response results in a large clinical and immunopathological spectrum manifestations. In Amazonian Brazil, ACL is caused by seven recognized species of *Leishmania*, six of them belonging to subgenus *Viannia* (*L.*(*V.*) *braziliensis*, *L.*(*V.*) *guyanensis*, *L.*(*V.*) *shawi*, *L.*(*V.*) *naiffi*, *L.*(*V.*) *lainsoni*, *L.*(*V.*) *lindenbergi*) and one within the subgenus *Leishmania (L.*(*L.*) *amazonensis)*. Amazon region of Pará is responsible for 41% of cases of ACL in Brazil. Due to the high occurrence of the disease and the diversity of species, the identification of the parasite is of paramount importance for the patient treatment and follow-up. This study aimed to identify strains isolated from patients with ACL by PCR-RFLP targeting sequences of *hsp70* gene (Graça *et al.*, 2012). The aim of this study was to identify strains isolated from patients with ACL by PCR-RFLP targeting sequences of *hsp70* gene

2 Methods

Twenty strains of *Leishmania* sp. were isolated in RPMI culture medium from cutaneous lesions of ACL patients care at Reference Center for Diagnosis of Leishmaniasis, Evandro Chagas Institute, Belem-Pará state, Brazil. DNA extraction was performed with phenol-chloroform followed by precipitation with sodium acetate and ethanol method. DNA was quantitated and subjected to PCR. PCR products were purified and cleaved by the restriction enzyme *Hae*III to detect polymorphisms in this sequence and compare with the reference strains belonging to subgenus *Viannia* and *Leishmania*.

3 Results

Our results showed that six of the twenty species of *Leishmania* were identified as *L. (L.) amazonensis*, five as *L. (V.) braziliensis,* three as *L.* (*V.*) *guyanensis* and six as *L. (V.) shawi shawi.*

4 Conclusions

PCR-RFLP assay targeting heat shock protein (*hsp70)* sequences showed to be a good discriminatory method, allowing the identification of the major species causing ACL in the Amazon region of Para state, Brazil. The demonstrated capacity of *hsp*70 PCR-RFLP to distinguish among closely related *Leishmania* species belonging to *Viannia* subgenus has a great value in this region, since it had not yet efficient molecular tool for differentiation of these species. In view of our findings, we could suggest the HSP70 PCR-RFLP as a possible diagnostic tool to replace MLEE in routine *Leishmania* typing. *Supported by: FAPESP2014/50315-0; LIM50/HC-FMUSP*

C1168 PROTEIN PHOSPHATASE PP2C PARTICIPATE IN THE DEVELOPMENT OF THE FLAGELLUM IN LEISHMANIA MEXICANA PROMASTIGOTES

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1 Background

Leishmania mexicana is the causative agent of cutaneous leishmaniasis. This parasite has a digenetic life cycle, moving between the invertebrate vector as (promastigotes) and vertebrate hosts as (amastigotes). Promastigotes have a long flagellum that emerges from the flagellar pocket, an organelle that is involved in the endocytic and exocytic pathways of the parasite,while amastigotes are characterized by the presence of a short flagellum. *Leishmania spp* flagellum also, plays a key role in attachment to the insect epithelium, and binding to host cells in order to establish the infection. There are some studies about different membrane proteins that regulate diverse functions in the flagellum such as kinases and phosphatases that regulated phosphorylation and dephosphorylation events however, little is known about specific phosphatases that participate in the formation of the flagellum of protozoos

2 Methods

We generated Knock-out parasites in PP2C protein by homologous recombination (KO-PP2C parasites). Two alleles were replaced by resistance genes (puromycin and hygromycin).To confirm that the copies were replaced in the parasites, a Southern-blot was performed. PCR and Western-blot assays were done to identify the gene *LmxM 25.07.50* and phosphatase PP2C, respectively. KO-PP2C parasites were monitored *in vitro* culture and the flagellum formation was observed at different culture days and also the phosphatase activity was tested in total extracts of these parasites.

3 Results

KO-PP2C and Wild type (WT) parasite,s the gene of encoded to PP2C was amplified. Interestingly, KO-PP2C parasites did not show flagellum in the first and second day of the culture, however, the flagellum appeared in the third day of the culture. Additional studies by Fluorescence M and Transmission Electron Microscopy in WT parasites showed that the PP2C was localized along the flagellum and flagellar pocket, we made a Western blot, using parasites with or without flagellum. The results of KO-PP2C parasites without flagellum did not show PP2C protein, however in parasites with flagellum, the protein was detected. The enzymatic activity of PP2C protein did not have correlation with the flagellum.

4 Conclusions

Our results show that PP2C protein of *L. mexicana* promastigotes correlates with the presence or absence of the flagellum in these parasites. It is important to known the specific participation and mechanism of PP2C in the formation of the flagellum. This work was supported by DGAPA-PAPIIT IN220816.

C1186 GENE EXPRESSION IN LESIONS OF PATIENTS WITH MUCOSAL AND CUTANEOUS LEISHMANIASIS.

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1 Background

Tegumentary leishmaniasis (TL) is an important health problem in the state of Bahia - Brazil. Some clinical forms of cutaneous leishmaniasis are known for their difficulty in treatment among them mucosal leishmaniasis (ML). ML is a clinical presentation that appears after the initial treatment of cases of TL, and is difficult to diagnose and its late recognition makes treatment difficult. ML is found in a frequency of about 3 to 8% of TL patients. ML is a highly destructive form and compromises the nasopharynx with important tissue destruction. Its treatment requires the prolonged use of high doses of medication which is associated with important side effects. In this study, we evaluated the profile of gene expression in lesions of the forms cutaneous localized and mucosa in patients from an endemic area of Bahia, which are involved in the pathogenesis of the disease.

2 Methods

This is a cross-sectional study to characterize the immunopathological aspects of lesions of 15 patients with Localized Cutaneous Leishmaniasis (LCL) and 8 patients with Mucosal Leishmaniasis (ML) using nanostring, a modern approach and high-performance to characterize the transcriptome of lesions.

3 Results

Were analyzed the nanostring results samples of each of the tissues: 15 patients with LCL, 7 healthy donors, 8 patients with ML and 8 with healthy mucosa. Among the evaluated genes, 28 were upregulated both in the LCL as the ML, as ICAM-1, Caspase 1, CXCL-10 and Granzymes A and B. However, some 100 genes were identified alone in LCL or ML. The analysis of canonical pathways showed upregulation of the pathway of the TREM-1 and NK-kB. On the other hand, there is a negative modulation of pathways related to activation of the LXR / RXR.

4 Conclusions

In the LCL and ML lesions there is an upregulation of genes which favor increased expression of inflammatory mediators, apoptosis and recruitment of lymphocytes and monocytes to the lesion site. On the other hand, there is suppression of genes that negatively modulate the immune response. As a consequence, there is an intense inflammatory response that appears to contribute to the pathogenesis of cutaneous leishmaniasis.

C1201 ASSOCIATION OF LEISHMANIAVIRUS WITHIN LEISHMANIA BRAZILIENSIS CLINICAL ISOLATES WITH THE PARASITE'S SUSCEPTIBILITY TO OXIDATIVE AND NITROSATIVE STRESS STIMULI

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1 Background

The endosymbiont RNA virus, *Leishmaniavirus* 1 (LRV1), has been frequently identified in *Leishmania braziliensis* and *L. guyanensis*, which cause both cutaneous and mucocutaneous leishmaniasis in the New World. LRV1 presence in *L. guyanensis* was shown to influence disease severity in a murine model. Recently, we found an association of LRV1 with antimonial treatment failure in human *L. braziliensis* infection. It has been hypothesized that LRV1 may confer a survival advantage to its *Leishmania* host, including a diminished parasite sensitivity to oxidative/nitrosative stress conditions. Aiming at exploring this, we focused on *L. braziliensis* isolates with different LRV1 status and tested their susceptibility *in vitro* as intramacrophage amastigotes, to different types of stress

2 Methods

We selected 12 *L. braziliensis* clinical isolates from Peru (previously characterized as 6 LRV1+ and 6 LRV1-) coming from diverse endemic regions. Relative viral transcript levels were estimated using RT-qPCR, amplifying a segment of LRV1 capsid gene; parallel amplification of *Leishmania G6PD* gene was used for normalization (expressed as LRV1/G6PD transcripts). Mouse peritoneal macrophages were extracted and infected with the isolates at a 1:10 ratio. After 24 hours infection, intracellular amastigotes were exposed to different types of stress: indirect oxidative and nitrosative (trivalent and pentavalent antimony – SbIII and SbV) and direct nitrosative (SNAP – a nitric oxide donor), for 48 hours. The 50% inhibitory concentration (IC50) was determined using a colorimetric trypanothione reductase-based assay and sigmoid regression analysis

3 Results

The studied LRV1+ isolates showed a wide range of relative viral transcript levels (from 10 to 350 viral to parasite transcripts). Thus far, we have analyzed IC50s for two isolates from each virotype. For SNAP, the IC50s were 140 and 290 µM (LRV1+) vs 74 and 74 µM (LRV1-); for SbIII they were both >20 µg/ml vs 16 and >20 µg/ml, and for SbV they were 65 and 140 µg/ml vs 140 and 290 µg/ml

4 Conclusions

Thus far, our data suggest that LRV1+ *L. braziliensis* parasites tend to present higher tolerance against nitrosative (SNAP) stress stimulus. As it was previously reported, intrinsic parasite's tolerance to SbV seemed not to be differentially influenced by LRV1 presence. Overall, these results may reflect the parasite's distinct genetic background (non-isogenic isolates were used here) as well as LRV1 status

C1210 THE ROLE OF THE LEISHMANIA MEXICANA A600 PROTEINS IN AMASTIGOTES DEVELOPMENT

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1 Background

One of the foci of *Leishmania* research has been the study of genes that are preferentially expressed at the amastigote stage of the parasite life cycle. These genes may be involved in the parasite survival in the mammalian host and/or in the modulation of the host immune response, resulting in the disease. Elucidating their role may help understand the pathogenesis of leishmaniasis and provide potential targets for the control of the disease. The *Leishmania* genome is constitutively expressed, with very few genes differentially expressed between the promastigote and the amastigote stages. Among those genes are members of the *L. (Leishmania) mexicana A600* gene locus, a family of four tandemly linked genes (*A600.1*, *A600.2*, *A600.3* and *A600.4*) which are necessary for the parasite successful replication in macrophages. The A600 proteins were recently localized to the mitochondria of promastigotes and this study aimed at characterizing their function in amastigotes.

2 Methods

We used a fractionation approach to localize the A600.1 endogenous protein in amastigotes. Mitochondrial metabolic activities of the deletion mutants *L. mexicana* A600$^{-/-}$ amastigotes were assayed and compared to the activities of *L. mexicana WT* (wild-type) amastigotes.

3 Results

We found that the A600.1 endogenous protein was constitutively expressed between *L. mexicana* promastigotes and amastigotes and localized to the mitochondrial fraction of amastigotes. *L. mexicana* A600$^{-/-}$ amastigotes exhibited deficient mitochondrial activities in comparison to the *L. mexicana WT* amastigotes.

4 Conclusions

We concluded that the A600 proteins are essential for the maintenance of mitochondrial function on which the intracellular stage of *Leishmania* is dependent for survival.

C1237 A2 GENE CHARACTERIZATION IN LEISHMANIA LEISHMANIA AMAZONENSIS ISOLATED FROM DOG WITH VISCERAL CLINIC.

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1 Background

Recent studies identified the protozoan *Leishmania Leishmania amazonensis* in cases of leishmaniasis with wide spectrum of clinical manifestations in humans and dogs, ranging from diffuse cutaneous, cutaneous, mucocutaneous and visceral. A large number of molecular markers have been studied for identification of virulence mechanisms in leishmaniasis. Since the discovery of the gene/protein family A2, several evidences indicate that it is one of the most eligible candidates as visceralization factor. It is known that *L (L.) infantum* and *L (L.) donovani* have multiple A2 genes, on the other hand the species that cause cutaneous disease in the Old World have a single copy of A2 gene. The aim of this study was to amplify and sequence the A2 gene of a *L. (L.) amazonensis* isolate from dog with visceral clinic in 2011, in Londrina city, Paraná State, Brazil and to compare the pattern of fragments and sequences generated with *L. (L.) infantum*, *L. (L.) braziliensis* and *L. (L.) amazonensis* strains.

2 Methods

The DNA was extracted from the following isolates: MHOM/BR/75/M2903 (*L. infantum*), MHOM/BR/1987/M11272 (*L. braziliensis*), MCAN/BR/2011/cãoLV01 (*L. amazonensis* visceral isolate) and from an isolate of *L. amazonensis* non characterized by the isoenzymes technique. A PCR using the primers L2 and R3, already described in the literature targeting to A2 *Leishmania infantum* genes, was performed. The different fragments generated by each strain were cutted from the agarosis gel and submitted to Sanger sequencing.

3 Results

The PCR fragments generated for *L (L.) infantum* (320 and 550bp) and *L. (L.) braziliensis* (260bp) was the same already reported. *L. (L.) amazonensis* got a mixed pattern with 260bp band, two bands around 600bp; furthermore, the strain from dog with visceral clinic showed a different band from all others in the height 1500bp. Regarding the sequencing, we have not got any sequence with satisfactory quality, this difficulty has been reported by other authors, maybe due to non-specific amplification.

4 Conclusions

This is the first work of *L. amazonensis* A2 characterization. The design of new primers based on *L. amazonensis* and *L. mexicana* genomes is an alternative to allow the characterization of these proteins. However, studies using cloning and next generation sequencing are necessary to obtain representative sequences and clarify the role of A2 in visceralization cases of *L. amazonensis*.

C1255 CUTANEOUS LEISHMANIASIS IN CHILDREN: A CASE REPORT

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1 Background

Cutaneous leishmaniasis is a protozoaninfectioncaused by severalspecies of genus*Leishmania*. The areas with the highest incidence of cutaneous Leishmaniasis in Paraguay correspond to the new population settlements in forested areas. All age groups are affected; children represent about 10% of the total of the cases.

2 Methods

Descriptive case report study

3 Results

We report a 14 year-old female patient from Mariano Roque Alonso, Central Department, which is a non-endemic zone. She presented two ulcerated lesions on the legs. The diagnosis of cutaneous leishmaniasis was made by the histological study of these lesions and Polymerase Chain Reaction. The patient was treated with Amphotericin b deoxycholate and at the end of therapy showed clinical improvement of the lesions.

4 Conclusions

Cutaneous leishmaniasis is a rare disease in children. The Polymerase Chain Reaction is a sensitive and specific test that with the histological study helps the early detection of the cases for its subsequent effective treatment.

C1304 PHISIOLOGICAL ROLE OF CHOLINE/ETHANOLAMINE KINASE OF L. BRAZILIENSIS

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PECET Colobia

1 Background

The biosynthetic pathways of membrane lipids have been considered as a promissory therapeutic target because of its importance in the cellular structure and physiology. Phospholipids (PLs) are the main component of the cell membranes in *Leishmania* being the phosphatidylcholine (PC) the most abundant PL species. The published genomes of several species of *Leishmania* have revealed the presence of the genes coding for the enzymatic components of the classical synthesis pathways for PC known as the Kennedy pathway in which the choline or choline/ethanolamine (C/EK) kinases catalyzes the first step inside the Kennedy pathway and it has been considered as a therapeutic target in several models like cancer and malaria. In this work we aimed to determine the physiologic effects of the overexpression of the C/EK of *L. braziliensis* (C/EKLbra) in *L. amazonensis* and its possible contribution in the parasite response to several antileishmanial agents.

2 Methods

The full ORF of the C/EKLbra was isolated by PCR from the gDNA of the *L. braziliensis* (UA301) strain and cloned into the integrative vector pIRmcs3(-). The construct C/EKLbra-pIRmcs3(-) was then linearized using *SwaI* and transfected into promastigotes of *L. amazonensis* (PH8 strain) by electroporation. The transfected promastigotes were cloned and characterized and further used for the infection of the macrophage cell line U937 for the drug sensitivity studies.

3 Results

We successfully overexpressed the ORF of the C/EKLbra in *L. amazonensis* cells as probed by Q-PCR and western blot. We found that the overexpression of the enzyme does not affect significantly the growth neither the infectivity of the promastigotes. Importantly the overexpression of the enzyme induced a reduction of the sensitivity of the intracellular parasites to glucantime.

4 Conclusions

No major effects in the cell biology of *L. amazonensis* upon overexpression of the C/EKLbra were observed, supporting previous studies, which indicate that *Leishmania* uses preferably alternative routes as the trimethylation of the phosphatidylethanolamine (PE) for the biosynthesis of PC. Nevertheless, our findings suggest a secondary biological role of the enzymatic components of the Kennedy pathway in response of *Leishmania* parasites to different antileishmanial compounds adding up a new layer of complexity to response of *Leishmania* to antimonials and suggesting an additional biochemical strategy for the development of resistance in the parasite.

C1305 EVALUATING PROTEIN KINASE B/AKT AS THE TARGET OF MILTEFOSINE FOR TREATING LEISHMANIA INFECTION

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1 Background

Miltefosine is currently indicated for the treatment of *Leishmania* infections. Other than *L. donovani* infections, infections by other *Leishmania* species are variably susceptible to Miltefosine. The basis for the differences in susceptibility of *Leishmanias* to Miltefosine is not known. Moreover, the target of Miltefosine in the treatment of *Leishmania* infections is still controversial. This is relevant in light of concerns of the potential emergence of Miltefosine-resistant *Leishmania*. In light of the fact that Miltefosine has been shown to block protein kinase B (Akt) activation in mammalian tumor cells, we hypothesized that host Akt in infected macrophages is the target of Miltefosine in *Leishmania* infections.

2 Methods

Several approaches were developed to determine whether host Akt is the target of Miltefosine in *Leishmania* infections. First chemical inhibitors that selectively block Akt activation were evaluated for their effect on the efficacy of Miltefosine on infected cultures. Then cells stably transfected with a lentiviral vector expressing shAkt. Induction of an siRNA resulted in AKT reduction. If Akt is the target of Miltefosine in established infections, it implies that the activation of Akt is sustained in infected cells. Finally, we determined the distribution of PI(3,4,5)P in infected cells as well as its precursor lipid (PI(4,5)P), using PH-Akt(GFP) and PH-PLCγ(GFP), which are well studied probes for these lipids. Additional, lipid probes were used to fully track the phosphoinositide precursors of the aforementioned lipids.

3 Results

We confirmed that the EC_{50} of Miltefosine that kills *L. donovani* and *L. amazonensis* promastigotes is much higher than the EC_{50} of Miltefosine that is needed to clear intracellular infections. This was interpreted to mean that Miltefosine targets a host cell activity. Akt activity inhibition or Akt knockdown resulted in loss of Miltefosine efficacy. Inhibition of MapKinases or the TLR pathway had no effect on the efficacy of Miltefosine. It was then found that PI(3,4)P or PI(3,4,5)P, are displayed on *Leishmania* PVs (LPVs) suggesting that the LPV is a potential site for sustained Akt activation. Interestingly, the precursor of PI(3,4)P, PI4P which is Golgi-derived was also found on LPVs.

4 Conclusions

Taken together, our studies demonstrate that host Akt is the most likely target of Miltefosine in *Leishmania* infection. With this knowledge of the most likely target of Miltefosine, approaches to test for resistance can now be developed.

C1335 FIRST MOLECULAR CHARACTERIZATION OF LEISHMANIA SPECIES IN WAZIRISTAN: THE WAR EFFECTED TRIBAL ZONE OF NORTHERN PAKISTAN

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1 Background

War, infection, and disease have close relationship. Military operations against terrorist activity in the tribal areas of Khyber Pakhtunkwa (KP), Pakistan has caused the largest human displacement of local people to urban areas. A neglected consequence of this tragedy has been the recurrent epidemics of cutaneous leishmaniasis. Waziristan is a tribal area on Southern border and is under military operation against terrorism since last 4 years.CL is a major and fast increasing public health problem among the local Pakistani population of this area. The Internally Displaced People (IDPs) are main source of bringing CL to other urban areas of KP. Epidemiological and molecular studies could not be conducted in the past during Taliban regime.

2 Methods

During an epidemic outbreak of CL in Waziristan, a large-scale epidemiologic study was carried out from May 2014 to May 2015 to determine the prevalence and molecular diagnosis of the CL in local population. 15230 individuals were surveyed in 25 different villages. A total of 856 CL cases with active CL lesions and scars were identified and 432 skin biopsies were collected from active CL lesions, confirmed by Giemsa stained microscopy and kinetoplastic DNA (kDNA) and finally characterized by ribosomal internal transcribed spacer 1 (ITS1) amplification and subsequent restriction fragment length polymorphism (RFLP) analysis using *Haemophilus* III enzyme.

3 Results

The total prevalence of CL in local population was found to be 5.6%, with active lesions and scar prevalence of 3.4% and 1.6% respectively. The microscopic examination was positive for 224/432 (52%) samples, kDNA PCR positive samples were 423/432 (98%) while ITS1-PCR was positive in 357/432 (82.6%) samples. *Leishmania* species characterizationby ITS1 PCR-RFLP analysis confirmed *Leishmania tropica* in 345/357 (96.6%) of ITS-PCR positive samples. L. *major* was found in 12/357 of positive cases, all of them with travelling history to Afghanistan.

4 Conclusions

This study has resulted in identification and characterization of *L. tropica* species in Waziristan for the first time and confirmed the previous epidemiological reports from Southern KP province that were based on prevalence studies only. We here highlight the urgent need of a regional / national leishmaniasis (vectors and reservoirs) control program in the military operated area. As IDPs from these areas are continuous source of CL to the urban areas of Khyber Pakhtunkhwa, Pakistan.

C1357 RNA-BINDING PROTEINS ARE REGULATED BY ARGININE MONOMETHYLATION IN LEISHMANIA, IMPLICATIONS TOWARD PARASITE VIRULENCE

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1 Background

Arginine methyltransferases (PRMTs) catalyze the post-translational arginine methylation of a wide spectrum of proteins in different cellular processes. *Leishmania* spp. genomes encode five different PRMT homologs, including PRMT7, which is only described in a restricted group of eukaryotes and the only enzyme known to exclusively catalyse monomethyl arginine (MMA). In Ferreira *et al*., 2014 we demonstrated that *Lmj*PRMT7 levels are inversely proportional to parasite virulence.

2 Methods

To identify MMA-modified targets we isolated proteins which associate with wild-type PRMT7 or catalytically inactive forms via crosslinked immunoprecipitation and quantitative mass spectrometry. To evaluate the impact of arginine methylation on protein function, we endogenously tagged target RNA-binding proteins (RBPs) for immunoblotting and RNA immunoprecipitation (RIP) studies. To investigate the impact of PRMT7 levels upon monomethylation in *Leishmania* parasites, we performed methyl-SILAC analysis of wild-type versus PRMT7 knockout parasites.

3 Results

We find that ~4% of the *L. major* predicted proteome carry at least one MMA modification with a significant enrichment of RBPs associated with gene expression and translation. Comparative methyl-SILAC between wild-type and *Lmj*PRMT7 knockout parasites (Δ*prmt7*) revealed the loss of monomethylation on several specific target sites in the absence of the enzyme. Expression levels of three PRMT7 target RBPs reveal differential expression during promastigote development in wild-type versus Δ*prmt7* parasites. RIP experiments with *in vivo* crosslinking (CLIP) are underway which will expose the effect of MMA on RBP RNA-binding function and identify transcript targets potentially implicit in *Leishmania* spp. virulence.

4 Conclusions

Data found in past investigation and ongoing experiments highlight important distinctions between mammalian and trypanosomatids PRMT7 orthologues. Our results suggest *Lmj*PRMT7 epigenetically regulates *Leishmania* parasite virulence by controlling parasite gene expression through altering the function of *trans*-regulatory RBPs via MMA modification.

C1364 THE ZIP3 AND CDF1 TRANSPORTERS, TWO PROTEINS THAT ALLOW LEISHMANIA PARASITES TO ACQUIRE ZINC IN A HIGHLY REGULATED WAY

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1 Background

Transition metals such as zinc are essential to any organism, yet they can be deleterious if in excess. These antagonistic properties of metals are frequently exploited by hosts to control infections through a set of defensive strategies, known as nutritional immunity, which aim either at depriving pathogens of these crucial micronutrients or at directing metal-inherent toxicity towards infective microbes. To start investigating how *Leishmania* oppose nutritional immunity, we focus here on the mechanisms used by these parasites to acquire and maintain zinc homeostasis

2 Methods

A range of biochemical and molecular and cellular methodologies were used to analyse two *Leishmania infantum* proteins with homology to divalent metal transporters.

3 Results

We show that control of zinc homeostasis in *L. infantum* promastigotes involves two zinc transporters one (ZIP3) present at the cell membrane and another (CDF1) expressed in acidocalcisomes. Our data indicate that when the parasite faces zinc restriction, ZIP3 is upregulated and zinc is imported into the cell. On the contrary, if the concentration of zinc in the medium increases, ZIP3 is downregulated. Removal of the ZIP3 from the membrane is, however, a slow process indicating that a second mechanism must be in place to protect the parasite from zinc toxicity during the 6-12 hours that ZIP3 takes to decay. Evidence is presented that this is the role of CDF1 that is rapidly induced upon zinc addition to the medium, allowing for excess zinc entering *Leishmania* to be safely stored within acidocalcisomes. Using CRISPR-Cas9 we are analysing the importance of these two proteins along *Leishmania* development. We know now that ZIP3 is redundant in promastigotes grown in zinc-rich medium but becomes essential in zinc limiting conditions; on the contrary, CDF is essential under normal growth conditions as only one allele can be removed from the genome.

4 Conclusions

The ZIP3 and CDF zinc transporters cooperate ensuring that zinc in *Leishmania* is kept within limits that enable its participation in physiological processes without exerting toxic effects, irrespectively of the amount of metal present in the microenvironment. By comparing the infectivity of parasites in which the expression of ZIP3 and CDF was ablated/modified with that of wildtypes we expect to define the relevance of these transporters for *Leishmania* amastigotes and also to get insight on whether or not zinc nutritional immunity is a host strategy against infections by *Leishmania* (funding:Norte-01-0145-FEDER-000012)

C1391 ANTIGEN PROCESSING AND PRESENTION AS A MECHANISM TO MODULATE CANINE VISCERAL LEISHMANIASIS: AN RNA-SEQ APPROACH.

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1 Background

Dog is the main domestic reservoir of *Leishmania infantum* and shows a wide spectrum of clinical manifestations, varying from mild weight loss to severe hepatosplenomegaly and body spread lesions, classical signs of canine visceral leishmaniasis (CVL). The goal of this study was to evaluate the gene expression profile of spleen and peripheral blood cells from Leishmania-infected dogs and investigate microstructural alterations in spleen, liver, gut and skin in order to determine possible molecular pathways and histopathological process underlying the mechanisms related to disease progression and resistance.

2 Methods

21 animals were recruited to this study at Zoonotic Control Center in Natal-RN and a sub group was selected for transcriptomic studies.Total RNA was extracted and cDNA libraries were constructed and sequenced in an Illumina platform. The reads obtained were filtered and aligned against *Canis familiaris* genome using Bowtie. Differential gene expression was analyzed using the Cufflinks workflow, functional annotation and molecular features for each gene was obtained from Gene Ontology and KEGG database using STRINGdb. A fragment of liver, gut, skin and another spleen fragment were collected, processed and stained by HE and immunofluorescence for histopathological analysis.

3 Results

Approximately, 2,700 genes were upregulated in the spleens of symptomatics animals, as *CTLA4*, *CCL2* and *IL27*, furthermore, about 2,800 genes were downregulated in the same group of animals, as *BMP6*, *C1QB* and *C1QC*. After an enrichment analysis for molecular pathways associated to visceral leishmaniasis pathogenesis, we found that NK cells activation, toll-like and NOD-like receptors pathways is highly expressed in the spleen of symptomatic ones, while GPI-anchors synthesis and immuneprotesome activation related genes is downregulated in spleen of symptomatic dogs and upregulated in the spleen of asymptomatic animals. Additionally, we observed that in symptomatic animals, the cytoarchitecture of spleen is altered, where white and red pulp delimitations is partially lost. We still observed an intense inflammatory infiltrate in the liver, gut and skin of these animals.

4 Conclusions

Taken together, these results suggests that misregulation of mechanisms classically described as acting in innate immunity is involved in CVL pathogenesis and an efficient antigen presentation maybe decisive for resistance to clinical signs development and tissue integrity during *L. infantum* infection in dogs.

C1421 THE TRYPANOSOMATID SERINE/THREONINE PROTEIN KINASE "JEAN3" MAY BE INVOLVED IN DRUG RESISTANCE AND INFECTIVITY.

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1 Background

Protein kinases are known crucial mediators of the transduction of environmental signals and the coordination of intracellular processes. Different protein kinases have been described to be critical for proliferation and/or viability of the clinically relevant stages of the parasites. Our group has identified "Jean3", a constitutively expressed Ser/Thr protein kinase in the trypanosmatid species causative agents of human pathologies. We have studied Jean3 expression during the growth cycle of *Leishmania major* and evaluated its implication in key processes such as infectivity and drug resistance.

2 Methods

Using the expression vector pXG-*LmJean3* we generated Jean3-overexpressing *L. major* parasites. The expression of *Jean3* in the WT and overexpressing strains was measured by RT-qPCR.
In vitro assays with murine peritoneal macrophages were performed to determine the infectivity index of the overexpressing parasites. For the cytokine expression determination in the host cells, Bone Marrow Derived Macrophages (BMM) were also infected.
MTT-based drug screening assays were executed to determine the EC_{50} values of amphotericin B (AMB), miltefosine (MIL), and paromomycin (PMM).
In silico docking was conducted to discover numerous chemical compounds that may interact with this novel kinase.

3 Results

Our analysis confirmed the constitutive expression of Jean3 in *L. major*. Jean3-overexpressing parasites were less infective in *in vitro* and showed lower EC_{50} values for AMB and MIL compared to controls.
In silico docking revealed that PMM interacts with Jean3. This result was confirmed as Jean3-overexpressing strain showed a higher EC_{50} value with respect to the control. Therefore, Jean3 overexpression seemed to induce PMM resistance. To validate our hypothesis, G418 a structurally related aminoglycoside was tested, obtaining very similar results. All these data may support the implication of Jean3 in PMM resistance phenomenon.
Finally, more studies are ongoing to analyze the host's immune response during BMM infections with the strains overexpressing *Jean3*.

4 Conclusions

Infectivity and drug resistance remain critical in the development of new strategies for leishmaniasis eradication. Our results suggest Jean3 kinase to be involved in these processes and particularly in PMM resistance. To date, although no clinical resistance has been reported for PMM, more studies are needed to understand this process.

C1425 A PUTATIVE AMINOACYL-TRNA-PROTEIN TRANSFERASE IS ESSENTIAL FOR LEISHMANIA MAJOR SURVIVAL

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1 Background

The N-end rule pathway leads to regulated proteolysis as an adaptive response to the external stress and it is ubiquitous from bacteria to mammals. An aminoacyl-tRNA protein transferase is the first enzyme and a key component of this pathway; it catalyzes the conjugation of a specific aminoacid to the protein N-terminal residue, addressing the protein to the targeted proteolysis.

2 Methods

In silico analysis was conducted to investigate the possible function of the protein coded by the LmjF.21.0725 gene. Immunofluorescence cell imaging using an antibody anti-Lmj-aa-tRNA-protein transferase was conducted to identify levels and distribution of the protein in *L. major*. Gene replacement and plasmid transfection were conducted using established protocols for *Leishmania* transfection. FACS analysis was used to evaluate kinetics of loss of the plasmid in absence of drug pressure.

3 Results

Although the pathway has not yet been described in *Leishmania*, we have identified a putative homolog of an aminoacyl-tRNA-protein transferase, coded by a gene located on chromosome 21 of *L. major*, Lmj21.0725. *In silico* analysis suggests that LmjF.21.0725 might be a homologue of the bacterial Leucyl, phenylalanyl-tRNA protein transferase. Comparative sequence analysis showed its high conservation among different species of *Leishmania.* Using immuno fluorescence cell imaging, we demonstrated the cytosolic localization of the protein, present at low levels in promastigotes. The knockout of both alleles encoding LmjF.21.0725, by homologous recombination was only achieved when the heterozygous transfectant expressed LmjF.21.725 as a transgene, from a plasmid. Additionally, the kinetics of loss of the plasmid in absence of drug pressure evidenced the essentiality of the gene for promastigotes survival. We are currently investigating the enzymatic activity of the protein and the effect of different forms of stress over the levels of the protein.

4 Conclusions

In our study we provide evidence for the existence and essentiality of this hypothetical protein for the parasite survival. Investigation of the putative aminoacyl-tRNA protein transferase and of a possible N-end rule pathway in *Leishmania major* may contribute to the understanding of targeted proteolysis roles in the parasite.

C1449 NADH OXIDIZING ENZYMES INVOLVED IN MITOCHONDRIAL NADH/NAD+ BALANCE IN LEISHMANIA

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1 Background

Regulation of mitochondrial NADH/NAD+ ratio is essential to attain a suitable metabolic status compatible with cell survival. Leishmania parasites have several enzymes involved in the oxidation of mitochondrial NADH namely the canonical complex I, fumarate reductase (FDR) and the alternative NADH dehydrogenase (NDH2). The expression and the relative contribution of these different systems for the maintenance of mitochondrial redox balance are yet unclear.

2 Methods

Expression of the different enzymes was evaluated in both *Leishmania infantum* and *L. major* through western blot analysis of total protein extracts, BN-PAGE analyses of crude mitochondrial preparations and oxygen consumption assays with intact parasites. Subcellular localization was addressed by a combination of immunofluorescence studies and western blot analysis of protease accessibility upon digitonin fractionation. Gene deletion was generated by homologous recombination.

3 Results

L. infantum FRD and NDH2 proteins are expressed in both the promastigote and amastigote stages of the parasite, while complex I activity was not detected. In *L. major*, all the 3 NADH oxidizing enzymes are expressed in promastigotes although FRD is expressed in higher levels than in *L. infantum* and NDH2 is less expressed in *L. major*.

NDH2 and FRD proteins are located in the mitochondrion. Moreover, overexpression of *Li*NDH2 was found to increase the basal oxygen consumption of intact parasites, thus demonstrating the involvement of this enzyme in the respiratory chain. Parasites overexpressing *Li*FRD display respiration rates similar to the wild type though less sensitive to KCN, a complex IV inhibitor.

Several attempts have been made to delete the *Lifrd* gene without any success until now. Moreover, we found that *Li*NDH2 is essential in *L. infantum*, including in the disease-causing stage, the amastigote. In fact, i) deletion of both *ndh2* chromosomal alleles is only possible upon previous complementation with an episomal copy of the gene, and ii) knockout promastigotes and amastigotes do not lose the *Lindh2* episome after multiple passages in culture in absence of drug pressure, in contrast to what happens with a control episome that is lost after few cycles of parasite replication.

4 Conclusions

L. infantum NDH2 is essential in both forms of the parasite. Present efforts are being directed towards the investigation on the essentiality of these proteins in both *L. major* and *L. infantum*.

C1484 AN OPTIMISED PROTOCOL PIPELINE TO ISOLATE LIFECYCLE STAGE-SPECIFIC POPULATIONS OF LEISHMANIA MEXICANA PARASITES.

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1 Background

Asynchronous lifecycle progression within axenic culture of *Leishmania* spp. parasites often results in mixed populations of different promastigote stages. The difficulty of obtaining pure populations and distinct methods for cell culture and harvests confound clarity and application of results in this research field. Historically, many investigations have profiled lifecycle stages based upon morphology and approximations of cell culture density rather than specific molecular markers and cell counts. It is generally agreed in the field that standardization of methods, reagents and definitive markers of lifecycle stages will benefit all and there is both an increasing need and ability to validate specific stages for direct comparisons.

2 Methods

We have collected, utilized, optimised and refined *in vitro Leishmania* spp. culture methods from the literature to concisely isolate large scale populations of distinct lifecycle stages for characterization. We offer a protocol pipeline to harvest and validate distinct stages based upon proliferative capacity, macrophage infectivity, susceptibility to human serum and molecular markers.

3 Results

We have validated that our pipeline successfully, consistently distinguishes between promastigote populations through large and small scale proteomic and transcriptomic analyses which have verified and expanded known markers. In addition to promastigote stage isolation, we describe a simple, scalable, gradient-free 45% Percoll purification protocol for the isolation of intracellular amastigotes from mammalian cell cultures. 24 hours post-J774.2 macrophage infection with human serum-resistant *L.mexicana* metacyclic forms, utilisation of a gradient-free 45% percoll layer showed significantly higher yields than sucrose or 45/100% Percoll gradients. Amastigote sample purity was confirmed by both optical and transmission electron microscopy (TEM) showing confident isolations. qRTPCR confirmed the expression of stage-specific enriched RNA markers.

4 Conclusions

Inconveniently, some molecular markers vary according to *Leishmania* spp. and species-specific expression patterns have been reported previously and observed in our work. In contrast, the proliferative capacity of the different lifecycle stages appears consistent for all *Leishmania* spp. examined. Using this to distinguish parasites as well as available transcriptomic data to identify stage-specific RNA markers, the pipeline can easily be adapted to *Leishmania* species beyond *L.mexicana*.

C1506 IDENTIFICATION OF LEISHMANIA RNA VIRUS SPECIES IN ISOLATES FROM TEGUMENTARY LEISHMANIASIS PATIENTS

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1 Background

Tegumentary Leishmaniasis (TL) is an infectious disease caused by the protozoa *Leishmania*. The clinical manifestations may vary from Cutaneous Leishmaniasis (CL) to more severe lesions with mucosal destruction such as Mucosal Leishmaniasis (ML). The factors associated with the development of lesions and the severity of the disease are recently related to the Leishmania RNA virus (LRV) infecting the parasite, known as one important factor responsible for the exaggerated immune response in LM patients. Some studies have shown that the presence of the LRV1 within parasites is related to tissue destruction, leading to a worsening process of the lesions, due to an exaggerated response of pro-inflammatory cytokines induced by the dsRNA recognition via Toll Like 3. The LRV is a double-stranded RNA virus (dsRNA) with a 5.3 kb genome which encodes the major viral coat protein and a RNA-dependent RNA polymerase (RDRP). The virus has been described in a few species of parasite and then grouped into the LRV1 Type (divided in 14 subtypes (LRV1-1 to LRV1-14) with significant differences in their genome) for the virus strains found in the New World. Old World strains were grouped into the LRV2 type. The association between the type and subtypes of the LRV with the clinical manifestations of the disease are still not fully understood, therefore it is the main purpose of this study.

2 Methods

A total of 39 isolates of *Leishmania spp* were successfully cultured from TL patients treated at the Rondonia Reference Hospital for Tropical Medicine, CEMETRON. The presence of LVR1 was tested by RT-PCR and subsequent sequencing of the amplified fragments.

3 Results

The presence of LRV1 was detected in 24 of 39 strains distributed among species: *L. braziliensis* (n = 14), *L. guyanensis* (n = 9) and *L. shawi* (n = 1). Ten LRV1 samples were sequenced: 6 from *L. braziliensis*, 3 from *L. guyanensis* and 1 *L. shawi*.

4 Conclusions

Preliminary analyzes of the sequences, when compared with others available on the databases, showed a grouping according to the species of the parasite. The six LRV1 sequences from *L. braziliensis* remain in the same group, whereas *L. guyanensis* samples distance themselves from this group along with other sequences from *L. guyanensis* and approach to the *L. shawi* sequence. This preliminary result suggests that the viruses are related to *Leishmania* species. A greater number of isolates will be analyzed to reinforce the hypothesis.

C1514 ROLE OF LEISHMANIAL ACIDOCALCISOMAL PYROPHOSPHATASE IN THE CAMP HOMEOSTASIS IN PHAGOLYSOSOME CONDITIONS REQUIRED FOR INTRA-MACROPHAGE SURVIVAL

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1 Background

Exposure of *Leishmania donovani* to macrophage phagolysosome conditions (PC) (37^0C and pH 5.5) led to increased intracellular cAMP and cAMP-mediated responses, which help in intra-macrophage survival pre-requisite for infectivity. In the absence of typical orthologs for G-proteins and G-protein coupled receptors, we sought to study the precise mechanisms for positive modulation of cAMP production during exposure to PC.

2 Methods

Upregulation of receptor adenylate cyclase was studied by mimicking phagolysosome condition and the total pyrophosphate pool was also measured in the parasite. Moreover, transmission electron microscopy and fluoroscence microscopy were done to study the translocation of acidocalcisomes in the membrane vicinity for the regulation of adenylate cyclases by acidocalcisomal pyrophosphatases.

3 Results

Amongst two promastigote-stage specific membrane bound receptor adenylate cyclases (LdRAC-A and LdRAC-B), LdRAC-A appeared to function as a major cAMP generator following PC exposure. Pyrophosphate (PPi), an energy storage compound as well as a by-product of cAMP biosynthesis by adenylate cyclase was found to be decreased following PC exposure. This may be due to microtubule and microfilament-driven translocation of acidocalcisomes near plasma membrane vicinity with concomitant increase of acidocalcisome membrane pyrophosphatase (LdV-H^+PPase) and acidocalcisomal soluble pyrophosphatase (LdVSP1). Episomal over-expression and conditional silencing demonstrated regulatory role of V-H^+PPase on cAMP trigger and consequent induction of resistance to macrophage-derived pro-oxidants and parasite killing. Furthermore, immunofluorescence analysis revealed possible co-localization of LdV-H^+PPase and LdRAC-A during PC exposure.

4 Conclusions

Collectively, these results suggest that translocation of acidocalcisome in membrane vicinity functions as a trigger for LdRAC-A-driven cAMP generation through depletion of PPi pool by LdV-H^+PPase.

C1526 THE HYDRO-EXTRACT OF ZINGIBER OFFICINALE CAN SILENCE IMPORTANT GENES OF LEISHMANIA MAJOR [MRHO/IR/75/ER]

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1 Background

Background: Leishmaniasis is a complicated disease induced by an obligate intracellular parasite from the genus *Leishmania*. Although experimental studies regarding the production of an effective vaccine to control Leishmaniasis have been extensively conducted over the past decades, there is still no vaccine against any form of leishmaniasis for general human use. Since vaccines against this parasite are not yet in sight, its control relies mostly on chemotherapy, so, there is an urgent need to develop new and better drugs to combat this infectious disease, The present study was carried out to investigate the hydro-extract of *Zingiber officinale* can silence important genes of *Leishmania* major[MRHO/IR/75/ER].

2 Methods

Methods: First, the promastigotes of *Leishmania major* [MRHO/IR/75/ER] were separately incubated with serial concentrations of HEZO for 24, 48, and 72^{h} at $37?^{C}$. Then, the cell viability of promastigotes was evaluated by MTT assay. On the other hand, the relative expression of *Cpb* and GP_{63} genes was detected by quantitative-real time PCR.

3 Results

Results: The increase of concentration and incubation time led to decrease of cell viability. Moreover, the expression of *Cpb* and GP_{63} genes was decreased with increase of concentration. Interestingly, the increase o incubation time could not affect the expression.

4 Conclusions

Conclusions: It could be concluded that HEZO can lead to death of *Leishmania major* promastigotes. Also, this extract can silence both important genes, e.g. *Cpb* and GP_{63} in *Leishmania major* [MRHO/IR/75/ER].

C1585 PROTEOLYTIC ACTIVITY IN THE EXOPROTEOME OF LEISHMANIA MEXICANA AMASTIGOTES

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1 Background

Leishmania survival requires extracellular factors to interact and modify host environments. Proteases have been suggested as keys in the survival of parasite during its life cycle. Structural differences between Leishmania and eukaryotes proteases advised them as potential chemotherapeutic targets. Even though several promastigotes exoproteome proteases have being characterized in Leishmania, little is known about amastigote exoproteome proteases becoming interesting its identification and characterization. Our aim is to study the presence of proteases in *L. mexicana* exoproteome using an axenic amastigotes model.

2 Methods

L. mexicana Bel 21 strain was cultured at 25°C in LIT media plus 5% fetal bovine serum (FBS). Reaching stationary phase, one volume of fresh LIT media plus 10% FBS was added and incubated at 35°C for 10 days for amastigote-like transformation. Exoproteome was obtained incubating 2 x10^9 parasite/ml in RPMI media plus 5% FBS at 35°C for 24 h. Supernatant with the exo-proteins was recovered removing parasites by centrifugation. Proteolytic activity was visualized in Coomassie Blue staining zymograms, employing 12% SDS-PAGE/0.1% gelatin-copolymerized gels, incubated in Buffer PD (Buffer phosphate 100 mM, pH 7, DTE 2 mM) plus $MgCl_2$ 0.5 mM, $ZnCl_2$ 0.1 mM and $CaCl_2$ 0.1 mM for 24 h at 37°C. For identifying metallo- and cisteine-proteases, zymograms were developed in presence and absence of EDTA and E64. The effect of divalent cations on the exoproteome proteolytic activity was evaluated in a similar assay but incubating the gel in Buffer PD with one of the above cations at a time.

3 Results

We report presence of proteolytic activity in *L. mexicana* amastigotes exoproteome. Protease activity was detected as a band group of high-molecular weight (MW) (200-100 kDa) and five discrete bands of low-MW (50-25 kDa). E64 inhibited the low-MW activities whereas EDTA was stimulatory. The result indicates that these activities are due to presence of a cysteine protease family. Interestingly, the stimulatory effect of EDTA suggests that low-MW activities are inhibited by divalent cations. The study of individual cations in presence/absence of EDTA showed that Zn^{2+} entirely accounts for the effect.

4 Conclusions

Proteolytic activity was evidenced in *L. mexicana* amastigote exoproteome. Cysteine-zinc sensitive proteases were found among this activity.

C1621 CHARACTERIZATION OF THE ENOYL-COA HYDRATASE/ISOMERASE PROTEIN OF LEISHMANIA INFANTUM

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1 Background

Most treatments currently available against leishmaniasis present several disadvantages such as toxicity, drug resistance and recurrence. Therefore, there is a need to develop new treatments or strategies able to prevent the onset of the disease. One of these possible strategies is vaccination.

We have selected a specific gene that encodes for a *L. infantum* enoyl-CoA protein (ECH) (LinJ. 29.2420), over-expressed during the most infective stage of the parasite, at metacyclic promastigotes, isolated through negative selection with peanut agglutinin (PNA) (Alcolea et. al, 2009). This expression profile may be related with the infectivity of the parasite.

The ECH has both hydratase and isomerase activities which are involved in the ????-oxidation pathway. The ECH hydratase catalyzes the syn hydration of *α,β*-unsaturated-CoA. The ECH isomerase catalyzes the conversion of cis-3-enoyl-CoA or trans-3-enoyl-CoA into trans-2-enoyl-CoA.

2 Methods

This gene has been cloned using the restriction enzymes BamH I and Hind III, in the expression plasmid pQE30 in *E. coli* cells, and optimal expression conditions of the encoded protein were 2h at 37°C. To obtain *L. infantum* transfected parasites the gene was also cloned in the pIRmcs3 plasmid using the enzymes Bgl II and Ned I. A polyclonal antibody was obtained by infecting two New Zealand rabbits, using 600µg of purified protein.

3 Results

The recombinant protein has been purified by affinity chromatography using a Ni^{2+} column. This protein forms hexamers, made up of two trimers. The expression of the ECH has been measured along the biological cycle of *L. infantum* from procyclic to metacyclic promastigotes.

Using the I-Tasser, PyMOL and BlastP software's together with the PDB database we predicted a protein 3D model. Using ClustalW software we were able to analyze nucleotide and protein homology. The nucleotide sequence was aligned with orthologous sequences, giving low percentages of homology with the mammalian proteins, and very low with the human one (6%).

4 Conclusions

The enoyl-CoA gene overexpressed on the most infective stage of *L. infantum* promastigotes has been cloned and the recombinant protein has been purified using native and denaturant conditions. The protein expression and the use of parasite transfectants will lead us to assess the relation of the protein with the infectivity of the parasite. The homology percentages and the predicted protein structure suggest that this could be a good candidate as a therapeutic target.

C1659 IDENTIFICATION OF LEISHMANIA SPECIES IN PATIENTS ATTENDED AT THE NATIONAL INSTITUTE OF HEALTH

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1 Background

In Peru, leishmaniosis is a serious problem in public health. There are endemic areas on the coast, highland and jungle. This disease is caused by a parasite of the *Leishmania* genus and presents clinically as cutaneous leishmaniosis (LC) and mucocutaneous leishmaniosis (CML), according to the affected areas of the body.
The High Resolution Melting (PCR-HRM) technique is based on the amplification of a conserved region of parasitic kDNA, which allows the identification of *Leishmania* species in real time. This is rapid and reliable method to carry out in parasite-host association studies, both in the human and vector of endemic areas of leishmaniosis.
Objective: Identify *Leishmania* species in samples from lesions of patients attended at the National Institute of Health, employing the PCR-HRM technique.

2 Methods

The identification of species of *Leishmania* was performed of 133 samples of lesions compatible with leishmaniosis, of patients who were attended at the National Health Institute from 2006 to 2011. For the analysis we used Stata v. 13.0.

3 Results

Of the 133 patients, 64.7% were male, between 18-24 years old (38.3%), 95.5% had a cutaneous clinical form and 4.5% had a mucocutaneous clinical form.
The species identified were 37% *Leishmania (Viannia) guyanensis*, 27% *L. (V.) peruviana*, 14%, *L. (V.) braziliensis*, 10% *L. (Leishmania) amazonensis*.
The evolution of the lesions were 3 to 6 months (69.8%), the 48.1% lesions compromised the upper extremities, 63.2% had a single lesion, 28.9% had a lesion diameter of 20 to 30 mm. The majority of cases came from the jungle area (60.9%).

4 Conclusions

In this study, we found the species *L. (V.) braziliensis, L. (V.) guyanensis, L. (L.) amazonensis*, were identified in mucocutaneous forms.
It was evidenced dispersion of species of *Leishmania* in new geographic areas not previously reported as *L. (V.) braziliensis* in La Libertad department; *L. (V.) lainsoni* in Ancash, Junín and Lima; *L. (L.) amazonensis* in Lima, Loreto, Cusco and San Martin; *L. (V.) guyanensis* in Ayacucho, La Libertad and Loreto.

C1669 MOLECULAR IDENTIFICATION OF CUTANEOUS LEISHMANIA IN SMEARS PREPARED FROM PATIENTS REFERRED TO HEALTH CENTERS OF ABARKOUH, ARDAKAN, BAFGH AND KHATAM, IRAN BY DIGESTION OF THE RDNA-ITS1 GENE

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1 Full text

2 Background

Leishmania genus is cause aspectrum of diseases, ranging in severity fromspontaneously healing skin lesions to fatal visceraldisease. The disease is a significant health problem inmany parts of the world including Iran. To date, there are no Vaccines against Leishmaniasis and control measuresrely on to alleviate disease and on vector control to reduce transmission. The aim of this study was Molecular identification of Cutaneous Leishmaniain smears prepared from patients referred to health centers of Abarkouh, Ardakan, Bafgh and Khatam, Iran by digestion of the rDNA-ITS1 gene.

3 Methods

In this study, through culture and microscopic slides using PCR-RFLP method was studied strains isolated from patients with leishmaniasis. RFLP analysis was used to determine the species. Enzymes used for RFLP, Hae ??? (BsuRI) is. To check product quality and product RFLP PCR and agarose gel electrophoresis was used for final data analysis SPSS 21 statistical software was used.

4 Results

Total results positive Cutaneous Leishmaniasis patients referred to health centers, most of the cases belonging to the city duck with 21 cases (8/36%) and the lowest at 7% (2/12%) owned the city seal. 10 (11.1%) of the samples were positive by microscopic molecular methods were negative and 15 cases (6/16%) of the samples that were negative by microscopy positive was diagnosed with molecular methods using statistical tests chi-square, between the microscopic and molecular methods was significant relationship ($p = 0.000$).

5 Conclusions

PCR-RFLP technique is an effective, quite high sensitivity and specificity method to determine Leishmania parasite Species in Giemsa stained.

C1739 BIOLOGICAL CHARACTERIZATION OF THE GEN LPAL13_220016300 IN LEISHMANIA PANAMENSIS

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1 Background

Leishmania during the infection process undergoes a morphological change from promastigote to amastigote, which is induced by the temperature and pH of the phagolysosome. This process involves biochemical changes characterized by the regulation of the genic expression. In this work we evaluated the differential regulation profile of the Leishmania panamensis stages by 454 sequencing. We selected the LPAL13_220016300 gene, as it presented 13.2 times more reads in amastigotes than promastigotes. This gene was annotated as gene coding for a hypothetical protein. Therefore, we investigated some biological features of this protein in Leishmania.

2 Methods

We made a computational characterization of the protein through on-line programs. Furthermore, differential expression of protein by Western blot and RT-qPCR was confirmed. We evaluated the level of transcripts at the moment of the Infection and during the differentiation of amastigotes to promastigotes. Finally, cell localization of the protein was determined in the two stages of the parasite.

3 Results

Bioinformatics analyzes showed a MAK16-L28e family domain and signal localization nuclear (SLN) sequence. Also, this protein contains a conserved zinc motif and shows a phosphorylation target for CK2. The expression analysis showed that the amastigotes presented higher expression, supporting the mRNA obtained by RNAseq and qRT-PCR. During the infection and differentiation processes the gene is also differentially expressed in amastigotes. Finally, the protein was found in nuclei in both stages.

4 Conclusions

This study suggests that this protein is probably involved in the process of adaptation to the macrophage environment, since its levels are altered at the time of infection and these are reestablished when reversing the phenotype to promastigote. The mechanism of action of this protein is still unknown, but the hypothesis arises that this protein, having a protein- (protein-DNA-RNA) interaction mediated by the zinc domain, is directly or indirectly affecting the expression of some genes involved in the process of adaptation and differentiation of the parasite.

C1771 SENSITIVITY OF PCR IN PERIPHERAL BLOOD FOR DIAGNOSIS OF CANINE VISCERAL LEISHMANIASIS IN ENDEMIC AREA OF BRAZIL

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1 Background

Visceral Canine Leishmaniasis is a serious and fatal zoonotic disease of wide distribution in Brazil, and has presented a very significant growth in recent years. For years treatment was prohibited, and the euthanasia was the main measure of control. Today with the release of the treatment, it is necessary to use a direct and indirect methods to accompany the different types of treatments. In this sense, the objective of the study was to evaluate the sensitivity of the Polymerase Chain Reaction (PCR), compared to the ELISA and/or DPP serological rapid test.

2 Methods

The study included 420 whole blood (with EDTA) samples from dogs from Campo Grande (Mato Grosso do Sul State), positive for Visceral Leishmaniasis in the ELISA and/or DPP rapid test, and clinical diagnosis. The samples were collected from May to July/2016. 300 µL of peripheral blood was used for the extraction of genomic DNA by the in house protocol with SDS 20%. PCR was performed with LEISH-1/LEISH-2 primers amplifying a 120-bp fragment. The amplification products were electrophoresed on agarose gel and stained with ethidium bromide.

3 Results

The sensitivity of PCR in peripheral blood was 50.2%, while the combination of serological tests was 100.0%.

4 Conclusions

For the diagnosis of Canine Visceral Leishmaniasis the combination of positive serological tests (ELISA and/or DPP), and clinical examination are more indicated than peripheral blood PCR. Acknowledgments to FUNDECT for financial support.

C1776 VIRTUAL SCREENING IDENTIFICATION OF NOVEL SELECTIVE INHIBITORS FOR LEISHMANIA (LEISHMANIA) MEXICANA 6-PHOSPHOGLUCONATE DEHYDROGENASE

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1 Background

The arsenal of drugs available against Leishmania infections is limited, generally are associated with potentially severe side effects and, until now, their mechanisms of action are not very clear. Furthermore, drug-resistant Leishmania parasites have developed in most endemic countries. To address an urgent need for new, safe and inexpensive anti-leishmanial drugs, we used *in silico* approaches with *Leishmania (Leishmania) mexicana* 6-phosphogluconate dehydrogenase enzyme (Lm6PGDH) involving homology modeling, molecular dynamics and structure-based methodologies. Lm6PGDH is a key enzyme of the oxidative branch and a potential drug target. Differences in the structure of the enzyme when compared with the human counterpart, were exploited for the evaluation of small molecule inhibitors through *virtual screening* by molecular docking.

2 Methods

Structure-based virtual screening was performed by docking a library of 780 compounds at the active site of *L. (L.) mexicana* and *Homo sapiens* 6PGDH enzymes using Autodock Vina and Vegazz software, followed by a refinement stage of the binding mode based on molecular dynamics simulation. ZINC and Malaria Box databases were filtered to include only compounds with 0.7 similarity to inhibitors previously reported for Tb6PGDH. The Lm6PGDH enzyme was obtained through homology modeling and molecular dynamics simulations.

3 Results

Thirteen selective promising molecules against Lm6PGDH were obtained. Compounds with higher affinity for the Lm6PGDH enzyme interact with residues Y194, R454(B) and H460(B) at the active site and could be important for the complex stability. Significant structural differences at the Lm6PGDH and Hs6PGDH enzymes active sites were identified and can be further exploited for the development of new drugs.

4 Conclusions

Structural differences that were found in the enzymes active sites clearly suggest that 6PGDH can be selected as a potential target for the development of new therapeutic drugs

C1784 CHARACTERIZATION OF A GLYCOSOME ASSOCIATED AMASTIGOTE SPECIFIC HYPOTHETICAL PROTEIN IN LEISHMANIA DONOVANI

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1 Background

Leishmania donovani, a protozoan parasite is the causative agent of fatal disease visceral leishmaniasis (VL). In the *Leishmania* life cycle, motile Promastigote forms that reside in the gut of the sand fly vector are transmitted to a mammalian host during a blood meal. Host macrophages ingest the parasites, which must differentiate into the non-motile, amastigote form to persist in the macrophages. Expression of amastigote specific genes have major role in allowing the parasite to proliferation in the macrophages. With current drugs against VL yielding drug resistant parasites and with no available vaccines against human VL, elimination of this disease has become a major challenge. With the focus towards development of drug or vaccine candidates, we aimed to characterize one amastigote stage specific gene named 'Ld30b' in *L. donovani*.

2 Methods

Recombinant Ld30b protein was expressed in E.coli using 'Sumo' expression vector and was purified by ni-NTA affinity chromatography. The polyclonal antibodies raised against the recombinant protein in mice, were used for detection of protein in the parasite lysate by Western blot analysis as well in protein localizations studies. Episomal expression of Ld30b fusing with HA-tag was attempted in the parasites to look for dominant negative effect in its function.

3 Results

The anti-30b antibodies transcribing identified a 14 kDa endogenous protein in the parasite lysate correlating to its known coding sequence through Western blot analysis. Both the recombinant proteins and the endogenous proteins of Ld30b existed as multimeric form (pentamer) in the non-denaturing poly-acrylamide gel electrophoresis. Co-localization Immunofluorescence analysis (IFA) using anti-30b and Anti HGPRT (Glycosome marker) suggested that the protein is localized in Glycosome organelles. Episomal expression of this protein in the parasite caused arrest of cell growth. Cell cycle analysis identified both the promastigotes and axenic amastigotes cells arresting in 'G0' cell cycle phase.

4 Conclusions

We have identified Ld30b, an amastigote specifically expressing hypothetical protein, to be a multimeric glycosome associated protein involved in cell division cycle. In the current post-genomic era, studying such genes that are specifically expressed at the intra-cellular stage with novel function in parasite virulence will unravel novel regulatory pathways for the development of drugs/vaccine candidates against Leishmaniasis.

C1835 DETECTION OF LEISHMANIA (LEISHMANIA) INFANTUM CHAGASI IN DOMESTIC FELINE (FELIS CATUS) OF THE ENDEMIC REGION OF BRAZIL.

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1 Background

Visceral leishmaniasis has spread in Brazil and in the municipality of Araguaína, located in the northern state of Tocantins, and had in 2010 and 2011 canine infection rates of 47.71% and 51.37%, respectively. Studies have reported their occurrence in domestic cats in different regions of Brazil. The objective of the research was to evaluate the infection in domestic cats in the city of Araguaína, considered an endemic region of the zoonosis.

2 Methods

Random blood samples were collected from 55 individuals from the Zoonosis Control Center of the municipality between March 2015 and April 2016. The samples were submitted to a DNA extraction procedure by GenElute Mammalian Genomic DNA Miniprep Kit (Sigma-Aldrich®), according to the manufacturer's recommendations. Molecular detection of Leishmania infantum chagasi was performed using standard PCR using primers RV1 (5'-CTTTTCTGGTCCCGCGGGTAGG-3 ') and RV2 (CCACCTGGCCTATTTTACACCA 5'-3') using the methodology described by Lachaud et al. (2002), with modifications. Of the 55 animals, 34 (61, 8%) were females and 21 (38.2%) males and at clinical examination, 34 had at least one of the following clinical signs: cutaneous lesions (18/55; %), lymphadenopathy (26/55, 47, 27%) and ocular lesions (4/55, 7.27%).

3 Results

Of the 55 animals analyzed, only one animal (male, adult, with no apparent symptoms) was detected as positive (1/55, 1.82%). The remaining 54 cats were negative (54/55, 98.18%). This result, although low, demonstrates the presence of the agent in the species investigated.

4 Conclusions

Considering the complexity of the multifactorial nature of leishmaniasis, the results point to the importance of monitoring the health of these animals and the need for specific control measures aimed at the prevention of infection and its potential transmission for humans.

C1883 KINETIC CHARACTERIZATION AND DESIGNING OF INHIBITORS AGAINST GLCNAC-6-PHOSPHATE DEACETYLASE ENZYME FROM LEISHMANIA DONOVANI

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1 Background

Leishmania spp. comprises an important group of trypanosomatid parasites that are transmitted by sand fly vectors and cause a range of diseases in humans. *Leishmania* resides primarily in macrophages throughout the infection in their mammalian hosts. Amastigote form of *Leishmania* residing in macrophages has to face the hostile environment of parasitophorous vacuole. The parasitophorous vacuole is glucose deficient nutrient limited acidic vacuole. Recent studies have shown that amino sugars like N-acetyl glucosamine(GlcNAc) and glucosamine (GlN) released from the turnover of host glycans, glycoproteins, and proteoglycans inside the parasitophorous vacuole are used as major carbon and energy source by amastigotes of *Leishmania*. Hexokinase (Hxk), GlcNAc-6-P deacetylase(NAGD) and GlcN6P deaminase (GND) are the three enzymes involved in GlcNAc catabolic pathway from simpler prokaryotes to higher eukaryotes. Genes for all these three enzymes are present in *Leishmania*. Mutants of pathogens, like *Leishmania, Candida albicans, Vibrio cholera, Magnoportha,* for GlcNAc catabolic pathway show the attenuated virulence both in cell lines and mice. So we have designed the inhibitors for NAGD to block the GlcNAc catabolic pathway.

2 Methods

NAGD was cloned in pET-28a (+). Expressed protein was purified using the Ni-NTA matrix. The Modified colorimetric assay developed by Leloir etal was used for the estimation of N-acetylglucosamine-6-phosphate. Using the standardized colorimetric assay for the GlcNAc-6-P deacetylase enzyme K_m and V_{max} values were determined. Insilico tools like PYMOL, Maestro, LigPrep, CASTP server, Glide Docking, etc. were used for selecting the inhibitors. Inhibitors were selected by the docking score.

3 Results

K_m value for the enzyme was found to be 1.17mM while V_{max} value obtained was 0.06552 µM/mnt. Selected inhibitors were checked for their inhibitory role against the enzyme under in-vitro conditions, and they were found to block the enzyme activity. IC_{50} values for the inhibitors were determined. Inhibitors were found to be working in nanomolar and micromolar range.

4 Conclusions

Enzymes are important drug targets. These inhibitors can be used to block the GlcNAc catabolic pathway which will deprive the parasite of the energy source inside the macrophage. Thus in a way these inhibitors can be used to attenuate the virulence and pathogenicity of *Leishmania*.

Vector biology and leishmania-sand fly interactions

C0067 DETECTION OF LEISHMANIA(V) GUYANENSIS DNA IN RHIPICEPHALUS (BOOPHILUS) MICROPLUS REMOVED FROM PECARI TAJACU LIVING IN THE JUNGLE

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1 Background

Several reports have previously reported the presence of *Leishmania infantum* in. "*Rhipicephalus sanguineus*" and show the possibility that "*Rhipicephalus sanguineus*" transmit leishmaniasis to a variety of hosts.

2 Methods

Eighty-one individual DNA extractions were performed using ticks collected from three *Tapirus terrestris* and three *Pecari tajacu* hunted in Madre de Dios, Peru.Afterwards, the ticks were prepared for kDNA PCR and HRM PCR to identify *Leishmania* sp., collected from *Tapirus terrestris* "tapir or sachavaca" and *Pecari tajacu*. The ticks were taxonomically classified.Using molecular tools, we identified kDNA of the pathogenic *Leishmania* sp in three wild ticks of *Rhipicephalus (Boophilus) microplus species,* collected from *Pecari tajacu* "sajino" hunted in the jungle of Madre de Dios.

3 Results

The HRM analysis showed that one of them had a curve compatible with *Leishmania (V.) guyanensis*.More steps are necessary in order to ascertain that wild ticks, mainly *Rhipicephalus (boophilus) microplus,* are vectors of *Leishmania* sp.

4 Conclusions

The results showed the presence of DNA of *Leishmania (V.) guyanensis* in *Rhipicephalus (Boophilus) microplus* presumably acquired after biting *Pecari tajacu.* Thus it is important to design future studies that may conclusively link the tick as a true vector, and *Pecari tajacu* as host, of leishmaniasis.Usually, humans are exposed to ticks while they are in the jungle, or when they are near domestic animals such as dogs that may have acquired ticks while being in the forest before returning to urban area.

C0076 LEISHMANIASIS IN TURKEY: FIRST CLINICAL ISOLATION OF LEISHMANIA INFANTUM / LEISHMANIA DONOVANI HYBRID STRAIN FROM A HUMAN VL AND CANINE LEISHMANIASIS CASE AND DNA OBTAINED FROM SAND FLIES (PHLEBOTOMUS TOBBI) IN THE NATURE

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1 Background

Visceral Leishmaniasis (VL) is mostly caused by *Leishmania infantum* or *L. donovani* in the Old World. Kala-azar is a zoonotic infection, dogs act as the main reservoirs and the vectors are the female *Phlebotomus*. In this study, we aimed to investigate the clinical propagation of the disease in humans and dogs infected with *L.infantum/donovani* hybrid and search for parasite DNA in the *Phlebotomus* using molecular methods.

2 Methods

27 year old male patient presented; fever for 6 weeks, chills-shivers, night sweating, weakness, leg pain, loss of appetite, epistaxis, weight loss, splenomegaly, LAP and pancytopenia. The physical examination of the dog revealed; conjunctivitis, blepharitis, weight loss, fatigue, loss of appetite, skin ulceration, onychogryposis, epistaxis, alopecia, LAP and splenomegaly.
Bone marrow samples of the patient and lymph node aspirates of the dog were inspected for *Leishmania* amastigotes under light microscopy. Furthermore the samples were cultured in enriched NNN medium.
By implementing light traps, 52 *Phlebotomus* were caught. The female flies' tissues were crushed for DNA isolation. *Leishmania* molecular identification were executed by primers and probes specific to ITS-1 and cytochrome B gene region of *Leishmania* parasites employing real time online PCR and melting curve analysis using the DNA obtained from the patient, dog and Phlebotomus.

3 Results

Leishmania amastigotes were detected in the slides prepared from the bone marrow and lymph node aspirates while promastigotes were present in the enriched NNN medium. *Phlebotomus* species were *P.similis*, *P.neglectus/syriacus*, *P.papatasi* and *P.tobbi*. Molecular analysis showed a similar *Leishmania* strain in the patient, dog and *P.tobbi.* Melting curve analysis revealed 2 piques in all three samples thus leading to the conclusion that the infecting strain was a hybrid of *L.infantum* and *L.donovani.* Sequence analysis of the obtained DNA were compatible with both *L.infantum* and *L.donovani*.

4 Conclusions

According to our findings it is assumed that *L.infantum* and *L.donovani* have formed a hybrid strain which is causing severe VL in both humans and dogs and the natural vector of this strain is *P.tobbi.* These findings are attributed to the mass population movements from *Leishmania* endemic countries into Turkey. The molecular and other experiments with the *L. infantum/donovani* hybrid strain are still ongoing.
Acknowledgement: We would like to thank Dr. Albert Bart and Prof. Tom van Gool from Amsterdam University and CBU Parasite Bank and Air Liquide company.

C0082 IDENTIFICATION OF THE COMMON PALESTINIAN PHLEBOTOMINE SAND FLIES USING MOLECULAR BIOLOGICAL METHODS

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1 Background

The 12S rRNA PCR followed by a reverse dot blot (RDB) assay and restriction fragment length polymorphism (RFLP) were assessed for identifying the most common Palestinian species of *Phlebotomus*

2 Methods

The 12S rRNA PCR yielded a product of approximately 165 bp for all the phlebotomine sand flies examined. A biotin-labelled 12S rRNA amplicon was hybridized to phlebotomine genus-specific and species-specific oligonucleotide probes immobilized onto a membrane.

3 Results

The phlebotomine species could be distinguished by using six probes: one that was genus-specific that hybridized to the sand flies of all the different phlebotomine species; one that detected sand flies of the species *Ph. Papatasi*; one that detected sand flies of the species *Ph. sergenti*; one that detected sand flies of the species *Ph. neglectus*; one that detected sand flies of the two species *Ph. neglectus and Ph. perfiliewi*; and one that detected sand flies of the species *Ph. tobbi*. Digestion of the amplicon common to all the phlebotomine species examined with the restriction enzyme Mn1I produced two fragments one of 150 bp the other of 16 bp. Digestion of the amplicon specific to *Ph. sergenti* with the restriction enzyme Mn1I produced three fragments of 106, 43 and 16 bp. Attempted digestion of the amplicon common to all the phlebotomine species examined with the restriction enzyme Hinf1 did not lead to its fragmentation. However, it did digest the amplicon specific to *Ph. papatasi*, producing three fragments of 109, 30 and 16bp, and the amplicon specific to *Ph. mascitti*, producing two fragments of 141 and 26 bp.

4 Conclusions

This new method provides what appears to be a useful means for large scale molecular biological identification of phlebotomine sand fly species caught in foci where leishmaniases are endemic, aiding epidemiological surveillance of the vectors and their control.

C0086 DEVELOPMENT OF PHLEBOTOMUS ARGENTIPES COLONY AND ITS APPLICATION IN XENODIAGNOSTIC STUDIES

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1 Background

Visceral Leishmaniasis is one of the most severe forms of vector borne infectious disease. It is transmitted by the bite of *Leishmania donovani* infected sand flies. In India, VL is endemic in Muzaffarpur district of Bihar state. The known vector of the disease is *Phlebotomus argentipes*. For conducting any experimental disease transmission study, the crucial requirement is a specific pathogen free vector colony.

2 Methods

Requisite for initiating and establishing a permanent sand fly colony were: construction of a proper insectary facility, procurement of equipment and supplies, as well as training of personnel to perform the complex and labor-intensive procedures required to support the colony. From March through mid-December 2015, sand flies were collected from human dwellings and cattle sheds using 30 light traps for 254 nights (7,620 trap nights). A total of 68,601 flies was collected (37,397 males and 31,204 females). From 13,348 females set up in isoline vials for oviposition, 2,598 clutches averaging 28 eggs each were harvested, approximately 90% of which hatched. Progeny were reared according to standardized methods, providing a continuous critical mass of F1 males and females to stimulate optimal social feeding behavior. To insure the purity of the colony as *P. argentipes* and to certify it free of pathogens, we performed PCR. To check its potencial to transmit parasites, we performed artificial membrane feeding experiment. We used serial diluted parasites and mixed with fix volume of blood to know the limit of parasite that required to develop sand fly infection.

3 Results

Presently, the colony produces 1500-2000 blood fed females per week. We found more than 20 parasites/sand fly successfully developed infection.

4 Conclusions

Prior to this endeavor, no laboratory colony of this vector existed in India that met the stringent biosafety requirements of this human-use study. Having this kind of colony, open several opportunity to disease transmission related studies.

C0108 THE ECO-EPIDEMIOLOGY OF CUTANEOUS LEISHMANIASIS CAUSED BY LEISHMANIA TROPICA IN PALESTINE: CASE STUDY; SPATIAL AND TEMPORAL PATTERNS OF SAND FLY ACTIVITY AND DISTRIBUTION IN THE CITY OF TUBAS

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1 Background

Leishmaniasis is still considered as a neglected disease by the World Health Organization. We are applying an eco-health approach that integrates public health measures, epidemiology, and disease ecology to study CL caused by *L. tropica* in the West Bank. This study aims to evaluate the density of sand flies within an urban landscape and between the urbanized area and adjacent hyrax colonies.

2 Methods

The study was conducted in Tubas District, located in the northeast part of the West Bank. There, we conducted sand fly trapping in a hot spot for Leishmania infections in July and September, 2016. We examined the effect of distance from hyrax colonies by quantifying sand fly abundance along three 0.5 km long trapping transects running from a rocky area containing hyrax colonies out across an area of inhabited houses. Five traps were placed along each transect, with 100 m between traps. Two 6-night trapping sessions were conducted using CDC light traps. The trapped sand flies were counted, and each fly was sexed and identified to species. *Leishmania* parasite detection was conducted on the phlebotomus females using PCR amplification of the internal transcribed spacer (ITS1), and the blood from recently consumed meals identified to species using the Cytochrome b PCR and Reverse Line Blotting (RLB) technique.

3 Results

A total of 1,051 *Phlebotomus* sand flies from 9 species were captured, of which 470 (5.2 per trap/night) were captured in July, and 581 (6.5 per trap/night) were captured in September. These included individuals of *Phlebotomus sergenti* (the most common), *Phlebotomus alexandri, Phlebotomus arabicus, Phlebotomus perfiliewi, Phlebotomus syriacus, Phlebotomus papatasi Phlebotomus tobbi, Phlebotomus kazruni, and Phlebotomus simici*. Sandflieswere captured in greater numbers (presumably reflecting higher densities) in stations located in the periphery and edges facing the wadies of the study site close to hyrax colonies. Sandflies captured next to hyrax colonies were infected with *Leishmania tropica*.

4 Conclusions

Living next to the city edges, facing the wadies, open green areas, and close to host colonies increase the exposure to infected vector (sand-fly). This case study was conducted to quantify the distribution of sand fly vectors across their habitats. Such knowledge can allow us to better identify areas of greater risk of infection and to focus our efforts in particular areas to control sand flies and encourage behaviors in local residents that increase personal protection.

C0155 NEW INSIGHTS INTO SANDFLY SALIVA: THE GLYCOSIALOME OF LUTZOMYIA LONGIPALPIS

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1 Background

Sandfly saliva modulates *Leishmania* infection by influencing local immune responses at the site of the bite. Although sandfly salivary proteins have been well studied, the sugars remain overlooked. Considering glycans play important roles in protein function and immunogenicity, they may have a role in the saliva's modulating effects and therefore pathogen transmission. Additionally, glycosylation can determine the half-life in blood of insect salivary proteins. Here we studied glycoproteins in the saliva of *Lutzomyia longipalpis*, the vector of visceral leishmaniasis, to understand their role in the transmission of *Leishmania*.

2 Methods

Sandfly saliva was dissected from 5-day old female sandflies and analysed by SDS-PAGE coupled to LC-MS before and after deglycosylation with N-glycanase. Enzymatically released sugars from salivary glycoproteins were fluorescently tagged and analysed by HPLC before and after glycosidases, in combination with highly sensitive LC-MS/MS. Recognition of salivary glycoproteins by receptors of dentritic cells (DC-SIGN) and macrophage (CTLD4-7-Fc) was tested.

3 Results

Analysis of saliva from *Lu. longipalpis* females yielded a list of glycoproteins, including salivary endonucleases and yellow related proteins. We show the N-glycan composition of *Lu. longipalpis* mostly consists of high mannose-type sugars, with Man5GlcNAc2 being the most abundant species. In addition, low abundantly mannosylated glycans containing an unknown modification were also detected. Sandfly saliva glycoproteins were succesfully recognised by the macrophage receptors, suggesting a possible interaction between salivary glycans and host cells.

4 Conclusions

We have previously found mannose-type sugars in tsetse saliva, which hints at conserved pathways of salivary protein glycosylation across blood feeders. Exposed mannosylations of salivary glycoproteins interact with immune cells such as macrophages. We are currently testing if these cells are activated by sandfly salivary glycans, as well as determining the glycosialome of other haematophagous arthropods.

C0209 LINB13 FROM LUTZOMYIA INTERMEDIA SALIVA ACTS AS A VECTOR EXPSOURE MARKER AND AS A RISK MARKER FOR CUTANEOUS LEISHMANISIS DEVELOPMENT CAUSED BY LEISHMANIA BRAZILIENSIS

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1 Background

Sand fly saliva contains immunogenic proteins that elicit the production of specific anti-saliva antibodies. This immunogenicity can be exploited to monitor for exposure to sand fly bites and, possibly, disease development. Efforts have been made to characterize sand fly salivary proteins and evaluate their utility in serological testing. Herein, we expressed salivary proteins from *Lutzomyia intermedia*, the main vector of *Leishmania braziliensis* in Brazil, and probed for the immune response of individuals residing in a Cutaneous Leishmaniasis (CL) endemic area.

2 Methods

We cloned and expressed 11 *Lutzomyia intermedia* recombinant salivary proteins. In the screening phase, the recombinant salivary proteins were tested against a panel of immune sera (n=89). In the validation phase, a selected salivary molecule was tested in a larger cohort of sera (n=264) from house-hold contacts of CL patients. Finally, we did a prospective analysis to access risk marker for CL development in this same endemic area.

3 Results

Initially, we identified five salivary proteins (Linb13, Linb21, Linb26 and Linb38) that were recognized in a similar way compared to *Lu. intermedia* salivary gland sonicate (SGS), the gold standard of vector exposure. Subsequent ROC curves showed that, among these five antigens, Linb13 displayed the highest sensitivity (87.3%) and specificity (73%, $p<0.001$) indicating that Linb13 was the best marker at predicting ELISA positivity against *Lu. intermedia* SGS. IgG4 against Linb13 was the principal isotype found in immune sera and no cross reactivity was observer between Linb13 and *Lutzomyia longipalpis* saliva. Next, to evaluate Linb13 as a putative marker of CL development, we prospectively evaluated anti-Linb13 serology in parallel to disease manifestation in the larger cohort 264 residents. We observed a higher ($p<0.001$) anti-Linb13 IgG response in individuals who developed CL compared to those who did not. During 5-year follow-up, 38/264 (14.3%) household contacts developed CL. 28 of 136 (20.5%) who were seropositive to Linb13 developed CL, compared with only 10 of 128 (7.8%) who were seronegative. Thus, individuals seropositive to Linb-13 are 2.6 times more likely to develop CL caused by *Leishmania braziliensis* (relative risk, 2.6; 95% confidence interval, 1.3-5.2; $P < .01$).

4 Conclusions

Linb-13 is a powerful epidemiological maker to access human exposure to sand fly bites and it also acts as a risk marker for CL development.

Source of funding: CNPq, FIOCRUZ, INCT-DT

C0284 STUDYING THE TRANSMISSION OF LEISHMANIA INFANTUM BY RHIPICEPHALUS SANGUINEUS IN DOGS: AN INTERVENTIONAL STUDY USING PARASITOLOGY, SEROLOGY AND MOLECULAR METHODS

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1 Background

Rhipicephalus sanguineus is the most widely distributed tick in the world, which is partly due to its biological flexibility and the global distribution of its major host i.e. domestic dog. In Mediterranean region, canindae are principal reservoir hosts of *Leishmania infantum*, which is usually transmitted by the phlebotomine sand flies. In this study, we evaluated *Rhipicephalus sanguineus* potential of transmitting *L. infantum* to uninfected dogs.

2 Methods

Five dogs with clinical manifestations of *Canine Visceral Leishmaniasis*, high anti-*Leishmania* antibody titers and concurrent *Rhipicephalus sanguineus* infestations, were selected from endemic areas of Canine Visceral Leishmaniasis (CVL) of Iran. At least, twenty live ticks were removed from each infected dog. After morphological identification, the ticks were divided into two groups; ticks belonging to the first group were dissected for parasitological examinations and Semi-nested PCR assay,and those of the second group were selected for experimental transmission of CVL caused by *L. infantum* to uninfected dogs. Nearly, 67% of ticks were infected by *L.infantum* using the Semi- nested PCR. All other parasitological tests of ticks were negative. Following experimental tick infestations, all uninfected dogs were kept for 9 months and examined monthly for clinical and serological tests.

3 Results

Monthly Clinical examinations and serological tests of the investigated dogs revealed negative results. Nested-PCR test results which were performed on biopsy samples of spleen and liver of dogs were also negative.

4 Conclusions

Based on this interventional study, *L.infantum*-positive *R.sanguineus* ticks were unable to transfer *L.infantum* from infected dogs to healthy ones. The detection of *L. infantum* DNA in ticks collected from naturally infected dogs by nested- PCR does not prove their vectorial competence. Further studies on infected ticks are required to determine their possible role in the transmission of *Leishmania infantum* to healthy dogs.

Keywords: *Rhipicephalus sanguineus*, *Leishmania infantum, Canine Visceral Leishmaniasis*

C0310 YELLOW-RELATED PROTEINS IN SAND FLY SALIVA AND THEIR ROLES IN LEISHMANIA-VECTOR-HOST INTERACTIONS

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1 Background

Sand fly saliva contains anti-hemostatic components necessary for successful bloodfeeding and immunomodulatory components enhancing *Leishmania* infection in the host. In addition, some salivary proteins, including Yellow-related proteins (YRPs) are antigenic and induce a specific IgG antibody response in repeatedly bitten hosts. These antibodies can serve as a marker of host exposure to sand flies and for analyses of the risk of *Leishmania* transmission or the effectiveness of vector control campaigns. Here we prepared the recombinant form of YRP and four other salivary proteins of *Phlebotomus orientalis*, main sand fly vector of *Leishmania donovani* in East Africa, and tested them with sera of domestic animals from visceral leishmaniasis foci. In addition, we studied variability and structure of all 33 sand fly YRPs known up to date.

2 Methods

Antigenic proteins from *P. orientalis* saliva were identified by immunoblot with canine sera followed by mass spectrometry. Five recombinant proteins were expressed in bacterial expression system and purified in Ni-NTA columns. They were used as antigens in ELISA with four host species. In parallel, phylogenetic relationship of sand fly salivary YRPs was determined, as well as the putative glycosylation sites and hypothetical amine-binding ability. 3D models of YRPs were constructed based on the previously described template YRP of *Lutzomyia longipalpis*.

3 Results

Out of five recombinant proteins tested, the YRP determined as the most suitable antigen, based on its high correlation with the whole salivary gland homogenate, specificity, sensitivity, and positive/negative predictive values. The bioinformatical analyses confirmed close relationship among YRPs from *Phlebotomus* species and higher variability in *Lutzomyia* species. *Sergentomyia* YRPs are very distinct from those of other sand flies studied. All sand fly YRPs proteins share the same folding as a six-bladed β-propeller with a tunnel through their structures and all of them are hypothetically able to bind biogenic amines which suggest their anti-inflammatory role during bloodfeeding and *Leishmania* transmission.

4 Conclusions

YRP of *P. orientalis* is the most promising candidate for detecting anti-sand fly saliva antibodies in different hosts and can be utilized in larger epidemiological studies. Distribution of YRPs in groups based on the predicted glycosylation patterns or amine-binding amino acids corresponds with the branches identified in sand fly phylogenetic tree.

C0311 BLOODMEAL DIGESTION IN SAND FLIES AND LEISHMANIA DONOVANI DEVELOPMENT: NO DIRECT EFFECT OF PROTEINASES ON PARASITE MORTALITY.

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1 Background

Leishmania development in sand flies is confined to the alimentary tract and is closely connected with bloodmeal digestion. Previously, it has been published that activities of sand fly midgut proteases are harmful to *Leishmania*, especially to amastigote-promastigote transition forms. Our experiments with four *Leishmania*-sand fly pairs gave opposite results.

2 Methods

We compared various parameters of bloodmeal digestion in four sand fly species that are either susceptible (*Phlebotomus argentipes* and *P. orientalis*) or refractory (*P. papatasi* and *Sergentomyia schwetzi*) to *L. donovani*. Next, we evaluated *in vitro* the effect of semi-digested midgut content on different life stages of *L. donovani*. Various morphological forms of parasites, including macrophage-derived amastigotes and transition forms, were incubated for 2 h with midguts dissected at various intervals (6-72h) post bloodmeal (PBM) or with commercially available proteinase. Their viability was determined using flow cytometry.

3 Results

Females of *P. argentipes* and *P. orientalis* significantly differed in time course of bloodmeal digestion despite the fact they are both natural vectors of *L. donovani.* Females of *P. argentipes* digested fast with a high peak of chymotrypsin activity, while *P. orientalis* digest considerably slower, having low peaks of proteolytic activities. However, both vectors were similarly susceptible to experimental infection with *L. donovani* and even 2 promastigotes were sufficient for the establishment of mature late-stage infections. The mortality of various *Leishmania* forms was observed after exposure to the sand fly midgut lysates *in vitro*: in all sand fly species, significant mortality effect was found at the end of their bloodmeal digestion. Surprisingly, the most susceptible stages were promastigotes, while mortality of transforming parasites and amastigotes was significantly lower. Parasites were also susceptible to killing by rabbit blood in combination with proteinase, but resistant to proteinase activity.

4 Conclusions

Significant mortality of *Leishmania* incubated *in-vitro* with sand fly midgut lysates was observed in susceptible as well as refractory parasite-vector combinations. The mortality correlates with the time course of bloodmeal digestion and increases with the degree of digested blood. We propose that *Leishmania* are susceptible to killing by products of bloodmeal digestion but not directly by midgut proteases.

C0329 TRANSCRIPTOMIC ANALYSIS OF THE EARLY EVENTS RESULTING FROM STIMULATION WITH SAND FLY SALIVA: THE CASE OF LUTZOMYIA INTERMEDIA VS. LU. LONGIPALPIS

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1 Background

Leishmania parasites are transmitted to the mammalian hosts by the bite of phlebotomine sand flies. During this process, not only parasites but also sand fly salivary products are delivered to the host and such salivary products display both pharmacological as well as immunomodulatory properties. In parallel, we have shown that immunity to *Lutzomyia intermedia* or *Lu. longipalpis*, vector of *L. braziliensis* and *L. infantum*, respectively, have different outcomes in human *Leishmania* infection. Therefore, the aim of this study was to characterize the initial human response to salivary antigens from *Lu. intermedia* and from *Lu. longipalpis.*

2 Methods

Human peripheral blood cells were stimulated with salivary gland sonicate of *Lu. intermedia* or *Lu. longipalpis* sand flies collected in the field. After two and six hours, cells were collected for RNA extraction and reverse transcription. cDNA libraries were sequenced in SOLiD platform. Sequences were mapped to the human genome using Bioscope and differentially expressed genes (DEGs), in comparison to non stimulated cells, were identified using Cuffdiff. Next, clusterization and statistical analysis packages were used o identify a transcriptomic signature associated with stimulation with sand fly saliva.

3 Results

Stimulation with *Lu. intermedia*, the vector of *L. braziliensis*, induced the upregulation of 244 genes and the downregulation of 133 genes, at six hours. Functional enrichment showed an upregulation in pathways associated with chemotaxis, regulation of cell activation and cytokine production. Stimulation with *Lu. longipalpis,* the vector of *L. infantum*, induced the upregulation of 181 genes and the downregulation of 189 genes, at six hours. Functional enrichment also showed an upregulation in pathways associated with chemotaxis and regulation of cytokine production and leukocyte activation. In parallel, we observed a suppression in pathways associated with the regulation of apoptosis.

4 Conclusions

We identify a common transcriptomic signature induced upon stimulation of human leukocytes with sand fly salivary molecules. Analyses are underway to investigate whether unique signatures can also be identified, that could be associated with the outcome observed upon human exposure to these sand fly species.

Source of funding: CNPq and FAPESB.

C0336 LEISHMANIA INFECTIONS AND BLOOD SOURCES OF FEEDING OF PHLEBOTOMINES SAND FLIES IN ENDEMIC AREA OF VISCERAL AND TEGUMENTARY LEISHMANIASIS IN BRAZIL

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1 Background

The aim of this work was to identify the species of *Leishmania* sp. and blood sources of phlebotomine sand flies in the urban and rural peridomiciliary environment of municipality of Caxias, State of Maranhão, highly endemic area of leishmaniasis, localized in northeast of Brazil.

2 Methods

Sampling of sand flies with CDC (Center of Disease Control - USA) type light traps was performed in alternate months, from March 2013 to February 2015, in 20 randomly selected houses, 10 in urban and 10 in rural areas. To identify *Leishmania* sp. was used digestion of the ITS1 amplicon with the restriction *enzyme HaeIII* and for the blood source was used the conserved region of the cytochrome *b* gene digested with *Hae*III and *Mbo*I. The PCR-RFLP products were compared with samples of blood of different vertebrate hosts.

3 Results

The most abundant phlebotomine species were *Lu. longipalpis* and *Lu. whitmani.* Seven species of *Leishmania* were detected: *L. infantum* in *Lutzomyia longipalpis*, *Lu. whitmani* and *Lu. termitophila*; *L. infantum/L. braziliensis* in *Lu. longipalpis*, *Lu. whitmani* and *Lu. trinidadensis*; *L. shawi* in *Lu. longipalpis*; *L. mexicana* in *Lu. longipalpis*; *L. braziliensis* in *Lu. Longipalpis* and *Lu. whitmani*; *L. guyanensis* in *Lu. longipalpis* and *Lu. termitophila*; *L. amazonensis* in *Lu. longipalpis* and *L. lansoni* or *L. naiffi* in *Lu. longipalpis* (the technique used does not separate these two species). *Lu. longipalpis* and *Lu. trinidadensis* were infected with *Leishmania* sp. In total 39.6% of the females fed preferentially on chickens, 35.4% in dog, 10.3% in rodent, 8.8% in man, and a smaller proportion in pig, horse, opossum and cattle. In the urban area the preferred hosts were 44.9% chicken, 28.9% dog, 10.6% rodent and 9.0% man, and in the rural area were 46.5% dog, 30.5% chicken, 9.8% rodent and 8.6% man. *Lu. longipalpis* was the most opportunistic species, feeding blood of all hosts surveyed. In *Lu. longipalpis* and *Lu. whitmani* were observed mixed feeding in man, dog and rodents (dog/chicken, dog/rodent and rodent/chicken). The presence of *Leishmania* sp. was more detected in females that feed in dog, rodent and man.

4 Conclusions

The study shows the concomitant circulation of *Leishmania* species that cause visceral and tegumentary leishmaniasis in the study area and the epidemiology complexity with the possible participation of several species of *Leishmania*, sandflies and reservoirs. This explains the occurrence of autochthonous human cases of both clinical forms of leishmaniasis in Caxias, MA.

C0387 HOST RESPONSE AGAINST LUTZOMYIA WHITMANI SALIVA AS A MARKER OF EXPOSURE AND PROTECTION AGAINST LEISHMANIA BRAZILIENSIS

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1 Background

Cutaneous Leishmaniasis (CL) has *Leishmania braziliensis* as the main agentin the Americas. *Lutzomyia intermedia* and *Lutzomyia whitmani* have been implicated as vectors of *L. braziliensis*. Although the role of *Lu. intermedia* has been explored in *L. braziliensis* transmission, there are no studies on the involvement of *Lu. whitmani* in CL. The aim of this study is to explore the role of *Lu. whitmani* in *L. braziliensis* transmission.

2 Methods

Mice were exposed to salivary gland sonicate (SGS) from Lu. whitmani and the we evaluated both the humoral an cellular response to SGS. In parallel, we also maintained chickens in areas of *Lu. whitmani* transmission to evaluate the possibility of using these animals as sentinel hosts. Finally, we evaluated human exposure to Lu. whitmani in areas of *L. braziliensis* transmission and a possible association with CL development.

3 Results

Mice immunized with *Lu. whitmani* SGS developed a humoral immune response to the antigens, characterized by the presence of IgG. Mice also developed a DTH response and we detected the presence of IL-4, IL-10 and IFN-g following in vitro stimulation. Importantly, mice immunized with *Lu. whitmani* SGS developed smaller lesions and displayed a lower parasite burden upon a challenge infection with *L. braziliensis*. In the endemic area, we also observed the presence of positive serology to *Lu. whitmani* SGS in chickens naturally exposed, indicating that these animals can be used to monitor areas of *L. braziliensis* transmission. Finally, we also detected the presence of anti- *Lu. whitmani* SGS antibodies in individuals residing in a CL endemic area. Moreover, individuals presenting a positive Leishmania Skin test had lower levels of antibodies to saliva compared to individuals with active disease.

4 Conclusions

Salivary antigens from *Lu. whitmani* are immunogenic and the elicited immune response is characterized as Th1/Th2. This immune response modulates CL development caused by *L. braziliensis.* Naturally exposed individuals, in a CL endemic area, develop antibodies to *Lu. whitmani* SGS and the presence of such antibodies maybe associated with protection.

C0436 BLOOD MEAL IDENTIFICATION IN FIELD-CAPTURED SAND FLIES FROM THE VISCERAL LEISHMANIASIS ENDEMIC REGION OF INDIA

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1 Background

Blood-meal identification of hematophagous insects provides information on host-feeding preferences or host feeding patterns in natural conditions. In the present study, we investigated the blood meal analysis among the blood fed female Phlebotomus *argentipes* collected from the VL endemic regions of Muzaffarpur district, Bihar, India.

2 Methods

Sand flies were collected by light traps and processed individually for DNA isolation and further PCR.To discriminate animal host using blood meals, a region of the mtDNA, *CytB* gene was amplified. It amplifies a 623bp product, that was restriction digested by HaeIII, as each mammalian host have a specific cutting site.

3 Results

Initially we collected total 35 female *P. argentipes* from mixed dwelling, out of which 26 was blood fed. After *CytB* specific PCR, we found 18 samples positive for confirmed blood meal as they showed 623 bp amplified product. After restriction digestion with Hae III, we found, 4 samples (22.2%) with human blood and remaining 14 (77.8%) with dog blood.

4 Conclusions

Sand flies collected from mixed dwellings, were used for identification of blood meal host. Mixed dwellings are the places, where human and cattle both live. Identification of blood meal from such location will give significant proof about sand flies preferred blood meal source.

C0448 DETECTION AND MOLECULAR IDENTIFICATION OF LEISHMANIA SPP FROM PHLEBOTOMUS LONGICUSPIS, PHLEBOTOMUS SERGENTI AND LESIONS OF CUTANEOUS LEISHMANIASIS IN THE NORTH-EASTERN OF MOROCCO

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1 Background

Cutaneous leishmaniasis (CL) is an infectious disease caused by various species of *Leishmania* and transmitted by several species of sandflies. CL is one of the most neglected tropical diseases and constitute a major health threat during the past 20 years in Morocco. The main objective of this study was to detect *Leishmania* infection in *Phlebotomus longicuspis, Phlebotomus sergenti* and in human skin samples, in Taza province, a focus of cutaneous leishmaniasis in North-Eastern of Morocco.

2 Methods

An entomological survey of Phlebotomine sand flies (*Diptera: Psychodidae*) was carried out in three sectors of Taza province from July to October 2015. A total of 3672 sand flies were caught during three consecutive nights using CDC light traps placed in houses and in animal shelters. Leishmania species were detected and characterized by ITS1 PCR-RFLP and kDNA nested PCR within *Phlebotomus sergenti, Phlebotomus perniciusis* and also in positive stained smears of 9 cutaneous leishmaniasis patients.

3 Results

Morphological identification allowed the identification of 13 species: 11 belonging to genus *Phlebotomus* and 2 species in the genus *Sergentomyia*. The sand fly diversity was analyzed by the ecological indices determinant: specific richness (S), Pielou index (J'), Simpson diversity index (D) and Shannon-Weiner index (H'). *Phlebotomus longicuspis* was the most abundant species, accounting 64.08% of the total collected sandlies, followed by *P. sergenti* (20.1%) and *P. perniciosus* (8.45%).
By kDNA nested PCR and ITS1-PCR-RFLP, *Leishmania tropica* and *Leishmania infantum* were found in *Phlebotomus sergenti and in Phlebotomus longicuspis* respectively. Eight out of 9 patients were found to be infected by Leishmania *infantum* whereas one patient was infected by and *Leishmania tropica*

4 Conclusions

This is the first molecular detection of *Leishmania tropica* and *Leishmania infantum* in *P. sergenti* and in *P. longicuspis* respectively in this CL focus.This study underlined also the predominance of L. infantum and its vector in this region where Leishmania tropica has always been considered the causative agent of cutaneous leishmaniasis for more than 20 years

C0454 DENATURING GRADIENT GEL ELECTROPHORESIS (DGGE) REVEALS MIDGUT FLORA DIFFERENCES BETWEEN DIFFERENT POPULATIONS OF PHLEBOTOMUS PAPATASI (DIPTERA: PSYCHODIDAE) IN TURKEY

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1 Background

Both female and male Phlebotomine sand flies feed on natural sugars, nectar and sap, and only females feed on different sources of blood. Although the role of midgut flora on sand fly-pathogen interactions has been previously shown, there are few reports on bacterial microbiota of *P. papatasi* and no data available for natural *P. papatasi* populations in Turkey. Therefore, we aimed to investigate microbiota of the specimens of different populations of *P. papatasi* using Denaturating Gradient Gel Electrophoresis (DGGE).

2 Methods

Sand flies were collected from four different towns located in western part of Turkey. The specimens from the laboratory *P. papatasi* colony were also included in the study. After sterile washing steps, sand flies were dissected and guts were separated. Pools were generated using 10 specimens from each population. DNA was extracted using Qiagen Blood&Tissue Kit and amplified with universal bacterial primers 27F/1492r. PCR products were used as template for second amplification for DGGE analysis with 341F (GC Clamps) /518R primer set. DGGE was performed with 35%-40% gradient gel and DNA bands were cut with sterile scalpels and sequenced after incubation step at 4°C for overnight.

3 Results

DGGE analysis showed that all specimens, including males, bear bacteria in their gut. Sequence analysis revealed the existence of 17 different organisms (one bacteria not identified in genus level) in the studied pools (*Variovorax* sp., *Bosea* sp., *Brevundimonas* sp., *Ochrobactrum* sp., *Cryptococcus* sp., *Bacillus cereus*, *Erwinia aphidicola*, *Ishikawella* sp., *Klebsiella* sp., *Serratia marcescens*, *Stenotrophomonas maltophilia*, Uncultured Alpha *Proteobacterium*, Uncultured *Proteobacterium*, Uncultured *Pantoea* sp., Uncultured *Thauera* sp., *Wolbachia* sp.). *Wolbachia* sp. was the most abundant bacterial phylotype, presented in all studied pools. *Bosea* sp. and *Variovorax* sp. not detected on laboratory colony but detected in the field-collected specimens.

4 Conclusions

Midgut microbiota identification of sand flies is an important step to improve our understanding of infectious agent transmission by sand fly bite. In our study, *Wolbachia* sp. and *Klebsiella sp.* were detected first time in sand fly midgut. In Turkey, this is the first study to reveal midgut flora of *P. papatasi* sand flies using molecular methods, which allows identifying uncultured bacteria.

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C0521 NATURAL LEISHMANIA INFANTUM CHAGASI INFECTION IN SYLVATIC PHLEBOTOMINE SAND FLIES SPECIES FROM AN URBAN-FOREST TRANSITION AREA IN CAMPINAS, SÃO PAULO STATE, BRAZIL

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1 Background

Autochthonous canine cases of visceral leishmaniasis have been reported since 2009 in Sousas and Joaquim Egídio districts located in the municipality of Campinas, in northern of state of São Paulo, Brazil. The main purpose of this study was to investigate natural infection by *Leishmania infantum chagasi* in phlebotomine sand flies in a focus area of the municipality of Campinas.

2 Methods

The collections were performed monthly using CDC miniature light traps, from April 2014 to March 2015. Phlebotomine sand flies were identified morphologically and all 186 female sand flies were separated according to date, collection site and species, and tested by Leishmania genus-specific PCR, using ITS-1 as the target sequence, and then by a PCR targeting a kDNA fragment specific for *Leishmania infantum chagasi*.

3 Results

A total of 477 individuals were collected of which 291 were males and 186 females. Six species known as incriminated or suspected vectors were identified: *Nyssomyia whitmani*, *Migonemyia migonei*, *Pintomyia fischeri*, *Nyssomyia neivai*, *Pintomyia pessoai* and *Lutzomyia longipalpis.* However, PCR analyses detected *Leishmania* DNA in unexpected phlebotomine sand flies species, showing infection in *Expapillata firmatoi* and *Pintomyia monticola* by *L. i. chagasi*.

4 Conclusions

This is the first report of presence of *L. i. chagasi* DNA in *Ex. firmatoi* and *Pi. monticola*. The anthropophilic behavior of both species associated with the detection of *Leishmania* DNA suggests their possible role in the transmission cycle of visceral leishmaniasis in the studied area. However, more studies need to be performed to assess the role of these species as a vector in the study area.

C0559 FIRST ISOLATION OF UNKNOWN LEISHMANIA PARASITES FROM A IDIOPHLEBOTOMUS LONGIFORCEPS SAND FLY COLLECTED IN AN ENDEMIC AREA OF LEISHMANIASIS OF THAILAND

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1 Background

Autochthonous leishmaniasis cases in Thailand are increasing dramatically. The disease was found in both immunocompetent and in immunocompromise hosts especially AIDS patients.The disease is transmitted to the vertebrate hosts through the bite of infected female sand fly. In Thailand, data of sand fly vectors for *Leishmania* is limited. Aim of this study is to investigate the potential vectors of leishmaniasis from Chiang Rai, northern regions of Thailand by isolation and PCR of the parasites from sand flies.

2 Methods

Sand flies were collected using CDC light traps. Individual female sand fly was dissected under a stereoscope for the observation of the natural *Leishmania* infection. The head and genitalia were mounted on slides for morphological character identification. The midgut was examined for live promastigotes of *Leishmania*, positive sample was then used for culturing and PCR for detection of *Leishmania* species.

3 Results

Total of 830 female sandflies dissected, one *Idiophlebotomus longiforceps* was positive for numerous *Leishmania*-like grouped promastigotes in the midgut. The phylogenetic tree constructed from sequences of internal transcribed spacer 1 (ITS1) and heat shock proteins (hsp70) were identified the parasite are closely related to *Leishmania* spp..

4 Conclusions

The natural *Leishmania* spp. infection in *Id. longiforceps* was the first report in Thailand. *Id. longiforceps* might be a potential vector of *Leishmania* spp in endemic area. Information obtained from the study would be applied to develop the effective control strategies for leishmaniasis in Thailand.

C0580 TESTS FOR VISCERAL LEISHMANIASIS DIAGNOSTIC DO NOT IDENTIFY ANIMALS WITH LEISHMANIA INFANTUM INFECTIOUS TO THE VECTOR LUTZOMYIA LONGIPALPIS

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1 Background

One of the main difficulties controlling reservoirs for zoonotic visceral leishmaniasis caused by *Leishmania infantum* is correctly identifying dogs which actually represent a risk for the population. A study was performed to assess several tests and an in house polymerase chain reaction specificity and sensitivity to detect dogs able to infect the vector *Lutzomyia longipalpis* vector.

2 Methods

We studied 99 animals with visceral leishmaniasis, diagnosed through the direct investigation of the parasite in a sample of bone marrow, popliteal lymph node and skin, using NNN (Novy, MacNeal, Nicolle) culture medium enriched with Schneider's. The serological tests performed were: Enzyme-Linked Immunosorbent Assay (ELISA), Immunofluorescence antibody *test* (*IFAT*), Dual-path platform fast test (TR DPP), rK39 immunochromatographic test (ICrK39), *Fast* Agglutination Screening Test (FAST™) e Direct Agglutination Test (DAT). PCR was performed using whole blood collected on filter paper and specific primers were used to amplify the ITS-1 region of rDNA. In the xenodiagnosis, 55 females of L. longipalpis were used, with an average of 27 dissected insects. The females were first generation, with five days of birth and deprived of any food source, which were placed in contact with the inner face of the ear for 45 minutes.

3 Results

All tests had poor performance diagnosing infectiousness, as it can bee seen by the very low areas under the curve, with 53% for FAST, 51% for ICrK39 and PCRsp, 50% for RT DPP®, 48% for RIFI 40 and ELISA, 46% for DATTM and 45% for RIFI80. Nearly all tests were reagent for dogs with negative xenodiagnosis, what lead to very low specificities, with the greatest value for PCRsp with 28%. From the 99 animals, 88 has some symptom and 11 were assymptomatic. When subjected to xenodiagnosis, 46 infected at least one specimen of L. longipalpis, 39 (44%) of which were symptomatic and seven (64%) were asymptomatic, but did not present a significant difference. The proportion of infected insects among the 99 dogs was 7%. For asymptomatic dogs, 16% and for symptomatic dogs, 6% (p=0.018). From all symptoms found, only dermatitis was statistically related to the chance of the dog being infective (infecting at least one sand fly), with a 9.3 odds ratio of being infectious.

4 Conclusions

The study shows that there is a imperative demand for the development of new technologies to identify dogs transmitting *L. infantum* for eventual public health application.

C0581 EVALUATION OF THE ECOLOGICAL CHARACTERISTICS IN THE VECTOR OF CUTANEOUS LEISHMANIASIS IN A NEW FOCUS OF MOHAMMAD ABAD, KERMAN, SOUTH-EAST OF IRAN

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1 Background

Following the massive earthquake of Bam in 2003,Mohammad Abad villagehas become a new focus of anthroponotic cutaneous leishmaniasis. The main goals of this study were to evaluate thefauna, seasonal activityand the host preference of sand fliesand to determine themain vectors of ACL in Mohammad Abad, Kerman province, south-east of Iran.

2 Methods

Sand flies were collected from indoors and outdoors bysticky paper traps from May to November.Regarding the importance of host preference and its impact on leishmaniasis control, blood meal was analyzed by the restriction fragment length polymorphism (RFLP) method.The cytochrome b mitochondrial genomic regions (mtDNA) and enzymatic digestion of*xhoI*and *Hae III*were used for the diagnosis ofhuman blood feeding.In thedetection of leptomonad (promastigote) contamination in sand flies, anested PCR method and high resolution melt (HRM) analysis techniques were exploited.

3 Results

A total of 920 sand flies were identified as belonging to 14 species in two genera;*Phlebotomus*spp. (5species) and *Sergentomyia*spp. (9species). Themost frequently occurring species was*Phlebotomussergenti* (67.39%),followed by*Phlebotomuspapatasi* (19.34%). The highest indoor collection was realized in mid-July, and that of outdoor collection ensued in early August.A total of 250 sand flies were collected for host preference analysis, and120 sand fliesbelonged to the species of *P. sergenti*following RFLP confirmation.Twenty nine*P. sergenti*sandflies (32.5%)were identified to have fed on human.Nested- PCR andHRM analysesconfirmed these sandflies had been infected with*L.tropica*.

4 Conclusions

The present study has confirmed *P.sergenti* as the main Phlebotomine sand fly vector for ACL infection caused by*L.tropica* in southeastern Iran.

C0583 XENODIAGNOSIS IN CATS INFECTED WITH LEISHMANIA INFANTUM IN BRAZIL

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1 Background

Infection by *Leishmania infantum* in felines has been reported in several countries, including Brazil, occurring mainly where visceral leishmaniasis (LV) is endemic in dogs and humans. The most frequent clinical manifestations in cats are nodules on the skin, ulcers and alopecia, suggesting a high parasite load on the skin and possibility of vector infection. The objective of this study was to verify the infectivity of sandflies in cats infected with *L. infantum*.

2 Methods

The study was developed at the Animal Sanity Laboratory of the Universidade Federal do Piauí. Included in the study seven felines parasitized by *L. infantum*, diagnosed using samples of bone marrow, popliteal lymph node and skin through the methods of NNN (Novy, MacNeal, Nicolle) culture medium enriched with Schneider's and Polymerase Chain Reaction and Restriction Fragment Length Polymorphism using *HaeIII* enzime, for identification of the specie. Three animals were submitted to xenodiagnosis, two from the University Veterinary Hospital and one from a private clinic in Teresina, Piauí State. In the clinical evaluation of the three animals, cat I had alopecia, lesions on the face and nose and slimming, cat II nodules on the edge of the ears, ulcers on legs and slimming and cat III alopecia in the ears and face and otitis. In the xenodiagnosis 60 female of *Lutzomyia longipalpis* were used per animal. The insects came from the phlebotomine colony established since 1995. First-generation females, maintained at 26°C and 92% humidity, five days after hatching and deprived of any food source and submitted to xenodiagnosis for 60 minutes. The insects were dissected on the fifth day after xenodiagnosis to investigate promastigote forms of the parasite in the digestive system.

3 Results

In the xenodiagnosis it was observed that two cats were able to infect the vector. In cats I and II the infected insect index was 15% and 20% and feed rate was 87% and 100%, respectively. In cat III the feeding rate was 100%, however, all insects died up to the fourth day after xenodiagnosis. In all infected insects, metacyclic promastigotes were detected in the stomach.

4 Conclusions

With the results of the xenodiagnosis we conclude that felines, in addition to acting as a source of diet for the vector, are able to infect *Lutzomyia longipalpis* and is likely to be responsible for transmitting of the infection to other animals and humans.

C0620 PHLEBOTOMUS ARGENTIPES, THE VECTOR OF LEISHMANIASIS IN SRI LANKA; STUDY ON SUSCEPTIBILITY PATTERNS

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1 Background

Phlebotomus argentipes is the known vector of *Leishmania donovani,* the causative agent of the disease leishmaniasis. The same species and the vector have also been recognized in India, Nepal, Bangladesh with VL as the resultant disease. Developing resistance to insecticides is a major challenge to develop an effective vector control programme. However, there is a major gap in knowledge regarding insecticide susceptibility pattern in sand fly populations in Sri Lanka.

2 Methods

Based on the leishmaniasis cases and the vector prevalence, Galgamuwa in North-Central Province and Pannala in North-Western Province were selected as study locations for this study.
Collected sand flies were reared using established methods in the insectory of Department of Parasitology, Faculty of Medicine, University of Colombo.
Identification of *Phlebotomus argentipes* was confirmed using standard taxonomic keys.

Ph. argentipes were exposed toDDT (4% and 0.4%),malathion (5% and 0.5%), deltamethrin (0.05% and 0.005%) and propoxur (0.1% and 0.01%) insecticides using WHO (World Health Organization) standard guidelines. The results were validated with the control samples using *Abbott's formula*.

3 Results

There were no difference observed in the number of knockdown and mortality in the village of Pannala and Galgamuwa. After one hour exposure, the knockdown was 64% , 75%, 67% , 66% for DDT 4% , Malathion 5% , Deltamethrin 0.05% and Propoxur 0.1% respectively (N=100; 5 replicates).
All flies died during 24 hour recovery period and no flies were recovered suggesting susceptible colony population.
For DDT 0.4%, Malathion 0.5%, Deltamethrin 0.005% and Propoxur 0.01% (N=100; 5 replicates), all flies were alived after one hour exposure and after 24 hour recovery period. Hence, the population is resistant at low concentrations of tested insecticides.

4 Conclusions

The results of susceptibility test revealed that the flies were susceptible to DDT 4% , Malathion 5% , Deltamethrin 0.05% and Propoxur 0.1% whereas resistant to DDT 0.4%, Malathion 0.5%, Deltamethrin 0.005% and Propoxur 0.01%.
Research work is continuing to better establish the susceptibility patterns of *Ph. argentipes* in Sri Lanka.

C0683 EXPERIMENTAL INFECTION OF PHLEBOTOMUS PERNICIOSUS BY BIOLUMINESCENT LEISHMANIA INFANTUM USING A MURINE MODEL AND ARTIFICIAL FEEDER

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1 Background

Leishmaniasis is a protozoan disease caused by digenetic *Leishmania* parasites circulating between vector sandflies harboring extracellular promastigotes and mammalian hosts carrying intracellular amastigotes. The goal of this study was to screen experimental infection of *Phlebotomus perniciosus* with *Leishmania infantum* using murine model and artificial feeder.

2 Methods

Phlebotomus perniciosus coming from Dr. Molina insectarium were maintained and colonized under controlled conditions. *L. infantum* strain MON-1 with transgenic modification-expressing luciferase (LUC-parasites) was maintained and passaged routinely. BALB/c mice were infected by theses parasites. They were imaged using the Photon Imager. For this, Luciferin solution was injected to mice via intraperitoneal route to monitor the emission of *Leishmania* bioluminescence. Ten minutes later, the mice were anesthetized by isoflurane 5% and put directly in the imaging chamber. The infected mice previously confirmed by imaging were anesthetized individually by ketamine and placed for 1 hour in the cage directly in contact with 200 female *P. perniciosus*. Moreover, in a comparative analysis, 10mL human blood containing 2.10^6/mL or 1.10^7/mL LUC-promastigotes were proposed for 1 hour via artificial feeder to 200 females. For molecular analysis, each blood-fed sample was extracted by Chelex method. RT-PCR was implemented for detection and quantification of *L. infantum* targeting kDNA minicircle.

3 Results

Of 1840 newly emerged females, 428 fed on mice infected by bioluminescent *L. infantum*. After death, each fly was analyzed individually by RT-PCR. Only a single female was *Leishmania* positive at six days post blood meal. A total of 1070 females offered blood meals via an artificial feeder containing infected human blood with *Leishmania* parasites as follow: A blood meal containing 1.10^7/mL LUC-promastigotes was offered to 270 females, of which 75 (28%) were engorged. Of the engorged flies, 44 (59%) were positive by RT-PCR, with a relative average of 50551 *Leishmania* parasites. A second group of 800 female sandflies was offered a blood meal infected with 2.10^6/mL promastigotes, of which 57 (7%) females fed successfully. Of the blood-fed flies, 22 (39%) were positive with a relative average of 6487 parasites.

4 Conclusions

There was a wide variability in infection intensity of *P. perniciosus* and their acquired *Leishmania* parasite burden. The artificial feeder seems to be an efficient method to obtain high rate of infectivity.

C0695 THE EFFECTS OF CLIMATE AND LAND USE CHANGES ON VECTORS OF LEISHMANIA TROPICA DISTRIBUTION AND SEASONALITY: AN ECO-EPIDEMIOLOGICAL PERSPECTIVE

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1 Background

Climate changes and land-use changes are known to enhance factors dictating vector borne diseases occurrence. Cutaneous leishmaniasis (CL) caused by *Leishmania tropica*, transmitted by *Phlebotomus sergenti* and *Phlebotomus arabicus*, has been detected during recent years in Israel, as infection has emerged in new urban and rural foci. It is assumed that the disease is more prevalent in the vicinity of the main reservoir host, rock hyraxes, in den sites (i.e., rock pile or caverns). The combined effects of land use and climatic variables may alter the suitable conditions and creation of enhanced habitats both for vectors and reservoir on *L. tropica* vectors' abundance. This research strives to identify these combined effects on the potential dynamics and distribution of the disease.

2 Methods

1. Monthly sand flies trappings were conducted during 2015-20016 in two focal regions of *L.tropica* in northern Israel. Trappings were conducted in hyrax den sites across a broad topographic and climatic gradient, Using advanced statistical models, vector abundance was related to temporary and periodic climatic variables in a micro and macro-habitat scales.
2. land-use effects on vector abundance were investigated in the vicinity of a hyper-endemic region of the east Galilee (Israel). We compared vector abundance and night activity levels among 5 common land-use categories using a high resolution sampling design – rock piles; open field; garden; watered and non-water grove.

3 Results

1. Vectors abundance, male & female, was found to be positively and exponentially correlated with dusk temperatures within rock piles, and to periodic (2 weeks) average dusk temperatures (best fitted model–R^2=0.67).
2. Major presence of both vector species, were observed in rock piles. Female of *P.sergenti* demonstrated a larger spatial distribution range in various habitats, and with longer night activity times than males.

4 Conclusions

Vectors display a high correlation with environmental variables such as climate and habitat type. The contemporary environmental scenario, comprised of increasing minimum daily temperatures, combined with a rapid agricultural and urban development in open fields, enhances conditions for *L.tropica* vectors and reservoir species. Consequently, this led to vector temporal and spatial expansion and to high exposure to *L.tropica* close to human developing area. We suggest that this trend will probably accelerate in the following era of climatic and land use changes.

C0751 HYALURONIDASE: A LUTZOMYIA LONGIPALPIS SALIVARY PROTEIN AS POTENTIAL VACCINE CANDIDATE AGAINST LEISHMANIA MAJOR INFECTION

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1 Background

Sand fly salivary components facilitate blood meal acquisition and have been shown to enhance pathogen transmission.LuloHYA, a salivary protein from *Lutzomyia longipalpis*, is an enzyme that degrades hyaluronic acid (HA), a constituent of the extracellular matrix of vertebrates. It has been referred as a spreading factor since it increases tissue permeability for other salivary components that serve as anti-haemostatic, vasodilatory or anti-inflammatory agents. Our goal was to characterize LuloHYA and study its physiological role in blood meal intake and parasite establishment.

2 Methods

Hyaluronidase from *Lu. longipalpis* was cloned, expressed in mammalian cells and biochemically characterized by HA turbudimetric assay. Vaccination studies were conducted with C57BL/6 and a homozygous mutant mice strain (B6.129S2-*Ighm*^{tm1Cgn}/J) which lacks mature B cells. Mice were challenged ID in both ears with 1000 *L. major* parasites and 1 salivary gland extract (SGE). Ear lesion thickness was measured every week for up to 8 weeks and parasite load was determined by limiting dilution assay. Cross-reactivity between anti-LuloHYA antibodies and *L. major* was tested by ELISA and immunofluorescence. The specific role of LuloHYA in feeding success was investigated by a blood feeding assay on mice. Mice that had circulating antibodies against LuloHYA or preimmune IgG as controls were offered to sand flies. The number of engorged flies in both groups were compared.

3 Results

LuloHYA is most active at pH 6 and lower ionic strength (25-100 nM). Both SGE and LuloHya specifically cleaves HA but do not alter other components of the extracellular matrix such as chondroitin sulfate, dextran sulphate or heparin. The vaccination experiment demonstrated that LuloHYA confer protective immunity against *L. major* infection. C57BL/6 animals immunized with LuloHYA controlled lesion development while lesions in control animals remained ulcerated. This protective immunity was abrogated when B6.129S2-*Ighm*^{tm1Cgn}/J mice were used indicating that anti-LuloHYA antibodies play a significant role for protection. No cross-reactivity was observed between anti-LuloHYA antibodies and the parasites. Furthermore, blocking LuloHYA with antibodies resulted in an impaired and delayed sand fly blood feeding.

4 Conclusions

This work highlights the relevance of the vector salivary hyaluronidase in both blood feeding and parasite establishment and further suggests the inclusion of this protein as a component for an anti-*Leishmania* vaccine.

C0795 OBSERVATION ON DESERT-TYPE KALA-AZAR CONTROL FOR AIRPLANE DEINSECTIZATION

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1 Background

It has been difficult to control the desert-type kala-azar.

2 Methods

Xiahe forest farm of Bachu county was as treatment area of desert-type kala-azar and the third township of Jiashi county as control area in desert-type kala-azar epidemic areas of Tarim Basin,Xinjiang China. The treatment area: the insecticide were sprayed by AT-402B plane to kill pests (*Apocheima cinerarius*) in 3200 hectares of populus euphratica forest during 7 to 25 of Apr in 2009. The control area: courtyard and indoor of the patient's residence and within around 10 ones were sprayed by insecticide during sandflies seasons except for from 2008 to 2009 and from 2014 to 2016, conducted on mosquito nets to protect children in 2015. The data of desert-type kala-azar patients came from the number published period 1996-2016. Incidence (a number of patients /10^5 populations) = the number of patients ×10/ million of total populations.

3 Results

On the treatment area: the incidence of desert-type kala-azar was at 160/105-260/105 before airplane deinsectization of 2009,and the one followed at 60/ 105 after 7 years of the pest control consecutively; On the control area: the incidence was at 264/105 and 246/105 from 2008 to 2009, after 2010 followed at 60/ 105,but rose again to 193/105,535/105 and 289/105 in 2014,2015 and 2016(to June).

4 Conclusions

The airplane deinsectization could significantly reduce the number of the patients in desert-type kala-azar epidemic areas, which maintaining 60/10^5 of follow was more than 7 years; and reduce the incidence of animal leishmania between the reservoir hosts. It was not obvious effect for deinsectization of the indoor and courtyard and mosquito nets use to control desert-type kala-azar in the epidemic areas.

C0926 FIRST MOLECULAR DETECTION OF LEISHMANIA INFANTUM IN SERGENTOMYIA MINUTA (DIPTERA, PSYCHODIDAE) IN ALENTEJO, SOUTHERN PORTUGAL

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1 Background

Phlebotomus perniciosus and *P. ariasi* are proven vectors of *Leishmania infantum* in Portugal. However, the occurrence of *Sergentomyia minuta* infected with *L. tropica* was observed in Algarve, southern Portugal, by molecular techniques. The*Alentejo region*, situated at the north of *Algarve,* presents human and canine leishmaniasis cases caused by *L. infantum*. Nevertheless, few studies related to phlebotomines were carried out in this region, despite the impact of this zoonosis in the country. In this context, we investigated the heterogeneity of sandfly species in the Alentejo region, namely in Évora and Beja districts, to further understand the epidemiology of leshmaniasis in this area.

2 Methods

Following species identification,the sand fly fauna and rates of natural infection by *Leishmania* were evaluated. Specimens capture was performed monthly, from May to November 2016, by CDC light-traps, in domestic and peridomestic biotopes. Molecular diagnosis was performed individually in non-blood-fed females, using a multiplex PCR with specific primers for the *Leishmania* genus (kDNA) and for a constitutive sandfly gene (*cacophony*). After amplification, all samples were reanalyzed through PCR product hybridization with a species-specific biotinylated probe corresponding to the conserved sequence of kinetoplast minicircles to confirm the infection by *L. infantum*.

3 Results

A total of 147 sand flies were collected, from which 109 were males and 38 females. Among the females, the most frequent species was *S. minuta* (65%), followed by *P. perniciosus* (29%), *P. sergenti* (4%) and *P. ariasi* (2%).For the evaluation of natural infection indexes, to date, 28 non-blood-fed females out of the 38 captured, were processed and analyzed by PCR. Positive results for the presence of *L. infantum* DNA were identified in only one specimen (3.57%) of *S. minuta*, collected in a peridomestic biotope.

4 Conclusions

These preliminar results suggest that *S. minuta* is a potential *L. infantum* vector in the Alentejo region, since this sand fly is able to fed in vertebrate hosts, including dogs, that are the main *L. infantum* reservoir in Portugal. Further studies are still required to confirm such conclusions. As perspectives, we are planning new captures in the same region, from May to November 2017, for better understanding *L. infantum* transmission cycle in the Alentejo area of Portugal.

C0944 COMPARISON OF THE INFECTIOUSNESS OF DOGS INFECTED BY LEISHMANIA INFANTUM, WITH AND WITHOUT CLINICAL PRESENTATION, ON SKIN SITES WITH LESIONS AND HEALTHY SKIN SITES

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1 Background

Canine visceral leishmaniasis (CVL) is a zoonotic disease caused in the Americas by *Leishmania infantum*, which is transmitted by the bite of infected sandflies of the species *Lutzomyia longipalpis*. In Brazil, dog is considered the main domestic reservoir of visceral leishmaniasis. Thus, it is important to study their participation in the transmission of the parasite to the vector. In the literature, divergent results are found regarding the infectiousness of dogs with clinical signs of CVL and those without clinical presentation. This divergence may be due to different methodologies applied in the studies, such as clinical classification of the dogs and anatomic sites on the animal, where the xenodiagnosis was performed, with no description concerning skin lesions. It is known that the parasite in the skin of the infected dog does not disseminate throughout the whole skin of the animal, staying on the site where it was inoculated by the sandfly bite. Hence, in these sites, the parasites cause skin lesions that might act as source of infection of *L. infantum* to naïve *Lu. longipalpis*. Thus, the aim of this study was to compare the infectiousness of clinically sick and healthy dogs, infected by *L. infantum*, on sites presenting skin lesions with healthy skin sites.

2 Methods

The xenodiagnosis was performed using 20 female sandflies in naturally infected dogs from an endemic area in Brazil. For each dog, two xenodiagnosis were done, one on a site of the skin with lesions (border of ulcer, induration and depigmentation) and another on a healthy skin site, on dogs with clinical (7) and subclinical (8) infection. *Leishmania* infection was detected on the second day post feeding, by direct evaluation of the presence of promastigotes in the midgut.

3 Results

Among the clinically sick animals, 42.9% (3/7) transmitted *L. infantum* to the vector. These animals presented positive xenodiagnoses on sites with skin lesions, wherein two transmitted only on the sites with skin lesions while one could transmit from both places evaluated, with and without lesions. The grade of infectiousness varied between 8.3% (one among 12 dissected) and 78.6%. In the other hand, there was no transmission in the group of dogs with subclinical infection.

4 Conclusions

Our results indicated that sites of the skin with lesions of CVL were a better source of *Leishmania* infection to sandflies, showing that lesions are important places to be evaluated in the determination of the infectiousness of dogs infected by *L. infantum*.

C0946 LEISHMANIA (VIANNIA) NAIFFI LAINSON AND SHAW 1989: A PARASITE EXCEEDING CONVENTIONAL ECOLOGICAL BARRIERS IN THE AMAZON BASIN

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1 Background

Leishmania-vector systems are assumed to be specific relationships between parasites and phlebotomine hosts that are related to such factors as co-host association, co-evolution, and vector/parasite co-speciation. In this respect, *Leishmania* (*Viannia*) *naiffi*, a species causing self-limiting American cutaneous leishmaniasis in the Amazon Basin, has been found in several phlebotomine species but so far it has only been found in one wild mammal, the nine-banded armadillo *Dasypus novemcintus*. This present work investigates the putative eclectic vector repertoire of *L.* (*V.*) *naiffi*, as well as its public health implications.

2 Methods

Early data about phlebotomine *L.* (*V.*) *naiffi*-infections were considered from the literature. Present data resulted from our entomological surveys carried out in the Brazilian Amazon forested areas from Serra do Navio (Amapá State-AP, Brazil) and Belterra (Pará State-PA, Brazil). Captures were performed using CDC light traps set at ground and canopy level, 'Shannon' captures and aspiration from tree bases. Attempted isolations from *Leishmania*-positive dissected flies were made *in vitro*/*in vivo*. Isolates were characterized by the indirect immunofluorescence reaction with a battery of species-specific monoclonal antibodies, isoenzyme electrophoresis, and a polymerase chain reaction-restriction fragment length polymorphism of the RNA Polymerase II gene with TspRI and HgaI endonucleases.

3 Results

L. (*V.*) *naiffi*-infections have so far been recorded in 7 phlebotomines:*Psychodopygus ayrozai*, *Ps. davisi, Ps. hirsutus hirsutus*, *Ps. paraensis, Ps. squamiventris squamiventris*, *Ps. s. maripaensis* and *Lutzomyia tortura.* Present findings include infections in 2 *Ps. s maripaensis* captured in a Shannon and a canopy CDC in AP, inone *Ps. wellcomei*/*Ps. complexus* from a PA canopy CDC, and in a *Nyssomyia anduzei* from an AP canopy CDC. This means that *L.* (*V.*) *naiffi* has now been found in a total of 9 different phlebotomine species. Themajority of the infections were in species of *Psychodopygus*,but also in a *Nyssomyia* and a *Lutzomyia* species.

4 Conclusions

These results indicate that in the same biome distinct, but not closely-related sand fly species, may be acting as *L.* (*V.*) *naiffi* vectors. The variety of highly anthropophilic phlebotomine species naturally infected with *L.* (*V.*) *naiffi* in the Amazon Basin has without doubt important epidemiological consequences and may be responsible for high infection rates of this parasite in man in some Amazonian biomes.

C0965 EVALUATION OF DIFLUBENZURON ON THE IMMATURE STAGES OF COLONIZED LUTZOMYIA LONGIPALPIS (DIPTERA: PSYCHODIDAE: PHLEBOTOMINAE), THE MAIN VECTOR OF AMERICAN VISCERAL LEISHMANIASIS IN BRAZIL

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1 Background

Alternative vector control methods aimed at reducing the population of phlebotomines in areas of transmission of American visceral leishmaniasis have been proposed, focusing not only on adults, but also on potential immature breeding sites. The present work aimed to evaluate in laboratory conditions the effects of the insect growth regulator 'diflubenzuron' on the biology of immature forms of colonized *Lutzomyia longipalpis* from an endemic area of ??Amazonian Brazil.

2 Methods

A population of *L. longipalpis* was used in laboratory (Barcarena F73), according to previously established methodology. Five serial dilutions (500, 250, 125, 62.5 and 31.2 ppm) of diflubenzuron (1% + vehicle q.s. 100%) were performed to impregnate circular filter papers (grade 1, qualitative 8cm) on a single occasion, during the evolution of the immature phases. Each impregnated filter paper was placed in a 9cm petri dish used for sowing 40 eggs, 3 days post-oviposition. The control group was treated with type II water. A survival curve was constructed from the mean of two replicates of the daily immature counts until adult emergence. Chi-square was applied between each other and control L1-pupa survival rates, considering significance if *p*-value <0.05.

3 Results

There was no inhibition at hatching (egg/L1) as also on pupa/adult emerging. The survival rates during L1/L2, L2/L3, L3/L4, L4/pupa molts and overall immature forms (L1/pupa) were, respectively, at 500ppm: 37.5, 13, 50 and 100% (L1-pupa: 2.5%); 250ppm: 30, 50, 33.3 and 50% (L1/pupa: 2.5%); 125ppm: 67.5, 62, 64.7 and 90.9% (L1-pupa: 25%); 62.5ppm: 60, 75, 94 and 88.2% (L1/pupa: 37.5%); 31.2 ppm: 82.5, 48.4, 75 and 100% (L1/pupa: 30%); control group: 62.5, 64, 93.7 and 100% (L1/pupa: 37.5%). Significant differences were observed between overall immature survival rates (L1/pupa) on concentrations 500ppm, 250ppm and control group ($p<0.05$).

4 Conclusions

The results showed that the lowest survival rates of individuals occurred during the L1/L2 and L2/L3 molt for all test colonies and the lowest overall immature survival rate (L1/pupa) occurred at concentrations of 500ppm and 250ppm. In spite of these two higher concentrations tested have been prove to be growth-inhibitory for colonized *L. longipalpis* under laboratory conditions, further studies on component safety and ecological impact are required in the field.

C1092 DETECTION OF LEISHMANIA IN SAND FLIES BY MULTIPLEX POLYMERASE CHAIN REACTION, IN SOUTHERN BRAZIL

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1 Background

The technique of multiplex Polymerase Chain Reaction (multiplex PCR) has been used in the diagnosis of leishmaniasis and in the determination of the natural rate of *Leishmania* infection in sand flies. The aim of this study was to standardize the multiplex PCR for the detection of *Leishmania* genus in the sand flies of endemic regions of Paraná River islands, northwest of Paraná state, Brazil.

2 Methods

The Sand flies collections were carried out in ten islands: Bandeira, Carioca, Fina, Mutum, Japonesa, São José Catarino, Santa Rosa, Floresta, Chapéu Velho and Cruzeiro, from June 2012 to October 2014. On Mutum Island, where a large number of permanent inhabitants live, the collection was performed once a month, from 6:00 p.m. to 6:00 a.m., for 13 months. In other islands, one collection in each season of the year was performed during one year, from 7:00 p.m. to 7:00 a.m.. Sand flies were collected with Falcão light traps, identified and conserved in tubes containing isopropanol, ten females per tube, for subsequent DNA extraction. The insects were macerated in lysis buffer, and DNA extraction was carried out with Chelex® 100 Resin. Two pairs of primers were used for multiplex PCR. The first pair was A1 and A2, which amplify a fragment of 110-120 bp of the conserved region from the minicircle of the kinetoplast (kDNA) of the genus *Leishmania*. The second was 5Llcac and 3Llcac, which amplify a fragment of 220 bp from the *IVS6* gene region of the cacophony in insects of the genus *Lutzomyia*. The product of multiplex PCR was analyzed by agarose gel eletrophoresis.

3 Results

Nyssomyia neivai was the predominant species, representing 99.9% of the collected specimens. An amount of 220 females (22 pools) of sand flies of *Ny. neivai* was submitted to the multiplex PCR. All pools showed the 220 bp fragments for sand fly DNA detection, indicating the absence of Taq DNA Polymerase inhibitors. No one of the pools was detected with *Leishmania* infection.

4 Conclusions

The studied islands still conserve native forest, maintaining the enzootic cycle of *Leishmania*. *Ny. neivai* was already detected with natural *Leishmania (Viannia)* sp. infection. This fact demonstrates the importance of this species in the epidemiology of cutaneous leishmaniasis.

C1096 LUTZOMYIA SPECIES AS PUTATIVE VECTORS OF LEISHMANIA IN THE SOUTHEASTERN PERUVIAN AMAZON

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1 Background

Cutaneous Leishmaniasis (CL) is an important health problem in the New World affecting civilian and military populations exposed in endemic settings. The Peruvian region of Madre de Dios located near the border with Brazil is one of the most endemic CL regions in South America with more than 4,451 reported cases during the last five years. However, little is known regarding the diversity and distribution of sand fly vectors in this area. We aimed to characterize the sand fly fauna in this region and identify putative *Leishmania* vectors.

2 Methods

Sand flies were collected in 2014 and 2015 in the district of Iñapari (Villa Primavera), Iberia (Flor de Acre and Arca Pacahuara), and Las Piedras(Mavila) using CDC light traps (18:00-06:00) inside and outside the houses and Shannon trap (18:00-21:00) outside the houses for anthropophilic sand flies. Shannon Diversity Index was determined using Past v3.0. Collected specimens were identified and non-blood-fed females were selected for *Leishmania* infection screening using kDNA PCR and FRET probes-based Nested Real Time PCR for species identification.

3 Results

We collected 10,897 specimens of the genera *Lutzomyia* (58 species) and *Brumptomyia* (two species). Our study determined widespread distribution and abundance of *Lu. auraensis* (24%), *Lu. whitmani* (19.4%)and *Lu. yucumensis* (15.8%) in the region. Villa Primavera showed the highest sand fly diversity. We found 10 *Lutzomyia* species infected with *Leishmania* species: *Lu. whitmani, Lu. llanosmartinsi*, *Lu. yucumensis, Lu. auraensis, Lu. migonei, Lu. sherlocki, Lu. shawi, Lu. walkeri and Lu nevesi*. We identified *L. (V.) guyanensis* in *Lu. whitmani* and *L. (V.) braziliensis* in *Lu. llanosmartinsi* and *Lu. davisi*.

4 Conclusions

Our study confirmed a high sand fly diversity in Madre de Dios, with differences in sand fly abundance and species composition across sites. Moreover, we identified 10 *Lutzomyia* species naturally infected with *Leishmania* spp., having identified *L.* (*V.*) *guyanensis* and *L.* (*V.*) *braziliensis* in three of them. The high abundance of putative vectors may explain why this region has a high prevalence of human cases in Peru.

C1136 INVESTIGATIONS ABOUT THE ROLE OF URBAN LANDSCAPE IN TRANSMISSION OF VISCERAL LEISHMANIASIS.

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1 Background

Visceral leishmaniasis (VL) caused in Brazil by Leishmania infantum is a zoonosis transmitted by the phlebotomine Lutzomyia longipalpis. Was restricted To the rural area of ??the Northeast. Since the 1980s, urbanization has been
And is dispersed throughout the country. Although the females of the vector are hematophagous, Regularly fed to plants, and vegetable sugars can be Modulate the transmission of Leishmania, thus, the association of plants with
LV in cities can help explain the phenomena of urbanization and dispersal. The objective was to verify the association of LV with Through Remote Sensing (SR) and Molecular analysis of plant food content lU. Longipalpis.

2 Methods

The Municipalities chosen for the research were Teresina (PI) and Belém (PA). The identification of Species of the municipality of Teresina was Of floristic survey. For the preparation of a molecular library Plant, primers were used for two plastidial DNA genes, Ribulose-1,5-biphosphate and K maturase. The sandflies were captured Using CDC-type traps and landscape analysis by SR were
Used high resolution satellite images.

3 Results

The most Representative of Teresina were: Mangifera indica; Azadirachta indica e Licania tomentosa. The extraction of plant DNA was performed successfully and the The minimum possible concentration of detection by conventional PCR was
0.1 ng / µl; Both Lu.longipalpis samples and plant samples, Were positive for the two primers used. This result Shows the possibility of identifying the plant food content of the Insects from the comparison between the sequences obtained in these and the
Contained in the plant molecular library.

4 Conclusions

The study will Gains in technology and strategies for controlling urban diseases, while To evaluate the possibility of landscape intervention for the control of LV and Of other urban diseases transmitted by insects.

C1149 IDENTIFICATION OF THE SAND FLY LUTZOMYIA LONGIPALPIS PLANT FEEDING PREFERENCE USING DNA BARCODE TECHNIQUE

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1 Background

Little is known about the feeding behavior of hematophagous insects that require sugar from plants to complete their life cycles. We studied plant feeding of *Lutzomyia longipalpis* (*L. longipalpis)*, a known vector of *Leishmania infantum/chagasi* parasites, in a tropical city with high incidence of visceral leishmaniasis.

2 Methods

L. longipalpis were collected using CDC traps at a single location in the city. The DNA barcode technique was applied to identify plant food source of wild-caught sand flies using specific primers for a locus from the chloroplast genome, ribulose diphosphate carboxylase (*rbcL*). DNA samples from all trees or shrubs within a 100-meter radius from the trap were collected to build a barcode reference library for the *rbcL* gene. The distance from the trees or shrubs to the trap and the average expansion of the tree or shrub crowns were assessed.

3 Results

The results indicated that DNA from nine of the 14 plant families represented near the collection site were found within the digestive tracts of sampled phlebotomine sand flies. While plants from the Anacardiaceae and Meliaceae families were the most abundant at the sampling site (25.4% and 12.7% of the local plant population, respectively), DNA from these plant families was found only in few flies; in contrast, despite its low abundance (2.9% of the local plant population), DNA from the Fabaceae plant family was detected in 94.7% of the sand flies. The proportion of sandflies testing positive for DNA from a given plant family was not associated with factors such as tree abundance, tree distance from the trap, or average crown expansion of trees from a given family.

4 Conclusions

The data, therefore, suggest that there may indeed be a feeding preference of *L. longipalpis* for plants in the Fabaceae family.

C1189 AN ECOLOGICAL AND PHYSICOCHEMICAL APPROACH TO THE NATURAL BREEDING SITES OF LEISHMANIA VECTORS IN NORTHERN COLOMBIA

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1 Background

Leishmaniasis are diseases caused by *Leishmania* parasites, traditionally these have been associated with selvatic and rural environments, nevertheless some social, economic and ecological factors has favor the appearance of autochthonous cases in periurban and urban settlements. The municipality of Ovejas is considered as the main focus of visceral leishmaniasis in the country, where *Lutzomyia evansi* is the vector of both LC and LV and in spite of this insect is the most abundant species little is known about its natural breeding sites. The characterization of natural breeding sites is essential to establish vector control measures in urban and peri-urban areas where leishmaniasis cases are being reported

2 Methods

1 kg of soil samples were taken around base of the tree, leaf litter, tree hollows, soil from coarse woody debris, poultry and pig shelters. The larvae were recovered by direct search under the stereoscope. Subsequently, these soil samples were incubated until hatching of remaining larvae. The trees associated to each breeding site were identified, and physical and chemical characteristics of the soil were analyzed

3 Results

616 soil samples were collected from 466 potential breeding sites in the periurban area of Ovejas, Sucre. 422 *Lutzomyia* immatures were retrieved from samples collected at the base of trees (n=101), leaf litter (n=1) and fallen trees (n=1). *L. evansi* was the most abundant specie (n=222), followed by *L. rangeliana* (n=53), *L. c. cayennensis* (n=22), *L. atroclavata* (n=21), *L. micropyga* (n=12), *L. trinidadensis* (n=4), *L. dubitans* (n=3), *L. gomezi* (n=2) and unidentifiable *Lutzomyia* specimens (n=83). Natural breeding sites of *L. evansi* were the base of the tree of *Cordia dentata* (n=53), *Astronium graveolens* (n=3), *Guazuma ulmifolia* (n=2), a few unidentifiable trees (n=5); and leaf litter. The analysis of the physical and chemical variables of the soil indicate that inmature sandfly stages are developed in soils that have an average pH of 7.4, with silty texture, rich in nutrients, with a good Ca-Mg ratio, cationic exchange capacity and Ca saturation. The abundance of immatures in a microhabitat was determined by the texture (silt) and the Ca-Mg ratio in the soils

4 Conclusions

Most of the natural breeding sites were found associated with the base of *C. dentata*, providing the necessary conditions for the development of the immatures in the vicinity of the dwellings. The abundance of immature recovered is related to some physical and chemical conditions of the soils

C1229 CLUSTER RANDOMISED TRIAL AND DEVELOPMENT OF A SANDFLY SEX PHEROMONE LURE TO REDUCE LEISHMANIA INFANTUM INFECTION.

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1 Background

New vector control tools are needed to combat leishmaniasis. A semi-synthetic version of a sex aggregation pheromone (3-methylgermacrine-B) of male *Lutzomyia longipalpis* has shown to be attractive in previous studies. Here, we present results from a cluster-randomised trial in Brazil to test this pheromone deployed with insecticide as a lure-and-kill intervention to reduce *Leishmania infantum* transmission to the reservoir (dogs). We also present results of further developments of the pheromone lure and deployment strategies through spatial modelling.

2 Methods

A cluster randomised trial was conducted in Brazil, whereby the pheromone and insecticide was compared to a sham control arm, and a positive control (deltamethrin-impregnated collars fitted to dogs) arm, deployed in 14 stratified communities per arm and followed-up over 2 years. The primary intervention effects were quantified by measuring the number of uninfected dogs that seroconverted over the course of the trial.
Using parameters derived from the trail and empirical estimates elsewhere, a stochastic, spatial, individual-based, transmission model for zoonotic visceral leishmaniasis was developed, to identify key characteristics needing further development, and to predict the most efficacious deployment scenarios.

3 Results

A reduction in canine incidence was attributed to the pheromone + insecticide trial arm that was relatively consistent across the levels of analysis, though the errors were broad. The performance of the pheromone followed similar patterns as the collar arm. Spatial models identified key pheromone components, currently under-going further development, to improve its efficacy in community-wide deployment.

4 Conclusions

These data were generated from the first trial of a synthetic vector pheromone applied in public health control, showing significant progress to becoming a novel vector control tool to combat zoonotic visceral leishmaniasis in the Americas.

C1279 SEROLOGICAL INVESTIGATION OF VISCERAL LEISHMANIA INFECTION DYNAMICS IN HUMAN AND DOGS IN WELKAIT DISTRICT, WESTERN TIGRAY, ETHIOPIA

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1 Background

Visceral Leishmaniasis (VL) is becoming a major public health problem both in terms of geographical spread and incidence in Ethiopia although failed to attract the attention it deserves. Magnitude, public health impact and dynamics of VL in Ethiopia are not well studied. Therefore, this study was conducted on Welkait district, unattached area with favorable environmental situation, to determine Human-reservoir leishmania infection dynamics, and to assess potential risk factors and community awareness in the study area.

2 Methods

A cross sectional study was employed in purposively selected sub-districts from November 2015 to June 2016 and house to house survey was carried out. A total of 663 study units were participated in the study among which 329 humans and 46 dogs were contacted for serological survey. ITleish and Leishmanin skin test (human) and only ITleish (dogs) were used for serological investigation. Two separate pre-tested questioners were used to obtain required information (for risk factors and KAP assessment).

3 Results

The overall sero-prevalence of human VL in the district was 8.8%. Environmental and host risk factors significantly associated with human VL infection were sub-districts, sex, re-settlement, sleeping outside and dog ownership. A sero-positivity of 4.35% was also recorded from dogs which was found statistically significant (p-value= 0.000) with the infection outcome of their owners. Overall score of participants with 59% knowledgeable, 95% positive attitude and 53% respondents with good practice had recorded. Furthermore, socio-demographic characteristics: sex, educational status and history of travel to VL endemic areas were found to have a statistically significant association (p-value < 0.05) with the individuals' knowledge on VL.

4 Conclusions

Detailed research on sand fly, circulating leishmania species and role of dogs in its dynamics are mandatory.

C1280 COMPOSITION AND DISTRIBUTION OF PHLEBOTOMINES OF MEDICAL IMPORTANCE IN THE MUNICIPALITIES OF TIERRALTA AND VALENCIA, DEPARTMENT OF CÓRDOBA (COLOMBIA).

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1 Background

Ecoepidemiological studies of cutaneous leishmaniasis and continuous monitoring of insect vectors have generated a knowledge base that can be used to implement control and prevention strategies targeted at the disease transmission dynamics in the transmission focal areas of Colombia. In this study, the presence and spatial distribution of phlebotomines of medical importance were determined in the municipalities of Tierralta (El Loro and Tuis Tuis villages) and Valencia (Guadua and Mieles villages), which are in the department of Córdoba (Caribbean coast of Colombia). In the department of Córdoba, the municipalities of Tierralta and Valencia have the highest numbers of cases of CL.

2 Methods

Entomological samples were collected via CDC traps located in the intradomicilary and peridomiciliary areas. Active searches were also undertaken with a mouth aspirator to collect adult phlebotomines resting on buttresses. Spatial distribution maps of the *Lutzomyia* species were then generated.

3 Results

A total of 1677 *Lutzomyia* individuals corresponding to 12 species were identified. Among these species, *Lutzomyia panamensis* was the most abundant (80, 18%). A high richness of *Lutzomyia* species, which are recognized as vectors of leishmaniasis in Colombia, is highlighted. The composition of the intradomicilary and peridomiciliary phlebotomines varied significantly ($F = 0.9962$; $df = 11.45$; $p = 0.02895$). The first records of *Lutzomyia carpenteri, Lutzomyia camposi, Lutzomyia dysponeta, Lutzomyia atroclavata* and *Lutzomyia yuilli yuilli* for the Córdoba department are highlighted.

4 Conclusions

The spatial distribution shows that *Lutzomyia panamensis* and *Lutzomyia gomezi* are dominant and associated with higher concentrations of housing. This study provides basic information on new records of phlebotomines for the department of Córdoba, and the results suggest that greater vector-human contact occurs in the peridomiciliary environment and a high number of species of *Lutzomyia* associated with the transmission of leishmaniasis are present in Colombia.

C1282 CURRENT COMPOSITION AND DISTRIBUTION OF PHLEBOTOMINES IN A HISTORIC FOCUS OF VISCERAL LEISHMANIASIS IN THE DEPARTMENT OF CÓRDOBA, COLOMBIA

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1 Background

The municipality of San Andrés de Sotavento (Córdoba) has been considered a historic focus of leishmaniasis since the 90's. Nowadays, some settlements in this municipality report sporadic cases of visceral and cutaneous leishmaniasis. San Andrés de Sotavento has undergone changes in landscape clearly associated with the expansion of the agricultural frontier and urbanism. As a consequence, a decrease in the remnants of gallery forests near the dwellings is observed, which were considered as a determinant biomarker that favoured the abundance and distribution of vector species, from which *Lutzomyia evansi* stands out. The latter justifies carrying out an entomological follow-up of *Lutzomyia* species (main risk factor), which can be comparative in composition and distribution with respect to the domicile, in order to generate useful information that impacts the vector control strategies in this locality.

2 Methods

The sandflies samplings were collected with CDC type traps in the intra, peri and extra domicile. Adult collection was also included with Shannon-type traps. Nocturnal activity pattern were evaluated. The spatial distribution of phlebotomines was estimated.

3 Results

The total number of sandflies collected was 3451. A greater abundance in the peri-domicile is observed with respect to intra-domicile and extra-domicile. Nine species of the genus *Lutzomyia* have been identified. *Lu. evansi* continues to be the abundant species with 92.17% of the current total. In the 1990s, *Lu. cayennensis* was the second in abundance, with great adaptability to the domicile. In this study, *Lu. panamensis* and *Lu. gomezi* that are the medically important species that continue in order of abundance after *Lu. evansi*. Another substantial difference is the absence of *Lu. serrana* in the taxonomic listing. Two peaks of activity were identified. The first between 20 and 21 hours. The second between 2 and 3 hours. The spatial distribution of phlebotomines suggests a greater presence and adaptability to the urban environment of species such as *Lu. evansi*, *Lu. gomezi* and *Lu. panamensis*.

4 Conclusions

High abundance of *Lu. evansi*, a change in the composition of the rest of the fauna of phlebotomines and a spatial distribution that demonstrates greater abundance of the species associated with houses. Activity peaks indicate an increased risk of intra-domiciliary transmission of visceral leishmaniasis in vulnerable children aged 0 to 5 years.

C1288 BACTERIAL DIVERSITY ANALYSIS OF IMMATURE AND ADULT MIDGUT OF LUTZOMYIA EVANSI, USING CULTURE-DEPENDENT AND CULTURE-INDEPENDENT METHODS

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1 Background

Lutzomyia (Lu.) evansi is a phlebotomine insect endemic to the Caribbean coast of Colombia with epidemiological significance for being the main vector of leishmaniasis. Although these insects can harbor pathogenic and non-pathogenic microorganisms in their intestinal microbiota, there is little knowledge on bacterial diversity present in the digestive tract of wild populations.

2 Methods

In this study, conventional microbiological methods and molecular tools, were used to assess the composition of bacterial communities associated with the digestive tract of immature and adult stages of wild populations of *Lu. evansi* from the department of Sucre (Caribbean coast of Colombia). We also determined the presence of *Wolbachia* endosymbiont bacteria and Leishmania parasite by PCR and DNA sequence analysis in *Lu. evansi* and other species. The antagonistic effect of *Pantoea ananatis*, *Ochrobactrum anthropi* and *Enterobacter cloacae* was evaluated against six species of human pathogenic bacteria and against stationary (Metacyclic-like) and exponential promastigotes (Procyclic-like) of *Leishmania infantum* (BCN-GFP strain) by co-culture assays.

3 Results

The culture-dependent technique showed that the intestinal bacteria belonged to *Acinetobacter*, *Enterobacter*, *Pseudomonas*, *Ochrobactrum*, *Shinella* and *Paenibacillus* were the dominant bacteria isolated in larvae and *Lysobacter*, *Microbacterium*, *Streptomyces*, *Bacillus* and *Rummeliibacillus* in pupae; *Staphylococcus*, *Streptomyces*, *Brevibacterium*, *Acinetobacter*, *Enterobacter* and *Pantoea* in adult stages. Endosymbiotic *Wolbachia* was found in three species: *Lutzomyia cayennensis*, *Lutzomyia dubitans* and *Lutzomyia evansi*. The highest percentage of inhibition was observed against exponential promastigotes with bacterial concentrations of 108 CFU/ml of *Enterobacter cloacae* (77.29% ± 0.6%) and *Pantoea ananatis* (70.17% ± 1.1%).

4 Conclusions

Significant variation in the bacterial communities associated with the digestive tract of *Lu. evansi*, depending of developmental stage and the blood intake of females. Evidence of *Wolbachia* infections in natural *Lutzomyia* populations encourage further investigation on the possible effects of this bacteria in *Leishmania* transmission. The growth inhibitory activity of exponential *Leishmania infantum* promastigotes shown by *Enterobacter cloacae* and *Pantoea ananantis* suggests that the presence of these bacteria in the vector intestine may affect the parasite development to metacyclic stages, infective to human hosts.

C1300 ARE FOOD DYES RELIABLE MARKERS TO DETECT PREFERENCES OF LUTZOMYIA LONGIPALPIS SANDFLIES BETWEEN DIFFERENT ATTRACTIVE TOXIC SUGAR BAITS?

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1 Background

Studies on attractive toxic sugar baits (ATSBs) frequently use coloured food dyes to indicate whether sandflies have ingested a particular bait. However, the potential for colour changes of these dyes in the sandfly gut post-ingestion have not been published. Should colour changes occur, leading to false positives or negatives, invalid conclusions about their ingestion could be reached affecting the progress of ATSB development. The purpose of this study was to identify differently coloured food dyes to use in ATSB studies that do not change colour following ingestion.

2 Methods

Uniformly-sized cotton balls were soaked in either 10% sucrose solution or 10% date juice solution dyed either with tartrazine (yellow food dye) or indigo carmine (blue food dye). Two different sugar sources were tested to ensure that any changes due to cross-reactions between specific sugar sources and the dyes could be identified. The balls were placed in eight 30cmx30cmx30cm cages. In a temperature controlled room (25.5^{o}C +/- 0.5^{o}C), approximately 40 *Lutzomyia longipalpis* starved for the preceding 48 hours were introduced in to each cage and left to feed for two consecutive nights (48 hours). At the end of the feeding period, each *Lu. longipalpis* was aspirated and placed on ice to kill. For comparison, a test using 198 *Culex quinquefasciatus* mosquitoes (141 exposed to tartrazine, 57 to indigo carmine) was performed. The crops of all individuals were dissected and observed under a compound microscope.

3 Results

Of the 65 *Lu. longipalpis* that ingested tartrazine (43 female, 22 male), 20% were yellow in appearance, 69.2% blue, 4.62% brown, 3.08% red, 1.52% green, and 1.52% transparent. The changes in colour from yellow occurred more frequently following ingestion of date juice (80%) than sucrose solution (53.33%). In the indigo carmine assay, 24 *Lu. longipalpis* took a sugar meal; 75% appeared blue, 12.5% red, 12.5% transparent, and 8.33% burst during dissection. The guts of all *Cx. quinquefasciatus* that fed on tartrazine dyed sugar sources appeared yellow after dissection, and all indigo carmine blue.

4 Conclusions

In no-choice assays utilizing either a 10% date juice or 10% sucrose solution dyed with 10% tartrazine, the guts of sugar-fed *Lu. longipalpis* showed very little conformity in appearance. These anomalies were not detected in *Cx. quinquefasciatus*, so may be specific to sandflies. While more replicates are required to validate these results, alternative dyes should be used to mark ATSBs in the interim.

C1358 ECOLOGICAL NICHE MODELLING OF NYSSOMYIA TRAPIDOI AND NYSSOMYIA YLEPHILETOR, VECTORS OF LEISHMANIA, WITH COMMENTS ON THEIR GEOGRAPHICAL DISTRIBUTION

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1 Background

The sand fly species *Nyssomyia trapidoi* and *N. ylephiletor* are vectors of *Leishmania* in the trans-Andean north-west region of South America and Central America.

2 Methods

In order to characterize the geographical distribution of both of these sand fly species, we performed ecological niche modelling based on occurrence records collated from the previously published research literature and unpublished data from museum collections.

3 Results

In total, our newly assembled database contains 323 occurrence records: 184 for *N. trapidoi* and 139 for *N. ylephiletor.* The two sand fly species have largely overlapping occurrences throughout southern Central America, especially in Panama and Costa Rica, where both are incriminated/suspected vectors of *Leishmania*, but differ in the northern and southern limits of their distributions: *N. trapidoi* is frequently encountered in Ecuador and Colombia, while only *N. ylephiletor* is found in Guatemala, Belize and Mexico. Our ecological niche models based on climatic variables accurately predicted – and discriminated between – the observed trans-Andean distributions of both *N. trapidoi* and *N. ylephiletor*, but also predicted the presence of both species in cis-Andean Amazonian regions, where they have not previously been reported. This apparent discrepancy between the observed and predicted distributions of both sand fly species could be explained by the Andes mountain range acting as a geographical barrier to their southward colonisation, although the possibility of their cryptic presence should also be considered.

4 Conclusions

The distribution maps we report can aid health authorities in the identification of potential risk areas for transmission of *Leishmania*, and in the organization of prevention and control programs, as well as indicating suitable habitat regions that could potentially be invaded by new sand fly species. Funding FAPEMIG (APQ-01811-12).

C1409 ENTOMOLOGICAL COLLECTION AND MOLECULAR IDENTIFICATION OF PHLEBOTOMUS ARGENTIPES IN ASSAM, INDIA

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1 Background

History of Kala-azar in Assam, India, dates back to eighteen century where many districts of the state were severely affected. Gradually disease was declined due to extensive use of insecticide (DDT) in malaria elimination / control programme. After 1950s very few kala-azar cases were recorded and presence of *Phlebotomus argentipes* the vector sand fly was almost wiped out. Following an outbreak of kala-azar in 2008 and occurrence of new cases in subsequent years, it was thought worthwhile to look for presence of vector *Phlebotomus argentipes* in the kala-azar resurgence areas.

2 Methods

Entomological collections of sand flies were done using sticky trap and manual aspiration methods (suction tube and flash light) from Kamrup district of Assam. Collected sand flies were morphologically identified, pooled and stored for further processing. Molecular techniques PCR and RFLP were used in this study. Briefly, DNA was extracted from sand fly pool using Qiagen DNA extraction kit. Species specific PCR targeting 18srRNA coding gene for *Phlebotomus spp*. was performed using primer sequences forward (5?-TCGAATCTATGGGTGGTGGT-3?) and reverse (5?-CACAATCCCAACC ACGAAG-3?) that amplify a region of 399bp. The amplified products were subsequently subjected to restriction digestion using *Hae III* restriction enzyme. Digested PCR products were analysed by electrophoresis on a 2% Agarose gel stained with Ethidium bromide and viewed under UV light.

3 Results

Of the 9 pools processed one pool was found positive for *P. argentipes*. The Hae III restriction enzyme digested the amplified products at two positions 27 bp and 158 bp generating three fragments of 27bp (invisible), 131bp and 241bp in the conserved region of *P. argentipes* while the remaining undigested products indicates presence of other species of the genus *Phlebotomus.*

4 Conclusions

In India several foci of kalaazar have been reported over a period of time and vector identification and incrimination are the important part in confirmation of indigenous transmission. Following the outbreak of kala-azar in 2008, it was thought worthwhile to identify the vector sand fly in the affected areas. Confirmation of presence of *P. argentipes* in the study area endorsed the local transmission of kala-azar. This warrants vector control measures implementation in the affected areas.

C1423 BEHAVIORAL AND MORPHOLOGICAL EVIDENCE FOR PHEROMONE COMMUNICATION IN MIGONEMYIA MIGONEI (DIPTERA:PHLEBOTOMINAE)

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1 Background

Mygonemyia migonei (França 1920) is a known vector of *Leishmania braziliensis*, the causative agent of cutaneous leishmaniasis in South America. Recently it has been suggested that this specie can also transmit *Le. infantum* thus contributing to the burden of visceral leishmaniasis infection. This study describes the ultrastructure of male sex pheromone disseminating structures on abdominal segments of *M. migonei* and attraction of female to males of the same species. Understanding the behavioral and observe the morphological evidence for pheromone communication may help us to understand the role of sex pheromone in this important vector.

2 Methods

A colony of *M. migonei* was established from adults collected in Baturité, Ceará, Brazil (04°19′41″S, 38°53′05″W). Adults were captured using CDC light traps and transferred to a holding cage (20 x 20 x 20cm) in an insectary. To determine the presence of pheromone gland disseminating structure, *M. migonei* adult males reared in the laboratory were sedated for 5 min at -100C and then fixed with 2.5% glutaraldehyde (pH=7.4). After fixation, the insects were washed in 0.1M cacodylate buffer, pH 7.2, and then fixed with 1% osmium tetroxide for 1 h. The samples were dehydrated by an increasing concentration of ethanol solution, dried in a critical point dryer, mounted on stubs and then coated with gold using a sputter coater. The material was examined under a JEOL - 5600 LV microscope. Experiments to determine whether female *M. migonei* sand flies were attracted to male *M. migonei* were carried out using a Y-tube olfactometer.

3 Results

Scanning electron microscopy showed that in *M. migonei*, these structures are located between abdominal tergal III to VI and appearing as small round cuticular crater elevations with a spined pore. Little is known of the mating behavior of this specie. Even with the presence of several pheromones disseminating structures in adult male of *M. migonei*, behavioral and chemical studies are necessary to verify the courtship behavioral in this species. Old females are attracted to a small and large group of males at the similar age in the presence of mice.

4 Conclusions

The attraction demonstrated in this study indicates that the host odor is likely to be an important component for a control measures effective based in semiocehmicals. We expect that the use of pheromone as a control tool in sandflies will help in reducing the use of insecticides.

C1431 LUTZOMYIA MIGONEI IS A VECTOR COMPETENT FOR LEISHMANIA (LEISHMANIA) INFANTUM

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1 Background

Leishmania (*Leishmania*) *infantum* is the most widespread etiological agent of visceral leishmaniases (VL) in the world, with significant mortality rates in human cases. In Latin America, this parasite is primarily transmitted by *Lutzomyia longipalpis*, but the role of *Lutzomyia migonei* as a potential vector for this protozoan has been discussed. Laboratory and field investigations have contributed to this hypothesis. However, proof of the vector competence of *L. migonei* has not yet been provided.

2 Methods

We evaluate for the first time the susceptibility of *L. migonei* to viscerotropic *L. infantum* strain (MHOM/BR/76/M4192) and dermotropic *L. infantum* strain (ITOB/TR/ 2005/CUK3) and compared with development of *L. longipalpis*. Females were fed through a chick-skin membrane on rabbit blood containing *L.* (*L.*) *infantum* promastigotes, dissected at 1, 5 and 8 days post-infection (PI) and checked microscopically for the presence, intensity and localization of *Leishmania* infections. In addition, morphometric analysis of *L.* (*L.*) *infantum* promastigotes was performed.

3 Results

High infection rates of both *L.* (*L.*) *infantum* strains tested were observed in *L. migonei*, with colonization of the stomodeal valve already on day 5 PI. At the late-stage infection, most *L. migonei* females had their cardia and stomodeal valve colonized by high numbers of parasites, and no significant differences were found compared to the development in *L. longipalpis*. Metacyclic forms were found in all parasite-vector combinations since day 5 PI.

4 Conclusions

Here we demonstrate that *L. migonei* is highly susceptible to the development of *L.* (*L.*) *infantum*. This, together with its known anthropophily, abundance in VL foci and natural infection by *L.* (*L.*) *infantum*, constitute important evidence that *L. migonei* is another vector of this parasite in Latin America.

C1448 SWIM LIKE YOUR LIFECYCLE DEPENDS ON IT: THE IMPACT OF FLAGELLAR MOTILITY ON THE SURVIVAL OF LEISHMANIA PARASITES.

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1 Background

Motility of *Leishmania* parasites is essential for survival during host transitions and is important for lifecycle progression. It is the presence of an appendage, at the anterior end of the promastigotes which allows parasite movement. This oscillating flagellum creates the force which enables the promastigote to be pulled through its environment. The environmental conditions of the parasite change significantly during the lifecycle and the parasite morphologically transforms to optimise its infection potential.
The details of parasite swimming behaviour remain obscure but are fundamentally linked to its infectivity. Our investigation focuses upon how flagellar structure adapts between promastigote forms and how this impacts function in distinct environmental conditions.

2 Methods

We have adapted a unique method of high-speed, three-dimensional imaging called digital inline holographic microscopy (DIHM) allowing us to examine the movements of *Leishmania mexicana* promastigotes. The data produced can be used to numerically refocus and create a high resolution reconstruction of parasite movement at different length scales.
Using stage-specific fluorescent markers to distinguish between the promastigote stages, we tracked parasites over multiple frames to gain information on the swimming patterns of these cells. This revealed how the parasite and host cells interact with each other and their environments in three-dimensions.

3 Results

Three-dimensional tracking of promastigotes demonstrates stage-specific differences in swimming behaviour in response to the presence of macrophages and in distinct biologically-relevant environments. At a higher magnification, the shape of an individual flagellum can be reconstructed allowing an in depth three-dimensional analysis and comparisons of how the flagellum structure evolves throughout its lifecycle. We have elucidated distinct characteristics in the flagellar structures of promastigotes and it is these distinctions which enablelifecycle progression and transmission.

4 Conclusions

DIHM has allowed us to mathematically quantify the relationship between the active bending of the flagellum and cell movements within contrasting environments of the promastigote-secretory gel in the midgut of the phlebotomine sandfly and during the infection of macrophages in a mammalian host. The swimming parameters have helped to determine which factors of promastigote movement are in response to distinct environmental pressures.

C1451 DISTRIBUTION, ABUNDANCE AND INFECTION OF SANDFLIES WITH LEISHMANIA PARASITES IN THE DEPARTMENT OF CORDOBA, COLOMBIA.

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1 Background

Leishmaniases include a spectrum of diseases caused by the flagellate protozoan parasite *Leishmania*, an obligate intracellular parasite transmitted to humans and other mammals by the bite of a female Phlebotomine sand-fly. In Colombia, nine *Leishmania* parasite species have been reported and 14 species of sandflies have been proven as vectors making it one of the three Latin American countries with the highest number of species. The aim of this study was to determine the presence and distribution of sand-fly species and identify their infection percentage in the Department of Cordoba, Colombia.

2 Methods

The study was developed in 12 different townships of the department of Cordoba, Colombia. In each township 24 houses were selected for sampling, where two mini CDC light traps were placed in the intra and peridomicile from 18:00h to 06:00h. Captured sandflies were preserved in 70% ethanol and taken to the lab for specie identification following taxonomic identification protocol described by Young & Duncan 1994 and Galati 2013. Molecular biology assays were performed in order to identifyinfected species. Conventional PCR using genetic targets HSP-70 and ITS-1 was used to identify the presence of the parasite followed by High Resolution Melting for *Leishmania* species identification.

3 Results

In total 18,664 Phlebotomine sandflies were captured. Fourteen Phlebotomine sand-fly species were recorded, with the most abundant species being *Pintomyia evansi* (72.15%), *Micropygomyia cayennensis* (9.39%), *Lutzomyia gomezi* (7.29%), *Psychodopygus panamensis* (4.62%), *Evandromyia dubitans* (3.15%) and *Pintomyia rangeliana* (1.54%). Molecular biology assays were performed in 8,653 females with Leishmania infection being detected in 5.79% of them. Leishmania species identified were as follows: *Leishmania infantum* present in 11.5% of the processed *Pi. evansi; Leishmania panamensis* present in 7.7% of the *Lu. gomezi*, 3.3% de las P*s. panamensis* y 2.2% de las *Pi. rangeliana* and finally *Leishmania guyanensis* present in *1.72%* of the *Mi. cayennensis.*

4 Conclusions

The township San Andres de Sotavento presented the highest number of captures with 75,5% followed by Sahagun and Moñitos. Furthermore, San Andres de Sotavento was also the township with the highest percentage of infection with 64.3% of the female sandflies analyzed being from this site. *Leishmania panamensis* was etected in sandfly species of the townships of Los Cordobas and Valencia.

C1453 CO-CIRCULATION OF SAND FLY-TRANSMITTED PHLEBOVIRUSES AND LEISHMANIA INFANTUM IN A ZOONOTIC VISCERAL LEISHMANIAIS FOCUS LOCATED IN ARID BIO-GEOGRAPHICAL AREAS OF CENTRAL TUNISIA.

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1 Background

In the Western Mediterranean basin, sand flies are vectors of several pathogens affecting humans and animals. In this study, we aimed to investigate phlebovirus and *Leishmania* parasites circulating in a focus of zoonotic visceral Leishmaniasis (ZVL) located in arid bio-geographical areas of Central Tunisia.

2 Methods

Sand flies were collected by using CDC light traps in the villages of Felta and Saddaguia located in Central Tunisia during September-October 2014 and 2015. Live sand flies were pooled with up to 30 specimens per pool and screened for phlebovirus and *Leishmania* by nested PCR in the polymerase gene and kinetoplast minicircle DNA, respectively. Dead sand flies were identified morphologically to species level.

3 Results

A total of 3191 and 2229 sand flies were collected in 2014 and 2015, respectively. Among 117 pools of sand flies collected in 2014, 4 were positive for phlebovirus yielding a minimum infection rate (MIR) of 0.12% (4/3191). Detected phleboviruses were identified: 1 Utique virus (UTIv), 1 Sand fly Fever Sicilian virus (SFSv), 1 Saddaguia virus (SADv) and 1 Toscana virus (TOSv). While UTIv and SFSv were detected in pools of males, TOSv and UTIv were detected in pools of female sand flies. Of a total of 52 pools of female sand flies, 3 were positive for *L. infantum* DNA on the basis of PCR amplification size, yielding a MIR of 0.1% (3/1370). Among 80 pools of sand flies collected in 2015, 3 were positive for phleboviruses consisting of 2 pools of TOSv and 1 pool of SFSv yielding a MIR of 0.13% (3/2229). All phleboviruses were detected in pools of female sand flies. Among 36 pools of female sand flies, 1 was positive for *L. infantum*, yielding a MIR of 0.1% (1/995). No co-infected pools with phlebovirus and *L. infantum* were detected. Sand flies of the subgenus *Larroussius* were predominant in this ZVL focus.

4 Conclusions

Our results provide good evidence of the co-circulation of phleboviruses and *L. infantum* in this ZVL focus. Dogs and human population living in this ZCL focus are co-infected with both *L. infantum* and phlebovirus. Since TOSv and *L. infantum* are transmitted by the same sand fly species of the subgenus *Larroussius*, humans and dogs are co-infected through co-infected or successive infected bites. Studies concerning the interaction between TOSv and *L. infantum* at the vector, reservoir and human level are needed.

C1467 ANTI-MICROBIAL PEPTIDES IN LUTZOMYIA LONGIPALPIS: POTENCIAL WEAPON AGAINST LEISHMANIA?

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1 Background

Antimicrobial peptides (AMPs) are abundant in nature, are found in animals, invertebrates and plants, and are part of an ancestral innate immune response repertoire. They can be active against virus, bacteria and parasites. Insects depend on AMPs for defense and for the control of their own microbiota. The first information on sand fly AMPS comes from studies in *Phlebotomus duboscqi*, after challenges with bacteria and *Leishmania major*. The identified defensin had activity against *Leishmania*, thus having a potential role in infection.

2 Methods

We have identified AMP genes in *Lutzomyia longipalpis*, and we are presently characterizing the putative role of these molecules in *L. longipalpis* microbiota homeostasis and responses to *Leishmania* and other pathogen infections.

3 Results

We have identified four *L. longipalpis* defensin genes and have investigated the expression of these genes in male and female insects fed on sugar. These genes had different expression levels both along time after eclosion at different days after sugar-feeding, as well as when males and females were compared. This indicates a potential functional difference for these four different defensins. Interestingly the peak of expression of most of them coincided with an increase in microbiota in the gut of the insect, suggesting a possible role on microbiota control for these AMPs. Both a cecropin and an attacin gene showed similar expression pattern. Males had higher expression of most immune molecules, which might be related to their exclusive sugar diet, which results in a faster growth of the microbiota, which might be controlled by increased levels of AMPs. Our group has also found a differential modulation of one defensin gene in relation to *L. longipalpis* infection with different bacteria and *Leishmania*, and different routes of infection. We have also found that upon *Leishmania* infection, there is an increase in the expression of different AMPs at approximately three days after infection, when the parasites have contact with the insect gut. Coincidently, at this point there is a decrease in parasite load in the gut. To confirm the role of AMPs in the control of parasite abundance, we are presently silencing the AMP genes previous to infections.

4 Conclusions

Alternative ways to control infective diseases, among them leishmaniasis, are necessary. AMPs have a potential role in *Leishmania* and microbiota control in *L. longipalpis*, and are thus good candidates for the development of these alternative actions.

C1470 IRRIGATION IN THE ARID REGIONS OF TUNISIA IMPACTS THE ABUNDANCE OF SAND FLIES POPULATION AND LEISHMANIA INFANTUM INFECTION

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1 Background

The current spread of zoonotic visceral leishmaniasis (ZVL) throughout arid areas of Central Tunisia is a major public health concern. The main objective of this study is to investigate whether the development of irrigation in arid bio-geographical areas in Central Tunisia have led to the establishment of a stable cycle involving sand flies of the subgenus *Larroussius* and *Leishmania infantum*,and subsequently to the emergence of ZVL.

2 Methods

Sand flies were collected from the village of Saddaguia, a highly irrigated zone located within an arid bio-geographical area of Central Tunisia by using modified Centers for Diseases Control (CDC) light traps. Morphological keys were used to identify sand flies. Collected sand flies were pooled with up to 30 specimens per pool according to date and tested by nested Polymerase Chain Reaction (PCR) DNA sequencing from positive pools was used to identify *Leishmania* spp.

3 Results

A total of 4915 sand flies (2422 females and 2493 males) were collected from Saddaguia in September and in October 2014. Morphological identification confirmed sand flies of the subgenus *Larroussius* to be predominant. PCR analysis followed by DNA sequencing indicated that 15 pools were infected with *L. infantum* yielding an overall infection rate of 0.6%. The majority of the infected pools were of sand fly species belonging to subgenus *Larroussius*.

4 Conclusions

Intense irrigation applied to the arid bio-geographical areas in Central Tunisia is at the origin of the development of an environment capable of sustaining important populations of sand flies of the subgenus *Larroussius*. This has led to the establishment of stable transmission cycles of *L. infantum* and subsequently to the emergence of ZVL.

C1475 SPATIAL VARIATIONS 2011-2015 OF LUTZOMYIA LONGIPALPIS AND NYSSOMYIA WHITMANI, THE LEISHMANIA SPP. VECTOR IN NORTHEASTERN ARGENTINA

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1 Background

Lutzomyia longipalpis has been reported in urban environments of Puerto Iguazú, Argentina since 2010, and *Nyssomyia whitmani* has been reported in the rural area since 1990, but in urban sites since 2011. We analyzed the variations in the spatial distribution of their abundance within the city of Puerto Iguazú in the years 2011 and 2015.

2 Methods

In December 2011 and November-December 2015 sandflies were sampled in 52 and 55 households respectively covering the whole urban area of Puerto Iguazú, for two or three consecutive nights with REDILA light traps. Traps were set according to the "worst peridomestic scenario" criterium. Spatial autocorrelation of abundance of each vector was studied using isotropic and anisotropic semivariograms. When autocorrelation was detected, abundance was interpolated using ordinary kriging according to semivariogram's parameters. Interpolated values of abundances for both periods were compared using QGis.

3 Results

The spatial distribution of each vector during 2011 remained similar in 2015 captures; *Lu. longipalpis* concentrated in more urbanized areas at the northwest of the city, and *Ny. whitman* in the center and southeast. *Lu. longipalpis* and *Ny. whitman* abundances resulted autocorrelated in both periods up to a distance of 700-1000 and 450 meters respectively.

4 Conclusions

The spatial analysis showed that the two studied species kept the 2010 distribution in 2015, *Lu. longiplapis* 76.4% and *Ny whitmani* 67.2%. *Lu. longipalpis*, is concentrated in the more urban anthropized area close to the main rivers, while *Ny. whitmani*, was closer to the southeast patches of forest, watherver is close to water bodies(2011 captures). This results will allow to focus intervention in risk areas and to reduce vector-human contact.

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C1496 SOIL VARIABLES AND PHLEBOTOMINAE INMATURE STAGE PRESENCE IN MISIONES, ARGENTINA

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1 Background

The location of the microhabitats where phlebotomine develop is one of the least-known aspects of its life cycle, since juvenile stages are difficult to detect in its terrestrial breeding sites. This project was developed in a rural area (Misiones, northeastern Argentina) where epidemic outbreaks of cutaneous leishmaniasis due to *Leishmania braziliensis* took place during 2004-2005 with *Nyssomyia whitmani* as its main vector. The farms in this zone have a chicken shed and a house typically built with high floors, microhabitats that have stable temperatures, high humidity and a resting place for domestic and even synanthropic animals. The objective of this work was to associate the presence of phlebotominae immature stages with soil physico-chemical characterization and adult phlebotomine abundance in microhabitats-potential breeding sites.

2 Methods

From November 2015 to April 2016, emergence traps (ETs) were set in two microhabitats of three farms: "Bellow house" and "Chicken shed". The sampled microhabitats were divided in squares of 0.5 x 0.5 m and the traps were maintained in the same site for 40 days. In April, a sample of soil of each square was collected and preserved for physico-chemical characterization.

3 Results

A total of 632 ETs were used to investigate 5.12 m^2 taking both microhabitats. Although the proportion of positive ETs for phlebotomine was low (4.95%), natural breeding sites were found in both microhabitats of the three farms. The phlebotomine production of Chicken shed (35.65 flies/m^2) was higher than the one of below house (4.97 flies/m^2). The samples of soil of "Chicken shed" presented a range of physico-chimical variables wider than that of Below house. Following a PCA, the "Below house" squares clustered at negative values of Axis 1, corresponding to low values of Organic Materia and Nitrogen (Soil variables).

4 Conclusions

Knowing the breeding sites of Phlebotomine will allow us to develop control strategies focused on larval stages.

C1523 UPDATED PHLEBOTOMINE SAND FLY (DIPTERA: PSYCHODIDAE) DISTRIBUTIONS IN SPAIN: PRELIMINARY FINDINGS

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1 Background

Phlebotomine sand flies (Diptera: Psychodidae) are able to transmit the protozoan *Leishmania infantum*, which causes canine leishmaniosis (CanL), a widespread zoonotic disease endemic in the Mediterranean basin. In Spain, both *Phlebotomus perniciosus* and *P. ariasi* are well known vectors of *L. infantum*. To implement appropriate control and focused measures, information on sand fly distributions is essential. This study updates sand fly distribution data and examines the factors affecting the distribution and density of phlebotomine species in the Iberian Peninsula and Balearic Islands.

2 Methods

Over the years 2013 and 2014, sand flies were collected across Spain using CDC-light traps. At each sampling site, the traps were left for an average of 13.8 hours. The study area included mainland Spain and the Balearic Islands. The number of sites surveyed in each bioclimatic area was proportional to the size of the zone such that the largest bioclimatic belts, Meso-Mediterranean and Supra-Mediterranean were the most surveyed (22 sampling sites each) followed by the Thermo-Mediterranean belt (6 sampling sites). Kruskall-Wallis and Wilcoxon signed rank tests were used to examine relationships between sand fly species as densities (number of sand flies per trap per hour) and the remaining variables. The variables recorded included bioclimatic zone, habitat and presence of animals.

3 Results

In this survey, 676 specimens of five species were captured and further identified as follows: 562 (83.13%) *P. perniciosus*, 64 (9.47%) *S. minuta*, 38 (5.62%) *P. ariasi*, 6 (0.89%) *P. sergenti* and 6 (0.89%) *P. papatasi*. Over the study, densities of *S. minuta* (Wilcoxon p=0.0084) and *P. ariasi* (Wilcoxon p=0.0269) increased in rural habitats. *P. perniciosus* showed a greater density increase in the Thermo-Mediterranean than Meso-Mediterranean zone (Kruskal-Wallis p=0.00584). When these results about sand fly distributions were compared with the findings of a literature review, we observed new records of *P. ariasi* in Burgos, *P. papatasi* in Huelva and *P. perniciosus* in Segovia.

4 Conclusions

P. perniciosus persists as the most likely abundant and frequent sand fly vector species in Spain. The new reports found reveal a lack of systematic surveys across our country indicating a need to enhance our efforts on mapping sand fly distributions.

C1536 MOLECULAR CHARACTERIZATION AND TRANSCRIPTOMIC ANALYSIS OF LEISHMANIASIS VECTORS IN SUDAN

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1 Background

Phlebotomine sand flies are responsible for the spread of *Leishmania* parasites. The sand fly midgut presents a number of biological barriers the *Leishmania* parasite must circumnavigate to proliferate and develop inside the insect vector. Sand fly midgut is therefore a fundamental organ representing a key target for interruption of *Leishmania* development and transmission. Despite this, few molecules in the midgut have been characterized. Acquiring a better understanding of the molecules present in this organ will illuminate the potential molecular interactions occurring between the *Leishmania* and the vector.

2 Methods

This study will be undertaken on *Phlebotomus orientalis* sand flies collected from endemic and non-endemic areas of visceral leishmaniasis in Sudan and compared with laboratory reared samples. The midguts of *P. orientalis* will be dissected, mRNA extracted from midguts using kits and cDNA libraries constructed using construction kits. Bioinformatics analysis will be used for sequenced midgut nucleotides, multiple sequenced alignment will be used to compare the data with known families of midgut proteins of other sand fly vectors. Protein sequences will be aligned to reconstruct maximum likelihood phylogenetic trees.

3 Results

The study may provide the first detailed description of *P. orientalis* midgut transcriptome. A variety of molecules will be obtained in high quality and data information will be deposited in an international gene data bank.

4 Conclusions

Overall, *P. orientalis* midgut proteins identified by transcriptome analysis can be further tested in order to explore their biological and pharmacological properties, which may enhance in the development of new and improved drugs/vaccines, prevention strategies and control policies for leishmaniasis.

C1580 THE VECTORIAL COMPETENCE OF PHLEBOTOMUS PERNICIOSUS TO LEISHMANIA INFANTUM ZYMODEMES ISOLATED FROM TUNISIA

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1 Background

In Tunisia, south Mediterranean basin, human visceral leishmaniasis (VL) and sporadic cutaneous leishmaniasis (CL) are considered as a significant human health problem affecting about 150 and 50 cases per year respectively. Three human zymodemes of *Leishmania infantum* (MON-1, MON-24 and MON-80) were identified. *Phlebotomus perniciosus* was suggested as vector of MON-1. Although *L. infantum* DNA was detected by molecular approach in wild collected *P. perniciosus* specimens no information about zymodemes is available.
The aim of this study was to investigate the intravectorial cycle of *L. infantum* MON-24 and MON-80 and to establish the potential role of *P. perniciosus* in the transmission of these zymodemes in Tunisia.

2 Methods

Promastigotes of L. infantum isolated from VL (MON-1) and L. infantum (MON-24 and MON-80) isolated from CL were used. Specimens of a reared P. perniciosus colony from Madrid, Spain, were infected with these zymodemes (three replicates with the three zymodemes were done). Female sand flies were infected by feeding them through a chicken skin membrane with a suspensions of promastigotes of each zymodeme (MON-1, MON-24 and MON-80) in heat inactivated rabbit blood (107 parasites/ml). Blood engorged females were immediately separated and maintained in a cage. The infection progress was followed by dissection of sand flies at 2, 4, 7 and 9 days post-feeding. Location of parasites was noted for each infected sand fly. Intensity of gut infection was graded to 4 categories weak (100-1000 parasites), heavy (>1000 parasites) and very heavy (>10 000 parasites). Statistical analysis was done by the Epi info program to confirm differences between zymodemes.

3 Results

All tested strains developed well in *P. perniciosus*, producing heavy late stage infection and colonizing the stomodeal valve (SV) of this species. Concerning *L. infantum* MON-80 and *L. infantum* MON-24, the parasites reach the SV as soon as the second day post feeding. Nevertheless, *L. infantum* MON-1 was found in the SV at 4 day post feeding. Furthermore, all infections produced metacyclic form of *Leishmania* at different rates with more than 1000 parasites in the SV.

4 Conclusions

Our study show, for the first time, that *P. perniciosus* can be a permissive vector for all zymodemes of *Leishmania infantum* (MON-1, MON-24 and MON-80) from Tunisia. This result could help to predict and to follow the spatio-temporal spreading of human leishmaniasis in Tunisia.

C1641 BIONOMICS OF COLONIZED SAND FLY PHLEBOTOMUS ARGENTIPES (DIPTERA: PSYCHODIDAE) IN BANGLADESH

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1 Background

Female sand fly *Phlebotomus argentipes* is the only vector of the Visceral Leishmaniasis (VL) in Bangladesh. No information about its bionomics in laboratory condition is available from the country. For the first time here we report the life cycle of the *P. argentipes* in laboratory condition in Bangladesh.

2 Methods

Trained ento-technicians collected *P. argentipes* by mechanical and mouth aspirators from the inner wall of cattle sheds of houses in non-endemic village of the sub-district Trishal. The insectarium had been set up by the help of ISCIII, Madrid, Spain and DNDi, Geneva, Switzerland. Sand flies were transferred to pots with humid plaster of Paris and were taken to the insectarium for colonization. We followed the procedures used for establishing and maintaining the colonies described by Molina et al. (2014) with some modifications. The sand flies were kept in an incubator, maintaining 12:12 (day:night) photoperiod and 27°C-28°C with a relative humidity of 75%. Randomly selected 20 laboratory breed sand flies at 1th and 2nd generation were tested for flebo, flavi, and densoviruses by RNA PCR in the Laboratory of Arbovirus and Imported Viral Diseases, ISCIII, Madrid, Spain.

3 Results

The number of sand flies available for colonization was 40 per week. The larvae of *P. argentipes* are motile and very active through all stages. The average duration of larval stages was 9.5 days (fed-eggs), 8 days (eggs-1st instar), 4 days (1st-2nd instar), 6 days (2nd- 3rd instar), 7 days (3rd-4th instar), 4.5 days (4th-pupae) and 10 days (pupae-adults). The total generation time was 49 days. The pupation of *P. argentipes* occurred mainly on the walls of the rearing pots, with few pupae remaining on the surface of larval food. The mortality of pupae was very low, with 83% developing into adults. 57% and 43% of emerging adult sand flies were male and female respectively. Copulation was much more frequent after the females engorged blood. Any copulation during blood feeding was observed. The mortality rates (apparent, real and irreplaceable) were highest in the 1st instar and lowest in the pupae stage. Maximum longevity of adult sand flies was about 19 days. Tested sand flies were free from germs.

4 Conclusions

The proportion of colonized adult sand fly in laboratory conditions is impressive (85%). Reduction of larval mortality at 1st instar should increase this proportion further. Insectarium based on sand fly colonies free from germs are appropriate for conduction of xenodiagnosis.

C1642 BLOOD MEAL PREFERENCES AND LEISHMANIA SPP. DETECTION IN SERGENTOMYIA MINUTA COLLECTED IN THE HUMAN LEISHMANIASIS FOCUS OF MADRID, SPAIN (2012-2016)

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1 Background

Entomological surveys conducted in the human leishmaniasis focus of Madrid (Spain) have showed a high density of *Sergentomyia minuta* (106.81 specimens/m^2). This species has been investigated by other authors for its possible implication in *Leishmania* spp. transmission in several leishmaniasis foci. Moreover, anthropophilic feeding behavior has been reported in this sand fly. In this work we carried out the blood meal identification and *Leishmania* spp. detection in *S. minuta* females collected in the focus.

2 Methods

Sand flies were collected monthly for five successive years (2012-2016) in the active season of sand flies by both sticky and CDC light traps. Blood meal identification of engorged sand flies was carried out by the amplification of a 359 bp fragment of the vertebrate cytochrome *b* gene followed by sequencing and comparison with Genbank® database by BLAST. Detection of *Leishmania* spp. was performed by amplification of a 120 bp fragment from kDNA and further confirmation by cysteine proteinase *b* (*cpb*) gene and internal transcribed region 1 (ITS1) PCR. Furthermore, ITS1 bands were purified and sequenced in order to be analyzed by BLAST. Sequences obtained in this study were aligned with *Leishmania* spp. sequences annotated in Genbank®.

3 Results

A total of 239 *S. minuta* females (n=212 fed and n=27 unfed) were analyzed. Six different blood sources were identified in 40 specimens: gecko (n=19), lizard (n=2), human (n=16), rabbit (n=1), hare (n=1) and cow (n=1). Detectionof *Leishmania* spp. by kDNA-PCR was positive in 40 engorged females (gecko, n=4; human, n=4; cow, n=1; unidentified blood source, n=31) and in 1 out of 27 unfed females. None of these specimens were positive by *cpb* PCR. ITS1 amplification was positive in 25 samples and 14 were successfully sequenced. BLAST results showed 94%-99% identity with *Leishmania* spp. isolated from *S. minuta* (LC028235) and *Leishmania* spp. isolated from lizard (KT990201, KT990199, KT990162 and KT990142).

4 Conclusions

This work reveals that blood preference of *S. minuta* is not only limited to reptiles but it also feeds on mammals, human included, more often than expected. On the other hand, *Leishmania* spp. detection showed a well-established cycle of this parasite in *S. minuta* from the studied area, some of them fed on mammals as human. More studies are needed in order to assess the possible transmission of this flagellate along the region.

C1646 TRYPANOSOMA SPP. DETECTION IN PHLEBOTOMUS PERNICIOSUS FEMALES COLLECTED IN THE HUMAN LEISHMANIASIS FOCUS OF MADRID (SPAIN)

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1 Background

Phlebotomine sand flies are known vectors of several pathogens like protozoan trypanosomatids such as *Leishmania* spp., bacteria as *Bartonella bacilliformis* and several arboviruses. Entomological surveys conducted in the human leishmaniasis focus of southwest Madrid have proven that *Phlebotomus perniciosus* is involved in the transmission of *Leishmania infantum* in the region. Moreover, direct xenodiagnosis assays proved the role of rabbits and hares as potential reservoirs in the focus area. In addition, *Trypanosoma* spp. has been detected in mammals such as rodents or rabbits. Concretely, several authors demonstrated that rabbits are also reservoirs of *Trypanosoma nabiasi*, which is transmitted by fleas. In this work we detected *Trypanosoma* spp. DNA in *P. perniciosus* females fed on rabbit.

2 Methods

A total of 155 engorged sand flies fed on rabbit were analyzed for *Leishmania* detection. Routine *L. infantum* amplification of a 120 bp fragment of kDNA and further confirmation by amplification of a 702 bp fragment of cysteine proteinase *b* (*cpb*) were performed. Moreover, amplification and sequencing of internal transcribed spacer 1 (ITS1) was carried out in those samples that showed a different ITS1 band size than the expected for *L. infantum* (311 bp). Studies of the small subunit ribosomal RNA (SSU rRNA) by amplification and subsequent sequencing were performed for further identification,

3 Results

In 20 specimens an ITS1 band of ?480 bp was found. In addition, 6 out of 20 samples were positive by SSU rRNA PCR (?940 bp). Moreover, 5 ITS1 and 4 SSU rRNA sequences were successfully sequenced and analyzed by BLAST. Co-infection of *L. infantum* and *Trypanosoma* spp. was determined in 2 samples.
ITS1 sequences analyzed by BLAST showed similarity with *Trypanosoma otospermophili* (86-88%) and *Trypanosoma kuseli (*86-87%). However, SSU rRNA sequence analysis revealed 99% identity with *Trypanosoma* spp. isolated from rabbits.

4 Conclusions

Natural infection of *P. perniciosus* sand flies by *Trypanosoma* spp. from the leishmaniasis outbreak of Madrid is reported in this work. The trypanosome was only detected in sand flies fed on rabbit. Hence, evidences suggest that this trypanosome could be identified as *T. nabiasi* considering that it is the only trypanosome described in this lagomorph. More investigation is needed for study the possible interaction between *L. infantum* and the trypanosome detected and the role of *P. perniciosus* in its transmission.

C1649 HIGH PROMASTIGOTES LOADS DETECTION BY REAL TIME PCR IN PHLEBOTOMUS PERNICIOSUS CAPTURED IN THE LEISHMANIASIS FOCUS IN SOUTHWEST OF MADRID REGION, SPAIN

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1 Background

Since 2010 a leishmaniasis outbreak has been notified in southwest of Madrid region. Entomological surveys have been conducted in the focus area since then to establish the density, distribution and phenology of vectors. Moreover, high *Leishmania* infection rates have been determined by molecular procedures and by dissection of the collected *Phlebotomus perniciosus* females. In this work a qPCR assay was applied with the aim of study parasite loads in these female sand flies.

2 Methods

Leishmania promastigotes isolated from a naturally infected sand fly captured in the focus (IPER/ES/2012/POL2FL7)were used to perform a standard curve with DNA extracted from promastigote dilutions exclusively and a second one mixing DNA from promastigote dilutions with 30 ng of DNA extracted from reared sand flies. A total of 135 DNA samples extracted from *P. perniciosus* females collected in the focus and previously analyzed by conventional kDNA-PCR (68 positive and 67 negative) were used in the determination of parasite loads by qPCR.
Statistical analysis was performed by Kruskal-Wallis in order to study differences among the three replicates of each standard curve and between parasite loads determined in each transmission period. Meanwhile, Mann-Whitney method was used to study differences between the two standard curves and between fed and unfed females.

3 Results

The limit of detection achieved in this qPCR assay was 0.001 parasite/reaction, which corresponds to 1 promastigote/ml from the original sample (1 µl was used per reaction). Moreover, results by qPCR did not showed differences with conventional PCR.A 16.17% (n=11) of the females presented over than 10.000 parasites/reaction, 23.53% (n=16) showed heavy promastigote loads (>1000 parasites/reaction), 30.9% (n=21) presented moderate loads (10-1000 parasites/reaction) and 29.4% (n = 20), low parasite loads (<10 parasites/reaction).
The number of parasites found in unfed females (n=33) was statistically higher than the number found in blood-fed females (n=35) (p-value<0.0001).

4 Conclusions

Results showed an elevated *Leishmania* promastigotes loads in nearly 70% *P. perniciosus* females collected in the human leishmaniases outbreak affecting southwest of Madrid region. Moreover, unfed sand flies showed higher parasite burden than blood-fed sand flies. This result supports the exceptional characteristics of this human leishmaniasis focus and the need to an intensive and continuous surveillance in the area.

C1651 OPPORTUNISTIC FEEDING BEHAVIOR AND LEISHMANIA INFANTUM DETECTION IN PHLEBOTOMUS PERNICIOSUS FEMALES COLLECTED IN THE HUMAN LEISHMANIASIS FOCUS OF MADRID, SPAIN (2012-2016)

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1 Background

Seasonal entomological surveys performed in the focus of human leishmaniasis in southwest of Madrid (Spain) have shown that *Phlebotomus perniciosus* is the only vector of *Leishmania infantum* in the area. Furthermore, molecular assays revealed that *P. perniciosus* feed mainly on rabbits (*Oryctolagus cuniculus*) and hares (*Lepus granatensis*). In fact, direct xenodiagnosis of leishmaniasis conducted on hares and wild rabbits from the affected area proved that these lagomorphs are infective to colonized *P. perniciosus* although in different proportions. In the present work, we carried out the determination of the feeding preferences and *Leishmania* detection in *P. perniciosus* females collected in an entomological survey carried out during the transmission periods 2012-2016 along the affected municipalities of the aforementioned focus.

2 Methods

A total of 737 blood-engorged *P. perniciosus* females collected by both sticky and CDC light traps in Leganés (n=530), Fuenlabrada (n=166), Getafe (n=34), Parla (n=4) and Humanes de Madrid (n=3) were studied. Blood-meal determination was performed by amplification of a fragment of 359 bp of vertebrate cytochrome *b* gene (*cyt b*) followed by sequencing and comparison with sequences deposited in GenBank®. Moreover, mixed blood meals were confirmed by RFLP-PCR of cyt *b* gene. *L. infantum* detection was carried out by amplification of a 120 bp fragment of kDNA and further confirmation by internal transcribed spacer 1 (ITS1) and /or cistein proteinase *b* (*cpb*) gene PCR.

3 Results

Blood meal source was successfully identified in 606 specimens (82.23 **%**). *P. perniciosus* females fed predominantly on hares (56.72%) followed by rabbit (13.57%). Blood from ten other vertebrates have been also identified. Moreover, 3 specimens with mixed blood meals were found. On the other hand, *L. infantum* DNA was detected in 112 sand flies (15.20%), most of them fed on hares (n=93). Furthermore, *P. perniciosus* females fed on rabbit (n=8), dog (n=2), human (n=2), with mixed blood source - rabbit/hare – (n=1) and unidentified blood source (n=6) were also found positive to *L. infantum*.

4 Conclusions

The diversity of blood sources observed in this study reveals an opportunistic feeding pattern in the *P. perniciosus* females from the focus. This behavior can be explained by the availability of the different vertebrates in each survey point and could influence *Leishmania* transmission along the affected area.

C1658 DEVELOPMENT OF A RAPID ASSESSMENT STRATEGY TO ESTIMATE LAND USE RISK FACTORS ASSOCIATED WITH DOMESTIC TRANSMISSION OF AMERICAN CUTANEOUS LEISHMANIASIS IN THE ANDEAN REGION OF COLOMBIA

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CIDEIM Colombia

1 Background

The domestic environment has become a risk factor for leishmaniasis transmission, due to the presence of phlebotomines in domestic and peridomestic environments, affecting all age groups. Phlebotomine presence and abundance may be associated with altered ecosystems. This project seeks to develop a rapid assessment strategy to estimate the risk of domestic transmission of American cutaneous leishmaniasis using, as a proxy, the phlebotomine abundance and species composition in relation to the land use around the house

2 Methods

Fifteen houses were randomly selected in four different foci (townships) located in the Andean region (Novillero, Santander; La Esmeralda, Cundinamarca; Honda Alta, Huila and El Cucal, Tolima). The habitat in the 100-m radius around each house was characterized at point locations at 10-m intervals along eight transects centered on the house. CDC miniature light traps were placed in an occupied bedroom (indoor), and two outdoors at a distance of 10-m (peridomestic). Species identification was done using the taxonomic keys. Blood sources were determined by amplification and sequencing of the *cyt b* gene; while analysis of infection with *Leishmania* was performed by PCR-Southern Blot. To evaluate the effect of land use on the abundance of phlebotomines in the domestic environment, descriptive, bivariable and multivariable analyses were performed.

3 Results

Thirteen species of sand fly were identified, of which the two most abundant were: *Pintomyia (Pifanomyia) quasitownsendi* and *Pi. (Pif.) longiflocosa*. The former comprised 99.5% of all phlebotomines caught in Novillero (615/618) and 48.3% in La Esmeralda (56/116). The latter comprised 35.7% in Honda Alta (20/56) and 99.2% in El Cucal (1700/1713). Both species were highly anthrophophilic, the blood sources being mainly human. One specimen of *Pi. (Pif.) quasitownsendi* was infected with *Leishmania (Viannia)*. The negative binomial regression model showed the average abundance of *Pi. (Pif.) quasitownsendi* decreased significantly with each meter of distance from the houses to sugarcane (IRR= 0.95; p= 0.003); while the abundance of *Pi. (Pif.) longiflocosa* increased significantly with each 1% increase in technified coffee crops (IRR= 1.08, p= 0.001).

4 Conclusions

Certain agricultural land uses, in particular monoculture, around the domicile favor the abundance of phlebotomines in the domestic environment and hence increase the risk of domestic transmission of cutaneous leishmaniasis

C1686 MORPHOLOGICAL AND MOLECULAR TAXONOMY OF PHLEBOTOMINE SAND FLY (DIPTERA: PSYCHODIDAE), IN LEISHMANIASIS ENDEMICS REGIONS OF TUNISIA.

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1 Background

Phlebotomine sandflies are considered as the natural vectors of *Leishmania* species, the causative agents of leishmaniasis a neglected tropical disease. In Tunisia integrated researches on Phlebotomine have shown that many species share several morphological characters could be mistaken for. An update of data, based on both morphological and molecular tools is needed to overcome the pending identification problems.

2 Methods

Using light traps and sticky paper we performed sandflies capture in different bioclimatic area, during three summer seasons (2008, 2010, 2012). The traps were placed where cases of leishmaniasis have been reported. Diagnose keys based on external and internal characters of head and genitalia were used to morphologically identify sandflies species. The thorax and the attached anterior abdomen were used for the molecular study of the 3'end of the mitochondrial gene. PCR products were next sequenced.

3 Results

A total of 16 species belonging to *Phlebotomus* (*P*) and *Sergentomyia* (*S*) genus have been identified. Taxonomic inventory made it possible to describe for the first time in Tunisia, malformations affecting genitalia of males *P. papatasi*. Molecular analysis shows that there was no relation between these malformations and genetics characters. *Phlebotomus* and *Paraphlebotomus* sub-genus are closely related and among the latter our results suggest that *P. alexandri* diverged very early and is closely related to *P. papatasi*. This result remains valid with other markers as previously described in literature. Our results also show a clear dichotomy between the two sympatric species, *P. chabaudi* and *P. Riouxi*, which leads us to consider that there are two valid typological species. Our results prove that Linnaean classification was not in conformity with the Hennigian one.
For *Larroussius* sub-genura, *P. perniciosus* was the most widespread species with a clear geographical delimitation between the typical and atypical forms.
Finally our study provides the first molecular identification of *S. clydei* in Tunisia, which has unambiguously distinguished it from male *S. christophersi*. In summary, our phylogenic analysis allows a clear discrimination between all species with significant bootstrap values.

4 Conclusions

Comparing our sequences with those available in gene bank, we place our sequence in relation to others fromMediterranean and Middle East regions.
Our study allowed as to overlay our sandflies distribution map with the available map of *Leishmania* parasites in Tunisia.

C1795 IDENTIFYING INFLUENTIAL FACTORS AFFECTING VISCERAL LEISHMANIASIS IN ENDEMIC AREAS OF BANGLADESH WITH GEOSPATIAL TECHNIQUES

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1 Background

Bangladesh Disease Control Unit of the Directorate General of Health Services estimated a total of 72949 Visceral Leishmaniasis (VL) cases and 207 associated deaths, in between the year 2000-2014, which shows severe endemicity in this region. The purpose of the study is to create an ecological niche model for *Phlebotomus argentipes* and to identify the environmental factors of VL in Bangladesh.

2 Methods

Three hyperendemic *upazilas* (sub-district), namely Fulbaria, Trishal and Gaffargaon of Mymensingh and one relatively low endemic *upazila* (Sreepur)of Gazipur districts constitute the study area. We used Maxent to develop the distribution model for *P.argentipes* and employed jackknife test of variable importance to identify the most important environmental variables. Nineteen bioclim variables, land surface temperature, NDVI, NDWI, slope, elevation and topographic wetness index (TWI), land cover, soil drainage, general soil type, soil moisture and soil reaction/pH were used as the environmental layers. Human (VL) cases were used as a proxy for presence location points of *P.argentipes*, obtained by GPS measurements in extensive field surveys. The model performance was evaluated by the area under the curve (AUC) of the receiving operator curve (ROC) and by a one-tail binomial test with the null hypothesis that the model does not predict the test/predicted presence points better than a random model.

3 Results

The model performed very well in both the AUC and the binomial test, establishing the precision and applicability of the niche model. Annual precipitation had the greatest percentage of contribution to the model development. Simultaneously, the jackknife test of variable importance showed that NDVI provided the most useful information while land surface temperature contributed the most unique information. Annual precipitation of 2080 mm, NDVI value of 0.68 and land surface temperature of 29.95^{o}C corresponds to the highest logistic probability of the presence of the vector. Five *unions* (sub-upazila) of Fulbaria and three *unions* in Sreepur and Trishal were identified to be high risk zones by the distribution model.

4 Conclusions

This first ever attempt of identifying environmental influence on VL distribution in Bangladesh provides a framework for tackling the ecological aspects of the disease. More focus can be given to the identified potentially risky areas in order to prevent a resurgence of the disease in coming years.

C1828 NOVEL METHOD TO QUANTIFY LEISHMANIA METACYCLIC PROMASTIGOTES TRANSMITTED TO THE SKIN BY SINGLE SAND FLIES.

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1 Background

The composition of the infectious dose has never been directly determined from single infective bites. To quantify the number of *Leishmania mexicana* and *Leishmania infantum* parasites and the proportion of metacyclic promastigotes transmitted to mice; we developed a strategy to quantify both *Leishmania* and metacyclic transcripts in a bite site.

2 Methods

A real-time reverse transcription quantitative PCR (RTqPCR) of small subunit ribosomal RNA (*ssrRNA*) and small hydrophilic endoplasmic reticulum-associated protein (*sherp*) was developed to analyse parasite and metacyclic transcripts in the same bite site.

3 Results

Despite high variation in the numbers of parasites delivered per bite, 76% of *L. mexicana* bites and 71% *L. infantum* bites contained ≥ 75% metacyclics. Analysis of multiple bites from individual infected flies revealed that approximately 60% could transmit ≥ 80% *L. mexicana* metacyclics for up to 10 consecutive bites. Meta-analysis of the 10th bite in relation to the vector's midgut infection revealed that sand flies were more likely to repeatedly transmit a high proportion of metacyclics if the midgut was significantly blocked with parasites and a parasite-derived glycan-rich plug - the promastigote secretory gel.
To test the impact of the proportion of metacyclics for infection, we mimicked 'high-quality' (100% metacyclics) and 'low-quality' (50% metacyclics), low dose *L. mexicana* transmissions to BALB/c mice using needles. Doses enriched for metacyclic promastigotes was associated with slower growing cutaneous lesions that harboured significantly more amastigotes compared to low quality infections and demonstrated greater transmission potential back to sand flies.

4 Conclusions

This new method for interrogating sand fly infection and *Leishmania* transmission highlights the efficiency of parasite transmission and reveals the composition of the infectious dose as an important infection determinant.

C1846 BITING MIDGES AS A POSSIBLE VECTOR OF THE AETIOLOGICAL AGENT OF CUTANEOUS LEISHMANIASIS IN GHANA

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1 Background

Leishmaniases are neglected tropical and sub-tropical diseases of public health importance in global medicine. New vector(s) is/are anticipated in the newly described species of *Leishmania* in the *Leishmania enriettii* complex (new subgenus *Mundinia*), as evidenced in previous work and recent laboratory procedures. The species of *Leishmania* responsible for cutaneous leishmaniasis in Ghana is yet-to-be named. The vector and the reservoir hosts are still unknown despite several efforts to collect and study sandflies in the endemic area recently. This work seeks to investigate the possibility that biting midges in the endemic area might be responsible for transmission by conducting laboratory experiments, after which field investigations will be mounted.

2 Methods

Two model vectors, *Lutzomyia longipalpis* and *Culicoides sonorensis*, were infected with the newly isolated *Leishmania* from Ghana, under the same conditions and kept for more than 10 days. The vectors were dissected daily to study the infections establishment in the vectors from day 1 to 10.

3 Results

Heavy infections characterised both vectors at the blood meal stage, until the blood meal were fully digested. Infections in *Lu. longipalpis* decreased to 0% 3 days after bloodmeal digestion. Infections in the *C. soronensis* were retained beyond 10 days to ≈80%, colonising the midgut and the stomodeal valve.

4 Conclusions

The human pathogenic *Leishmania* responsible for CL in Ghana heavily infected *Culicoides sonorensis*, colonizing the midgut and stomodeal valve up to and beyond day 10 at higher infectivity rate. This supports the idea that midges could be vectors of this new species of the parasite in Ghana, and successfully transmit the parasite. Since midges are repetitive feeders, a second blood meal could help the parasite to probably survive longer and trigger molecules that might help them develop to the metacyclic promastigotes. The different morphological structure of the *Culicoides* and sand fly needs to be studied in detail. Moreover, a gelatinous substance (PSG-like) appearing around the parasite in the midgut of the *Culicoides* requires further investigation.

C1865 ELEVEN YEARS OF PHLEBOTOMINE ENTOMOLOGICAL SURVEY IN SICILY

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1 Background

Phlebotomine sandflies transmit pathogens affecting humans and animals worldwide. Among over 800 species of sandfly recorded, 98 are proven or suspected vectors of human leishmaniases. Phlebovirus infections and diseases are present in large areas of the Old World, especially in the Mediterranean area, in which virus diversity has proven to be higher than initially suspected. Studies about sandfly fluctuation of population in a geographic area can be helpful to better understand spreading of leishmaniasis.

2 Methods

From January 2005 to December 2016 in the nine Sicilian provinces a total of 6042 capture were performed in rural animals breeding, for all the year monthly and approximately once a week. For sandfly capture, Onderstepoort-type blacklight traps were used from the sunset to the sunrise of the following day. The collected insects were stored in alcohol at 70% and transported to the laboratory for analysis.

3 Results

Among all, 1981 catches were positive for the presence of sandflies (32,8%). The first positivity were found in five provinces in January (Agrigento, Palermo, Caltanissetta, Messina, Syracuse) and in eight out of nine there were positivity until December. Agrigento and Caltanissetta provinces were positive every month monitored (100%) while the other had this prevalence CT 75%, EN 83%, ME 83%, PA 92%, RG 67%, SR 75%, TP 58%. Within the period 2005-2016, the number of sandflies per positive catches (SPPC) was low in the province of Ragusa, Syracuse and Messina (12,5; 17,04; 28,95), moderate in the province of Agrigento, Catania, Enna, Palermo and Trapani (147,14; 141,90; 239,97; 100,75; 118,03) and very high in the province of Caltanissetta (1254,3). The comparison of SPPC among three different 9-years time intervals (2005-2014; 2006-2015; 2007-2016) in the 4 provinces with the highest prevalence of sandflies positive catches showed a trend towards a significant reduction of SPPC in 2005, 2006 and 2007 compared to 2014, 2015 and 2016, respectively.

4 Conclusions

Our study shows a clear reduction in the abundance of sandflies within Sicily through the years; climatic factors as well as an awareness raising of farmers on prevention could be responsible in such modification. However, our results suggest a longest period of sandflies presence throughout the year in specific provinces, implying the necessity of a constant monitoring of their activity to develop adequate countermeasure for prevention of Leishmaniasis. Thanks to Pippo Bono, Gaspare Lo Bue, Nicola Galati.

C1875 ABUNDANCE AND DISTRIBUTION OF SANDFLY SPECIES PRESENT IN SICILY

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1 Background

Sandflies are bloodsucking insects belonging to the Diptera order (Psychodidae: Phlebotominae) and include several species vectors of human leishmaniases. Italian species of sandflies belong to *Phlebotomus* and *Sergentomya* genera. However, the detailed information about species circulation of sandflies in Italy is still fragmented. The aim of this work was to study the presence, abundance and distribution of sandfly species in Sicilian rural environments.

2 Methods

Sampling was carried out through the 2012 in close proximity to livestock farms in all the Sicilian provinces. Onderstepoort-type blacklight traps were set up for a night. The analysis concerned 512 catches. Insects were stored in 70% ethanol. Sandfly specimens were identified according to the morphological keys reported in literature and divided into males, unfed, engorged and pregnant females.

3 Results

A total of 100763 insects was collected (53.9% males, 46.1% females, out of these 0.3% gravid and 5.1% engorged females) and 5752 (5.7%) specimens were identified morphologically. Seven species were found. *Phlebotomus perfiliewi* (81,1%), *P.perniciosus* (15,5%) and *Sergentomyia minuta* (2,65%) were the most abundant species. *P. neglectus* (0,49%), *P. mascittii* (0,14%), *P. papatasi* (0,06%) and *P.ariasi* (0,001%) were found in a less amount. For the most representative species, the seasonal trend showed the highest abundance in June for *P.perfiliewi* and in July for *P.perniciosus* and *S.minuta*. Distribution and abundance of the different species in all the Sicilian provinces was evaluated.

4 Conclusions

Five of the seven species found in this study are Leishmania proven vectors. The two most abundant species, *P. perniciosus* and *P. perfiliewi*, are the main vectors of human leishmaniasis in Italy. The highest prevalence of P.perfiliewi is related to its zoophilic behaviour that increases the possibility to collect it in rural areas, while P. perniciosus is the main anthropophilic vector of Leishmania, as well as *P.papatasi* that is usually collected in urban areas.
Even if this study is focused on rural areas, it is relevant to integrate the knowledge about host-vector-pathogen interactions in different environments and to evaluate the possible risk of Leishmania spreading in endemic areas.
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C1879 DETERMINING THE POTENTIAL OF SYNTHETIC SEX PHEROMONE OF LUTZOMYIA LONGIPALPIS FOR VECTOR CONTROL.

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1 Background

Lutzomyia longipalpis is the South American vector of Leismania infantum the aetiologic agent of human and canine visceral leishmaniasis. Lu. longipalpis s.l. is a species complex, however it remains unclear which characters can be used to reliably identify member species. Sex pheromones analysis, suggests that in Brazil Lu. longipalpis is a complex of 5 sub species. Of the 5 known sex pheromone types, one is widely distributed Central and South America including Brazil and one occurs mainly in the North East of Brazil. The remaining 3 pheromone types have a very restricted geographical distribution. Sex pheromones offer significant potential for use in vector control programs. Our previous work demonstrated this potential but to take advantage of it, it is important to understand how a pheromone product might perform in the field and the limits to its possible usefulness.

2 Methods

We carried out studies to determine where in Brazil a synthetic sex pheromone might be used and the conditions under which it might be deployed. We tested if the sex pheromone of *Lu. longipalpis* in South Western Brazil would be attractive to *Lu. longipalpis* in the North East of Brazil (and vice versa) and then the extent to which each sex pheromone attracted its conspecific pheromone type in an area where the 2 pheromone types occur in sympatry. We also determined the extent to which host odour is an important element in the attractiveness of *Lu. longipalpis* sex pheromone in the field. Finally, we determined if the pheromone could be combined with LLIN rather than residual insecticide as a way of combining pheromone and insecticide in a lure-and-kill strategy that would be simple to deploy.

3 Results

The results showed that pheromone of one *Lu. longipalpis* population was not attractive to the sex pheromone of a different allopatric population. The results from sympatric populations were not as clear cut. The sex pheromone when used without host odour was strongly attractive. LLIN used along with sex pheromone provided an effective alternative to using insecticide delivered by residual spraying.

4 Conclusions

We conclude that the synthetic sex pheromone that is currently available could be used widely in South and Central America as part of an integrated vector management program. It may be possible to use pheromone by itself with insecticide delivered by LLIN and this could potentially make it a less expensive alternative to existing vector control options.

C1885 LUTZOMYIA LONGIPALPIS IN AMERICAN TEGUMENTARY LEISHMANIASIS ENDEMIC URBAN AREAS IN CORRIENTES, ARGENTINA

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1 Background

American tegumentary leishmaniasis (ATL) is a widespread infection in the province of Corrientes, Argentina. During 2015, ten clinically and parasitologically confirmed human cases of ATL, in which there was urban transmission, were recorded in the city of Corrientes. These cases corresponded to eight adults and two children.

Lutzomyia longipalpis appear currently adapted to domestic environments in urban areas of this city, and is classified as an area of transmission of canine visceral leishmaniasis. However,
only one human case was confirmed until now.

This urban area was selected for the analysis of seasonal patterns of *L. longipalpis*, because it was one of the first places where cases of human ATL were reported in the province.

2 Methods

Corrientes (27° 15 ' N and 58° 50´W) with a population of 376,400 inhabitants, ecologically it is classified as subtropical without dry station, with precipitations upper to 1,600 mm annual.

The study involved systematized entomological captures in one house in the urban zone, from February to December 2015. Sand flies were collected using two CDC light-traps during two consecutive nights of every week. The CDC traps were set 1 m above the ground and operated from 18 to 6 h. Sand flies were identified using Galati´s keys.

3 Results

During the capture period 3,163 specimens of *L. longipalpis*, 2,752 males (87%) and 411 females (13%) were collected. The highest population levels was during September with and average temperature of 20.0°C, and with the largest fraction of days without precipitation,

From February onwards, the numbers increased constantly until May and from October to December). No sample was collected in July,this was the coldest month of 2015 with an average daily low temperature of 13°C.

L. longipalpis specimens were collected in months when relative humidity surpassed 75%. No significant correlation was found between the monthly abundance of phlebotomine sand flies and the monthly averages of temperature, relative humidity or rainfall.

In addition, females were analyzed by polymerase chain reaction (PCR) that confirms the presence of *Leishmania amazonensis* (Mario Steindal, Universidad Federal de Santa Catarina, Brazil).

4 Conclusions

Based on the occurrence of autochthonous human cases of ATL and on the presence of *L. longipalpis that* are infected by *Le. amazonensis*, it is possible to hypothesize that this phlebotomine sandfly specie might be involved in the urban cycle of ATL in Corrientes.

C1886 NOVEL APPROACHES FOR CONTROL OF PHLEBOTOMUS ORIENTALIS, THE PRINCIPAL VECTOR OF VISCERAL LEISHMANIASIS IN EAST AFRICA

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1 Background

The sylvatic nature of *Phlebotomus orientalis*, the principal vector of visceral leishmaniasis throughout East Africa, pauses a tremendous challenge to control programs attempting to halt the transmission of the disease. As a component of KalaCORE program in E. Africa, the aim of this study is to understand the vector bionomics and evaluate cost-effective measures that can be used to suppress the transmission of the disease. Here, we present and discuss results of an extensive study on the distribution of the vector in relation to VL control. We also discuss how insecticide spraying of outdoor fences, insecticide treatment of animals and use of personal protection measures can impact the man biting rates of the vector.

2 Methods

The study was conducted in two main study areas; one among resident populations in 6 VL endemic villages in eastern Sudan and the other in agricultural farms in NW Ethiopia where seasonal and migrant populations contract the disease. The vector distribution was investigated using CDC light traps and sticky-paper traps set at fixed positions at indoor, outdoor, peridomestic and sylvatic sites of the villages and different microhabitats of the agricultural farms. A small trial was also conducted in 4 clusters of houses in a village in eastern Sudan to determine impact of deltamethrin spraying of courtyard fences in reducing the vector abundance.

3 Results

Our results demonstrate that the vector is rarely found at indoor sites and most transmission takes place in the outdoor sites of the courtyard, and the surrounding peridomestic and sylvatic habitats. The data indicates that the fences are already acting as a barrier against the vector resulting in highly significant reduction ($P<0.001$) of its abundance in peri-domestic habitats and the outdoor courtyard sites. Spraying of these fences with deltamethrin insecticide resulted in further reduction of *P. orientalis* both at outdoor and peri-domestic sites.

4 Conclusions

Our results show that the traditional methods of residual spraying of indoor sites has no impact on the vector. Development of improved house fences and spraying them with environmentally friendly pyrethroid insecticides may provide a better alternative to indoor IRS. Preliminary results also indicate that currently used wide-mesh LLIN have little impact on the vector and their use for VL control in Gedaref state should be reconsidered.

C1898 MOLECULAR CHARACTERIZATION OF NATURAL INFECTION IN LUTZOMYIA LONGIPALPIS WITH LEISHMANIA BRAZILIENSIS IN A REGION OF LAVRAS, MINAS GERAIS, BRAZIL.

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1 Background

Leishmaniasis is a neglected disease that affects a large part of the world population causing serious damage to health. They are caused by parasites of the genus *Leishmania* that have the females of sand flies as vectors, living alternately in these insects and also in vertebrate hosts. The disease is divided into two main types: tegumentary leishmaniasis and visceral leishmaniasis, which is the most serious type of disease that can cause death in the patients. In the Americas, cutaneous and mucosal leishmaniasis had an incidence, in 2014, of 19.76 cases per 100,000 inhabitants in 16 endemic countries. 75% of the cases were reported by Brazil, Colombia and Peru. For Visceral Leishmaniasis, 12 countries were endemic, and of the 48,720 cases reported in the period from 2001 to 2014, 96% were registered in Brazil. The present study aimed to identify the sandflies found in the Peri domicile of a residence in the city of Lavras-Minas Gerais, Brazil, and, through molecular analysis, to verify the occurrence of infection by *Leishmania* spp.

2 Methods

For the collection, CDC type light traps were installed in the Peri domicile of a residence, during three consecutive days per cycle, from February 17th to March 09th, 2016. The captured insects were screened and later identified by sex in a magnifying glass. Females were stored in liquid nitrogen, until the week prior the setting of the slides and molecular analysis. Afterwards, the females were taken to the laboratory of Archeology and Protozoology of the Renée-Rachou Research Center-FIOCRUZ / MG, where they passed through a protocol for setting of slides and later molecular analysis (PCR, RFLP) aiming at the identification of the sandfly species and research of *Leishmania* infection, respectively.

3 Results

35 females of the following species were captured*: Lutzomyia longipalpis*, *Lu. whitmani*, *Migonemyia Migonei*, *Evandromyia cortelezzii*, *Ev. Lenti* and *Brumptomyia* sp., according to the criterion proposed by Galati (2003). In PCR analysis five *Lu. longipalpis* were positive for *Leishmania* infection and, through the RFLP technique, *Leishmania* species were identified as *L. braziliensis*.

4 Conclusions

Infection of *Lu. longipalpis* by *L. braziliensis* was observed in this study, pointing out the need for further studies on the vector competence of *Lu. longipalpis* in the transmission of *L. braziliensis.*

C1911 STRESS ASSOCIATED PHAGOSTIMULATORY FACTORS AFFECTING THE LEISHMANIA DONOVANI TRANSMISSION IN PHLEBOTOMUS ARGENTIPES VECTOR OF INDIAN VISCERAL LEISHMANIASIS

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1 Background

Leishmania donovani is a causative parasite for visceral leishmaniasis in Indian sub continentbeing delivered by *Phlebotomus argentipes*. Through the experimental studies,the interaction of *L. donovani* (parasite) and female *P. argentipes* (host) has been depicted influencing the vector physiology or physical fitness upon the cycle of parasite transmission into its next host. Therefore, understanding the factors influencing the intake of parasite by the host served as the main objective for the present study. Also, it offers better understanding regarding host-parasite interaction correlating disease transmission to its next host.

2 Methods

Through the artificial blood-feeding technique *P. argentipes* were observed at the different concentration of ATP and different duration of starvation period for accessing its affect upon the parasite transmission in its host.

3 Results

Among the experimented ATP gradients i.e., 0.0M, 0.0025M, 0.005M, 0.01M and 0.02M, maximum rate of engorgement was observed at the concentration of 0.01M of ATP i.e., 60%, 58% and 62.5% in triplicate study; whereas the minimum feeding percentage i.e., 24.17% was observed at 0.0M ATP. For observing maximum rate of blood-feeding at different period of starvation of sand flies, the highest uptake of blood-meal by the female sand flies were observed at 36 hrs of starvation period in three separate trials i.e., 62.5%, 55% and 57.5%. However, above 36 hrs of starvation, rate of feeding percent declined by 22.5%, 17.5% and 20%. As per concerning parasitic infection, average parasite infection per gut was observed to be highest at the 5 x 10^5 parasitic concentrations along with optimum value of ATP and starvation period.

4 Conclusions

Starvation stress condition positively affects energy requirements of host which get fulfilled by the higher no. of ATP that positively acts as a phagostimulant so that percentage of feeding gets uplifted that further implicates upon the disease transmission.

C2024 VISCERAL AND CUTANEOUS LEISHMANIASIS OCCURRENCE AND TRANSMISSION DYNAMICS IN KENYA

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1 Background

Visceral and cutaneous leishmaniasis are of public health concern in Kenya. The disease is endemic in the Rift valley, Northern, Northeastern and Eastern regions of the country. The epidemiology of the disease depends on the interaction of sandflies, reservoir hosts and susceptible population. This study sought to determine the distribution and genetic variability of the vectors and parasite in leishmaniasis endemic regions in Kenya, and to identify sandfly host blood meal preference.

2 Methods

Vector sampling was done in three of 47 counties (Baringo, Nakuru and Marsabit). Leishmanaises hot spots were identified using health facility screening records. Trapping was done around termite mounds, vertisols, indoors, animal sheds and burrows using CDC light traps and sticky papers. Morphological and molecular identification of vectors was done using taxonomic keys and sequencing of the mitochondrial cytochrome c oxidase subunit 1 (COI) gene. Dissected midguts of sandflies suspected to harbor promastigotes were cultured in NNN media overlaid with complete Schneider's media, DNA extracted and subjected to High Resolution Melt analysis. Bloodmeal analysis was done by amplifying the mitochondrial COI gene.

3 Results

Vector density from each collection site was recorded. 14,000 sandflies were collected: Baringo (52.3%), Nakuru (8.6%), Marsabit (37.2%). Overall 40% were *P. martini*. In lower Baringo (Marigat), *P. martini* was the only vector trapped while upper region (East Pokot) *P. duboscqi* (40%) and *P. martini* (30%) were prevalent. In Gilgil (Nakuru), *P. guggiesbergi* (60%) a vector of CL was prevalent, *P. saevus* (6%) and *P. sergenti* (5%) were also identified. In Marsabit, two main vectors were collected, *P. martini* and *P. orientalis*. Outdoor trapping yielded highest number of sandflies. Ninety five percent of the *P. orientalis* were from acacia dried swampy cracked soils while *P. duboscqi* was collected along the river bed. Sandfly blooding feeding was diverse with most feeding on humans and rodents (hyraxes). *Leishmania tropica* and *L. donovani*parasites were isolated from *P. guggiesbergi* and *P. martini* respectively.

4 Conclusions

Vector distribution was diverse in the regions sampled. Leishmaniasis transmission could be occurring outdoors as people slept outside where most blood fed vectors were collected. Vector control strategies to target both indoor and outdoor sandfly biting must be implemented.

C2061 STICKY TRAPS WITH SEMIOCHEMICALS AND SLOW RELEASE SYSTEM TO MONITOR NEGLECTED DISEASES VECTORS

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Centro para el Desarrollo de la Investigacion Cientifica CEDIC Asuncion Paraguay

Full text

Vector Borne Disease Control Programs have as their main mission to reach a distance between human populations and their vectors. Therefore, this new challenge is a priority issue for the endemic areas of our continent where these diseases are a public health problem. Chemical compounds influence with relative success insect vectors such as triatomines and sandflies, however more information is needed to develop a tool that allows the early detection of their presence in the home environment.

Previous laboratory tests that were performed by this work team identified the attractiveness of isolated volatiles of *Triatoma infestans* in copulation from laboratory columns. In the pre-field tests, four of these volatiles were tested as pheromones in traps located inside experimental hencoops, simulating the domestic environment. Field studies were carried out in the endemic localities of the Paraguayan Chaco in order to compare this sticky trap in relation to the manual capture performed by the Control Program experts. More recently, trials have been initiated to determine the attraction of sandflies with the same pheromones by measuring abundance in CDC light traps modified by serial catches in urban and rural-urban areas.

The baits increased the sensitivity of the trap to capture *T. infestans* in percentages between 12.5% ??and 63.6%. The detection probabilities were estimated at $p \approx 0.40$-0.50 with bait and at barely $p \approx 0.15$ for the control traps. Hexanal, nonanal, heptanal and benzaldehyde showed great attraction inside triatomines while manual capture was always negative. Abundance trials in light traps have shown significant results when pheromones were incorporated.

More recently, through an associative project, the efficacy of the pheromone mixture and the increase of the effect of these semiochemical products are evaluated with the introduction of a slow release device of pheromones.

The relatively high sensitivity of baited traps in the early detection of *T. infestans* and the potential detection of phlebotomines and other vectors, indicates that the combination of these tools with community participation and vector control programs would optimize the approach of these diseases.

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Drug development: conventional and alternative. Clinical and experimental therapy in cutaneous and visceral leishmaniasis

C0085 NANOTHERAPEUTIC APPROACH AN ALTERNATE WAY FOR THE DELIVERY OF AMPHOTERICIN B IN THE TREATMENT OF VISCERAL LEISHMANIASIS

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1 Background

Amphotericin B (AmB) has been the first-line treatment for visceral leishmaniasis (VL), a neglected protozoan disease, especially in regions like Bihar, India, where resistance to antimonials is widespread. However, adverse drug reactions are a major limiting factor. We evaluated a novel nano formulation of AmB conjugated to amine-modified graphene (f-Gr) for safety and efficacy over conventional AmB. Further we also investigated the potential of functionalized graphene as a nanocarrier to improve the oral efficacy of AmB.

2 Methods

The f-Gr was prepared in a gentle one-step process of non-covalent (amine) functionalization with the help of amino acid L-cysteine. This f-Gr was further conjugated to AmB by peptide bond. The conjugate (f-Gr-AmB) was characterized by Raman spectroscopy, Fourier transform infrared spectroscopy, scanning electron microscopy, and transmission electron microscopy. In vitro toxicity and efficacy studies were done in J774A.1 macrophage cell lines and In vitro Efficacy studies were performed in Hamsters and toxicity studies in Swiss Mice.

3 Results

f-Gr-AmB was found to exhibit lesser cytotoxicity toward J774A.1 cells than AmB, and did not induce any hepatic or renal toxicity in Swiss albino mice. In vitro antileishmanial assay in J774A.1 cells showed significantly enhanced efficacy of f-Gr-AmB over AmB. Furthermore, percentage inhibition of amastigote replication in a hamster model of VL was significantly higher in the f-Gr-AmB treated group (87.8%) compared to the AmB group (70.4%).
In vivo antileishmanial results showed that AmB conjugated to amine modified graphene could significantly enhance oral efficacy, compared against Miltefosine during a 5 day course of dosage 10 mg/kg. At the oral dose of 10 mg/kg, f-Gr- AmB has a significantly greater antileishmanial activity (89 % parasite inhibition) than 5mg/kg Miltefosine (78 % parasite inhibition), the only oral drug available for VL.

4 Conclusions

These results suggest that f-Gr-AmB could be a safe and effective nanotherapeutic alternative to conventional AmB in the treatment of VL.

C0087 EFFICACY AND SAFETY OF AMPHOTERICIN B DEOXYCHOLATE VERSUS N-METHYLGLUCAMINE ANTIMONIATE IN PEDIATRIC VISCERAL LEISHMANIASIS: AN OPEN-LABEL, RANDOMIZED AND CONTROLLED PILOT TRIAL IN BRAZIL.

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1 Background

Despite their high toxicity, antimonials and amphotericin B deoxycholate are commonly used for visceral leishmaniasis (VL) treatment. There are few studies with pediatric population, showing conflictive data about efficacy and adverse events. The aim of this study was to evaluate efficacy and safety of amphotericin B deoxycholate vs. N-methylglucamine antimoniate for pediatric VL treatment in Brazil.

2 Methods

This was a randomized, open-label, 2-arm and controlled pilot clinical trial. Treatment naïve children and adolescents from 6 months to 12 year old with diagnosis of VL and without signals of severe illness were treated with N-methylglucamine antimoniate (20 mg/kg/day for 20 days) or amphotericin B deoxycholate (1 mg/kg/day for 14 days). All patients were diagnosed with positive direct examination and/or positive PCR for *Leishmania* spp performed in bone marrow samples. The primary efficacy end point was a VL cure 180 days after treatment completion. The analysis was performed with intention-to-treat (ITT) and per protocol (PP) analyses.

3 Results

One hundred and one volunteer were included in the trial. Efficacy was similar for both groups. The antimonials group had 51 individuals, who had a cure rate of 94,1 and 100% accord to ITT and PP analyses, respectively. Amphotericin B group had 50 individuals, with efficacy of 94 and 97,9% accord to ITT and PP analyses, respectively. All patients reported adverse events (AE). Serious AE were similar in both groups. Five individuals should be withdrawn from the study (3 in antimonials group and 2 in amphotericin B group).

4 Conclusions

Efficacy and adverse events rate of VL treatment with N-methylglucamine antimoniate or amphotericin B deoxycholate were similar in the pediatric population evaluated.

C0094 VALIDATION OF THE MEGLUMINE ANTIMONIATE INTRALESIONAL INFILTRATION TECHNIQUE FOR TREATMENT OF CUTANEOUS LEISHMANIASIS

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1 Background

Although meglumine antimoniate (MA) intralesional infiltration is an option for cutaneous leishmaniasis (CL) therapy widely used, specially in the old world, until now, there are few detailed descriptions available for the intralesional technique and several procedure variations, which makes it difficult to compare results. This study aims to validate a standardized MA intralesional infiltration guidance for treatment of localized CL

2 Methods

The first script including instructions for the procedure was drawn based both on 21 trials retrieved from literature and direct monitoring of procedure. Conflicting issues about the technique were decided by electronic consultation to experts. The script was submitted to a formal inspection computer-aided in order to identify readability, clarity, omission, redundancy and unnecessary information (content validation). For criterion and construct validation, professionals with or without prior experience with intralesional approach performed 118 intralesional infiltration procedures under the supervision of a same observer using a checklist.

3 Results

The influence of changes in critical conditions (compliance with the guidance's instructions and professional experience) on outcome conformity (saturation status achievement), tolerability (scale of pain referred) and safety (bleeding) was assessed. The median procedure length was 12 minutes (ranging from 3 to 35 minutes) and in 72% of them, patients classified the pain during infiltration as mild (up to 3 in visual analogue scale that goes from 0 to 10). The bleeding was also classified as mild (less than or equal to 3 on the Boezaart scale) in 96.6% of the procedures. 100% compliance with the guidance was observed in 66% of infiltrations. In spite of this, in 100% of the inspected procedures, lesion saturation was observed at the end of infiltration.

4 Conclusions

We proposed and validated a guidance for the intralesional therapy approach for CL, elaborated from a variety of sources, reflecting expert opinion as to best practice, current research, as well as insight from professionals with different backgrounds. The high percentage of achieving the desired outcome, the mild bleeding, the patient's tolerance and the reliability here presented confirm that the procedure is feasible. It was confirmed that the intralesional technique guidance is a reliable, robust and reproducible procedure even among professionals without previous training in this therapeutic approach.

C0109 BIOLOGICAL ACTIVITY OF SOME IRANIAN MEDICINAL PLANT EXTRACTS ON LEISHMANIA MAJOR PROMASTIGOTES, IN VITRO

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1 Background

Approximately 80% of all cutaneous leishmaniasis cases are rural cutaneous type caused by *Leishmania major* and Iran is endemic at least in 11 regions. 5-capacity antimony compounds are the most significant medicine applied against leishmaniasis with several obstructs such as delaying treatment and disease recurrence, relevant drug side complications and drug resistance. Hence, study on novel and natural compounds to substitute and /or complement the synthesize drugs in used is in urgent need to delete complications and enhance the efficiencies.

2 Methods

Medicinal plant extractsstarting with 1000µg/ml were examined using 96- well plates. Using Neubauer slide to count 10^6 *Leishmania major* promastigotes from cultured flasks enclosing 10% of BHI in RPMI1640 medium for every single well. MTT solution was added to each well, subsequent to 72h incubation and absorbance was recorded in 570 nm applying ELISA reader.

3 Results

IC_{50} of six plants extracts including *Allium hirtifolium*, *Ziziphus spina-christi*, *Punica granatum* peel, *Eucalyptus olida*, *Descurainia Sophia*, *Conocarpus erectus* were determined at 87, 112, 138, 142, 175 and 201 (µg / ml), respectively.

4 Conclusions

Selected plant extracts showed different biological inhibition activity on promastigotes. Promising activities encouraged a possibility of achieving better results through altering protocols method for plant extraction. The most active plant extract was related to *Allium hirtifolium* with IC_{50} of87µg/ml. Therefore, it is suggested to carry out further studies on *Allium hirtifolium* in order to purify its most efficient fragment applying *in vivo* and *in vitro* experiments.

Key words: *Leishmania major*, MTT method, Medicinal plant extracts, Iran

C0124 CINNAMOMUM CASSIA EXHIBITS APOPTOSIS MEDIATED ANTILEISHMANIAL ACTIVITY COMPLEMENTED BY THERAPEUTIC CURE IN VIVO

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1 Background

There is a dire need for drug discovery against visceral leishmaniasis (VL), a life-threatening protozoal infection, as the available chemotherapy is expensive, ineffective and have side effects coupled with emergence of resistance. Exploration of plants as alternate drug resources has rewarded mankind in the past and aimed in this direction, we investigated the antileishmanial potential of *Cinnamomum cassia* bark dichloromethane fraction (CBD).

2 Methods

Dichloromethane, ethanolic and aqueous fractions of *C. cassia* bark were prepared by sequential extraction that were appraised for their anti-promastigote activity along with apoptosis-inducing potential. The anti-amastigote efficacy of CBD was studied in *Leishmania donovani* infected peritoneal macrophages from BALB/c mice and nitrite level as a surrogate marker of nitric oxide (NO) production was also estimated. The *in vivo* leishmanicidal efficacy was evaluated in *L. donovani* infected BALB/c mice and hamsters and various correlates of host protective immunity ascertained. Toxicity profile of CBD was investigated *in vitro* against peritoneal macrophages, and alterations in liver and kidney functions were determined *in vivo*. The plant secondary metabolites present in CBD were identified by gas chromatography-mass spectroscopy analysis (GC-MS).

3 Results

CBD displayed significant anti-promastigote activity (IC_{50} 33.6 µg ml^{-1}) that was mediated via apoptosis. CBD also inhibited intracellular amastigote proliferation (IC_{50} 14.06 µg ml^{-1}) independent of NO production. The protection achieved was 80.91% (liver) and 82.92% (spleen) in mice and 75.61% (liver) and 78.93% (spleen) in hamsters, indicating its profound therapeutic efficacy. CBD exhibited direct leishmanicidal activity, as it did not specifically induce a T helper type (Th)-1-polarized mileu in cured hosts. This was evidenced by insignificant modulation of NO production, lymphoproliferation, DTH (delayed type hypersensitivity), serum IgG2a and IgG1 levels and production of Th2 cytokines (IL-4 and IL-10) along with restoration of pro-inflammatory Th1 cytokines (INF-gamma, IL-12p70) to the normal range. CBD was devoid of any toxicity *in vitro* and *in vivo.* Various CBD constituents that may have imparted the observed leishmanicidal efficacy were identified by means of GC-MS analysis.

4 Conclusions

Our study highlights the profound antileishmanial efficacy of *C. cassia* bark DCM fraction and merits its further exploration as a source of effective and safe antieishmanials.

C0139 SUCCESSFUL TREATMENT OF PENTAVALENT ANTIMONIAL RESISTANT CUTANEOUS LEISHMANIASIS PATIENTS BY USE OF LUCILIA SERICATA LARVAE AND LARVAL SECRETIONS

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1 Background

Pentavalent antimony compounds **(PAC)** used as the first-line drugs for the treatment of leishmaniasis. However, drug resistance is emerging worlwide. Liposomal amphotericin B is another option for the treatment of drug-resistant *Leishmania* strains. But the cost of the liposomal amphotericin B is expensive. Thus, our research group propose *Lucilia sericata* larvae and larval secretions' biotherapy are satisfying and cost-effective alternative for the treatment of **PAC** resistant cutaneous leishmaniasis cases especially in the low-resource settings.

2 Methods

Twelve patients diagnosed Cutaneous Leishmaniasis (CL) were undergone **PAC** therapy but the therapies were unsuccessful. Seven of the patients were Syrian refugees and Turkish people residing nearby the refugee camps. Five of the patients were females, and seven of them were males. Seven of them were children aged 4-14 years and five of them were adults aged between 29-54 years. The location of the CL; seven of the patients have lesion on the face, three of the cases on the upper extremity and two of them have lesions on lower extremity. All of the lesions were sampled and cultured in N.N.N. medium and also the samples stained and checked under microscope. The first stage larvae applied onto the lesions of the five patients' arms and legs directly for 72 hours. The cases with facial lesions, the larval secretions were used. The secretions were applied to the skin lesions once daily for ten days. The larval secretions were soaked onto the surface of gauze dressings and applied to the facial lesions. After every therapy, the lesions were checked for leishmania.

3 Results

The amastigote forms were seen at the preparations but there were no growth on cultures. The amount of amastigote forms were decreased exponentially after every treatment applications. There were no amastigote forms seen of the lesions after the sixth day of the direct-larvae treatment. The secretions-applied facial lesions were cleared out of parasites after the eighth day. The patients were fully cured at the tenth day with both methods.

4 Conclusions

Leishmaniasis as a disease of poverty needs low-cost solutions. The *L. sericata* larvae and the secretions treatment are cost-effective and suitable for low-resource settings. This method is suitable to apply with great success and low complication rates. This therapy is notable for the cure of cutaneous leishmaniasis. The larvae and the secretions are natural products without any kind of toxicities and side effects.

C0159 LEISHMANIA DONOVANI: AN IN VITRO STUDY OF ANTIMONY-RESISTANT AMPHOTERICINE B-SENSITIVE ISOLATES.

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1 Background

Sodium stibogluconate (SSG) has been the mainstay in the treatment of visceral leishmaniasis (VL) in Sudan since it was first introduced in the country five decades ago. The recommended regimen for the drug is 20 mg/kg/day for 28 days. The majority of Sudanese VL patients respond to this regime. Failure of response in some patients could be due to under-treatment in peripheral hospitals and/or underlying systemic diseases. Amphotericin B is a very potent anti-leishmanial drug but with profound nephrotoxicity. However, at a low total dose of 7 mg/kg it has been shown to have high cure rates with moderate toxicity.
This study was initiated in an attempt to determine the in vitro sensitivity of *Leishmania donovani* isolates from Sudanese VL patients with diverse clinical drug responsiveness to standard leishmanicidal drugs.

2 Methods

Eight isolates, six from antimony-resistant and two from clinically responsive patients were included in the study. Parasites were tested as promastigotes and four of them were selected to be tested as amastigotes using a murine macrophage-like cell line. The results indicated that the conventional promastigotes and amastigotes-screening assays did not correlate with the clinical picture of patients. In vivo unresponsiveness does not necessarily mean primary parasite resistance.

3 Results

The results indicated that the conventional promastigotes and amastigotes-screening assays did not correlate with the clinical picture of patients. Increasing concentrations of SSG diminished the incorporation of the [3H]thymidine in a linear fashion compared to the control values in both sensitive and resistant isolates. [3H]thymidine uptake was inhibited in all isolates in a linear fashion with increased concentrations of amphotericin B. In the macrophage assays, the four parasites tested were highly infective to the cells (80–99%) and exhibited moderate to high intracellular replication potentials.

4 Conclusions

Amphotericin B could be a suitable second line drug in patients unresponsive to pentostam and without concomitant diseases, if close hospital monitoring is available. Promastigotes sensitivity testing concentrations are virtually incomparable with the *in vivo* clinically curable doses and the amastigotes/macrophage test concentrations.

C0184 EFFICACY OF PENTAVALENT ANTIMONIATE INTRALESIONAL INFILTRATION FOR CUTANEOUS LEISHMANIASIS THERAPY: A SYSTEMATIC REVIEW

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1 Background

The mainstays of cutaneous leishmaniasis (CL) treatment are pentavalent antimony (SbV) compounds administered parentally, despite its recognized toxicity that requires frequent laboratory monitoring and complicates its use in areas with scarce infrastructure. Because of this, the WHO's Expert Committee on leishmaniasis have expanded the recommendations for the use of local therapies, including SbV intralesional infiltration (IL-SbV), as CL therapy alternatives even in the new world. However, the efficacy rate of this approach has never been compiled. The aim of this study was critically and systematically to assess the efficacy of IL-SbV for CL treatment.

2 Methods

The PRISMA guidelines for systematic reviews and the Cochrane manual were followed. The sources used were the MEDLINE and LILACS databases and International Clinical Trials Registry Platform of World Health Organization. The outcome of interest was clinical cure, defined as complete re-epithelialization of all lesions. The IL-SbV pooled cure rate was estimated for several subgroups and direct comparisons were performed when possible.

3 Results

Forty studies involving 5679 patients treated with IL-SbV infiltration were included. The pooled IL-SbV efficacy rate was 75% (95%CI 68-81%). In the old world, it was observed an overall IL-SbV efficacy rate of 75% (95%CI 66-82%) and cure rates significantly higher with sodium stibogluconate (SSG) than with meglumine antimoniate (MA): 83% (95%CI 75-90%) *versus* 68% (95%CI 54-79%), p=0.03. Studies directly comparing IL-SbV with topical paromomycin, IL hypertonic saline, radio frequency-induced heat therapy, topical trichloroacetic acid and cryotherapy showed no efficacy difference between these interventions. In the new world, the global IL-SbV efficacy was 77% (95% CI 66-85%) and unlike in old world, a significant difference favoring MA in relation to SSG was observed: 61% (95%CI 49-73%) *versus* 82% (95%CI 70-89%). Comparing different IL infiltration schedules, it was observed higher cure rates in patients submitted to IL-SbV treatments longer than 14 days.

4 Conclusions

Despite the high heterogeneity and low methodological quality of studies, by indirect comparison, these results show that the antimony infiltration efficacy rate is similar to that reported with antimony systemic use. The evidence gathered so far is insufficient to identify the ideal IL therapeutic regime, as well as to estimate the rates of adverse events and mucosal late complications.

C0195 WITHANIA SOMNIFERA CHEMOTYPE NMITLI 118R SIGNIFICANTLY AUGMENTS THE ANTILEISHMANIAL EFFICACY OF MILTEFOSINE IN LEISHMANIA DONOVANI INFECTED HAMSTERS

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1 Background

A combination therapy including a potential and safe immunostimulant with lower doses of effective drug has proven as a significant approach which is more effective than immunotherapy or drug therapy alone. Miltefosine is the only promising orally active antileishmanial drug, but due to its long half-life, there is risk of development of resistance. To overcome these problems, efforts are needed to develop combination therapy of miltefosine with effective immunostimulating agents where a decrease of parasitic burden and simultaneous enhancement of adaptive immunity can be achieved. *Withania somnifera* (L.) Dunal (Solanaceae), commonly known as Ashwagandha, is one of the most important medicinal plant in the traditional Indian medical systems. Pharmacological studies have established that root extracts of *W. somnifera* contain several bioactive constituents called withanolides. The plant has long been used for its several beneficial properties and recently as an immunomodulator.

2 Methods

25 day old *Leishmania donovani* infected animals (hamsters) were administered with NMITLI 118R (30 mg/kg × 5 days) either alone or in combination with ED_{50} dose of Miltefosine (10 mg/kg × 5 days. The treated animals were euthanized on days 30 and 60 post-treatment (p.t.) and checked for parasite clearance, delayed type hypersensitivity (DTH) response, cytokine and inducible nitric oxide synthase levels by real-time PCR, nitric oxide (NO) production, reactive oxygen species (ROS) generation, lymphoproliferative and antibody responses.

3 Results

The group of animals that received 118R and ED_{50} dose of miltefosine showed optimum inhibition of parasite multiplication (~80%) by day 60 p.t. The efficacy was well supported by the increased inducible NO synthase mRNA transcript, strong IFN-g and IL-12 mediated Th1 immune responses and significantly suppressed levels of Th2 cytokines (IL-4, IL-10 and TGF-β). Additionally, same therapy also induced significant increase in the level of NO production, ROS generation, *Leishmania* specific IgG2 antibody along with profound DTH and strong T-cell responses as compared with all the other treated groups.

4 Conclusions

Our results suggest that combination of chemotype 118R with ED_{50} doses of miltefosine may provide a promising alternative for the cure of visceral leishmaniasis with significant restoration of the host immune response.

C0224 DEVELOPING POTENTIAL DRUG FROM SECONDARY METABOLITES OF ACTINOMYCETES TO FIGHT AGAINST VISCERAL LEISHMANIASIS : HOLISTIC CHEMOINFORMATIC APPROACH

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1 Background

Increasing resistant against existing antileishmanial drives an urgent needs to develop an antileishmanial drug with excellent efficacy and safety profile. Around 80% secondary metabolites of actinomycetes have been used to treat various microbial diseases. Integrated and holistic approaches for *Leishmania donovani* are useful to identify new molecules that could lead to future antileishmanial drug.

2 Methods

Three sequential docking protocols (HTVS, SP & XP) were performed to screen the secondary metabolite compounds (n=9718) from actinomycetes source against five key enzymes of *Leishmania donovani* metabolic pathway. These enzymes were adenine phosphoribosyl transferase (PDB ID: 1QB7), trypanothione reductase (PDB ID: 2JK6), N-myristoyl transferase (PDB ID: 2WUU), pteridine reductase (PDB ID: 2XOX) and MAP Kinase (PDB ID: 4QNY). While, the binding energy of top ligands were predicted using the MM-GBSA module of Schrödinger suite.

3 Results

Standard precision mode of docking results into top 60 ligands having good binding affinity for all five aforementioned enzymes as compared to the miltefosine as a reference ligand. Extra precision (XP) docking of these top 60 ligands resulted into only 42 ligands showing better docking score as compared to miltefosine as reference ligand. Furthermore, top 42 ligands and miltefosine were subjected to MM-GBSA study to ensure the binding free energy.

4 Conclusions

On the basis of integrated and holistic chemoinformatic approach two ligands (PubChem CID: 71316092 and 85202755) were finalized, which qualifies all the screening parameters. These two ligands could be developed as oral antileihsmanial drug which have the potential to fight against *Leishmania donovani* parasites for the elimination of visceral leishmaniasis.

C0225 PENTAVALENT ANTIMONIAL RESISTANT CL CASES IN TURKEY, CLINIC, LABORATORY DIAGNOSIS, AND GENOTYPES: CASE SERIES

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1 Background

Every year 1,5 million cutaneous leishmaniasis (CL) are reported worldwide. For the last 50 years pentavalent antimonial compounds such as sodium stibogluconate (Pentostam®) and meglumine antimonate (Glucantime®) are used as the first choice for CL treatment. Nevertheless, in the recent years, resistance reaching %60's against pentavalent antimonial compounds are being reported from India, South America and Middle East. In this study, 10 autochtonous cases who received at least 2 regimens of pentavalent antimonial therapy without cure and/or had relapses were included with their clinical findings, laboratory diagnosis and genotyping data

2 Methods

Clinical materials, obtained from the lesions of the patients prediagnosed with CL, were inoculated in the enriched medium and NNN culture media. Then slides were prepared , stained with Giemsa ,and inspected under light microscopy for *Leishmania* amastigotes. *Leishmania* molecular identification were executed by primers and probes specific to ITS-1 gene region of *Leishmania* parasites employing RT online PCR and melting curve analysis using the DNA obtained from both the clinical materials of the patients and the culture media. Pentavalent antimonial therapy were administered to 10 CL patients

3 Results

Leishmania amastigotes were seen in all slides and leishmania promastigotes were observed in enriched media. For the NNN media, leishmania promastigotes were detected in 4 samples yet it was noted that there was no reproduction in the media. Molecular diagnosis by ITS-1 region revealed that all strains were *Leishmania tropica.* Seven patients received 2 and one patient received 3 regimens of meglumine antimonate therapy, 1 patient had 2 regimens of meglumine antimonate and one regimen of sodium stibogluconate and one patient received 2 regimens of meglumine antimonate and sodium stibogluconate therapy with no clinical cure of the lesion. 7 patients were evaluated as acute CL while 3 were chronic CL

4Conclusions

It is predicted that this study will be helpful for the scientists working on the field and the clinicians about the laboratory diagnosis and genotyping of pentavalent antimonial resistant CL cases. This study will also lay the grounds for the future studies about the treatment alternatives for resistant Leishmania strains.

Acknowledgement: The study was funded by TUBITAK (The Scientific and Technological Research Council of Turkey) (Grant No. 214S239)

C0226 MOLECULAR MODELING AND VIRTUAL SCREENING APPROACH TO DISCOVER POTENTIAL ANTILEISHMANIAL INHIBITORS AGAINST ORNITHINE DECARBOXYLASE

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1 Background

Visceral leishmaniasis (VL) is a tropical neglected disease, which encounters poorest of poor people living in Asia, Africa and Latin America; causes the mortality of more than 30,000 people worldwide. The armamentarium for the treatment of VL cases is limited and continuously facing decreasing of efficacy for existing drugs. Ornithine decarboxylase (ODC) is one of the interesting drug targets in Leishmania donovani, due to its association with redox metabolism. To search an antileishmanial compound showing the inhibitory effect against ornithine decarboxylase of Leishmania donovani.

2 Methods

In this study, we have modelled the three dimensional structure of ODC using Phyre2 (Protein Homology/analog Y Recognition Engine V 2.0), followed by validation using VADAR (Volume, Area, Dihedral Angle Reporter), RAMPAGE, ERRAT, Verify3D and ProSA (Protein Structure Analysis). In order to develop potential antileishmanial, we conducted a high throughput virtual screening of ZINC database ligands comprising of 135,966 compounds. Furthermore, QikProp, ADMET predictor and MM-GBSA was performed for ADME (Absorption, Distribution, Metabolism and Elimination), toxicity and binding energy prediction for top ligands, respectively. Finally, molecular dynamics simulation was performed to get potential antileishmanial compounds.

3 Results

Screening of zinc database compounds using high throughput virtual screening has given twelve compounds with good inhibition activity against ornithine decarboxylase. Furthermore, the molecular dynamics simulation work reveals that ZINC67909154 could be a potent inhibitor and this compound can be used to combat VL disease.

4 Conclusions

This study concludes that ZINC67909154 has the great potential to inhibit L. donovani ODC and would add to the drug discovery process against visceral leishmaniasis.

C0230 ANTILEISHMANIAL ACTIVITY OF ANALOGS COMPOUNDS OF N-METHYL-8-METHOXYFLINDERSINE AGAINST LEISHMANIA (V) PANAMENSIS

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1 Background

Leishmaniasis is a neglected disease, endemic in 98 countries around the world with a 300 million of people at risk to be infected. The Global prevalence is 12 million cases and the disease incidence is from 1.5 to 2 million cases per year. This disease has a clinical spectrum based in localized lesions and non-resolution disseminated lesions, which require treatments with side effects in vital organs. For all these reasons, new treatment alternatives are needed to its control. N-methyl-8-methoxyflindersine was identified as a quinolonic alkaloid with an antileishmanial activity. Seven analogs of N-methyl-8-methoxyflinderine were selected and analyzed considering their cytotoxic effects against human macrophages and antileishmanial activities against *L. (V) panamensis* promastigotes and amastigotes forms.

2 Methods

We evaluated the *in vitro* antileishmanial activity of seven analogs of N-methyl-8-methoxyflinderine against *Leishmania* promastigotes and intracellular amastigotes. The analogs of alkaloid compound were determined by the similarity of structure, through the Tanimoto index and manual comparison. The cytotoxic effects of alkaloids in human macrophages and viability of promastigotes forms of *L. panamensis* were determined using the alamar blue assay. The effect of compounds on intracellular amastigotes was assessed by fluorescent microscopy.

3 Results

Alkaloid analogs with structure similarity were:1,2,3,4-Tetrahydrobenzo[h]quinolin-3-ol,3-Ethyl-2-propyl-quinoline-4-carboxylic acid,2-Amino-1-methylimidazo[4,5-b]quinolone,Skimmianine,1H-quinolin-4-one,Berberine and tetrahydroberberine. These compounds were not toxic in human macrophages. However, the alkaloids compounds shown an inhibitory activity in a dose dependent manner in *L. panamensis* promastigotes (2.87 to 67.98 µg/mL).Results of intracellular amastigotes assay shown that 3-Ethyl-2-propyl-quinoline-4-carboxylic acid,1,2,3,4-Tetrahydrobenzo[h]quinolin-3-ol and Berberine, decrease the infected macrophages (22.15, 4.47 and 13.16 µg/mL, respectively) at dose dependent manner.

4 Conclusions

These compounds were not toxic to host cells. This study has identified alkaloids as prominent molecules in the development of leishmaniasis new therapies. *This project was financed by Colciencias project No.1101-569-34446.*

C0231 NOVEL CHEMOTHERAPEUTIC AGENTS TARGETING LEISHMANIA BRAZILIENSIS TRYPANOTHIONE SYNTHETASE FOR THE TREATMENT OF CUTANEOUS LEISHMANIASIS

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1 Background

The development of new anti-leishmania drugs is urgently needed. Enzymes involved in the redox metabolism of trypanosomatids such as Trypanotione Synthetase (TryS) are interesting targets for drug development as they are essential for parasite survival, are unique to trypanosomatids or have little similarity with host enzymes. The aim of this study is to evaluate the therapeutic effect of TryS inhibitors on *L. braziliensis* infection.

2 Methods

A high-through-put screening assay was developed and 400 compounds were tested against *Trypanosoma cruzi* TryS, *Trypanosoma brucei* TryS and *Leishmania infantum* TryS. J774 macrophages infected with *L. braziliensis* were then used to evaluate leishmanicidal effect of selected compounds. Bone marrow macrophages were infected with *L. braziliensis* and treated with TryS inhibitors to determinate EC_{50}. Macrophage response to treatment was assessed by nitric oxide ($NO^{\bullet}$) and superoxide ($O_2^{\bullet -}$) production using Griess test and parasite growth by count in Neubauer chamber.

3 Results

From the initial screening, we selected 16 compounds to evaluate their leishmanicidal effect. Among these compounds KuRK259 and KuRK258 displayed EC_{50} of 0.6 µM and 1.6µM, respectively, against *L. braziliensis*-infected macrophages. The compounds displayed selectivity indexes of >35 and >62, respectively, towards intracellular parasites. KuRK259 significantly inhibited promastigote rescue upon culture in Schneider medium and depleted intracellular trypanothione. However, parasite killing was not associated to $NO^{\bullet}$ and $O_2^{\bullet -}$ increase. Exposure to KuRK 259 was not toxic to macrophages but significantly inhibited promastigote growth.

4 Conclusions

TryS inhibitors are good candidates for development of drugs to treat CL caused by *L. braziliensis* and experiments are under way to prove their efficacy in a preclinical model of the disease.
Source of funding: CNPq, FIOCRUZ and Pasteur Institute International Network.

C0232 IN VITRO AND IN VIVO ANTILEISHMANIAL ACTIVITY OF THE QUINOLONIC ALKALOID COMPOUND: N-METHYL-8-METHOXYFLINDERSINE

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1 Background

Leishmaniasis is a parasitic disease responsible for one of the highest level of morbidity worldwide. The therapy for the control of intracellular pathogens responsible for the disease is still far from ideal due to multiple factors still unresolved (high toxicity, emergence of strains of parasites resistant to drugs, high costs associated with care of patient, routes of administration, among others). This highlights the need to develop alternative therapies for safer and more effective disease control.Here we evaluated the antileishmanial activity of compound N-methyl-8-methoxyflindersine, derived from *Raputia heptaphylla* (a species whose genus has been used to the treatment of cutaneous presentation of leishmaniasis in South America), in order to confirm if the activity evidencing through folk medicine, could be attributed to this molecule.

2 Methods

We evaluated the antileishmanial activity of N-methyl-8-methoxyflindersine compound through *in vitro* assays (testing the cytotoxicity activity against promastigotes and axenic amastigotes, and by developing tests to assess the control of infection *in vitro* using human dendritic cells) and *in vivo* assays (evaluating the efficacy of topical treatment of *Mesocricetus auratus* hamsters experimentally infected with *L. panamensis*).

3 Results

Among the most important findings in this study, we highlight the anti-leishmanial activity evidencing through *in vitro* assays where was observed an EC_{50} of 34.5 µM on promastigotes, EC_{50} of 121.7 µM on axenic amastigotes and EC_{50} of 23.5 µM on intracellular amastigotes (with a selectivity index of 3.2, calculated taking account the relation of the LC_{50} over the target cells of infection and the EC_{50} for the control of *in vitro* infection) and *in vivo* assays with the induction of clinical improvement in 83.3% of the individuals belonging to the group treated topically with the compound, compared with a 33.3% of animals that showed clinical improvement in the group treated with the cream without active ingredient.

4 Conclusions

The findings obtained in this study highlight the potential activity of this compound type quinoline alkaloid (a group of chemical compounds of great interest for its broad spectrum of biological activity). These results support the evidence collected by the native population of regions of South America, where the disease is endemic and who have used *Raputia* to treat the disease. This project was financed by Colciencias project No. 1101-569-34446.

C0249 THE ANTI-LEISHMANIAL EFFICACY OF ARTEMISIA DRACUNCULUS HYDROALCOHOLIC EXTRACT IN VITRO AND ITS EFFECTS ON IFN-? AND IL-4 RESPONSE

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1 Background

Leishmaniasis is a parasitic disease which appears with a range of symptoms including cutaneous, mucocutaneous, and visceral leishmaniasis. The present study sought to determine the anti-leishmanial effect of the extract of *Artemisia dracunculus*(Tarragon) compared to control treatment with pentavalent antimony(meglumine).

2 Methods

In this experimental study,*A. dracunculus* collected from West Azerbaijan province, Iran and dried in the shade at 35-40 °C and the ethanolic extract of the plant was prepared.Promastigote of *L. major* (MRHO/IR/75/ER) cultured in RPMI-1640 containing 10% FBS, incubated in 24±2°C. The effect of different concentrations of Artemisia's extract were compared with Glucantime ® in the stationery phase by MTT colorimetric assay and Trypan blue staining. The amount of inhibitory concentration (IC50) was calculated. Human peripheral blood mononuclear cells (HPBMCs) treated with *L.major* and production of IFN- γ and IL-4 cytokines measured at concentrations of 25, 20, 10 and 5µg/ml *A. dracunculus*.

3 Results

Treatment with the extract did not effect the survival of the parasites during the first 48 hours; however, on the third day (72 hours), all concentrations significantly reduced the number of parasites with an efficacy of more than 50% at 10 µg/ml ($p < 0.01$), 20µg/ml ($p < 0.001$), and 25 µg/ml ($p < 0.0001$). Moreover, IFN-γ and IL-4 secretion from the HPBMCs was significantly affected in a dose-dependent manner, compared to the control (no extract). The IFN-γ/IL-4 ratio, further confirmed this notion.

4 Conclusions

: The present results show that*A. dracunculus* extract can not only exert potent anti-leishmanial activity, but may also enhance cellular immunity to this parasite. Further studies are required to determine the main compound(s) responsible for these effects of the plant.

C0250 IN SILICO AND IN VITRO COMPARATIVE ACTIVITY OF GREEN COMPONENTS AGAINST LEISHMANIA INFANTUM

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1 Background

Leishmaniasis is an obligate intracellular protozoan parasitic infection caused by the genus *Leishmania*. Clinical manifestations of the diseaseexist ranging from the severe tegumentary ulcerative forms to the fatal visceral leishmaniasis. Leishmanial chemotherapies with classical and recent drugs have limitations such as toxicity, resistance and variable efficacy.
In silico and bioinformatics methods offer an interesting alternative ways to the troublous experimental approaches, which efficiently predict the details of ligands binding to targets. Pteridine reductase 1 (PTR1), trypanothione synthetase (TS) and arginase (ARG) are the three of several enzymes that are unique and essential for the *leishmania* parasite survival and have given the opportunity to use as drug targets.
In this study, we investigated in silico and invitro antileishmanial activity of main components of green tea, catechin, EC, GCG and EGCG against *leishmania (L.) infantum* promastigote and axenic amastigote in comparison to drugs control pentamidine and glucantime.

2 Methods

Green tea ligands, pentamidine and glucantime were docked using AutoDock 4.3 program into the active sites of *L. infantum* PTR1, TS and ARG which were modeled using homology modeling programs. The colorimetric MTT and DNA fragmentation assays were used to measures *L. infantum* promastigotes and amastigotes viability and programmed cell death at different concentrations of green tea compounds in a concentration and time-dependent manner and the obtained results were expressed as 50% and 90% of inhibitory concentration (IC_{50} and IC_{90}).

3 Results

In silico and In vitro assays showed that all the green tea compounds have antileishmanial activity. EGCG and ECG were the most active compounds against *L. infantum* promastigotes and axenic amastigotes with an IC_{50}s of 0.06 µM and 0.17 µM and IC_{90}s of 0.037 µM and 0.097 µM respectively. pentamidine displayed less growth inhibition in concentration of about 20 µM.

4 Conclusions

In silico and especially docking results were in accordance with the in vitro activity of these compounds in presented study and EGCG and ECG showed reasonable levels of selectivity for the *Leishmania* PTR1 and TS.

C0271 IN VITRO ANTI-LEISHMANIAL EFFECTS OF KELUSSIA ODORATISSIMA MOZAFF EXTRACT ON LEISHMANIA MAJOR

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1 Background

Plants used for traditional medicine contain a wide range of substances that can be used to cure a large namber of diseases such as infectious diseases. The present study aimed to evaluate the *in vitro* effects of aqueous, ethylacetate and butanol fractions of *Kelussia odoratissima Mozaff* (wild celery; mountain celery) extract on *Leishmania major* promastigote and amastigote.

2 Methods

The fractionation was carried out using liquid-liquid extraction method. Promastigotes were subjected to different concentrations (1280, 1000, 640, 320, 160, 80 and 40 ug/ml) of each extract for 24, 48 and 72 hours while amastigotes were subjected for 48 h.

3 Results

According to our findings, no significant anti-leishmanial effects was observed for the aqueous fractions ($p > 0.05$) on promastigote form of parasite. The highest anti-leishmania leffect (100% Growth inhibitory) was demonstrated when amastigotes were treated with the butanol fraction ($p < 0.001$) (IC_{50}=154.1 µg/ml).

4 Conclusions

The results indicate that the extract of this plant could be a candidate for treating cutaneous leishmaniasis. However, its anti-leishmanial potent should be evaluated on the parasite *in vivo*.

C0272 IN VITRO ANTI-LEISHMANIAL EFFECT OF FALCARIA VULGARIS EXTRACT ON LEISHMANIA MAJOR

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1 Background

This study is the first of its kind and aimed at evaluating the in vitro anti-leishmanial activity of aqueous, ethyl acetate and butanol fractions of *Falcaria vulgaris* on *Leishmania major* promastigotes.

2 Methods

The fractionation was performed using liquid-liquid extraction method. The experimentation for each fraction was carried out in 6 concentrations (1.28, 0.64, 0.32, 0.16, 0.08 and 0.04 mg/ml) and at 4 hours (6, 24, 48 and 72).

3 Results

According to findings, no clear anti-leishmanial effect was observed for the aqueous and ethyl acetate fractions ($p > 0.05$). The highest anti leishmanial effect was found in the presence of the butanol fraction and at hour 48 ($p < 0.001$). In addition, the lethal dose 50 (LC50) for the butanol fraction at hour 48 was equal to 343.8 µg/ml.

4 Conclusions

Then, anti-leishmanial effect of this plant together with its tissue repair effect poses it as a possible candidate for the treatment of cutaneous leishmaniasis. However, its anti-leishmanial effect should be evaluated on the amastigote form of the parasite in vivo.

C0275 SAFETY AND EFFICACY OF CURRENT ALTERNATIVES IN THE TOPICAL TREATMENT OF CUTANEOUS LEISHMANIASIS. A SYSTEMATIC REVIEW

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1 Background

Leishmaniasis is a prevalent disease in many countries, but there are few drugs available for the treatment. Studies of topical treatments for leishmaniasis were systematically reviewed, to evaluate the therapeutic efficacy, safety, and any adverse effects of these treatments.

2 Methods

A systematic literature review was conducted in the PubMed and Web of Knowledge databases, covering the period from 11 July 2004 to 8 July 2014. The methodology was defined by the PRISMA statement The papers identified in the databases involved 8 studies with a total of 1744 patients.

3 Results

The majority of studies were from Iran (4/8), covered a period of eight years (2003–2011), and included patients 4–85 years of age. The most frequent Leishmania species in the studies were L. tropica (4/8) and L. major (2/8). The treatments administered were thermotherapy, paromomycin and combinations, CO2 laser, 5-aminolevulinic acid hydrochloride (10%) plus visible red light (633 nm) (ALA-PDT), and cryotherapy. Six articles reported cure rates over 80.0%. Six studies reported on failure rates, 3 of them reporting rates lower than 10%. Four studies did not report relapses or recurrences, while the other studies reported low rates (1.8 to 6.3%). The most common adverse effects of the topical treatments were redness/erythema, pain, pruritus burning, edema, vesicles, and hyper- or hypopigmentation.

4 Conclusions

The findings reviewed here provide strong evidence that the new treatments thermotherapy, paromomycin and combinations, CO2 laser, 5-aminolevulinic acid (10%) plus hydrochloride visible red light (633 nm) (ALA-PDT), showed high cure rates with few adverse effects, relapses or recurrences, while cryotherapy showed a moderate cure rate. More studies are needed, with different species of Leishmania in different regions, to assess the overall effectiveness and safety of these treatments.

C0279 REPURPOSING BUTENAFINE, AN ANTIFUNGAL AGENT, IN AMERICAN CUTANEOUS AND VISCERAL LEISHMANIASIS (IN VITRO)

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1 Background

The production ofergosterol lipid, important for the *Leishmania* membrane homeostasis, involves different enzymes. This pathway can be blocked to azoles and benzylamine drugs, such as Butenafine. The aim of the present work was to evaluate the anti-leishmanicidal activity of this drug in three major species of Leishmania responsible for causing the American tegumentar leishmaniasis(*L. (L.) amazonensis* and *L. (V.) braziliensis*) and visceral leishmaniasis (*L. (L.) infantum*).

2 Methods

Promastigote forms of *L. amazonensis*, *L. braziliensis* and *L. chagasi* were incubated with butenafine for 48h, and its effect was compared with the leishmanicidal action of miltefosine, a standard drug. Ultraestructural alterations in *L. amazonensis* promastigotes after butenafine treatment were evaluated by transmission electron microscopy. Antiamastigote effect of butenafine was estimated in intracellular amastigotes of *L. amazonensis*, *L. braziliensis* and *L. chagasi*; 48h later the effective concentrations 50% (EC_{50}) were estimated. In addition, nitric oxide (NO) and hydrogen peroxide (H_2O_2) levels in supernatant of infected macrophages were analyzed. Miltefosine was used as standard treatment.

3 Results

Butenafine eliminated promastigote forms of *L. amazonensis, L. braziliensis* and *L. infantum*. In addition, butenafine induced alterations in promastigote forms of *L. amazonensis* that resemble programmed cell death. Butenafine as well as miltefosine presented mild toxicity in peritoneal macrophages, however, butenafine was more effective to eliminate intracellular amastigotes of *L. amazonensis* and *L. braziliensis* (EC_{50}s 29.8 and 38.4 µM, respectively) compared with miltefosine (~ 60µM); butenafine also eliminated amastigote forms of *L. infantum* (EC_{50} of 47.2 µM), but miltefosine was more effective (30.6 µM). Antiamastigote effect of butenafine was not associated with elevation in levels of NO or H_2O_2.

4 Conclusions

Taken together, data presented herein suggest that butenafine can be considered as a prototype drug able to eliminate *L. amazonensis, L. braziliensis* and *L. chagasi*, the etiological agents of severe forms of leishmaniasis, such as anergic diffuse, mucocutaneous and visceral forms of the disease. Supported by São Paulo Research Foundation (2015/18746-4 and 2016/00468-0) and HC-FMUSP.

C0298 IDENTIFICATION OF LEISHMANICIDAL COMPOUNDS FROM ESSENTIAL OIL OF TUNISIAN CHAMOMILE "MATRICARIA RECUTITA L" : INVESTIGATION OF THE MECHANISM OF ACTION

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1 Background

Leishmaniasis is among the most problematic protozoan diseases causing a considerable morbidity throughout the world. The treatment of these infectious diseases including *Leishmania infantum* remains a challenging public health need. In this context, essential oil (EO)from Tunisian chamomile "*Matricaria recutita L*", has several biological activities such as antimicrobial, antioxidant and anti-inflammatory, is evaluate for its activity against *L. infantum*, a bio-guided isolation and identification of keys molecules responsible for these effects as well as the investigation of their mechanisms of action.

2 Methods

EO was extracted from chamomile flowersusing Clevenger type apparatus. Its fractionation was guided by its leishmanicidal activity, it was injected over silica and sephadex gel column using different mobile phases. Most active fractions were analysed by NMR. Activity against *L. infantum* promastigotes and amastigotes was determined by a colorimetric assay based on alamar Blue reagent. LDH cytotoxicity detection kitwas used for the evaluation of drug-induced cytotoxic effects. Annexin-V/propidium iodine double staining assay was performed using the Tali™ Apoptosis kit and a Tali™ Image Based Cytometer for apoptosis determination. The SYTOX Green assay was performed to detect membrane permeability alterations of parasites. The collapse of an electrochemical gradient across the mitochondrial membrane during apoptosis was measured using a JC-1 Mitochondrial Membrane, Potential Assay Kit and ATP level was measured using a Cell Titer-Glo Luminescent Cell Viability Assay.

3 Results

Chamomile EO exhibits *in vitro* leishmanicidal activity on promastigotes forms with a low inhibitory concentration at 50% (IC_{50}) ranging from (12.258±2.007 µg/ml)for the EO to (9.51±0.050 µg/ml) for the most active isolated molecule: α-bisabolol. Its IC_{50} was 4.798±1.327µg/ml against amastigotes forms and the selectivity index was 6.656. α-bisabolol induces apoptosis after 24h with percentages of apoptotic cells: 17% (IC_{50}) and 20% (IC_{90}). Therefore, membrane damage occurs immediately after incubation with molecule, this was proved using confocal microscope and it decreased the mitochondrial membrane potential and total ATP level.

4 Conclusions

α-bisabolol possesses good leishmanicidal activity affecting the mitochondria function by decreasing its membrane potential and ATP content. This molecule could induce apoptosis via the mitochondrial pathway.

C0301 ANTI-LEISHMANIAL ACTIVITY AND GC-MS CHEMICAL PROFILING OF SCROPHULARIA STRIATA ESSENTIAL OIL

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1 Background

Leishmaniasis is protozoan disease that considered as one of the problems in public health. Due to toxicity and resistance of available drugs, development of new compounds is important. The aim of the present study was to evaluate in vitro effect of *Scrophularia striata* essential oil on *L. tropica* and *L. major*.

2 Methods

First, essential oil was prepared and analysed with GC/MS. After exposure of parasites to different concentrations of essential oil, viability of promastigotes and axenic amastigotes were investigated by colorimetric assay and the obtained results were expressed as 50% inhibitory concentration.

3 Results

Essential oil analysis showed that nonane, linalool and α-terpineol were the most abundant compounds. In the present study IC50 of *S. striata* was achieved 5.8 and 2.1% for promastigotes and axenic amastigotes of *L. tropica* and 6 and 4.5% for *L. major*, respectively.

4 Conclusions

Overall, according to *S. striata* low cost, availability and acceptable leishmanicidal effects, further investigation can be done.

C0323 INDUCTION OF APOPTOTIC LIKE DEATH IN LEISHMANIA AMAZONENSIS BY P CYMENE ISOLATED FROM TUNISIAN THYMUS CAPITATUS

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1 Background

Leishmaniasis, a vector-borne disease that is caused by a parasite *Leishmania*. This neglected disease is endemic in large areas of the tropics, subtropics and the Mediterranean basin. Clinically, Leishmaniasis,considered as Neglected Tropical Disease,is divided into three major categories – cutaneous, mucocutaneous, and visceral – and is. Even though, this illness *has been treated* successfully, the present therapy is compromised with the appearance of resistance forms and the high drugs toxicity. Thus an urgent need to identify new treatments to this disease has increased significantly. In the last decades, studies on the evaluation of the biological activities of Medicinal and Aromatic Plants have confirmed their interesting pharmacological properties. In the present study, the *in vitro* leishmanicidal activity of *p* cymene, isolated from Tunisian *Thymus capitatus* extract, against the *Leishmania* (L.) *amazonensis* was investigated.

2 Methods

The biological activity of the crude extract, fractions and pure molecules was evaluated against *Leishmania* (L.) *amazonensis* and macrophage cell line J774 using Alamar blue method. The action mode of isolated bioactive molecules was elucidated by studying the type of cell death with the Tali™ Apoptosis Kit – Annexin V Alexa Fluor® 488 and Propidium Iodide, the permeability of the plasma membrane, the mitochondrial membrane potential and the ATP levels in the treated parasites.

3 Results

p-cymene showed a strong leishmanicidal activity with an IC_{50} of 8.529 ng/ml ±1.470 and 2.214 ± 0.265 µg/ml against both amastigotes and promastigotes stage, respectively. The present molecule showed a selective activity toward the parasite with selectivity index of 250. Cells were treated with the calculated IC_{50} and IC_{90} of the tested molecule for 24h. The results pointed out that the present molecule at IC_{90} induced 30% of apoptotic cells. The spectrofluorimeteric data revealed that the *p* cymene at the IC_{90} could reduce the mitochondrial membrane potential and ATP production by 80% in treated parasites.

4 Conclusions

In the present study, we founded that the p cymene induced a pronounced decrease in the mitochondrial potential and therefore in the total ATP levels. Moreover, these events verify that the studied drug could induce apoptosis in the *Leishmania* cells through the intrinsic pathway.

C0325 AMERICAN TEGUMENTARY LEISHMANIASIS IN BRAZIL: ENCOURAGING RESPONSE TO TREATMENT WITH LOW DOSE OF MEGLUMINE ANTIMONIATE FOR PATIENTS INFECTED IN DIFFERENT BRAZILIAN REGIONS

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1 Background

Background. In Brazil, American tegumentary leishmaniasis (ATL) is caused by different *Leishmania* species - *Leishmania braziliensis* is the most prevalent. The clinical presentation and therapeutic response to *Leishmania* may be affected by the patient's individual response, the species involved and also by the genetic heterogeneity of the parasite. In Rio, de Janeiro, improved treatment response may result from *Leishmania braziliensis*' genetic homogeneity, whilst in other Brazilian regions heterogeneity may impair treatment response. Favorable results have been achieved by the Evandro Chagas National Institute of Infectious Diseases (NIID) of the Oswaldo Cruz Foundation, Rio de Janeiro, Brazil, through alternative doses to those recommended by WHO and the Brazilian Health Ministry. The objective of the present work was to compare responses to 5mg Sb^v/kg/day meglumine antimoniate treatment among patients from the state of Rio de Janeiro and other Brazilian states, aiming to evaluate which *Leishmania* species and *Leishmania braziliensis* subpopulations are involved.

2 Methods

Methods. Multi locus enzyme electrophoresis (MLEE) and sequencing of ITS-2 region of rDNA were used for *Leishmania* typing and investigation of genetic variability. The sample was composed of 108 patients, 36 patients who acquired ATL in other Brazilian states (OS group), and 72 patients who were infected in the State of Rio de Janeiro (RJ group).

3 Results

Results. *Leishmania braziliensis* was found in 100% RJ group patients; however, in OS group, *Leishmania braziliensis* was found in 18 (86%) of 21 isolates, including two isozyme variants. Within this group, *Leishmania amazonensis* was identified in one patient and *Leishmania guyanensis* in two. Higher genetic variability was found in OS group sample when compared with RJ samples – by analyzing the ITS-2 region. Use of 5mg Sb^v/kg/day meglumine antimoniate led to cure in 72% cases/108 patients. Considering additional treatments using the same dosage, 88.9% patients were cured with 5mg Sb^v/kg/day. If only 90 patients infected by *Leishmania braziliensis* are considered, 86% satisfactorily responded to 5mg Sb^v/kg/day meglumine antimoniate treatment.

4 Conclusions

Conclusion. Our results suggest that response to this therapeutic scheme is not related to higher or lower parasite genetic variability. Treatment using low dose of meglumine antimoniate, with less side effects for the patients, may be performed in other regions than the state of Rio de Janeiro.

C0326 SAFETY AND EFFICACY OF LIPOSOMAL AMPHOTERICIN B FOR TREATMENT OF COMPLICATED VISCERAL LEISHMANIASIS IN PATIENTS WITHOUT HIV, NORTH-WEST ETHIOPIA.

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1 Background

Visceral leishmaniasis (VL) is a protozoan disease that is invariably fatal if left untreated. The disease is found in 70 countries with incidence of 0.2 to 0.4 million cases. The mainstay of treatment in resource limited countries like Ethiopia is antimonials, while use of liposomal amphotericin B is reserved for treatment of complicated VL cases. The aim of this study was to assess the safety and efficacy of liposomal amphotericin B in HIV negative VL patients diagnosed with complications

2 Methods

A retrospective chart review was conducted involving records of patients admitted between January 2009 and December 2014. Baseline sociodemographic, clinical, and treatment outcome data were collected. The doses of liposomal amphotericin B and adverse events related to treatment were retrieved. Categorical and continuous variables respectively were analyzed by Chi-square and Mann-Whitney U tests. A p-value of less than 0.05 was considered statistically significant.

3 Results

A total of 147 patients with severe VL were treated with liposomal amphotericin B in total dose ranges of 20mg/kg to 35mg/kg. In the overall treatment outcome analysis, initial cure (30 days after start of treatment) was observed in 128 (87.1%), treatment failures in 10 (6.8%), interruptions in 2(1.4%) and deaths in 7 (4.8%) patients. Initial cure rate at high dose (24-35mg/kg total dose) was 96.7% (59/61) versus 80.2% (69/86) at lower doses (<24mg/kg); which was significantly higher (P< 0.01), OR=4.56: 95%, Confidence Interval (CI) = 1.17 – 20.78). Ten cases (11.8%) of treatment failure occurred in the low dose treatment group. The most common adverse events (AEs) were hypokalemia in 39 cases (26.5%) and infusion related reactions in 16 (10.9%). The frequency of hypokalemia and infusion related reactions were not significantly different between the low and high dose liposomal amphotericin B.

4 Conclusions

In HIV negative complicated VL patients, high dose of liposomal amphotericin B was found to have high cure rate at the end of treatment. The appropriate dose for better efficacy needs to be determined. Monitoring serum potassium level during treatment with liposomal amphotericin B should be an essential component of the clinical management of VL.

C0349 EVALUATION OF THE EFFICACY OF A PAROMOMYCIN AND CHLOROQUINE COMBINATION THERAPY IN EXPERIMENTAL MODELS OF CUTANEOUS LEISHMANIASIS

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1 Background

The antimalarial chloroquine (CQ) is clinically used in combination with doxycycline to cure Q-fever. Its therapeutic role in this context is thought to be related to its ability to accumulate and neutralize the pH in acidic cell organelles, such as macrophage phagolysosomes within which *Coxiella burnetii* bacteria multiply. As there is a similar cellular host-pathogen biology for *Leishmania* parasites which cause cutaneous leishmaniasis (CL), this study aimed to determine whether such an approach could be the basis for a new treatment for the disease.

2 Methods

In vitro synergy between the antileishmanial drugs paromomycin sulphate (PM), miltefosine and amphotericin B coincubated with 10 µM CQ was tested in standard 72 hours intracellular antileishmanial drug assays against *L. major* and *L. mexicana* amastigotes in peritoneal mouse macrophages (PEMs). Combinations which yielded significant results were then assessed against the same *Leishmania* species in BALB/c mice, intraperitoneally treated daily for 10 days with standard drugs alone or with 25 mg/kg CQ (n=3-5 per group). Lesion size and parasite load per gram of skin tissue (qPCR) were used as indicators of efficacy.

3 Results

Outcomes of *in vitro* assays were variable for the different drugs. Significantly, the addition of 10 µM CQ to PM reduced EC50s by over 5-fold against both *L. major (*from 91.70±11.2 µM to 16.88±1.55 µM) and more definitely against the normally insensitive *L. mexicana* (86.62±22.28 µM). Evaluation in the BALB/c–*L. major* model showed that 50 mg/kg PM combined with CQ resulted in a slightly faster healing of the lesion compared to PM in monotherapy (which shrunk to 0 mm by day 8 v*ersus* day 9), but parasite load at the end of treatment was not significantly reduced ($1.03\pm3.65\times10^6$ and $1.03\pm2.76\times10^6$ per gram of tissue, respectively). A similar phenomenon was observed in identically treated *L. mexicana* infected mice: the combination yielded a reduction in lesion size in comparison to the PM alone group, but day 10 parasite burden was not significantly different (3.85±0.10 mm *versus* 5.37±0.24 mm and $7.02\pm5.39\times10^6$ *versus* $3.43\pm1.19\times10^6$ parasites per gram, respectively).

4 Conclusions

While initial *in vitro* results for a PM and CQ combination to treat CL were promising, evaluation in *L. major* and *L. mexicana* murine models showed only moderately improved efficacy outcomes after administration of high doses of both drugs.

C0351 ACCUMULATION OF AMPHOTERICIN B IN LESIONS AND HEALTHY SKIN AREAS OF L. MAJOR INFECTED BALB/C MICE AFTER AMBISOMEÆ TREATMENT

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1 Background

The polyene antibiotic amphotericin B (AmB) in its liposomal form AmBisome® is used as a second line drug in the treatment of complex cases of cutaneous leishmaniasis (CL). However, little is known about its skin distribution or why it is more effective than other formulations. In this work, amphotericin B skin disposition after AmBisome® therapy was compared in *L. major* infected and uninfected mice.

2 Methods

BALB/c mice presenting with *L. major* lesions on the rump above the tail were intravenously administered doses of 0, 6.25, 12.5 or 25 mg/kg LAmB every 48 hours over a 10-day period (n= 5 per group). In the following experiment, infected as well as uninfected rodents were given the same 25 mg/kg regimen (n=3 per group). At the end of treatment, skin tissues from the rump (lesion site) and back (unaffected control site) were collected, homogenized and AmB was extracted and quantified with LC-MS/MS. Lesion size and parasite load per gram of tissue (qPCR) were used as indicators of efficacy.

3 Results

On the 10th and final day of treatment with 0, 6.25, 12.5 and 25 mg/kg AmBisome®, AmB levels found in lesion homogenates were 0.0 ± 0.0, 6.48 ± 1.50, 8.78 ± 1.98 and 68.96 ± 21.44 µg per gram of infected skin respectively. Over a dose range from 0 to 12.5 mg/kg AmBisome®, there was a direct correlation between intralesional drug accumulation and reduction in lesion size as well parasite load compared to the untreated controls. A separate experiment confirmed the intralesional AmB levels after the same regimen of 25 mg/kg AmBisome® to be relatively high (78.96 ± 24.90 µg/g). Compared to drug concentrations in the lesion-free back skin of the same *L. major* infected mice (5.70 ± 2.01 µg/g), this is a significant and more than 10-fold increase (p=0.0427). In the healthy back and rump skin tissues of uninfected mice, AmB levels of 30.29 ± 5.25 µg/g and 21.34 ± 4.58 µg/g were found, which are lower but not significantly different from those found intralesionally (p=0.2685).

4 Conclusions

AmBisome® shows a linear dose response up to 12.5 mg/kg in the BALB/c *L. major* model of CL. After a 10 day dose regimen of 25 mg/kg, intralesional AmB levels are 10-fold higher compared to healthy skin in the same animal and elevated (but not significantly) in contrast to skin tissues in uninfected mice.

C0359 MONITORING TREATMENT OF CUTANEOUS LEISHMANIASIS (CL) PATIENTS WITH QUANTITATIVE REAL-TIME PCR AND IDENTIFICATION OF LEISHMANIA PARASITES NOT RESPONDING TO PENTAMIDINE ISETHIONATE (PI) THERAPY

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1 Background

Monitoring treatment efficacy is essential in CL patient management and is a way to assess potential treatment failures due to parasites poorly responding to treatment. *Leishmania (Viannia) guyanensis* is the principle cause of CL in Suriname. This disease is treated with PI, but treatment failure has increasingly been reported. Therefore, the clinical efficacy of PI was evaluated in a controlled trial and parasite dynamics under treatment was monitored by a PCR-based method for a period of 12 weeks. It was also determined whether parasite loads at selected time points could predict the outcome of PI treatment. Furthermore, treatment failure due to other infecting *Leishmania* species was assessed.

2 Methods

Parasite loads of CL patients were determined in skin biopsies using quantitative real-time PCR before as well as six and twelve weeks after treatment with PI. Clinical responses were evaluated on week 12 and compared to parasite loads before initiation of the treatment as well as on weeks 6 and 12. In parallel, molecular species differentiation was performed.

3 Results

L. (V.) guyanensis was the main infecting species in 129 of 143 patients (about 90%). PI treatment led to a statistically significant decrease ($p < 0.001$) in the parasite counts at week 12 compared to before treatment and cured about 75% of these patients. Treatment failure was attributable to infections with *L. (V.) braziliensis*, *L. (L.) amazonen*sis, and *L. (V.) guyanensis* (1/92, 1/92, and 22/92, respectively, of evaluable cases). There was a substantial agreement beyond chance between the parasite load at week 6 and the clinical outcome at week 12, as indicated by the κ value of 0.608.

4 Conclusions

L. (V.) guyanensis is the main infecting species of CL in Suriname followed by *L. (V.) braziliensis* and *L. (L.) amazonensis*. Furthermore, patients' responses to PI can be better anticipated on the basis of parasite load six weeks after the treatment than on the basis of parasite counts before the treatment.

C0367 PHOTODYNAMIC VACCINATION: LIGHT INACTIVATION OF PHOTO-SENSITIZED LEISHMANIA WITH SINGLET OXYGEN AS SAFE AND EFFECTIVE VACCINES AND VACCINE CARRIERS FOR IMMUNO-PROPHYLAXIS AND THERAPY

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1 Background

Photosensitizers (PS) are light-activable to generate singlet oxygen and other ROS in the presence of oxygen for microbicidal photodynamic therapy (PDT). "Photodynamic vaccination" (PDV) is to use PDT-inactivated Leishmania as whole-cell vaccines against leishmaniasis, and as a universal carrier to deliver add-on vaccines against other diseases (Chang KP, et al. Parasit Vectors. 2016; 9:396). The efficacy of Leishmania for vaccine delivery makes use of their inherent attributes to parasitize antigen-presenting cells. PDT-inactivation of Leishmania provides the safety for their use. This is accomplished in two different ways: chemical engineering of PS to enhance their uptake by Leishmania for effective photo-sensitization, and transgenic approach to render Leishmania aminolevulinate (ALA)-inducible for cytosolic accumulation of uroporphyrin.

2 Methods

Porphyrinogenic Leishmania and their transfectants expressing vaccine candidates were prepared. These and control Leishmania were doubly PDT-inactivated as usual. Some were kept frozen to facilitate their storage and shipment. Frozen products were inoculated into advanced cancer patients for open label direct observation trials based on compassionate access with their informed consent according to Dr. Elliott's IRB-approved protocol.

3 Results

The transfectants expressed Western blot-positive vaccines and remained porphyrinogenic in response to ALA. Multiple inoculations of each subject with the frozen vaccines produced no clinically adverse effects. Two of the four subjects are still alive. Presented elsewhere are the results from assessing PDT-inactivated Leishmania as prophylactic and therapeutic vaccines in experimental and canine leishmaniasis, respectively.

4 Conclusions

The data point to the safety of the vaccines used and their potential contribution to prolonging the life of the vaccinees. Work is on-going to include additional subjects. This new modality for vaccine delivery provides the versatility to balance safety versus efficacy for evaluations.

C0368 ANTILEISHMANIAL EFFECT OF PERSIAN GULF BROWN ALGAE (SARGASSUM OLIGOCYSTUM) ON LEISHMANIA MAJOR (MRHO/IR/75 /ER) IN VITRO

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1 Background

Leishmaniasis with wide spectrum of clinical manifestation considered as a major public health problem in 88 countries worldwide.Current drugs has some limitations and disadvantages and showed vary level of effectiveness. Based on previous reports about antibacterial and antifungal effects of the Persian Gulf brown algae (Sargassum Oligocystum), therefore current study was aimed to assessment the effect of *S. Oligocystum* on *Leishmania major* (MRHO/IR/75 /ER) in vitro.

2 Methods

*S. Oligocystum*was prepared from Persian Gulf, extraction from them performed by methanol solvent using percolation method.*L. major* promastigotes cultured in RPMI 1640 media and in Stationary stage treated with different concentration of extracts, incubated at 25±1°C for 24, 48 and 72 h. MTT assay was employed for IC50 value. Finally morphological alterations were compared treated and untreated promasitoges.

3 Results

The results showed that extractcan inhibit the growth of promastigotesandabout48 h after treatment, in concentrations of 5mg/ml(IC50) of Sargassum Oligocystum while in glucantime-treated promastigotes as positive control group, IC50 was 21.96 µg/ml.Also extract exhibited a dose and time-dependent antileishmanial activity against *L. major* parasites. By adding these concentrations of the extract to the infected macrophages, the effect was separately evaluated that 24 h after proximity, some morphological changes were seen.

4 Conclusions

According to the results, the anti-leishmanial effect of the S. Oligocystumextracts may make it possible to use them in the treatment of cutaneous leishmaniasis as a complementary or alternative therapy; however, further studies are necessary and should be evaluated in cell culture and in vivo conditions to confirm it.

C0385 THE REPURPOSING OF THE ANTIDEPRESSANT CLOMIPRAMINE AS NEW ALTERNATIVE ON THE CHEMOTHERAPY AGAINST LEISHMANIA AMAZONENSIS

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1 Background

Leishmaniasis is a huge public health problem, caused by protozoan of genus *Leishmania*. The chemotherapy currently available for leishmaniasis is not completely efficient, mainly due to severe toxicity and emergence of resistant strains. On the pursuit of alternatives, the search of new uses for approved drugs, known as drug repurposing, arises as a cheaper and faster approach on drug discovery. Here, we evaluate the activity of the antidepressant Clomipramine against *Leishmania amazonensis* and describe its mechanism of action on the parasite.

2 Methods

The activity of Clomipramine was evaluated against *L. amazonensis* (WHOM/BR/75/JOSEFA) promastigotes and intracellular amastigotes inside macrophages J774.A1. The inhibitory concentrations were determined (IC_{50} and IC_{90}) for promastigotes and amastigotes, by direct counting, respectively at 72h and 48h. In order to evaluate the mechanism of action of Clomipramine, promastigotes were treated for 24h and stained with different fluorescent probes to assess various cell parameters: cell membrane integrity (Propidium Iodide), mitochondrial membrane potential (TMRE), reactive oxygen species - ROS (H_2DCF-DA; Amplex-Red), lipoperoxidation (DPPP), DNA fragmentation (TUNEL), Phosphatidylserine (PS) exposition (Annexin V-FITC) and autophagic vacuoles (dansylcadaverine). Looking for ROS involvement the same assays were performed in parasites pre-treated with the antioxidant *N*-acetyl-L-cysteine (NAC 20 µM).

3 Results

The antidepressant Clomipramine expressed IC_{50} values of 8.22 and 15.45 µM, respectively against promastigotes and intracellular amastigotes. Regarding the mechanism of action, our results suggested that Clomipramine act by a mitochondrial pathway by depolarizing the membrane potential. In addition, an intense oxidative stress was induced by Clomipramine, which expressed as an elevation in ROS levels. Such effect was inhibited when parasites were pre-treated with NAC. As consequence of this stress, the drug induced lipoperoxidation and DNA fragmentation at IC_{90}. Regarding the cell fate after treatment, the drug did not affect cell membrane integrity neither induced PS exposition, but induced intense autophagic vacuolization.

4 Conclusions

Taken together our results support the repurposing of Clomipramine as a potent anti-parasitic agent against *L. amazonensis.* The drug seems to kill the parasite by a mitochondrial pathway, with no induction of necrosis neither classic apoptosis, but with clear autophagy involve

C0399 TAMOXIFEN INDUCES APOPTOSIS IN LEISHMANIA MAJOR PROMASTIGOTES IN VITRO

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1 Background

Tamoxifen is an antagonist of the estrogen receptor and currently used for the treatment of breast cancer. The current treatment of cutaneous leishmaniasis with pentavalent antimony compounds is not satisfactory. Therefore, in this study, due to its antileishmanial activity, effects of tamoxifen on the growth of promastigotes and amastigotes of Leishmania major Iranian strain were evaluated in vitro.

2 Methods

Promastigotes and amastigotes were treated with different concentrations (1, 5, 10, 20, and 50 µg/ml) and time periods (24, 48, and 72 hr) of tamoxifen. After tamoxifen treatment, MTT assay (3-[4,5-dimethylthiazol-2-yl]-2,5 biphenyl tetrazolium bromide assay) was used to determine the percentage of live parasites and Graph Pad Prism software to calculate IC50. Flow cytometry was applied to investigate the induction of tamoxifen-induced apoptosis in promastigotes.

3 Results

The half maximal inhibitory concentration (IC50) of tamoxifen on promastigotes was 2.6 µg/ml after 24 hr treatment. Flow cytometry analysis showed that tamoxifen induced early and late apoptosis in Leishmania promastigotes. While after 48 hr in control group the apoptosis was 2.0%, the 50 µg/L concentration of tamoxifen increased it to 59.7%.

4 Conclusions

Based on the in vitro antileishmanial effect, tamoxifen might be used for leishmaniasis treatment; however, further researches on in vivo effects of tamoxifen in animal models are needed.

C0407 IN VITRO ANTILEISHMANIAL ACTIVITY OF NOVEL AZOLES (3-IMIDAZOLYLFLAVANONES) AGAINST PROMASTIGOTE AND AMASTIGOTE STAGES OF LEISHMANIA MAJOR

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1 Background

Leishmaniasis is a protozoan infectious disease widely distributed all around the world. First line drugs including antimoniales are insufficient due to resistance in endemic areas and high toxicity. Azole antifungals like ketoconazole (KCZ) are also used as antileishmanial agents for several decades.

2 Methods

In the present study, we evaluated in vitro antileishmanial effects of new azole antifungals namely 3-imidazolylflavanones (**IF**s) and their oximes (**IFO**s) against *Leishmania major* parasites.

3 Results

The obtained results showed remarkable effect of our compounds on promastigote and amastigote stages of *L. major*. In particular, the 4-chloro analog of flavanone (**IF-2**) and 3-chloro substituted flavanone oxime (**IFO-3**) with IC_{50} values ≤8.9 µg/mL were 8-fold more potent than KCZ (IC_{50}= 72 µg/mL) against promastigote form of *L. major*. In amastigote stage, the compounds **IF-2** and **IFO-2** decreased the mean number of infected macrophages (MIR) more than KCZ ($p<0.005$). In addition, compounds **IF-1**, **IF-2**, **IF-4**, **IFO-2**, **IFO-3** and **IFO-5** decreased the mean number of amastigotes per macrophages (MNAPM) significantly more than KCZ ($p<0.005$). All compounds decreased both MIR and MNAPM significantly more than control ($p<0.001$). Compounds **IF-2** and **IFO-2** withparasite survival of 7.70% and 20% had the highest inhibition on intracellular amastigotes. Although most of compounds displayed acceptable selectivity index, compound **IF-2** had the highest CC_{50} value (115.4 µg/mL)and SI (383.3).
We concluded that our new synthetic azoles displaying potent activity against *L. major* could be considered as new hits for drug development in the field of antileishmanial therapy.

4 Conclusions

We concluded that our new synthetic azoles displaying potent activity against *L. major* could be considered as new hits for drug development in the field of antileishmanial therapy.

C0420 EVALUATION OF THE CYTOTOXICITY EFFECT OF BIMETALLIC AL/TIO2 NANOPARTICLES ON LEISHMANIA MAJOR PROMASTIGOTES (MRHO/IR/75/ER) IN VITRO

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1 Background

The Cutaneous Leishmaniasis (CL) classified as a Neglected Tropical Disease (NTD). Treatment of the disease has remained insufficient since current antileishmanial drugs have several disadvantages such as toxicity,teratogenicity, costliness and drug-resistance. Therefore, there is an immediate need to search for new antileishmanial compounds. Titanium dioxide nanoparticles (Tio_2 -NPs) have been widely applied as a drug delivery agent.The basic antimicrobial mechanism of Tio_2-NPsis the generation of reactive oxygen species (ROS). Even though Leishmania parasites are sensitive to ROS. In this study, we evaluated the leishmanicidal effects ofBimetallic AL/Tio_2 Nanoparticles with mean particle size of 20 nanometers (nm) on *Leishmania major* promastigotes in vitro.

2 Methods

Bimetallic AL/Tio_2nanoparticles were spherical in shape with a particlediameter of 22.0± 2.0nm.The promastigotes(MRHO/IR/75/ER) were cultivated in RPMI1640 medium containing 100units/ml penicillin, streptomycin 100µg/ml and20%FBS in a 25 ± 1°C incubator.In order to evaluate the parasite survival, the multiplication of the promastigotes was determined by counting the cells by hemocytometer chamber before and after adding the nanoparticles, (in a range between 0.75 and 400µg/mL) after 24, 48 and 72h of incubation. Amphotericin B(AmB) at a concentration of 4µg/ml was a positive control. GraphPad prism5 was used to determine the IC50. Antileishmanial effects of different concentrations of bimetallic AL/Tio_2-Nps (0.75, 1.55, 3.12, 6.25, 12.5, 25, 50, 100, 200, 400µg/ml(on Viability percentage of promastigotes *L. major* were determined by the 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide (MTT) assay.

3 Results

IC50 of bimetallic AL/Tio_2-Nps after 24 hours of incubation was 25.8 µg/ml. also IC50 of control drug (AmB) was calculated 27.2 µg/ml.The percentage of cytotoxicity of promastigotes after adding bimetallic AL/Tio_2-Nps was 79.3% by MTT.

4 Conclusions

According to the MTT assay, the concentration of 400 µg/mlof Bimetallic AL/Tio_2nanoparticles with 79% cytotoxicity was promising against *L. major* promastigotes and further studies are recommended to evaluate the effects of this plant. The efficacy of examined Nps on this species of parasite is approximately equivalent to that of Amphotericin B; so, it may have prospective effects as an antileishmanial drug.

C0425 TARGETING OF CONTROLLED RELEASE AMPHOTERICING B PARTICLES TOWARDS LEISHMANIA PARASITES

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1 Background

Amphotericin B (AmB) is one of the drugs of choice for visceral leishmaniasis (VL) which is fatal if left untreated. However, AmB provokes severe adverse effects such as nephrotoxicity and infusion-related side effects that limit its use in clinical practice. Current marketed formulations are based on lipidic systems which are no-cost effective and require prolonged hospitalization. The development of parenteral cost-effective AmB systems would expand the treatment access.

2 Methods

Novel controlled release formulations based on different encapsulation systems such as albumin microspheres, drug aggregation state and particle size modifications were developed in order to improve AmB efficacy/toxicity balance. The toxicity of the novel formulations was assessed by studying the kinetics of the haemolytic process. Pharmacokinetic studies were performed both in healthy and VL-infected beagles.

3 Results

Larger particle size and drug encapsulation into albumin microspheres showed a reduction in haemolytic toxicity. Pharmacokinetic studies showed that the least hemolytic formulations were poly-aggregated AmB and AmB encapsulated into albumin microspheres. Both formulations exhibited a very low hemolytic activity which was similar to the marketed gold standard liposomal formulation (AmBisome®). However, when it comes to the pharmacokinetic profile, both formulations were significantly different from the gold standard, being characterised by a faster decrease from bloodstream (leading to a smaller area under the curve and large volume of distribution in tissues). This pharmacokinetic profile is useful for the treatment of several diseases specially in leishmanisis where the parasites are accumulated intracellularly in macrophages of the reticulo-endothelial system.

4 Conclusions

The effect of the encapsulation system, aggregation state and particle size played a key role in the toxicity and biodistribution of AmB in the dog. The dose administered, the effect of anesthesia and dog´s healthy state had also a significant effect on AmB pharmacokinetics. In terms of haemolytic toxicity and pharmacokinetic profile, poly-aggregated AmB have shown to be a promising cost-effective alternative for visceral leishmaniasis.

C0450 EVALUATION OF PHOTODYNAMIC THERAPY USING DIFFERENT PHOTOSENSITIZERS ON LEISHMANIA TROPICA PROMASTIGOTES

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1 Background

Leishmaniasis is transmitted by a vector called Phlebotomus and it is a common parasitic disease caused various clinical tables, from deadly internal organ involment to self-healing skin lesion, in 98 countries. *Leishmania tropica* is the most often agent of cutaneous leishmaniasis in Turkey. Photodynamic therapy (PDT) is an alternative treatment method which uses a photosensitizer activated in the presence of light and molecular oxygen to produce reactive oxygen species that eventually target cell death by apoptosis or necrosis. The purpose of this study was to determine the efficiency of Methylene blue (MB), chloroaluminum phthalocyanine (AlClPc), toludine blue (TB) and phenorforbide a (PPa) mediated PDT in exerting morphological and apoptotic changes in *Leishmania tropica* promastigotes.

2 Methods

Leishmania tropica promastigotes were incubated with increasing concentrations of MB, TB, AlClPc and PPa for one hour and then were illuminated with a white light source for 30 min. After incubation at 26°C for 18 hours, cell viability (analyzed using a hemocytometer and XTT colorimetric assay), morphological changes (determined by giemsa staining) and apoptotic markers (analyzed by DAPI staining and DNA fragmentation assay) of *Leishmania tropica* promastigotes were evaluated as a function of MB, TB, AlClPc and PPa concentration.

3 Results

Parasite viability was significantly different in between the groups treated with MB, TB and PPa, with or without irradiation. There were no statistically significant differences in cell viability of AlClPc treated *Leishmania tropica* promastigotes with and without irradiation. Also, DAPI staning results indicated that apoptotic bodies and nucleus fragmentation started to be visible in MB, AlClPc and PPa mediated PDT groups. Furthermore, DNA ladder pattern which is used to define apoptosis, was observed in irradiated MB, AlClPc and PPa groups, but not observed in irradiated TB, non-irradiated MB, AlClPc, TB and PPa.

C0452 IN VITRO LEISHMANICIDAL ACTIVITY OF TUNISIAN SEAWEEDS EXTRACTS AGAINST L.AMAZONENSIS AND L.INFANTUM

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1 Background

Researches on identification and investigation of new natural bio-active molecules have been increasing nowadays to substitute the synthetic molecules. Further they exhibit a promising potential on drug development. Recently, several studies reported the biological activities of many compounds isolated from photosynthetic marine organisms as anti-inflammatory, antioxidant, trypanocidal, microbicidal, anti-tumoral and leishmanicidal. Chemically, compounds from seaweeds have complex structures that could be used in drug development including drugs for leishmaniasis. Tunisian coastlines are an abundant resource of seaweeds with important species diversity; therefore, the aim of the present study was to evaluate the leishmanicidal activity of different seaweed species.

2 Methods

24 samples of seaweeds were harvested from 3 different geographical points of tabarka's coast (Tunisia). The sampling was made in two different seasons. In total, 14 different species belonging to the 3 phylums was identified. For each simple a successive extraction was held using 3 solvants (hexane, ethyl acetate and methanol). *In vitro* leishmanicidal activity was evaluated using the Alamar Blue method against two different genus of the parasite: *Leishmania amazonensis* and *Leishmania infantum*.

3 Results

For the same seaweeds species, the IC_{50} (the extract concentration resulting in 50% parasite growth inhibition) increase with the polarity. For all the tested species, the hexanic fractions were the most active with an IC_{50} between 8.2µg/ml and 331.09µg/ml for *L. amazonensis,* and between 5.68µg/ml and 208.36µg/ml for *L.infantum*. In the case of the green alga *Dasycladus vermicularis*, the IC_{50} values for *L.amazonensis* range from 35.48µg/ml to 221.4µg/ml and for *L.infantum* from 23.27µg/ml to 198.6µg/ml for hexanic and methanolic extracts, respectively. Statistical analysis revealed that the *L.infantum* was more sensitive than *L. amazonensis.* Further, this activity was variety and solvent dependant.

4 Conclusions

The hexanic extracts ofTunisian seaweeds exhibit a good leishmanicidal activity. Due to the solvent polarity, this property would be correlated to the presence of terpenoids compounds. Bio-guided purification will be necessary to isolate and purify the bioactive compounds from the most effective alga species.

C0487 ANTILEISHMANIAL ACTIVITY OF CARTHAMUS TINCTORIUS , PIMPINELLA ANISUM , CUMINUM CYMINUM, CINNAMOMUM VERUM EXTRACTS IN MICE INFECTED WITH LEISHMANIA MAJOR

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1 Background

Leishmaniasis is a parasitic disease transported by sand flies. In this study, we evaluated the efficacy of some medicinal plants (*Carthamus tinctorius* , *Pimpinella anisum* , *Cuminum cyminum, Cinnamomum verum*) and systemic glucantime in a comparative manner against the causative agent of cutaneous leishmaniasis in BALB/c mice.

2 Methods

The extract of aerial parts of plants were obtained by maceration. For in vivo testing, inbred mice were challenged with *Leishmania major* parasites and the resultant ulcers were treated with alcoholic extract based-ointments applied topically two times per day for a period of 20 days. Glucantime was injected intramuscularly at 20 mg/kg/day for 20 days . A group of 48 mice were randomly divided into 6 subgroups. The control group received the ointment void of extracts, whereas the reference group received glucantime only. The efficacy of treatments was evaluated by measuring ulcer diameter by kulis vernier, parasite burden in spleen and NO production in macrophages using Griess test.

3 Results

Our results indicated that plant extract based-ointments were effective in reducing ulcer size and parasite burden in spleens, but their effects did not differ significantly from that of glucantime. The plant extracts tested in this study were able to increase NO production that helped parasite suppression but was not significantly more effective as compared with glucantime.

4 Conclusions

Our findings indicate that the tested plant extracts are effective for treatment of cutaneous leishmaniasis in mice but further researches are required to recommend a potential plant extract as an alternative drug.

C0499 TOPICAL TREATMENT FOR CUTANEOUS LEISHMANIASIS ñ DRUG DISCOVERY

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1 Background

Cutaneous leishmaniasis (CL), a widespread parasitic disease, affects 0.7-1.2 million people every year. Current treatments for CL are unsatisfactory. Topical treatment could reduce adverse effects through local exposure, increase patient compliance and require less stringent follow up. Benzoxaboroles, a relatively new class of compounds showed promising activity when tested against a panel of five *Leishmania* species. A subset of these active compounds were submitted to an array of *in vitro* ADME studies and the three compounds with best profile were tested *in vivo* in a CL mouse model.

2 Methods

Benzoxaborole compounds were screened against intracellular amastigotes. Physicochemical properties that influence dermal exposure, such as log $P_{oct/water}$ and solubility, were determined and compared to literature values. Stability in and binding to mouse skin homogenate was also determined. Permeation of test compounds through reconstructed human epidermis (RHE) (Mattek) was measured. The disposition of promising compounds were further examined using BALB/c mouse skin in Franz Diffusion Cells (FDC). Sample analysis was performed using LC-MS/MS. The three compounds with the best overall skin disposition profile were then tested in BALB/c mice infected with *L. major* JISH118.

3 Results

The physicochemical properties of most compounds were indicative of good dermal exposure. Testing showed no remarkable instability, and most compounds were more stable than the controls (known substrates for skin esterases). Skin binding was variable, with a range of unbound fractions observed. Four compounds showed a better permeation profile than the model penetrant, testosterone when applied to the RHE. These results were confirmed in the FDC experiment. In the *in vivo* study, one compound showed good activity when administered topically, and a second compound exhibited good activity when administered orally.

4 Conclusions

The drug discovery approach applied to this project consisted of potency and selectivity testing followed by limited *in vitro* ADME testing to identify lead compounds with a good dermal exposure and disposition. Initial *in vivo* results are promising and suggest that (1) this approach will be useful in discovery of new CL drug candidates, and (2) the benzoxaboroles may provide good starting points for discovery of both topical and oral treatments for CL.

C0505 PHEOPHORBIDE A INDUCES AUTOPHAGY MEDIATED BY OXIDATIVE STRESS IN AMASTIGOTES FORMS OF LEISHMANIA AMAZONENSIS

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1 Background

Leishmaniasis is still considered a serious public health problem, due to its wide geographic distribution and high rates of morbidity and mortality. Considering the problems related to the drugs currently available for this disease it is necessary to develop new alternative therapies. Thus, the photodynamic therapy emerges as an alternative for the treatment of patients with leishmaniasis. The objective of this study was to evaluate the mechanism of action and predominant cell death after treatment of amastigote forms of *Leishmania amazonensis* with the photosensitizer compound pheophorbide *a* (pheid *a*).

2 Methods

Amastigote forms were obtained through the infection of macrophages by promastigote forms of *L. amazonensis*. After the 48-hour infection period, the infected macrophages were treated with 1 and 7 μM of pheid *a*, irradiated for 20 min by red LED´s with a power of 3 mW/cm^2 and then incubated for 24 h. Thereafter, the macrophages were lysed with a needle (30 G), the amastigotes were separated from debris by differential centrifugation. The supernatant was collected, and then analyzed by fluorometric assay. For mechanistic study in amastigote forms (1 × 10^6 cells/mL), the probes H_2DCFDA, Amplex Red Kit, propidium iodide and monodancylcadaverine were used to evaluate total reactive oxygen species (ROS), hydrogen peroxide (H_2O_2), plasma membrane integrity and formation of autophagic vacuoles, respectively. To evaluate ROS involvement, we performed the pretreatment of a group with the antioxidant *N*-acetyl-L-cysteine (NAC 20 μM).

3 Results

We found that pheid *a* caused an increase in the production of total ROS, and that this effect was significantly reduced in the presence of NAC. Pheid *a* also caused a significant increase in the production of H_2O_2 when compared to untreated controls. This compound was not able to alter the integrity of the plasma membrane of the parasite, however, caused an important induction in the formation of autophagic vacuoles.

4 Conclusions

Pheid *a* demonstrates great potency on the development of new topical formulation for treatment of cutaneous lesions caused by *Leishmania* sp. In addition, our study indicates that parasites appear to die by high oxidative stress that culminates in autophagic cell death.

C0508 IN VITRO SUSCEPTIBILITY OF A CLINICAL ISOLATE OF LEISHMANIA DONOVANI PROMASTIGOTES TO COMMIPHORA WIGHTII (GUGGULU): A PROTEOMICS STUDY

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1 Background

Visceral leishmaniasis, prevalent in 80 countries accounts for high DALYs (Disability Adjusted Life years).In India it's caused by *Leishmania donovani* (LD) and in epidemic form confined to the North-eastern state of Bihar. Currently available drug(s) have limitation with reference to drug resistance, toxicity and affordability so, it becomes imperative to explore novel anti-leishmanials from natural sources.
Commiphora wightii or Guggul gum (GU) has wide therapeutic applications and it is being used extensively for arteriosclerosis,hypolipidemia etc. In the present study GU was evaluated for its anti-leishmanial potential along with Amphotericin B (Amp B). A clinical isolate of LD obtained from a patient with relapse from Amphotericin B treatment, was evaluated**.** Various parameters for determining the mechanism of the inhibitory effect of GU and Amp B by flow cytometry was investigated. A whole cell proteomics study with a comparative (untreated and treated) in-depth differential expression analysis of LD was studied. This study highlights the anti-leishmanial effect of GU which shows promise as potential therapeutic for Leishmaniasis.

2 Methods

L.donovani (MHOM/IN/BHU1243) promastigotes were treated with GU or Amp B and inhibitory concentration (**IC_{50}**) was determined by MTT assay. The mechanism of inhibition was assessed by AnnexinV/PI staining for apoptosis, mitochondrial membrane potential, reactive oxygen species (ROS) generation by flow cytometry. The proteomics analysis was performed with LC-MS and data analysis was carried out to identify differentially expressed proteins using SWATH MS (Sequential Window Acquisition of All Theoretical Fragment Ion Mass Spectra).

3 Results

On analysis for the mechanism involved in growth inhibition of the LD treated with GU or Amp B, loss of mitochondrial membrane potential, phosphatidylserine externalization, elevated ROS was observed. The proteomics analysis resulted in identification of 1021 proteins which was significant (p value ≤ 0.05) along with a comparative differential expression analysis which resulted in identification of 582 proteins (treated and untreated) by SWATH MS.

4 Conclusions

This study highlights the potential of GU, already known for its medicinal properties in various ailments, inhibited LD growth with simultaneous effect on various metabolic pathways. Anti-leishmanial effect of GU along with Amp B on a clinical isolate of LD is being reported for the first time with an in-depth differential proteomics analysis.

C0534 EFFICACY OF AZOLES THERAPY FOR TEGUMENTARY LEISHMANIASIS: A SYSTEMATIC REVIEW AND META-ANALYSIS

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1 Background

Several controlled and uncontrolled studies addressing azoles antifungal drugs for cutaneous (CL) and mucosal leishmaniasis have been published with inconclusive results. We conducted a systematic literature review of studies evaluating the efficacy and toxicity associated to therapy with azoles for tegumentary leishmaniasis (TL).

2 Methods

PRISMA guidelines for systematic reviews and Cochrane manual were followed. Sources were MEDLINE, LILACS, IBECS databases and manual search of references from evaluated studies. The grey literature (*Google Scholar*) was also searched. We included all studies reporting cure rate after cutaneous or mucosal leishmaniasis treatment with systemic azoles drugs, regardless of their design. R software was used to perform one-group meta-analysis of study arms with the same drug to estimate global rates of success and adverse events with each drug. The outcomes of interest were clinical cure and adverse events. Cure was assessed according to current methodological recommendations for CL trials proposed by Olliaro et all, 2013.

3 Results

A total of 36 studies reporting outcomes after fluconazole (9), ketoconazole (13) and itraconazole (14) treatments, in total involving 1251 patients, were included. Twenty-one studies were performed in the old world and 15 studies in Americas. Of these, only 14 (39%) were randomized controlled trials (RCT). The overall definitive cure rate for all azoles drugs was 61% (CI95%: 52 to 69%). The efficacy rate was 61% (CI95%: 51 to 69%) and 67% (CI95%: 45 to 84%) for old and new worlds, respectively. If considered only RCT, the efficacy rate was 54% (CI95%: 41 to 67%); 56% (CI95%: 42 to 69%) for old world and 59% (CI95%: 22 to 88%) for new world. By indirect comparisons, the cure rates were similar among fluconazole, itraconazole and ketoconazole group arms, namely 65% (CI95%: 49 to 77%); 56% (CI95%: 43 to 69%) and 62% (CI95%: 40 to 79%) respectively. All studies addressing exclusively mucosal leishmaniasis form used itraconazole and showed a definitive cure rate of 52% (CI95%: 23 to 80%). Adverse events were reported for 14% (CI95%: 3 to 43%), 14% (CI95% 5 to 31%) and 16% (16%, CI95% 8 to 28%) of the patients treated with fluconazole, ketoconazole, itraconazole respectively.

4 Conclusions

Available evidence suggests that fluconazole, ketoconazole and itraconazole have similar and low efficacy rates for TL treatment. There is insufficient evidence to support the exclusive use of azoles therapy as single agent for TL treatment.

C0540 AQUAGLYCEROPORIN1 GENE EXPRESSION IN ANTIMONY RESISTANCE AND SUSCEPTIBLE LEISHMANIA MAJOR ISOLATES

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1 Background

The mechanism of antimony resistance in *Leishmania* has been studied extensively which showed decreased in?ux and/or increased ef?ux of the drug. Aquaporin-1 (AQP1) has been shown to mediate the uptake of trivalent antimony. Here we are tried to find the expression level of *AQP1* gene in resistant versus non-resistant clinical isolates of *Leishmania major* in Iranian patients.

2 Methods

Clinical isolates were obtained from 16 patients referred to Navab Safavi Clinical Center, Isfahan, Iran from October 2014 to December 2015. After diagnosis of cutaneous leishmaniasis using microscopic observation, biopsy was performed from lesion(s) of each patient and stored inside RNA later at -20 ºC. All patients were provided written informed consent to participate in this study before recording their information and sampling based on Helsinki declaration. Each patient was description with Glucantime and followed for three months. All sensitive and resistance isolates were considered and compared with *AQP1* gene expression using real time PCR that analyzed with delta delta CT.

3 Results

Out of 16 clinical isolates, four patients were resistant and 12 were non-resistant. The *AQP1* gene expression in resistant isolates were significantly more than the one in response failure isolates (P=0.001).

4 Conclusions

The significant over expression (0.5 fold) of *AQP1* in resistant versus non-resistant isolates may show different mechanism such as mutations. Mutations may change the physiological function of the aquaporin1 protein that may affect on its expression level.

C0571 EVALUATION OF ROCK INHIBITOR:A1LX EFFECTS ON LESION PROGRESS OF CUTANEOUS LEISHMANIASIS IN MICE

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1 Background

Leishmania sp. The causative agent of cutaneous leishmaniasis (CL) are obligate intracellular protozoa that infect, multiply and persist in macrophage. Macrophages are the host cells for duplication of*Leishmania,* the first line of defense against parasite invasion. Polarization of Macrophages (M) to M1 and M2-type in response to infections might in part describe the different activities of M (killing *vs.* proliferation) of the parasites, this polarized response leads to the stimulation of Th1 or Th2like responses, respectively. Rho-associated, coiled-coil-containing protein kinases(ROCKs) are involved in cytoskeletal rearrangement, angiogenesis, inflammation and also polarization of macrophages . As ROCK isoforms establish macrophage polarization into M1 or M2 subtype in the infected tissue and have been shown that enhance disease susceptibility and progression of the disease is also associated to the induction of the M2 macrophage phenotype, hereby in the current study, we aimed to inhibit the macrophage polarization to M2 with specific kinase inhibitor and evaluate its effect on disease outcome.

2 Methods

We induced an experimental leishmaniasis in Balb/C mice infected with *L.major* and performed Rock inhibitor A1LX intervention in early and late stages of the infection.

3 Results

The administration of Rock inhibitor A1LX showed a therapeutic effect on postponing the onset of the lesion and ameliorating the severity of disease, and also decrease in parasite load in tissue of footpad and lymph nodes.

4 Conclusions

Rock inhibitor A1LX ameliorates disease progression in CL, acting possibly through anti-inflammatory pathways.

C0576 IS TOPICAL ALOE VERA GEL EFFECTIVE ON CUTANEOUS LEISHMANIASIS? A DOUBLED-BLIND, RANDOMIZED, PLACEBO-CONTROLLED TRIAL

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1 Background

Aloe (Liliaceae) is a genus of plants which have been known for their medicinal value. The aim of this study was to determine the efficacy of topical Aloe vera with systemic Glucantime vs. topical placebo and systemic Glucantime in treatment of cutaneous leishmaniasis.

2 Methods

Seventy-two patients more than two years old with proven acute cutaneous leishmaniasis (ACL) were set in two groups. In the first group (A), topical Aloe vera 10% gel was applied twice a day and systemic Glucantime in a dose of 20 mg/kg/day was injected intramuscularly for three weeks. In the other group (B), topical placebo gel twice a day and systemic Glucantime (20 mg/kg/day intramuscularly) were applied for three weeks. The lesions were examined at the baseline, weeks 3, 6 and 12.

3 Results

There were no statistically significant differences in improvement rate ($P \geq 0.05$).Ten patients out of 15 with nodules in Aloe vera gel group showed considerable improvement compared with patients who used placebo gel. Although statistical differences were not significant, the decreasing trend was comparable between two treatment groups. At the last month of follow-up, using Aloe vera gel was as effective as the placebo group.

4 Conclusions

Our data indicates that Aloe vera group in initial phase of disease was more effective than placebo group in the same duration and complete and partial improvement was more frequent in the third month of follow-up in Aloe vera group than placebo group.

C0582 TREATMENT OF CUTANEOUS LEISHMANIASIS: WHAT IS NEW IN 2016?

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1 Background

Leishmaniasis is reported from 98 countries, cutaneous leishmaniasis (CL) is reported form 80 countries mostly developing ones. Treatment of cutaneous leishmaniasis (CL) is a major challenge of the endemic areas and the sole recommended chemotherapy is using antimonite derivatives which is costly accompanies side effect and not always effective

2 Methods

Search for new treatment options for CL continues. On the other hand, as CL is a self-limiting disease in many patients, any new approach should be evaluated in a well-designed, randomized controlled trial

3 Results

The available systematic reviews and meta-analyses on the treatment of different forms of CL will be reviewed and the latest treatment modalities for CL and an evidence based appraisal of them, as well as the requirement for a suitable clinical trial for CL will be presented

4 Conclusions

Unfortunately we still do not have a very effective, safe, and affordable treatment for cutaneous leishmaniasis (CL).

C0598 TREATMENT OF CUTANEOUS LEISHMANIASIS (PAPULONODULAR TYPE) WITH CO2 LASER AND TCA (TRI CHLOROACETIC ACID) 50%

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1 Background

Cutaneous leishmaniasis (CL) is a chronic parasitic diseases that is common between animals and human beings and transmitted by insect bite .it is endemic in Isfahan province of Iran. There many modalities of treatment for CL but the efficacy and safety of most of them are questionable. The aim of this study was to assess the efficacy and safety of CO2 laser ablation plus topical TCA acid (trichloroacetic acid) as a peeling agents.

2 Methods

24 patients with papulonodular CL entered the study. The age was 1-25 years and 15 female and 10 male. The patients entered the study after singing the informed content, we anesthetized the papulonodular lesions by 1% lidocaine and then ablated the whole lesion by CO2 laser that was Alma (CAL USA) and then peeled with 50% TCA. We did the procedure one time and followed the patients monthly for 6 months. After procedure the patients received healing cream only for 7-10 days. We took digital pictures before and after treatments and in follow up period.

3 Results

22 patient improved after 3 weeks of starting treatment and 3 patients need to repeat treatment one more .patients satisfaction was excellent. And scar was flat and good .we saw some durable erythema that improved spontaneously without any treatment.

4 Conclusions

treatment of CL with continues mode of CO2 laser +TCA peel is a safe and effective treatment for cutaneous leishmaniasis.

C0601 ANTILEISHMANIAL EFFECT OF NEW INDENO[1,5]NAPHTHYRIDINE COMPOUNDS

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1 Background

Leishmaniasis is a emerging disease in an increasingly globalized world. Current treatments against visceral leishmaniasis are plenty of undesirable side effects. New drugs and suitable drug targets are an urgent need for antileishmanial drug development. Based on the strong leishmanicidal effect of camptothecin (CPT) and indenoisoquinolines eukaryotic DNA topoisomerase I (Top) has been recognized as potential chemotherapeutic target for the development of new antileishmanial compounds. Due to the flat polyheterocyclic structure of CPT, we hypothesized that quasi-flat polycyclic heterocycles as indenonaphthyridine could also act as putative topoisomerase inhibitors.

2 Methods

The design of indeno-1,5-naphthyridine derivatives is based on an efficient and straightforward tandem three-component Povarov reaction of aldimines with electron-rich alkenes and subsequent tetrahydronaphthyridine dehydrogenation. By this methodology we accessed to 50 novel compounds. This series of compounds was assayed on iRFP-transfected L. infantum amastigotes obtained from BALB/c mice splenic explants. The antileishmanial effect of these compounds was determined recording the infared fluorescence emmited by the cultures. Selective indexes (SI) were calculated in non-infected macrophages using the Blue-Alamar method. The inhibition of relaxation activity of bisubunit Leishmania Topoisomerase I (LTopIB) was carried out according to previously reported method.

3 Results

The antileishmanial effect of the newly synthesized compounds was investigated on L. infantum iRF1.4 infected splenocytes (amastigotes). Fluorinated and 3-methoxylated derivatives of 4-phenyl ring substituted at 6 position showed submicromolar antileishmanial effect and interesting SI values. In addition, some of these compounds showed selective inhibitory effects on LTopIB-mediated relaxation assays compatible to those observed for the natural inhibitor CPT.

4 Conclusions

From these studies we can conclude that some of newly synthesized compounds exhibited strong antileishmanial effect on L. infantum amastigotes. The ex vivo infecting amastigote platform resemble better the actual parasite form that threat host cells. In addition, splenic explants have the advantage of containing all the immunological cell populations involved in host response. Nevertheless, authors consider that the more than one of magnitude of difference between the LTopIB inhibition and the antileishmanial effect, points to a secondary target of these compounds.

C0614 IMMUNOTHERAPY BY ALPHA DEFENSIN (HNP-1) USING THREE DIFFERENT METHODS OF GENE THERAPY, PEPTIDE THERAPY AND TRANSGENIC L. TARENTOLAE IN BALB/C MICE INFECTED WITH L. MAJOR

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1 Background

The incidence rate of cutaneous leishmaniasis is estimated between 1-1.5 million cases a year. Due to some obstacles (toxicity, drug resistance and permanent scars) in current therapy, considerable attention to develop new and effective treatment is highly demanded. Antimicrobial peptides (AMPs) have high therapeutic index, broad spectrum of activity, safety, and minimal risk of microbial resistance. Defensins are members of the large family of AMPs and act as immunomudolatory molecules. Among all, Human Neutrophil Peptide-1 (HNP-1), which is produced by neutrophils, is one of the most potent defensins. Our previous promising results in *in vitro* condition showed the effect of recombinant HNP-1on *L. major* promastigotes and amastigotes as anti leishmanial agents.

2 Methods

In this study, we further expand the idea to show the therapeutic effect of HNP-1 in three different models of gene therapy, transgenic *L. tarentolae* and peptide therapy in *L. major* infected BALB/c mice. To reach this point, we confirmed the proper expression of pcDNA-HNP-EGFP construct (for gene therapy) in COS-7 mammalian cells using by fluorescence microscopy and flowcytometry. Furthermore, recombinant *L. tarentolae* expressing HNP-EGFP as secretory form was prepared and integration of the expression cassette in the *ssu* locus was confirmed by diagnostic PCR. In addition, the HNP-EGFP expression was verified using western blot. Synthetic HNP-1 peptide provided commercially and folded by oxido-shuffling reagent, then the antibacterial and anti-parasitic activity of HNP-1examined against *E. coli* and *L. major*, respectively. The efficacy and immune responses as well as histopathological analysis of three different modalities in *L. major* infected BALB/c mice were assessed.

3 Results

Our results showed that immunotherapy with HNP-1 in all three modalities were effective against *L. major* infected BALB/c mice due to lower level of parasite burden, arginase and IL-4 production in compare to control groups. The high amount of IFN-γ and nitric oxide production, which is essential in killing of parasite, is another finding in tested mice. Among the three immunotherapeutic approaches, the gene therapy (pcDNA-HNP) was statistically more effective than other modalities.

4 Conclusions

Considering, there are a few *in vivo* studies about the impact of the AMPs on leishmaniasis, it could open a new window for further evaluation especially its combination with chemotherapy.

C0649 NOVEL NANOPARTICULATE SYSTEMS AS TOOLS TO ENHANCE THE ANTILEISHMANIAL PROPERTIES OF PAROMOMYCIN

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1 Background

Leishmaniasis is a neglected tropical disease with high mortality and morbidity. Chemotherapy continues to be the primary method to fight this pathology, however treatment options are limited. Toxicity associated to most of the therapies, prolonged treatment durations and emergence of resistances encouragethe development of alternative therapeutic strategies. Paromomycin (PRM) was approved for treatment of visceral Leishmaniasis (VL). However, its biodistribution profile is not ideal. Recently, we were able to enhance its therapeutic effect in a VL murine model, after association to liposomes, due to a preferential targeting to liver and spleen [1]. In the present work, its antileishmanial effect was evaluated in a *L. major* cutaneous Leishmaniasis (CL) murine model using appropriate lipid systems for parenteral and topical application. Moreover, aiming at the development of an alternative oral formulation for PRM, a biodegradable protein-based nanocarrier was constructed to be tested in a VL model.

2 Methods

PRM-nanoliposomes for parenteral administration were prepared by the dehydration-rehydration method [1]. For topical application, ethosomes with a mean size below 200 nm were designed [2]. For oral administration, PRM nanoparticles were prepared by a simple coacervation procedure using casein in the presence of lysine as a stabilizing agent [3]. All these nanosystems were physicochemically characterized and tested in CL and VL murine models. Therapeutic effect was evaluated in terms of parasite burden (PB). For CL model, skin lesion size was also assessed.

3 Results

The topical application of PRM-ethosomes was able to contain lesion size. Parenteral administration of PRM-liposomes displayed superior therapeutic effects: lower PB in liver and spleen and higher reduction on lesion size. PRM-casein nanoparticles were gastro-resistant, being only released under simulated intestinal conditions. The biological evaluation in VL murine model is on course.

4 Conclusions

The obtained results demonstrate the potential of novel PRM nanoparticulate systems as valuable strategies for treatment of VL and CL. For each clinical manifestation of Leishmaniasis, the most appropriate nanoformulation should be addressed.

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C0680 COMPARISON OF PROTOCOLS IN METHYLENE BLUE-MEDIATED PHOTODYNAMIC INACTIVATION ON CUTANEOUS LEISHMANIASIS IN A MURINE MODEL USING REAL TIME BIOLUMINESCENCE

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1 Background

Leishmaniasis is a chronic disease developed by parasites of the genus *Leishmania*. Cutaneous leishmaniasis (CL) has several different forms including destructive and ulcerated lesions. The available treatments are limited because of side effects, resistance and toxicity. Photodynamic therapy (PDT) has been explored as an alternative treatment once it is less expensive and no reports about resistance have been described. The aim of this work was to evaluate two protocols of methylene blue (MB)-mediated PDT on CL induced in mice using real time bioluminescence. In addition, we monitored lesion size progression and hyperalgesia.

2 Methods

Promastigotes of *L. (L) amazonensis* transgenic line expressing luciferase were used. Twelve BALB/c mice were infected in the left footpad with 1.10^6 promastigotes. After 4 weeks, mice were randomly assigned to experimental groups (n=4): Control (non-treated), G1 and G2 submitted to one and two PDT sessions, respectively. The second session was performed 24h after the first. PDT was performed using a red LED (λ= 660±22 nm), MB (100μM) and 150J/cm^2 fluence. Disease progression was evaluated once a week by measuring lesion size with a caliper and hyperalgesia with von Frey filaments. Parasite burden was evaluated through luciferase detection by bioimaging, every day, in the first 96h and then for the next 4 weeks. Prior to imaging, mice received 75mg/kg luciferin, intraperitoneally. Results were quantified with proper software, expressing the number of photons/s/cm^2. Statistically significant differences were considered when $p<0.05$.

3 Results

Statistically significant lesion size reduction was observed after 2 weeks post PDT compared to G1 and control groups. In 4 weeks, G2 demonstrated to be clinically more effective and no ulcer signs were detected. Hyperalgesia revealed a positive outcome after 2 weeks, with 50% nociceptive stimulus decrease. In 3 weeks there was a 75% reduction in G2, suggesting that 2 sessions were more efficient. Parasite burden was lower in the first 24h and 72h in both treated groups. Besides, G2 demonstrated statistically significant parasite burden reduction 96h after PDT compared to control.

4 Conclusions

The present study demonstrated that PDT reduced lesion size and hyperalgesia in BALB/c mice, suggesting that therapy probably influenced the inflammatory process. However, further studies are required to deep understanding about PDT mechanisms in cutaneous leishmaniasis treatment.

C0681 ANTILEISHMANIAL ACTIVITY AND IMMUNOMODULATION OF LIPOSOME-ENCAPSULATED FULLEROL IN MURINE MODEL OF VISCERAL LEISHMANIASIS

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1 Background

Visceral leishmaniasis (VL) is a systematic parasitic disease which leads to high rates of morbidity and mortality in humans worldwide. Pentavalent antimonial drugs, such as Glucantime® (Glu), are still the first choice for the treatment of leishmaniasis in several developing countries. However, their use is limited by their serious side effects, for example, pancreatitis, nephrotoxicity, cardiotoxicity and hepatotoxicity. Fullerol (Ful) is a spherical water-soluble carbon nanostructure, with high antioxidant and immunomodulatory effects. In this study, our aim was to evaluate the antileishmanial activity of Ful *in vitro* and *in vivo* models of VL as well as its possible immunomodulatory effect *in vivo*. Ful was evaluated *in vivo* in both free and encapsulated form in liposomes (Ful Lipo).

2 Methods

In the *in vitro* cytotoxicity and antileishmanial assays, peritoneal macrophages of BALB/c mice infected or not with the amastigote form of *Leishmania infantum* were treated with increasing concentrations of Ful (free form) (0.015-0.15 mg/mL). In the *in vivo* tests, BALB/c mice were infected with *L. infantum* and treated intraperitoneally, with either Glu (120mg Sb/kg/day), Ful (0.05mg/kg/4days), Ful Lipo (0.05mg/kg/4days), Ful Lipo (0.2mg/kg/4days), empty liposomes or saline (control group). After treatment, the parasite load was measured by qPCR and the cytokine profile in the liver (IL-4, IL-10, TNF-α and IFN-γ) was evaluated by Elisa assay.

3 Results

Ful exhibited *in vitro* antileishmanial activity with IC_{50} of 0.042 mg/mL. In the *in vivo* assay, there was a significant parasite reduction in both the liver and spleen in the groups treated with Ful Lipo (0.05 and 0.2 mg/kg/4days, $p < 0.001$) and Glu ($p < 0.05$), when compared to the control group. Increasing the dose of Ful Lipo to 0.2 mg/kg/4days resulted in significantly higher parasite suppression when compared Glu, with no parasite detected in the liver. In addition, Ful Lipo at 0.2 mg/kg/4days was found to induce a pro-inflammatory immune response in the liver, with a significant increase of TNF-α and IFN-γ ($p < 0.05$).

4 Conclusions

The treatment with liposome-encapsulated fullerol showed a high efficacy against VL that seems to be related to pro-inflammatory action.

C0691 PHASE II CLINICAL TRIAL WITH INTRALESIONAL INFILTRATION OF MEGLUMINE ANTIMONIATE FOR THE TREATMENT OF CUTANEOUS LEISHAMNIASIS IN THE AMERICAS

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1 Background

Cutaneous leishmaniasis (CL) is a world-wide health problem and to date there is no highly effective and minimally toxic therapy for the disease. In 2010, the World Health Organization Expert Committee on Leishmaniasis recommended the inclusion of local treatments among the acceptable therapeutic alternatives for New World leishmaniasis; however, the meglumine antimoniate (MA) intralesional infiltration is still supported by weak evidence in the Americas. The aim of this study is to provide evidence about efficacy and safety of a standardized MA intralesional infiltration technique for the treatment of localized CL.

2 Methods

This study is a one arm phase II trial conducted at a reference center for CL treatment in Brazil. CL cases with parasitological confirmation presenting less than 4 lesions were eligible to be treated with weekly pentavalent antimony intralesional infiltration by using a standardized and validated technique, up to a maximum of 8 infiltrations. Cure was assessed according to current methodological recommendations for CL trials proposed in 2013.

3 Results

A total of 38 patients (22 men and 16 women) were included and had initial cure assessed until now. The age of patients ranged from 14 to 75 (median, 42.5) years and the median duration of lesions was 3 months. Thirty-four of the 38 patients (90%) had ulcerated lesions. The total number of lesions was 43 and they were distributed in the limbs (80%), head (14%) and trunk (7%). Initial cure, defined as full re-epithelialization and disappearance of any other signs of inflammation, was achieved in 33 of the 38 patients (87%). Overall, patients received 6 ± 2 infiltrations during an average treatment period of 37 ± 14 days. Adverse events where scarce, mainly local and related to the injection site, laboratorial events were negligible, suggesting the possibility of bypassing regular laboratorial monitoring with this approach to CL therapy.

4 Conclusions

Our results confirm that MA intralesional infiltration has an acceptable efficacy for CL treatment. This therapeutic approach represents a simpler monitoring regimen and a safer alternative to the current systemic antimony treatment for CL, widely used in public health in the Americas.

C0716 EFFICACY TRIAL OF TOPICAL NANO-LIPOSOMAL AMPHOTERICIN B FOR THE TREATMENT OF CUTANEOUS LEISHMANIASIS CAUSED BY LEISHMANIA TROPICA

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1 Background

Cutaneous leishmaniasis (CL) is the most common form of leishmaniasis, 90% of CL occurred in 9 countries including Iran. Leishmaniasis still is a major health problem in many endemic foci such as Iran, the disease is mainly seen in 14 of the 22 countries of EMRO region including Iran. CL in Iran is either anthroponotic CL (ACL) caused by *L. tropica* and Zoonotic CL (ZCL) caused by *L. major*, up to 25% of reported CL in Iran is ACL caused by *L. tropica* with increasing incidence rate in most parts of the country. The highest incidence of ACL in Iran is in Mashhad, Khorasan Razavi province and Bam, Kerman province. In 2003, the number of CL patients in Khorasan province was 5,399 and 3,673 was from holly city of Mashhad, in 2009 the reported number of ACL patients in Khorasan Razavi was 5,860. Treatment of ACL is very difficult. The aim of this study was to evaluate the safety and efficacy of topical liposomal Amphotericin B in addition to national standard treatment for the of anthroponotic cutaneous leishmaniasis caused by *Leishmania tropica*

2 Methods

The potential candidate patients were interviewed, physically examined, their lesions were checked by a dermatologist. Skin samples were collected from the patients for direct smear, culture and PCR and those who met inclusion/exclusion criteria were included in the study, all of the patients received the national standard treatment which is using by weekly application of liquid nitrogen and weekly intra lesional injections of meglumine antimonite (Glucantime). In addition, the patients were double blind randomly assigned to receive either topical liposomal form of nano liposomal form of Amphotericin B 0.4% (SinaAmpholeish 0.4%) or placebo twice a day for 28 days

3 Results

A total of 207 CL suspected patients with 451 lesions were screened from the patients referred to the Mashhad leishmaniasis clinic, Khorasan Razavi Province Health Center during the trial, age ranges of the patients were from less than a year to 87 years old. 120 (M=68 (56.7%), 28, F= 52 (43.3%) patients (age 12-60 years) with 226 CL lesions were included. The results showed that the treatment was safe and well tolerated. All the patients healed and there was no relapse

4 Conclusions

No side effect due to Glucantime injections or topical SinaAmpholeish 0.4% or placebo has been seen during the study period. The data have been entered the computer, verified, cleaned and is waiting for the monitor to check and then the codes will be broken and the efficacy will be presented

C0732 EVALUATION OF ANTI LEISHMANIAL EFFECT OF AGROSTEMMA GITHAGO ON LEISHMANIA MAJOR PROMASTIGOTE BY MTT ASSAY

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1 Background

Resistance to the first-line anti leishmanial drugs, pentavalent antimonials, are increasing around the world. Second-line drugs such as Amphotricine B and Miltefosine have also side effects and expensive for patients. According to the anti-cancer and cytotoxic effects of *Agrostemma githago* extract, this study was conducted to evaluate the anti leishmanial effect of *Agrostemma githago* in comparison Glucantime on *Leishmania major* promastigotes by MTT assay.

2 Methods

A number of 2.5×10^6 *Leishmania major* promastigotes in BHI medium in stationary phase were added to each well of the 96 well culture plate. *Agrostemma githago* extract was added to the wells at different concentrations (1, 2, 3, 4, 6, 8, 12, 16 mg/ml). Glucantime was used as a control group. Cells were incubated for 48 hours in 24°C. After centrifuged for 10 minutes in 4°C, supernatant were discarded and 50 µl of MTT were added. After 3 hours in 24°C and centrifuging in 4°C, the supernatant was removed and 100 µl of DMSO was added. After 15 minutes the plate was read by ELISA reader in 570 nm. Furthermore, Trypan blue assaywas performed to evaluate agrostemma extract effect on *Leishmania major* promastigotes.

3 Results

MTT results displayed that increasing concentrations of Agrostemma extract could significantly reduce cell viability of *Leishmania major* promastigots compared to the control group ($p<0.05$). Also, inhibitory effect of Agrostemma extract was dose dependent. IC_{50} of the Agrostemma and Glucantime were calculated 0.365 and 71.01 mg/ml, respectively. Moreover, trypan blue assay showed that increasing concentrations of the extract reduced number of live *Leishmania major* promastigots.

4 Conclusions

Aqueous extract of *Agrostemma githago* has stronger inhibitory effect than Glucantim on *Leishmania major* promastigots. Although more expended studies are needed to evaluate its effect in “in vivo” conditions and on host cells.

C0745 DEVELOPMENT, SYNTHESIS AND EVALUATION OF PHTALAZINES DERIVATES AGAINST LEISHMANIA BRAZILIENSIS. SUPEROXIDE DISMUTASE AS TARGET.

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1 Background

Treatments for Leishmaniasis are based on outdated drugs with high toxicity, ineffectiveness and resistance. Currently, new compounds with antiparasitic activity designed to affect specific enzymatic pathways are investigated. Examples include inhibitors of superoxide dismutase (FeSOD), an enzyme responsible for maintaining the oxidative balance of the parasites against the host immune system

2 Methods

22 phthalazine derivatives were synthesized and evaluated on the viability of *Leishmania braziliensis* promastigotes and on mouse bone marrow-derived macrophages (BMDM) by MTT assay. The two compounds with the best index of selectivity were selected to determine EC50 per dose response curve. EC50s were also determined for intracellular amastigotes, using infections with BMDM. The inhibition of the selected compounds on the enzymatic activity of SOD was evaluated by xanthine oxidase reaction.

3 Results

We demonstrated the effectiveness of 3 phthalazine derivatives on the viability of promastigotes, although only two were innocuous for BMDM cells. The EC50 of derivatives II4I and II8b was determined in 3.4µM and 0.12µM, respectively. These compounds had a selectivity index of 25 and 160. When evaluating the antiparasitic activity on the clinically important stage of the disease, we found that the EC50 for amastigotes/cell of II4l was 0.12µM and EC50 for infected macrophages for this same derivative was 0.54µM. In the same way we observed for the derivative II8b the EC50 for amastigotes/cell was 0.59µM, and EC50 for infected macrophages had a value of 1.42µM. When evaluating the possible interaction of these two derivatives on the enzymatic activity of SOD, was observed an inhibition that could partially explain this toxic effect.

4 Conclusions

Through a rational design and biological screening we found 2 phthalazine nitroheterocycles-functionalized with antiproliferative activity on *Leishmania braziliensis* stages, both with indexes of selectivity greater than 50 on *in vitro* infection models. It was possible to demonstrate their direct inhibition on the enzymatic activity of SOD that could partially explain the antiproliferative effect, even in intracellular amastigote. Due to the promising results obtained in this study, we focused on the design of *in vivo* assays, which allow us to validate these compounds as molecules of interest for the treatment of cutaneous leishmaniasis

C0754 IN VITRO SCREENING OF ANTI-LEISHMANIAL ACTIVITY OF HEDERAGENIN DERIVATIVES

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1 Background

Leishmaniasis is a neglected tropical disease endemic in 88 countries that affect approximately 12 million people. The drugs currently available for *Leishmania* treatment are almost the same as the ones used 50 years ago. These drugs are limited due to their toxicity, the presence of biological resistance in several *Leishmania* species, the length of treatment and their high cost. Thus, it is important to continue the search for a new, cheaper, effective and less toxic treatment and their high cost. In this study, we evaluated the potential of 60 hederagenin derivatives to inhibit the intracellular growth of *Leishmania infantum* amastigotes parasitizing macrophages DH82, *in vitro*.

2 Methods

The ELISA test was employed to measure the abilityinhibitory activity of these compounds at to inhibit amastigote . proliferation. The cytotoxicity of the active compounds were evaluated by metabolic activity, using MTT assay.

3 Results

Hederagenin and eight derivatives: compounds 3, 4, 22, 32, 34, 44, 49 and 52 were able to inhibit the intracellular amastigote proliferation with the respective IC50 values: 61, 9.7, 12, 18, 38, 32 11, 2 and 6 µM. These compounds showed better results than the positive control, salt antimony III (80 µM) and also low toxicity against Macrophage DH82, BGM and HepG2 cells. Moreover, hederagenin derivatives 3, 4 and 49 demonstrated excellent anti-leishmanial activity at micromolar level and high selectivity indexes: 6.4, 6.9 and 22.5, respectively.

4 Conclusions

These results denotes the potential of hederagenin derivatives, in special the 3, 4 and 41 compounds as promising candidates for the treatment of leishmaniasis. More studies are necessary to investigate theefficiency and safety of these compounds *in vivo*.

C0777 EVALUATION OF A POSSIBLE SYNERGISTIC EFFECT OF MEGLUMINE ANTIMONIATE WITH PAROMOMYCIN, MILTEFOSINE OR ALLOPURINOL ON IN VITRO SUSCEPTIBILITY OF LEISHMANIA TROPICA RESISTANT ISOLATE

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1 Background

Pentavalent antimonials are still the first choice treatment for leishmaniasis, but with low efficacy and resistance is emerging. In the present study, the effect of meglumine antimoniate (MA, Glucantime) combined with paromomycin, miltefosine or allopurinol on *in vitro* susceptibility of *Leishmania tropica* resistant isolate was evaluated.

2 Methods

The drugs were obtained from commercial sources and diluents of each drug in medium were prepared on the day of experiment. J774 A.1 murine macrophage cell lines were attached to the cultured on slide and incubated at 37 °C with 5% CO_2 for 24 h. Then the stationary phase promastigotes were added to the cells and after 4 hrs of incubation different concentrations of MA, paromomycin, miltefosine or allopurinol were added and incubated for an additional of 72 h. Then the slides were dried and fixed with methanol, stained by Giemsa and studied under a light microscope. Drug activity was evaluated by assessing the macrophage infection rate and the number of amastigotes per infected macrophage was done by examining 100 macrophages. The experiment was done in triplicates

3 Results

Various concentrations of MA along with paromomycin, miltefosine or allopurinol significantly inhibited ($P<0.01$) the proliferation of *L. tropica* amastigote stage in the macrophage cell line as compared with MA alone or positive control.

4 Conclusions

Combination of Glucantime with paromomycin, miltefosine or allopurinol showed a synergistic effect on the clinical isolate of *L. tropica in vitro*. Use of combination therapy is a new hope and a logical basis for therapy of the patients with cutaneous leishmaniasis. Further investigations are needed to evaluate the therapeutic effects of these drugs on the CL patients.

C0781 ELIGIBILITY CRITERIA AND OUTCOME MEASURES ADOPTED IN CLINICAL TRIALS OF TREATMENTS OF CUTANEOUS LEISHMANIASIS; A SYSTEMATIC LITERATURE REVIEW COVERING THE PERIOD 1991-2015

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1 Background

Deficiencies and inconsistencies in the design of clinical trials for treating cutaneous leishmaniasis (CL) have made it difficult to construct evidence-based treatment guidelines which address the needs of the affected populations. We conducted a systematic review of the eligibility criteria and outcomes adopted in treatment trials of CL conducted during 1991-2015 to complement previous systematic reviews and further inform the design of CL treatment trials.

2 Methods

We conducted a systematic review of the literature in 14 databases using pre-specified key-words; two researchers independently assessed eligibility and methodological quality criteria and extracted the information. We conducted a qualitative synthesis a report absolute and relative frequencies of eligibility and outcome criteria. Comparisons were made for studies conducted before and after the Consolidated Standards of Reporting Trials statement (CONSORT), 2001.

3 Results

Out of 2238 non-duplicate papers identified, we analysed eligibility and outcome criteria used in the 106 clinical trials in CL meeting inclusion criteria published during 1991-2015. They enrolled 11531 participants in 243 treatment groups and were 61% on OWCL and 39% on NWCL. 74% were published after the launch of CONSORT in 2001.

On recruiting subjects, demographic characteristics and parasitological confirmation of CL were taken into account in >69% of the studies (19% with species identification), while <50% specified disease characteristics such as duration of illness, number, size and anatomical location of the lesions. Equal opportunities were offered to both genders in 95% of cases. On assessing treatment outcome, the primary outcome was defined in 90% of studies, which was in 82% based on clinical evaluation (complete re-epithelialization for ulcers); the most common duration for the follow-up time was 6 months. Study reporting generally improved after 2001.

4 Conclusions

This study provides further evidence of the variable quality of treatment trials in CL, explores the granularity of the methods and results sections of papers over a 25-year period, and assesses the adequacy of reporting before and after the publication of the CONSORT statement. Lack of standardisation, compounded with the small number of trials relative to the magnitude of the disease in its multiple forms, and with the range of treatments tested explains why evidence to inform treatment guidelines is generally weak for CL.

C0785 COMPARATIVE STUDY OF SOLID LIPID VERSUS POLYMERIC NANOPARTICLE N-HALOMETHYLATED QUATERNARY AMMONIUM CARRIERS FOR THE TREATMENT OF LEISHMANIASIS.

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1 Background

The formulation of an active pharmaceutical ingredient is often an obstacle in drug development because of the multiple factors to be considered, In recent years, efforts to employ nanoparticle technologies have been growing exponentially for diverse applications in pharmaceutical industry, the development of has been beneficial due to: the possibility of helping to overcome problems associated with poor solubility in water, the protection of therapeutic molecules, modification their blood circulation and tissue distribution, and the ability to focus their action on specific targets. The new treatments to treat leishmaniasis are aimed at obtaining nanomedicines. This work compared therapeutic effectiveness between two nanoformaltions as N halymetil carriers for the treatment of leishmaniasis

2 Methods

Polymeric nanoparticles were prepared by nanoprecipitación while solid lipid nanoparticles (SLN) were by emulsion–solvent. Selection criterias for each formulation were particle size, Z-potential and encapsulation efficacy. The chosen formulations were characterized physicochemical, stability, differential cytotoxicity and *in vitro* antileishmanial activity.

3 Results

For polymeric nanoparticles C6 presented interaction with different types of Resormer® polymers, only 7525 presentation was stable; additionally, the presence of the SPAN 60 surfactant generated particles larger than 1000 nm, Tocopherol greatly improved the drug encapsulation efficiency as well as the particles size and appearance. SLN particle produced by the three lipids were very similar in their characteristics: size within the nano-range scale with a narrow polydispersity index, and a high encapsulation efficiency of bioactive compound (>60%). All nanoformulations revealed reduced cytotoxicity activity when compared with free-C6 in different cells lines, as well as they demonstrated potent activity against Leishmania *panamensis in vitro* test.

4 Conclusions

Both c6 nanoformulations are potential candidates as therapeutic agents against cutaneous leishmaniasis.

C0786 IMMUNO-CHEMOTHERAPY AGAINST EXPERIMENTAL VISCERAL LEISHMANIASIS: PARTNERSHIP OF DIHYDROEPIANDROSTERONE AND LOW DOSE MILTEFOSINE

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1 Background

Visceral leishmaniasis (VL) is the only tropical disease, which is being treated by non-leishmanial drugs. Chemotherapy remains the only tool against VL in the absence of effective vaccines and vector control programs. However, none of the drugs are ideal for treatment due to high toxicity, resistance issues, prohibitive prices, treatment duration or parenteral mode of administration. Therefore, use of immunomodulatory agents in combination with standard drugs may augment their effectiveness, especially in immunocompromized patients and reduce the adverse effects associated with single-drug therapy.

2 Methods

We investigated the detailed therapeutic and immunological parameters involved during combination therapy with an immunomodulator, dihydroepiandrosterone (DHEA) (1 mg/kg × 5 days, subcutaneous route) and low dose miltefosine (2.5 mg/kg × 5 days, oral route) against *Leishmania donovani*/ Balb/c mouse model of VL. The selected dose of miltefosine for combination therapy was 8-times lower than the curative dose (20 mg/kg) of miltefosine. The liver and splenic parasite burden was evaluated by Giemsa staining. Th1/Th2 cytokines, nitric oxide level and cell proliferation were determined by ELISA, Griess reaction and XTT assay, respectively on day 7 post-treatment (i.e. day 28 post-infection).

3 Results

Our results demonstrated that animals treated with 1 mg/kg dose of DHEA showed 52.8 and 54.6% inhibitions in hepatic and splenic parasite load, respectively and when co-administrated with lower dose of miltefosine, superior level of protection (~100% inhibition in parasitic burden) was observed. The curative effect of adjunct therapy was associated with significant ($p<0.001$) enhancement in the secretion of Th1 cytokines, IFN-γ and IL-12 along with extreme ($p<0.001$) down-regulation of Th2 cytokine, IL-10, which further elevated the secretion of nitric oxide in splenocytes isolated from treated animals when compared with untreated group. The significant T cell proliferative response were also observed in lymph nodes and splenocytes of mice treated with DHEA plus low dose miltefosine and this was supported by the high levels of IFN-γ and IL-12 during combination therapy.

4 Conclusions

Our results suggested that DHEA plays crucial role in the immune activation in immunocompromized host and when given in combination with low dose of miltefosine, generates strong Th1-biased immune response thereby minimizing both dose and duration of miltefosine.

C0787 COMPARISON OF GROWTH OF LEISHMANIA TROPICA IN THREE TYPES OF MURINE MACROPHAGE

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1 Background

Leishmaniasis is a major health problem in some endemic foci in different countries including Iran *Leishmania tropica* is the causative agent of anthroponotic cutaneous leishmaniasis (ACL). Although control of ACL depends on treatment of the patients but available drugs are not fully effective. Studies on various aspects of L. tropica are solely done on cell culture and but . and studies on so far no animal model is developed for, so ACL is still a major the world's fifth-valence antimony compounds. In this study, peritoneal macrophages ,femour macrophages and J774 A.1 macrophages mouse cell line shows the influence of Glucantime on it infected with new standard strain IR/09/khamesipour-Mashad *Leishmania tropica* be examined and effectof drug on them.

2 Methods

J774 A.1 macrophages mouse cell line, femurs and peritoneum macrophages were attached to the slide and incubated at 37 0C,with 5% CO_2 for 24 hr. *L. tropica* promastigotes in stationary phase were added to the macrophages and incubated for another 24 hrs , then different concentrations of Glucantime (MA) were added and incubated for additional 72 hours. The slides were stained by Giemsa. The slides were checked under light microscope. The number of infected macrophages and the number of amastigotes per 100 macrophages were recorded. To ensure the results of the experiments were repeated three times.

3 Results

The result shows the concentration of 50µ/ml MA withJ774 A.1 macrophages mouse cell line, femurs and peritoneum (P?0.005) significantly inheibited on invitro growth of Leishmania tropica amastigote stage

4 Conclusions

Various concentrations of MA plus either J774 A.1 macrophages mouse cell line, femurs and peritoneum macrophages significantly inhibited the growth of*L.tropica* in same concentrations. In J774 A.1 macrophages mouse cell linethe number of infected cells and amastigotes were less thanfemurs and peritoneum macrophages but the best concentration was same for everyone.

C0788 ANTI-LEISHMANIAL EVALUATION OF TETRAHYDROQUINOLINE DERIVATES: VOLUME REGULATION AND STEROL BIOSYNTHETIC PATHWAY OF LEISHMANIA BRAZILIENSIS.

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1 Background

Leishmaniasis is considered by the WHO as one of the six parasites disease with the highest rates of morbidity and mortality worldwide The first line drugs used (Glucantime® and Pentostan®) generate serious side effects, as well as resistance phenomena. In this study a serie of tetrahydroquinolines was synthesized de novo via straightforward synthesis based on the $BF_3{\cdot}OEt_2$-catalyzed imino Diels-Alder reaction.

2 Methods

Ten tetrahydroquinolines were evaluated on *Leishmania braziliensis* promastigotes viability, and cytotoxicity toward mouse bone marrow-derived macrophages (BMDM) using MTT assay. The effect of the selected compound on intracellular amastigotes, was evaluated by infection protocol using BMDM macrophages. To study the action mechanism, we evaluated the sterol biosynthetic pathway by GC/MS and cell volume recovery (RVD) by light scattering

3 Results

All compounds evaluated shown an appreciable biological activity on parasite viability and low BMDM cytotoxicity. The most prominent compound (JS) show an EC50: 6µM, without affect BMDM cells (selectivity index: 50). On intracellular amastigotes we found a decrease in infected macrophages percent, with an EC50: 0.8 µM (selectivity index: 370). The sterol biosynthetic pathway has been extensively validated as an important chemotherapeutic target and we were able to show an accumulation of squalene (81.28%), concordant with a significant depletion of remainder sterols, and an accumulation of exogenous cholesterol. The compound JS (6µM) affected significantly the RVD of *L. braziliensis* under hypo-osmotic stress compared with untreated controls.

4 Conclusions

Selective effectiveness of quinoline structures has been demonstrated on several occasions, including previous work in our laboratory. In this study we demonstrate the effect of JS on *L. braziliensis* promastigotes and intracellular amastigotes, without affecting the viability of its host cell. We also corroborate an alteration in sterol biosynthesic pathway, associated with quinoline structure, shown by an accumulation of squalene. This membrane alteration could be involved in the significantly effect on RVD shown under hypo-osmotic stress. Studies on *in vivo* models will be carried to validate this compound as a promise molecule in the treatment of cutaneous leishmaniasis

C0794 PHYSICOCHEMICAL CHARACTERIZATION AND DEVELOPMENT OF A TOPICAL FORMULATION OF TS-45, A NEW MOLECULES WITH ACTIVITY IN CUTANEOUS LEISHMANIASIS.

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1 Background

The development of new treatments for leishmaniasis focuses on the identification of chemical entities that are effective, less toxic and easier to administer. Thus, topical formulations for cutaneous leishmaniasis represents an interesting alternative for reducing toxicity associated with leishmanicidal treatments with systemic administration. This work aimed to development and characterization of a new molecule called TS-45 and investigate its physicochemical properties and potential for topical delivery, and validation of a quantification method.

2 Methods

The characterization of the physicochemical properties of the TS-45 active ingredient was through experimental tests and the characteristics relevant to the development of a topical formulation. Tests included infrared spectroscopy, solid state tests such as thermal analysis (DSC, TGA), powder X-ray diffraction (XRDD), particle size, crystal habit, birefringence, density. Stability tests were also established on di-methyl sulfoxide, hygroscopicity, Log P and Log D, aqueous solubility and on acetonitrile, Isopropyl Alcohol, Methanol); (Oleic acid, cetiol V, isopropyl myristate and mineral oil). Topical formulations were developed and characterized in order to improve the dermal permeability of the active substance and were evaluated in vitro in Franz and Enhancer cells.

3 Results

Solid-state tests showed that the TS-45 compound exhibited characteristic of crystalline solids. The compound has low affinity and solubility in water and in polar solvents and little hygroscopicity. In the *in vitro* tests, the formulations showed increase in dermal permeability with respect to the active alone.

4 Conclusions

In this research, for the promising molecule against cutaneous leishmaniasis, the TS-45 compound, some of its intrinsic physicochemical properties were determined and the evaluation of the formulations promises to have good permeability for topical administration

C0802 COMBINATORY EFFECT OF MEGLUMINE ANTIMONIATE PLUS PAROMOMYCIN, ALLOPURINOL OR MILTEFOSINE ON IN VITRO GROWTH OF AMASTIGOTE STAGE OF LEISHMANIA TROPICA

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1 Background

The first choice treatment for leishmaniasis is still meglumine antimoniate derivatives but the efficacy of the drugs particularly against *L. tropica* is not acceptable and resistance is emerging. In the current study the effect of meglumine antimoniate (Glucantime, MA) combined with either paromomycin, allopurinol, or miltefosine was evaluated on *L. tropica* amastigote growth in *in vitro* model.

2 Methods

J774 A.1 macrophage mouse cell line was attached to the slide and incubated at 37 ^{0}C,with 5% CO_2 for 24 h. *L. tropica* promastigotes were added to the macrophages and incubated for another 4 hr and then different concentrations of Glucantime and paromomycin, allopurinol or miltefosine were added and incubated for additional 72 h. The slides were then dried, fixed, stained by Giemsa and checked for presence of amastigotes under a light microscope. The number of infected macrophages and the number of amastigotes per 100 macrophages were recorded. The study was performed in triplicate.

3 Results

The results showed that in comparison with Glucantime alone, various concentrations of MA combined with different drugs significantly inhibited *in vitro* growth of *L. tropica* amastigotes (MA plus paromomycin; $p \leq 0.009$), MA plus miltefosine;$p \leq 0.008$ and MA plus allopurinol; $p \leq 0.010$).

4 Conclusions

Various concentrations of MA plus either paromomycin, allopurinol or miltefosine significantly inhibited the growth of *L.tropica* compared with MA alone. Further studies are required to evaluate the combinatory effect of these drugs in *in vivo* conditions.

C0803 POLYMERIC NANOPARTICLES FOR THE ADMINISTRATION OF ANTILEISHMANIAL MOLECULES

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1 Background

Scientist have explored various new therapeutic methods to combat the problems associated with the current antileishmanial drugs. Decreasing the pharmacological compound sizes to nanoscale can change significantly their physical properties, bringing benefits in the bioavailability and absorption of the molecules. Therefore, nanomedicine has emerged as a feasible and effective solution to the problems evidenced by the high percent of drugs with low water solubility or relevant toxic effects. This work determined the therapeutic effectiveness of polymeric nanoparticles developed by nanoprecipitation method in three compounds with antileismanial activity demonstrated previously

2 Methods

Compounds selected for polymeric nanoparticles development trough nanoprecipitation method were alendronate (commercialy available) and E and TC2, two new discovered molecules. For the three compounds was determined their solubility in water and other solvents; in addition, the quantification method was developed according to the physicochemical characteristics of each compound. Excipients were selected through the observation of the characteristics of nanoparticle in Scanning Electron Microscopic and Zetasizer Nano S. Formulations were also characterized according to their pH's stability and encapsulation efficiency. In last, antileishmanial activity of nanoformulations was tested *in vitro*

3 Results

E and TC2 showed low solubility in water and polar solvents while alendronate was highly soluble in water. E and TC2 were quantified using UV-visible method while alendronate was quantified by titrimetric method. Tocopherol greatly improved the drug encapsulation efficiency as well as the particles size and appearance, while Span 60 ® produced an uneven appearance in the samples. The nanoparticles were stable to storage condition and pH variations. No one compound was cytotoxic in the U-937 cells; the effective concentration showed by the encapsulated compounds was higher than that showed by free compounds.

4 Conclusions

Nanoprecipitation is a useful method for the efficient selection of excipients to be used in a formulation. This method produces polymeric nanoparticles which may constitute a promising alternative for the administration of different compounds with leishmanicidal activity

C0809 PHYSICO-CHEMICAL, TOXICOLOGICAL AND BIOPHARMACEUTICAL CHARACTERIZATION OF N-HALOMETHYLATED A QUATERNARY AMMONIUM FOR THE TREATMENT OF LEISHMANIASIS.

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1 Background

Physico-chemical, biopharmaceutical and toxicological characterization of an active pharmaceutical ingredient are decisive steps in the development of a drug. Previous studies have identified a halomethylated quaternary ammonium salt as promissory compound with leishmanicidal activity, its antiprotozoal activity is related to structural similarity with the choline, capable of interfering with the synthesis of phosphatidylcholine. The present work is aimed at the physicochemical, toxicological and biopharmaceutical characterization of a halomethylated quaternary ammonium salt was called C6.

2 Methods

The first step was the physicochemical characterization of compound C6. Bioinformatics tools were used to characterize properties related to possible oral administration. Other properties relevant were characterized experimentally. The acute and subacute toxicity, teratogenic, the pharmacokinetic and intestinal absorption profile, were developed according to international guidelines.

3 Results

C6 fulfilled the Lipinski rules, a high correlation was demonstrated between the most stable *in silico* three-dimensional conformation, and experimental crystalline structure available. C6 showed moderate solubility in water and polar solvents, additionally pH did not affect the solubility, a possible polymorphism was identified. At toxicological level, some gastrointestinal alterations of slight degree while that effect teratogenic was absent. C6 demonstrated intestinal absorption and pharmacokinetic profile was monocompartimental after oral administration.

4 Conclusions

The preclinical profile of compound C6 shows promising results to be a compound that can be administered orally.

C0828 ACTIVITY OF 2-HYDROXY-3-PHENYL-1,4-NAPHTHOQUINONE AGAINST LEISHMANIA (VIANNIA) BRAZILIENSIS IN VITRO AND IN VIVO

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1 Background

Leishmaniasis are neglected diseases with different clinical outcomes and are caused by protozoa of the genus *Leishmania*. In Brazil, the main agent of cutaneous and mucosal leishmaniasis is *Leishmania (Viannia) braziliensis*. The current therapeutic arsenals available for these diseases are toxic, expensive and parasite resistance is a growing problem. Given this scenario it is necessary to expand the alternatives for treatment of leishmaniasis. Naphthoquinones are bioactive molecules with antitumor and antiprotozoal activities. The aim of this study was to evaluate the effect of 2-hydroxy-3-phenyl-1,4-naphthoquinone on *L. (V.) braziliensis in vitro* and in experimental models of infection.

2 Methods

Promatigotes were incubated with 2-hydroxy-3-phenyl-1,4-naphthoquinone (25-800µM) for 96h/28ºC and the number of viable parasites were counted daily under a microscope. Human monocytes (THP-1 cell line) were treated with naphthoquine (50-1600µM) overnight and cell viability was measured by MTT assay. Peritoneal macrophages from BALB/c mice were infected with promastigotes (5:1) and treated with naphthoquinone for 48h. The number of infected cells and amastigotes were counted under a microscope. BALB/c mice were infected on the footpad and treated for two weeks (3 times/week) by intralesional route after one week of infection. Hamster golden were infected on the footpad and treated for 5 weeks (3 times/week) by intralesional route after 9 weeks of infection. The lesion size was measured weekly and parasite load estimated by limited diluition.

3 Results

This naphthoquinone showed activity *in vitro* on promastigotes (IC50 = 101 ± 34µM) and intracellular amastigotes (IC50 = 193 ± 19µM) with low toxicity for human monocytes (CC50 = 767 ± 76µM). The treatment of infected macrophages with the naphthoquinone did not induce the nitric oxide production, but increased the production of cytokines (IL-6, TNF, MCP-1 and IL-10). *In vivo* 2-hydroxy-3-phenyl-1,4-naphthoquinone was capable of controlling the lesion size and significantly decrease the parasite load on BALB/c mice. On infected and treated golden hamsters the reduction of the lesion was small, but the parasite load in the infected footpad and draining lymph node was significantly reduced.

4 Conclusions

These data show that 2-hydroxy-3-phenyl-1,4-naphthoquinone has activity against *L. (V.) braziliensis in vitro* and *in vivo*, therefore it is a promising molecule to continued preclinical studies.

C0832 ANTILEISHMANIAL AND TOXICITY ACTIVITY OF TOPICAL FORMULATION OF MILTEFOSINE IN EXPERIMENTAL MODELS

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1 Background

Leishmaniasis is a neglected disease affecting mainly people living in poverty. In Colombia about 11 million people are at risk of acquiring the disease. Current treatments are not ideal given their variable effectiveness, toxicity, cost and adverse reactions. Topical treatments applied directly on lesions could be an excellent option in some cases of cutaneous leishmaniasis (CL). The objective of this work was to evaluate a topical formulation containing miltefosine (MIL) in BALB/c mice

2 Methods

A semi-solid formulation was prepared with (f-MIL) and without (no-f-MIL) miltefosine and *in vitro* and *in vivo* antileishmanial activity were determined. Mice infected with *L. (Viannia) braziliensis* and *L. (V.) panamensis* were treated topically on the lesions for 20 days with f-MIL and no-f-MIL. Measures of lesions (nodules and ulcers) and histopathological analysis were performed. Dermal irritation, physical alterations and biochemical profile were evaluated. The contact hypersensitivity was determined by pre-treatment/challenger with f-MIL and no-f-MIL. F-MIL-treatment induced in *vivo* genotoxicity was determined by comet and micronucleus (MN) assay. Cyclophosphamide and ethylmetanesulfonate were used as control.

3 Results

A stable physicochemical f-MIL was obtained, it was active against *Leishmania* intracellular amastigotes (IC_{50}: 2.13±0.3 mg/mL) and promastigotes (IC_{50}: 2.00±1.1 mg/mL). Toxicity in THP-1 cells was of CC_{50} 9.89 ±1.5. F-MIL applied topically *in vivo* healing *L. (V.) braziliensis* and *L. (V.) panamensis* infected mice. No-f-MIL treatment was ineffective (lesion area from 25 to 51 mm^2). In the histopathological analysis no leukocyte infiltrate or intracellular parasites were present. F-MIL did not induce signs of irritation. No changes in weight, food intake, serum levels of urea and transaminases were observed. Compared with the positive control (1-fluoro-2,4-dinitrobenzene), neither increase in ears thickness (0.15 versus 1.0 mm) nor histological inflammatory changes were induced in f-MIL-sensitized animals after f-MIL-challenger. DNA damage was observed after treatment with serial doses of f-MIL. The% of DNA damage was 22.0-28.8% (Control: 74.38%) and for MN was 0.5-3.2 (Control: 175-200 MN) in polychromatic erythrocytes.

4 Conclusions

The topical formulation containing miltefosine was active *in vivo* and *in vitro*. We conclude that formulation containing miltefosine is safe and an appropriate candidate for topical treatment of CL.

C0842 CALOPHYLLUM BRASILIENSE TREATMENT REDUCES THE PARASITE LOAD IN LYMPHY NODE AND SPLEEN IN INFECCTION BY LEISHMANIA (LEISHMANIA) AMAZONENSIS IN VIVO

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1 Background

The research for new natural compounds with potential against *Leishmania* has increased. *Calophyllum brasiliense* has been used to treat several diseases and studies showed its antileishmanial property. The aim of this study was to evaluate the leishmanicidal activity of *C. brasiliense* in BALB-c mice infected with *Leishmania (Leishmania) amazonensis*.

2 Methods

The Ethics Committee on animal use of the Universidade Estadual de Maringá (process number 3085250715) approved this project. BALB/c mice were infected in right footpad with 1 x 10^6 *L.L.a*/mL. The treatment started 21 days post-infection and continued for six weeks. Animals were divided into five groups: topical treatment with 1% of *C. brasiliensis* dichloro extract incorporated into a hydrogel constituted by 20% of F-120 and 0,20% of carbopol 934P (daily); intraperitoneal treatment with the same formulation of the topic one (daily); amphotericin b treatment (5 mg/kg/day for 10 consecutive days and 15 alternate days); infected and non-treated (positive control) and non-infected. Footpad edema was weekly monitored using plethysmometer and pachymeter. Lymph node and spleen were removed, weighed and diluted in serial dilution. After 21 days of incubation at 25ºC, samples were analysed for the presence of promastigotes. The title was the last dilution with at least one parasite. Number of parasites/g in tissue was calculated and results were compared between treated and non-treated animals.

3 Results

There were no reduction in treated footpad edemas in comparison to the positive control group. Interestedly, the topic and intraperitoneal treatment with *C. brasiliensis* decreased the parasite load in lymph node by 73% and 87%, respectively. These also happened in the parasite load of spleen, being 94% in topic treatmentand 78% in intraperitoneal treatment. Both treatment with *C. braziliensis* demonstrated to be more efficient than amphotericin b treatment for decreasing parasite load in spleen (57%). In lymph node, the reduction of parasite load was higher in intraperitoneal treatment with *C. brasiliensis*than in amphotericin b group (80%).

4 Conclusions

Although the topic and intraperitoneal treatment with *C. brasiliensis* did not improve footpad edema, they reduced the parasite load in spleen and lymph node. This supports further investigation of *C. brasiliensis* as antileishmanial drug because of its systemic effect.

C0873 ALTERNATIVE MEDICINE AS A NEW TREATMENT OPTION AGAINST VISCERAL LEISHMANIASIS.

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1 Background

Visceral leishmaniasis is a neglected but typically fatal vector-borne protozoan disease. Till now the disease lacks a completely effective and non-toxic treatment. This led us to investigate, an area of alternative medicine i.e. homeopathy which uses ultra low doses of highly diluted substances originated from plants, animals or minerals.

2 Methods

In the present study, *in vitro* antileishmanial activity of Iodium, and Arsenicum album, with Φ (mother tincture), 6C and 30C potency was checked by exposing the parasite to different dilutions of drugs and percentage growth inhibition was determined by using trypan blue dye exclusion test. Further, *in vivo* studies of these drugs with 30C and 200C potency was carried out in inbred BALB/c mice. Animals were infected intravenously with 10^7 promastigotes of *L. donovani* and both preventive and therapeutic studies were performed.

3 Results

Our findings demonstrated that the homeopathic drugs were more effective against promastigotes of *L. dononani* as compared to the standard drug sodium stibogluconate (SSG). Maximum growth inhibition was observed at highest dilution i.e. 1:64 of mother tincture of Iodium (55.93 ± 1.34%) against promastigotes of *L. donovani* respectively after 72 hours of incubation. In the *in vivo* studies, the parasite load decreased significantly in both the preventive and therapeutic groups of both the drugs. However, Iodium was found to have better leishmanicidal activity than the Arsenicum as demonstrated by lesser hepatic parasite load and higher delayed type hypersensitivity responses. Of the two potencies tested, 30C was found better (82.48% reduction in LDU) but showed mild hepatotoxicity in terms of increased levels of SGOT and SGPT levels. The results were further supported by the histopathological studies. The concentration of Urea and Creatinine were found to be in normal range in all the treated animals.

4 Conclusions

Thus, the current study proved the antileishmanial potential of the two homeopathic drugs. However, further studies are required in higher animal models before coming to a conclusion.

C0876 MICELLAR SYSTEMS OF P-123 AND F-127 FOR ANTILEISHMANIAL DRUG- LOADED NANOCARRIERS

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1 Background

Nowadays, leishmaniasis is classified as one of the most neglected diseases. The available treatments with meglumine antimoniate (Glucantime) and amphotericin B in Brazil have side effects and this in association with their administration complexity led to a large number of therapeutic withdrawals. The research for new drugs is extremely important and the field of selective delivery system as micelles is fundamental to improve leishmaniasis treatment, being P-123 and F-127 the main ones in drug delivery. Previous studies showed P-123 and F-127 are no significantly cytotoxic to red cells and *Leishmania (Leishmania) amazonensis.* F-127 has low cytotoxicity to macrophages, but it was not observed for P-123. Although, this cytotoxicity results to macrophages seem to be a risk, those can be used in topical formulations, lysing infected macrophages. The aim of this study was to investigate the internalization of P-123 and F-127 into LLa and macrophages, as well as their stability in RPMI 1640 media, validating the use of those micelles as antileishmanialdrug carriers.

2 Methods

The Ethics Committee on animal use of the Universidade Estadual de Maringá (process number 9199240715) approved this project. Peritoneal cells of BALB-c mice and *L.L.a* promastigotes were exposed to rhodamine B-labeled P-123 and F-127 at the concentrations of 40, 20, 10, 4 mg/mL and 20, 10, 5, 2 e 1 mg/mL, respectively. After 30 minutes, they were observed in fluorescence microscopy. P-123 and F-127 at the concentrations of 80, 40, 20, 10 and 5 mg/mL were diluted in RPMI 1640 media; 30 minutes later micellar diameters were analysed by dynamic light scattering and repeated in 24 hours, 48 hours and one week.

3 Results

In the internalization assay, macrophages and LLa intensely presented red fluorescence, indicating cellular penetration because of specific biological membrane interactions. In the stability assay was observed that carrier systems were stable in high concentrations and in low concentrations markable alterations happened.

4 Conclusions

P-123 and F-127 have potential to be antileishmanial drug- loaded nanocarriers.

C0888 CYTOTOXIC AND MORPHOLOGIC EVALUATION OF METHANOLIC EXTRACTS OF CARICA PAPAYA SEEDS ON LEISHMANIA MEXICANA PROMASTIGOTES.

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1 Background

Drugs currently used for leishmaniasis treatment are highly toxic, little tolerated by patients and having limited effectiveness. The development of new drugs is an urgent task. *Carica papaya L.* is used as a traditional herbal medicine for leishmaniasis in regions where the disease is endemic. However, the compounds responsible for the curative activity have not been reported.
With the purpose of identifying metabolites with leishmanicidal activity, the effect of methanolic extracts of *C. papaya L.* seeds and their fractions were evaluated on promastigote cultures of *Leishmania mexicana* (BEL 21).

2 Methods

Seeds were washed with distilled water, desiccated and pulverized. The powder was extracted with methanol for 48 h, filtered and roto-evaporated at 50ºC. The sediment was resuspended in DMSO:H_2O (1:1) and stored at -10ºC for cytotoxic assays or dissolved in isopropanol and adsorbed to silica gel for chromatography. This was performed by sequential elution with isopropanol, ethanol, methanol and methanol:H_2O (8:2). Fractions were evaporated at 35ºC and the sediment resuspended in DMSO:H_2O (1:1). Promastigotes were incubated at 25ºC for 48h, in LIT medium containing penicillin (100 U/ml), streptomycin (100 µg/ml), fetal bovine serum (FBS, 5%) and different concentrations of crude extract or fractions. In order to evaluate parasite survival the inhibitory effect of extracts was estimated by the MTT method and by cell counting in a hemocytometer. For selectivity assays, macrophages (J774) were incubated in RPMI medium with antibiotic plus 10% FBS and different extract concentrations for 48 h at 35ºC, in 5% CO_2 and 90% humidified-air atmosphere. Cell morphology changes were observed by phase contrast microscopy.

3 Results

The findings showed that promastigotes are susceptible to methanolic extracts, showing an IC_{50} of 0.2±0.05 mg/ml. These extracts induced cell granulation, rounding of parasites, bizarre flagellar movement and flagellum loss. Extracts up to 2 mg/ml showed no toxic effects on macrophages. A selectivity index of 37 was found. Fractions were less inhibitory than crude extracts, suggesting that components or metabolites present in the extract can be acting additive- or synergistically.

4 Conclusions

The results reveal that methanolic extracts of *C. papaya* seeds have selective effect on *Leishmania* promastigotes survival and induce marked morphological changes.
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C0905 LEISHMANICIDAL ACTIVITY OF ORGANIC EXTRACTS OBTAINED FROM CLEOME SERRATA, CNIDOSCOLUS TEHUACANENSIS, CNIDOSCOLUS CHAYAMANSA, ROMERO OFFICINALIS AND MOUSSONIA DEPPEANA

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1 Background

Leishmaniasis is endemic in 98 countries and is classified as an emergent/re-emergent disease. It is caused by about 20 species and it has several manifestations as cutaneous, mucocutaneous and visceral. It is estimated that there are 20 million infected people, 350 million are at risk, and 60,000 people die by year. In Mexico, has been reported in 22 states and is known as "gum collector's ulcer". In Campeche Mexico, over a two-year period, 76% of people with skin lesions were diagnosed with cutaneous leishmaniasis, caused by *L. mexicana*. Recently, cases of co-infection of leishmaniasis/HIV/AIDS have been reported. Antimoniun pentavalent drugs such as sodium stibogluconate and meglumine antimoniate are the first line drugs used for leishmaniasis treatment; pentamidine isothionate, amphotericin B, miltefosine and paramomicin sulphate are alternatively used. Leishmaniasis treatment has several problems such as parasite drug-resistance and the secondary effects.

2 Methods

In this study, we evaluated the leishmanicidal activity of organic extracts obtained from medicinal plants, *Cleome serrata, Cnidoscolus tehuacanensis, Cnidoscolus chayamansa, Romero officinalis and Moussonia deppeana.* The organic extracts were prepared maceration process at room temperature from aerial part and were tested against *L. mexicana.* The activity of organic extracts against promastigotes and amastigotes of *L. mexicana* was carried out using the resazurin method. The cytotoxicity of organic extract was tested using the macrophage cell line J774.2

3 Results

The organic extract from *C. serrata* and *R. officinalis* were the most active against *L. mexicana* promastigotes (IC_{50} = 4.61 and 6.93 µg/mL). On the other hand, *C. tehuacanensis* and *C. serrata* organic extractswere the most active against *L. mexicana* amastigotes with IC_{50} values of 8.73 and 13.02 µg/mL. Extracts from *C. serrata, C. tehuacanensis, C. chayamansa* (CC_{50}= 129.04, 125.01, 121.39µg/mL)were less cytotoxic than that from *M. deppeana* and *R. officinalis (*98.08 and 47.35 µg/mL). Interestingly, *C. serrata* and *C. tehuacanenis* organic extracts showed the highest Selectivity index (CC_{50}/IC_{50}) against *L. mexicana* promastigote and amastigote, respectively.

4 Conclusions

C. tehuacanensis and *C. serrata* extracts are the most active leishmanicidals and are candidates to continue investigating as a source of active compounds against *Leishmania*.

C0910 PHYTOTHERAPY OF LEISHMANIA DONOVANI INFECTED MICE BY BAUHINIA VARIEGATA: AN INNOVATIVE APPROACH IN TREATMENT OF LEISHMANIASIS

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1 Background

The therapy of visceral leishmaniasis (VL) is limited by resistance, toxicity, decreased bioavailability of the existing drugs, non-availability of vaccines and down regulation of cell-mediated immunity. Thus, we envisaged combating the problem with plant-derived antileishmanials that could concomitantly mitigate the immune suppression of the infected host. In this direction, we investigated the antileishmanial efficacy of *Bauhinia variegata* in murine model of VL. This plant has been widely used in traditional medicine and is considered to have medicinal properties to treat various diseases such as cancer, hepatitis and HIV.

2 Methods

Inbred BALB/c mice were infected intravenously with $1x10^7$ promastigotes *of Leishmania donovani* and kept for 30 days. These animals were then administered with 500 mg/kg body wt. of hydroethanol extract of *B. variegata* (HEEBV) orally for 14 days and checked for parasite clearance, delayed type hypersensitivity (DTH) response, histopathological analysis, biochemical and cytokine assay. Sodium stibogluconate (SSG) and Amphotericin B were used as reference drugs. In order to identify the antileishmanial compounds present in the HEEBV, LC-MS analysis was carried out.

3 Results

LC-MS analysis of HEEBV revealed the presence of some components with known antileishmanial activity along with other compounds. Out of these lupeol was checked for its efficacy against this parasite. The animals treated with HEEBV and lupeol revealed a significant reduction in parasite load with heightened DTH response. Moreover, the liver and kidney function tests were found to be in the normal range. Histopathological studies of liver, kidney and spleen revealed damage in the tissues when SSG and Amphotericin B was given but no damage was observed in HEEBV and lupeol treated mice. Animals treated with HEEBV and lupeol showed enhancement in Th1 cytokines (IFNγ, IL12) with fall in disease-associated Th2 (IL10, IL4) cytokines.

4 Conclusions

Thus, from the present study we accentuate that *B. variegata* and lupeol could be employed as effective therapeutics agents against *L. donovani*. Further studies are required in higher animal models to prove its leishmanicidal efficacy.

C0919 PHOSPHORYLATION OF H2A AS DOUBLE-STRAND DNA DAMAGE BIOMARKER IN LEISHMANIA INFANTUM TREATED WITH TOPOISOMERASE POISONS

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1 Background

Topoisomerases are pointed as targets for design and synthesis of new antileishmanial drugs. TopIB poisons stabilize the TopIB-DNA bond preventing the ligation step, yielding single strand breaks (SSBs) that may evolve to double-strand breaks (DSBs) when the replication fork collides with them, triggering apoptotic processes. Eukaryotic DNA is packaged into nucleosomes, arranged by H2A, H2B, H3 and H4 histones. In Leishmania the induction of DSBs undergoes histone H2A phosphorylation on a threonine residue placed within 1 Mb flanking nucleosome. This process is mediated by members of phosphoinositide 3-kinases (PI3-K) family, like ATM-, ATR-, and DNA-dependent protein kinases, which presence has been pointed in trypanosomatids. The current manuscript describes for the first time how TopIB camptothecins (CPT) and indenoisoquinolines are responsible for DNA damage and time-dependent phosphorylation of Leishmania H2A.

2 Methods

DNA cleavage induced by CPTs and indenoisoquinolines was assessed by agarose gel electrophoresis in the presence of etidium bromide. H2A phosphorylation was evaluated both by confocal microscopy and flow cytometry. In addition, cell extracts from promastigotes treated with the compounds were resolved in SDS-PAGE gels. Phosphorylated H2A histone was identified by immunoblotting using a specific antibody. DSBs was measured using SDS/K precipitation assay of previously treated promastigotes.

3 Results

Fluorescence microscopy was used to visualize the consequences of exposure to CPT analogues on leishmanial DNA. Anti-γH2A fluorescence microscopy revealed signals in approximately 10% of unperturbed wild-type cells and substantially increased proportion of cells with these signals following exposure to DNA damaging agents (Fig. 3A). Ten-micromolar final concentration of CPTs and 1 mM indenoisoquinolines were incubated during 30 to 120 min, stepwise 30 min with Leishmania promastigotes. H2A phosphorylation was partially prevented by wortmannin and caffeine thus pointing to the involvement of members of PI3-K.

4 Conclusions

Leishmania parasites were able to phosphorylate H2A histone after DNA insults produced by the exposure to Top poisons partially via PI3-K. However, further investigation is required to identify any effectors that may interact with these histone modifications.

C0920 EXPLORING THE ROLE OF HYDROETHANOLIC EXTRACT OF RHODIOLA IMBRICATA EDGEW. AND ITS BIOACTIVE COMPONENT, SALIDROSIDE FOR EFFECTIVE THERAPY OF EXPERIMENTAL VISCERAL LEISHMANIASIS

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1 Background

Visceral leishmaniasis (VL) is a deleterious form of leishmaniasis that chiefly impinges the poorest sections of the society. VL is usually lethal due to the secondary infections, organ failure and immunosupression. The standard therapeutic options are crippled due to life-threatening side-effects. In this sense, immunomodulatory plants offer ample sources to develop new antileishmanial agents. In the present study, antileishmanial and immunomodulatory activity of *Rhodiola imbricata* Edgew., and one of its bioactive component, Salidroside has been evaluated.

2 Methods

Inbred BALB/c mice were infected with promastigotes of sodium stibogluconate (SSG) sensitive and resistant strains of *Leishmania donovani.* After 30 days of infection the animals were treated orally with hydro-ethanolic extract of roots of *R. imbricata* and Salidroside. For controls, the standard drugs SSG and amphotericin B were used. The hepatic parasitic load was assessed in terms of Leishman Donovan Units. Cell-mediated and the immunological facet of the plant were analyzed by delayed type hypersensitivity responses (DTH) and levels of various Th-1/Th-2 cytokines. Further, the safety of extract and component was assessed through biochemical and histopathological studies.

3 Results

Reduction of the hepatic parasite burden, heightened DTH response and elevated levels of Th-1 type of cytokine levels (IFN-gamma and IL-2) was more in infected animals treated with Salidroside than in the infected animals treated with the plant extract. This indicated that Salidroside has more immunomodulatory and leishmanicidal efficacy than the extract. Moreover, normal biochemical parameters and histology of liver, kidney and spleen demonstrated the safety of this plant extract and component as compared to animals treated with SSG and amphotericin B which showed hepatoxicity and nephrotoxicity respectively.

4 Conclusions

The study demonstrated that hydroethanolic extract of *R. imbricata* and Salidroside showed antileishmanial and immunomodulatory activity. The promising antileishmanial activity shown by root extract of *R. imbricata* and Salidroside may provide a new lead in the treatment of VL.

C0932 INDIVIDUAL-BASED MODELLING OF INTRACELLULAR LEISHAMANIA INFANTUM AMASTIGOTES

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1 Background

Assays with macrophage-amastigotes models are appropriate for the screening of anti-leishmanial drugs. Nevertheless, the complex dynamics of such cultures hinders the collection of quantitative and objective data. Individual based Modelling (IbM) is an appropriate mathematical approach for the interpretation and quantification of these cultures. IbMs consider each cell as a unique and discrete entity with its own attributes. As a result of IbM simulations, system dynamics emerge from the behaviour of individuals and their interaction.
The aim of this study is to build an IbM that helps analysing the dynamics of amastigotes-macrophages cultures. Previously, we need to develop experimental methodologies to provide the required quantitative information.

2 Methods

Murine macrophage cell line RAW 264.7 and murine (Balb/c) peritoneal cells were infected with *Leishmania infantum* promastigotes (MHOM/ES/2016/BCN-877). Infections were carried out using 10:1 (promastigotes-macrophages ratio) in Lab-tek™ chambers in RPMI 1640 medium supplemented with 10 % Fetal Bovine Serum. After 2, 4, 8, 24, 48 and 72 h of incubation at 37 ?C and 5 % CO_2, the wells were detached and the slides stained with Giemsa. Several images from the slides were taken in order to observe and quantify the system. The IbM was built accordingly and implemented in NetLogo.

3 Results

A common time pattern was established for both types of macrophages although fewer murine peritoneal macrophages became infected and the intensities of infection were reduced. Intracellular amastigotes were detected at 24 h and reached their maximum peak at 72 h. The compilation of individual information of cells together with IbM simulations allowed the interpretation of the behaviour experimentally observed as well as the quantification of different parameters such as the probability of infection, the time to transform promastigotes into amastigotes, the amastigote duplication time or the number of amastigotes that cause the lyses of macrophages.

4 Conclusions

IbMs state rules governing the behaviour of the individuals and return the emerging dynamics of the collective. This is useful in the study of processes linked to changes in individual properties and heterogeneity at the cell scale, as in the case of evaluating infected macrophages cultures. This IbM reproduced the overall dynamics of experimental cultures, and may be used for understanding and evaluating *in vitro* assays of leishmanicidal activity of drugs

C0934 IN VITRO ACTIVITY OF CHLOROPHYLL A AND B USED IN PHOTODYNAMIC THERAPY ON LEISHMANIA (LEISHMANIA) AMAZONENSIS

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1 Background

American tegumentary leishmaniasis (ALT) is considerated by The Health World Organization a public health problem because of its important clinical manifestations. For decades, the first line of leishmaniasis treatment has been the pentavalent antimonial, even with its side effects and contraindications. Based on this, studies has been done with the purpose to obtain alternative therapy, one of these is photodynamic therapy. It uses photosensitising agents that release reactive oxygen species leading to cellular death in presence of light reducing side effects. Chlorophyll has the required characteristics to be used in photodynamic therapy as high light abortion and long time in excited form. The aim of this study is to investigate the antileishmanial activity of chlorophyll in photodynamic therapy on promastigotes forms of *Leishmania (Leishamania) amazonensis* (L.L.a).

2 Methods

Promastigote forms were exposed to Chlorophyll A and B, in the concentration of 0.395 µM to 0.0001 µM and 2.31 µM to 0.001 µM in 96-well plates, respectively. The half plates was illuminated with red light emitting diode (LED) for 30 minutes and the other half was not illuminated. Both types of plates were incubated for 24h at 25ºC. The parasite viability was analysed by XXT method and absorbance was measured in spectrophotometer at 450/620 nm. Following, the lethal dose for 50% of parasites (LD50) was calculated.

3 Results

chlorophyll A and B had leishmanicidal activity in presence and absence of light although the LD50 of chlorophyll dropped in approximately 3 folds in presence light, being the LD50 without illumination of 0.15 µM and 0.055 µM under illumination.

4 Conclusions

chlorophyll A and B can be a promising compound in the treatment of ALT because of their antileishmanial activity. Furthermore, they are also an option of photosensitizing drug for photodynamic therapy, since chlorophyll A and B have their activity increased after illumination.

C0936 PHOTODYNAMIC THERAPY WITH HIPERICIN AGAINST PROMASTIGOTES OF LEISHMANIA (LEISHMANIA) AMAZONENSIS

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1 Background

Although pentavalent antimoniates have several systemic side effects, invasive administration, contraindications and therapeutic fails, it still to be used as a first line of treatment for leishmaniasis. These treatment difficulties has inspired the search for alternative drugs and photodynamic therapy is one of those. The hipericin is a promising photosensitizing agent with antitumoral and antimicrobial activity and it has a local action dependent of illumination. The purpose of this study was to evaluate the antileishmanial activity of Hipericin in photodynamic therapy against promastigotes of *Leishmania (Leishmania) amazonensis.*

2 Methods

At the concentrations of 100 to 0.04 µM, hipericin was added in 96-well plates with *L. (L.) amazonensis* promastigotes. After 4 hours, half of plates was illuminated for 30 minutes with with light emitting diode, and another was not illuminated. All plates were incubated for 24h at 25ºC. Leishmanicidal activity was done by the XTT method and a spectrophotometer was used to measure absorbance at 450/620 nm and lethal dose for 50% of parasites (LD50) was determine.

3 Results

Hipericin did not have any antileishmanial activity without illumination, but it had a DL50 of 11.82 µM in presence of light.

4 Conclusions

Hipericin has leishmanicidal activity and it is dependent on light exposition. Further studies are necessary.

C0954 POTENTIAL THERAPEUTIC OF 17-AAG IN VITRO AGAINST LEISHMANIA INFANTUM ISOLATES FROM PATIENTS REFRACTORY TO ANTIMONY TREATMENT

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1 Background

Visceral leishmaniasis (VL) is a severe infectious disease caused by *L. infantum* in America. If not treated properly, the VL can lead to death. Pentavalent antimonials have been the treatment of choice for the past 70 years. However, their use is associated with major side effects, in addition to the emergence of treatment failure. Thus, given the growth of treatment refractory cases, it is urgent to develop new anti-*Leishmania* compounds that are safe and available compared to what exists today. Previously, we showed that 17-(allylamino)-17-demethoxygeldanamycin (17-AAG), a Heat Shock Protein 90 (HSP90)-specific inhibitor, reduces *L. amazonensis* infection *in vitro* and *L. braziliensis in vitro* and *in vivo*. In this study, we analyzed the leishmanicidal activity of 17-AAG against clinical isolates of *L. infantum.*

2 Methods

Two isolates of *L. infantum* obtained from antimony-refractory patients and two isolates from antimony-responsive patients from Brazil were used. Promastigotes were treated with 17-AAG alone (1-10 μM) or 17-AAG (0.25 a 3 μM) associated with the trivalent antimony (active form of the drug). After 48 h, parasite viability was evaluated by direct counting of live motile parasites using a Neubauer chamber.

3 Results

We observed that treatment with 17-AAG was effective to reduce parasite viability in the isolates obtained from antimony-refractory and responsive patients. The concentration of 2 μM significantly reduced the parasite viability in both isolates from responsive patients and in one isolate from antimony-refractory patient. Moreover, an increasing concentration of 17-AAG maximized killing effects in a dose-dependent manner in all isolates. The half maximal inhibitory concentrations (IC_{50}) were 2.8 and 3.3 μM, for isolates obtained from responsive patients and 3.4 and 4.5 μM for isolates obtained from refractory patients. Next, we compared the efficacy of the association between 17-AAG and trivalent antimony. The compounds association showed synergism and low concentrations of 17-AAG, 0.25 and 0.5 μM, displayed significantly lower parasite numbers when combined with trivalent antimony.

4 Conclusions

We demonstrated that 17-AAG killed *L. infantum* promastigote from antimony-refractory patients and its synergism action with antimonial was able to reduce the concentration of 17-AAG needed to kill *L. infantum*.

C0975 COMPARATIVE STUDY OF CARDIOTOXIC EFFECTS IN PATIENTS WITH AMERICAN CUTANEOUS LEISHMANIASIS

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1 Background

Cutaneous Leishmaniasis (CL) is a world wide spread disease. Among its treatments there are the pentavalent antimonial derivates, like meglumine antimony, whose cardotoxicity is observed, mainly by alterations on the electrocardiogram (EKG). In this context, miltefosine rises as an effective alternative against several species of *Leishmania* and other protozoa. Despite its importance there isn't an option which combines both, high efficacy and low toxicity.

2 Methods

This is a retrospective cohort study in which we retrieveled and analysed electrocardiographic registries found in medical records from patients with CL, treated on the Dermatology Service from Brasilia University Hospital. Were included patients with age between 18 and 70 years who had been diagnosed with CL by the medical team of this department. The patients were divided in two groups, according to the treatment received: meglumine antimony (MG) and miltefosine (M). We compared the relative risk and proportions of patients with electrocardiographic alterations during the treatment.

3 Results

We searched for 111 medical records from patients accompanied between 2008 and 2013 and retrieveled epidemiological, therapeutical and electrocardiographic data from 53 of them. At the seventh day of treatment, 11,4% of individuals in group MG and 33,3% in group M presented with an altered QTc, while on the fourteenth day, 26,6% of group MG presented the same alteration against none in group M. This tendency persisted and, at the twenty-first day, 35,3% of group MG presented an altered QTc while there were no alterations in group M. The relative risks (RR) of the tested variables were evaluated and a difference was found at the seventh day of treatment. Patients treated with miltefosine presented higher frequency of QTc>440, with RR = 0,2571 (Confidence interval 95% = 0,07 to 0,94; p = 0,0485).

4 Conclusions

The results of the present study suggests that there is cardiotoxicity in the beginning of miltefosine treatment observed by an increase on QTc, which reduces along the treatment, while meglumine antimony presents a time dependent cardiotoxicity, increased with the duration of treatment. Therefore, our results suggest that miltefosine is less cardiotoxic when compared to meglumine, reducing the morbidity and mortality attached to increased QTc.

C0933 POLYGLYCEROL-DOXORRUBICIN CONJUGATE UPTAKE IN PRIMARY SPLEEN CULTURE MACROPHAGES INFECTED WITH L. INFANTUM.

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1 Background

Current leishmanicide drugs have difficulties to reach the parasite. The combination of these drugs with polymeric nanoparticles or liposomes could overcome these limitations. Both nanoparticles and liposomes allow the selective uptake by macrophages, even polymeric nanoparticles drive drug to the phagolysosome. Moreover in polymer-based drug delivery systems a drug covalently conjugated to form a prodrug and it release requires the splitting of the linker between the nanoparticle and the drug, this linker can be pH-sensitive or enzymatically-cleavable. Here, doxorubicine (DOX)-loaded polyglycerol nanoparticles are assessed against *ex vivo* spleen *L. infantum* infections with the aim of delivering drug inside the acidic parasitophorus vacuole where the intracellular parasite resides.

2 Methods

Free and conjugated DOX with a pH-sensitive hydrazine linker (PG-DOX(pH)-PEG), enzymatically-cleavable linker (PG-DOX(enz)-PEG) and conjugate to a non-cleavable linker is used as a control (PG-DOX(non)-PEG) were provided by M. Calderon. Uptake of nanoparticles was assessed by both confocal microscopy and flow cytometry. Efficacy and cytotoxic effect of nanoparticles were measured by standard methods in experimental in vitro infections of murine Raw 264.7 and J774.2 macrophages.

3 Results

DOX conjugated nanoparticles, bearing a pH-sensitive hydrazine and cathepsin-cleavable linker were able to liberate DOX inside the macrophage phagolysosomes. Confocal microscope analysis showed co-localization of the nanocarriers with intracellular amastigotes. Cytotoxicity of both nanoparticles against established macrophage cell lines (Raw 264.7 and J774.2) decreased when the pH or enzyme cleavage nanoparticles were used.

4 Conclusions

New nanostructures designed to deliver drugs to the phagolysosome where the intracellular parasite resides, might help to combat this infectious disease in developing countries.

C0993 USE OF POLY(AMIDOAMINE)S FOR THE DELIVERY OF ANTILEISHMANIAL DRUGS

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1 Background

Delivering drugs to a selected site with minimal exposure for sensitive adjacent healthy cells is the aim of the nanomedicine field. Encapsulation of drugs in targeted nanovectors is applicable to infectious disease treatment as a potentially essential tool. Polyamidoamines (PAAs) are a family of biodegradable synthetic polymers exhibiting a combination of properties imparting them a potential in the biomedical field. The objective of this research has been to determine by confocal fluorescence microscopy the subcellular localization of the PAAs AGMA1 and ISA23 administered to macrophages infected with *Leishmania infantum*.

2 Methods

Intracellular amastigotes were obtained by infection of RAW 264.7 cells with *L. infantum* (MCAN/ES/92/BCN722) promastigotes stained with NHS-rhodamine prior to infection. 48 h post-infection, the fluorescently labeled PAA-based nanoparticles AGMA1-FITC and ISA23-FITC (with mean diameters of 96.5 and 280.7 nm respectively) were incorporated and different organelle-specific dyes were used in order to reveal the localization of polymers inside infected macrophages, using laser scanning confocal microscopy.

3 Results

ISA23-FITC is observed in the cytoplasm of 94% of macrophages, while a low 2% penetration is observed for AGMA1-FITC.

4 Conclusions

ISA23-based nanoparticles are potentially useful for the encapsulation and delivery of antileishmanial drugs.

C1035 NOVEL CHALCONE-THIOSEMICARBAZONES WITH ANTI-LEISHMANIAL ACTIVITY

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1 Background

The parasitic protozoans of the genus *Leishmania* are the causative agents of several forms of cutaneous, mucocutaneous, and visceral leishmaniasis in any parts of the world. The available antileishmanial drugs still rely on the highly toxic, cause serious side effects and require long-term treatment.There is an urgent need to search for new, safe and efficient antiparasitic agents for the treatment of leishmaniasis. Chalcone-thiosemicarbazones is a class of compounds with a wide range of biological activity and, simultaneously, substances containing the thiosemicarbazone or chalcone group present activities against many pathogens, including *Leishmania sp*.
In this present work, 5 chalcone-thiosemicarbazones (CT-H, CT-Me, CT-F, CT-Br, CT-Cl) were prepared, and evaluated for the anti-leishmanial activity.

2 Methods

The compounds were obtained, in good yields, after condensation reaction of chalcones with thiosemicarbazones in acid medium, and all were fully characterized. The antileishmanial activity of these compounds was assayed in promastigotes of *Leishmania amazonensis* (MHOM/BR/77/LTB0016 strain), *L. infantum* (MHOM/MA67ITMAP263 strain), and intracellular amastigotes forms of *L. amazonensis*. Further, the cytotoxicity of these compounds was evaluated on non-infected murine macrophage cells. Promastigotes were cultured with and without the drugs in Schneider´s medium at 2°C, using pentamidine isethionate as reference drug. After 24h incubation, parasite viability was determined using the MTT assay. Anti-amastigote activity was assayed in mouse-infected macrophages cultured in DMEM medium at 34°C and 5% CO_2. The macrophages were infected with *L. amazonensis* promastigotes and cultured for 1h. Anti-amastigote activity was determined by counting the number of intracellular parasites after 72h of treatment.

3 Results

The results showed that CT-Cl derivative was the most active compound for promastigotes of *L. infantum* and *L. amazonensis*, with IC_{50} values 3.97±0.46 µMand 5.22±0.75 µM,respectively. The other compounds also showed a good *in vitro* anti-leishmanial activity with IC_{50} in the range of 4.34 to 14.80 µM. The results of assays with intracellular amastigotes of *L. amazonensis* indicated IC_{50} < 3.125 µM. In addition, all these derivatives were less cytotoxic than pentamidine.

4 Conclusions

In conclusion, these results reinforce the chalcone-thiosemicarbazone derivatives as potential new candidates for leishmaniasis treatment.

C1037 IN VITRO ACTIVITY OF PLANT EXTRACTS AND FLAVONOIDS AGAINST LEISHMANIA BRAZILIENSIS

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1 Background

Plants are very rich in bioactive molecules and can be source of new drugs. The aim of this study was to evaluate the in vitro effect of several brazilian plants extracts against *Leishmania braziliensis*, the most prevalent species that causes tegumentary leishmaniasis in Brazil. We tested *Solanum lycopersicum* (Sl), *Colacasia esculenta* (Ce), *Psidium guajava* (Pg), *Aloe vera* (Av), *Kalanchoe pinnata* (Kp) cultivated under different light conditions and isolated flavonoids. The experiments were performed on amastigostes and promastigotes forms of the parasite.

2 Methods

Promastigotes of *L. braziliensis* were cultivated in the presence or absence of 500µg/ml of each extract for 96h/28°C, when the parasite viability was measured by MTT (Triazolyl Blue Tetrazolium Bromide) reduction assay. For the amastigotes assay, monolayers of *Swiss* mice or hamster peritoneal macrophages were infected with *L. braziliensis* promastigotes (5 parasites/macrophage) and incubated in the presence or absence of extracts (500µg/mL) for 48-96h/37°C/5%CO_2

3 Results

The extracts did not present activity against promastigotes forms. Most extracts had inibitory activity in intracellular amastigotes and Sl was the best, with 73,3% inhibition at 125µg/mL. Pg was the second best with 57,9% and Av with 56% inhibition. At 96h time Kp cultivated in habitat was the best inhibitor with 69,4% followed by Kp cultivated under blue light (61,9%), white light (61,9%) and UV light (48%). Kp majoritary flavonoid presented inhibition of 79,6% at 100µg/mL (IC_{50}: 8,35µg/mL) and quercetin inhibited 79% of infection index at 300 µM (IC_{50}: 20,9 µM) and selectivity index of 22. Previous treatment of macrophages with quercetin reduced the infection index in 65% at 300µM. None of the extracts tested was able to estimulate nitric oxide production by macrophages, suggesting direct action on the parasite.

4 Conclusions

These data demonstrate that extracts of Sl, Pg, Av (decoction) e Kp (habitat) are the most active against *L. braziliensis* amastigotes and isolated flavonoids are more active than the extract. We are currently evaluating the activity of these extracts on citokyne production and parasite death by apoptosis.

C1039 ANTILEISHMANIAL THERAPEUTIC POTENTIAL OF BIODEGRADABLE MICROPARTICLES CONTAINING CHENOPODIUM AMBROSIOIDES L. (MASTRUZ)

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1 Background

Chenopodium ambrosioides L., popularly known as mastruz, is commonly used for the treatment of leishmaniasis in Brazil. Previous studies showed *in vivo* and *in vitro* anti-*Leishmania* activity of the hydroalcoholic extract (HE) of mastruz leaves. In an effort to identify novel therapeutic alternatives for the treatment of leishmaniasis, the present study evaluated the antileishmanial effect of the HE and its fractions.

2 Methods

For this purpose, the molecular affinity between the total proteins from *Leishmania amazonensis* and HE or glucantime was evaluated by surface plasmon resonance (SPR). Subsequently, the leishmanicidal potential of HE was assessed in promastigote culture. Then it was evaluated the effect of HE and fractions as well as the biodegradable microparticles formed by poly lactic-co-glycolic acid (PLGA) containing encapsulated HE in macrophages infected with *L. amazonensis* amastigotes. The results indicated that HE showed potential for binding to *Leishmania* proteins similar to Glucantime®. The fractions Hexane (FHex), chloroform (FCI), ethyl acetate (FAc) and hydroalcoholic (FHA) were evaluated for cytotoxicity against promastigote forms *in vitro*.

3 Results

The results showed that FHex and FCl were the most effective fractions considering the IC_{50}. The cytotoxic action on murine macrophages was tested. The FHex and FCl fractions presented greater cytotoxic action on macrophages (≥50%), compared to FAc and FHA, which maintained cell viability above 80%. The *in vitro* treatment of macrophages with FAc and FHA fractions increased nitric oxide. The rate of infection after treatment with HE, FAc and FHA (250 µg/mL) was reduced by 60%, 36% and 52%, respectively, which was accompanied by increased NO production. The chemical analysis of the fractions showed a predominance of compounds of quercetin and kaempferol derivatives in the two active fractions, indicating that anti-*Leishmania* and immunostimulatory activities may be associated with these compounds. The encapsulated HE was more effective than the encapsulated glucantime in inhibit the phagocytic index, when tested for anti-*Leishmania* activity in infected macrophages.

4 Conclusions

These results reaffirm the medicinal potential of *C. ambrosioides* as an anti-L*eishmania* agent and provide the first evidence of activity immunostimulatory and anti-*Leishmania* of the flavonoids of this plant species, compounds which are candidates for use in pharmaceutical formulations for clinical use.

C1044 ACTIVITY OF PTEROCARPANQUINONE LQB-182 ON LEISHMANIA. (VIANNIA.) BRAZILIENSIS IN VITRO AND IN VIVO.

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1 Background

Leishmaniasis are a complex of diseases with a wide spectrum of clinical manifestations caused by the genus *Leishmania*, which *Leishmania (Viannia.) braziliensis* is the most distributed species in the New World. The therapeutic arsenal is restrict, toxic and expensive and facing this panorama, the search for new potential drugs becomes necessary. Pterocarpanquinones are synthetic hybrid molecules created from a naphthoquinone and pterocarpan. The aim of this study was to investigate *in vitro* and *in vivo* the activity of pterocarpanquinone LQB-182 against *L. (V.) braziliensis*.

2 Methods

Stationary-phase, L. braziliensis promastigotes were incubated with pterocarpanoquinone LQB-182 (20 µM) for 96h at 26ºC, and and the number of parasites was counted daily in a Neubauer chamber.BALB/c mice peritoneal macrophages were incubated with several concentrations of LQB-182 (0-300 µM) for 72h at 37°C/5%CO_2 and cell viability was measured by MTT reduction assay. Monolayers of BALB/c mice peritoneal macrophage were infected with *L. braziliensis* promastigotes (ratio of 5 parasites/macrophages) and subjected to treatment with the LQB-182 for 72h/ 37ºC/ 5%CO_2, and number of infected cells were counted under optical microscopy. *L. braziliensis* infected BALB/c mice were treated from the seventh day of infection with LQB-182 administered intralesionally (three times a week) for two weeks.The size of lesion was measured weekly and parasite load estimated by limited diluition.

3 Results

Our analysis *in vitro* showed LQB-182 activity on promastigotes (IC_{50}=10,98 ± 1,44 µM), intracellular amastigotes *(*IC_{50} =17,01±2,46µM) forms and low toxicity to BALB/c peritoneal macrophages (CC_{50} = 303,6 µM). The nitrite and reactive oxygen species quantification on culture supernatants showed that was no increase of production of this radicals. However, we observed that macrophages treated with LQB-182 phagocytosed more fluorescent beads than untreated controls ($p<0,05$). *In vivo*, LQB-182 was capable of controlling the lesion size and significantly decrease the parasite load ($p<0,001$) when administrated by intralesional route. The analysis of biochemical parameters showed an increase of hepatic enzymes, above the reference values.

4 Conclusions

This data show that pterocarpanquinone LQB-182 was selectively active *in vitro* against the parasite, and also effective *in vivo*. therefore it a promising molecule to continue pre-clinical studies.

C1046 LEISHMANICIDAL ACTIVITY OF NOVEL BENZIMIDAZOLE DERIVATIVES DESIGNED AND SYNTHESIZED AGAINST LEISHMANIA MEXICANA ARGINASE

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1 Background

The identification of noveltherapeutic targets and the development of new drugs for the treatment of leishmaniasis are urgently needed since chemotherapy currently available has several problems including many adverse side effects, and parasite drug resistance to all known drugs. Arginase (ARG) is considered a therapeutic target because it is important for parasite survival and proliferation.In an effort to have alternative drugs for the treatment of cutaneous leishmaniasis, eight molecules (compounds **1**-**8**) selected from a previously reported virtual screening study of a ZINC database in LmARG were synthesized. Their inhibitory activity on LmARG and their anti-leishmanial activity against different *Leishmania* species representative of cutaneous, mucocutaneous and visceral leishmaniasis were also evaluated.

2 Methods

1-**8** were synthesized by straightforward reductive amination method. Spectrometric and spectroscopic data obtained were consistent with the expected structures. The inhibition of recombinant LmARG activity by **1**-**8** was determined using the QuantiChom™ Arginase Assay Kit.The anti-leishmanial activity (IC_{50}) of **7** and **8** was evaluated against the promastigote and amastigote of different *Leishmania* species by resazurin method. Citotoxicit of 1-8 was evaluated using the macrophage cell line J744 by the resazurin method.

3 Results

All compounds with the exception of **1** inhibited recombinant LmARG activity in a range of 18.14 to 76%. In addition, **7** and **8** were less cytotoxic than miltefosine and showed high activity against *L. mexicana* promastigotes. Compounds **7** and **8** were selected to search for their anti-leishmanial activity against different *Leishmania* species. Compounds **7** and **8** showed leishmancidal activity mainly against the promastigote and amastigote of cutaneous and mucocutaneous *Leishmania* species. Importantly, **8** showed the highest Selectivity Index against all *Leishmania* species tested.

4 Conclusions

The inhibitory activity of **7** and **8** on recombinant LmARG is according to the expected activity from de virtual screening study.The inhibitory effect of **7** and **8** on ARG activity, indeed accounts for their *in vitro* anti-leishmanial activity. These compounds can be used as a scaffold for the development of more potent agents against LmARG.

C1068 EVALUATION OF ANTILEISHMANIAL ACTIVITY OF A 3PACK KIT (ANTISEPTIC-KERATOLYTIC-DRUG CREAM) AS AN ALTERNATIVE FOR THE TREATMENT OF CUTANEOUS LEISHMANIASIS IN BALB/C MICE

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1 Background

Cutaneous leishmaniasis (CL) lesions are characterized by elevated (crater-like) nodules or ulcers with thickening of the corneal stratum (SC) by hyperkeratosis. Usually they are co-infected with bacteria and fungi that must be treated before applying any medication. Topical administration of the compounds in cream-like dosage could maintain a localized effect resulting effective for CL. This study focuses mainly on the evaluation of the therapeutic potential of the 3PACK Kit for the treatment of CL in *L. (V.) braziliensis* BALB/c mice

2 Methods

Infected mice were placed in three groups: negative control, pentamidine (PMD) 3% cream and PMD 6% cream groups. Treatment was performed for 30 days, twice daily. Lesions were measured every 7 days. The antiseptic was applied all the time of the treatment and the keratolytic was applied every 12 h at the first five days. A microscopic and histopathological analysis was done on skin fragments from the lesions

3 Results

Compared with the control group, treatment with 3% of PMD cream cure the ulcer completely (0 versus 6.48 mm^2). In one mouse the nodule decreased completely at the end of treatment, but 20 days after an increase to 15.54 mm^2 was observed. In the others, the nodule size remained constant after 20 days of treatment (11.3 versus 43 mm^2). Half of the group (2/4 mice) treated with 6% PMD cream had no lesion at the end of treatment. However, presence of parasites was observed under microscopic and histopathological analysis. Irritation signs at the site of application were observed.

4 Conclusions

The 3PACK Kit could be a good strategy for CL treatment. The induction of clinical cure but not a parasitic one indicates probably a low leishmanicidal action of the PMD in *in vivo* conditions, or a low permeation of the PMD cream at the infected dermis.

C1074 MEGLUMINE ANTIMONIATE INTRALESIONAL IN LOCALIZED CUTANEOUS LEISHMANIASIS: A RETROSPECTIVE COHORT STUDY.

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1 Background

According to Pan American Health Organization (PAHO), systemicallypentavalent antimonials are the first choice for the treatment for Cutaneous Leishmaniasis (CL). However, this therapy may cause serious adverse effects, such as cardiac, renal and hepatic damage. Intralesional meglumine antimoniate (MA) injection is therapeutic option, although scientific evidence is limited. This paper aims to evaluate cure and adverse effects of patients with localized CL treated with intralesional MA compared with standardized treatment.

2 Methods

This is a retrospective cohort study performed in a referral centre. It was selected a sample with 46 patients with localized CL, 14 cases and 32 controls. Cases were those treated with intralesional MA, consisting of injection of Sb+5 81mg/ml, in the dosage of 1ml/cm2 of lesion, 1 application per week, for 4 weeks. Controls were treated with 10-20mg Sb5+/kg/day intravenously or intramuscularly for 20 days. The exclusion criteria were discontinuation of treatment for more than 7 days, more than 2 dermatological lesions, concomitant mucosal leishmaniasis or recurrent Cutaneous Leishmaniasis.

3 Results

The percentage of male subjects was higher in both groups, corroborating data literature. The median age was higher in the cases (48.5) compared to the controls (25) ($p = 0.003$), possibly because intralesional treatment was indicated in subjects with more comorbidities. The cure rate was lower in case group ($p = 0.067$), but the statistical tests showed only a tendency for this association. The adverse effects does not show significant difference between groups (p=0,761). Besides the lower dose, intralesional treatment has systemic side effects such as hepatic enzymes elevation and electrocardiographic changes. There was no difference between groups in interruption of the treatment.
Studies of this modality treatment are not standardized and have little concrete safety and efficacy data. The main advantage of this technique would be less side effects and similar efficacy. Contrary to the literature, an equivalence of adverse effects between groups was observed.

4 Conclusions

Despite the limitations of the present methodology, the treatment with intralesional injection of MA was similar to the standardized one. However, it has not been shown to have fewer side effects. Researches still are needed to elucidate efficacy and safety of this therapy.

C1076 IN VITRO AND IN VIVO INTERACTIONS BETWEEN CURRENT ANTI-LEISHMANIAL DRUGS

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1 Background

Combination of synergistic drugs in a topic formulation could be a strategic alternative for cutaneous leishmaniasis (CL) treatment. Current drugs include pentavalent antimony, amphotericin B, miltefosine (MIL), paromomycin sulfate (PM) and pentamidine isethionate (PMD). The uses of those drugs are associated with toxicity and side effects. The objective of this work was to evaluate drug interactions between MIL, PM, PMD, ketoconazole (KET), azithromycin and 8-aminoquinoline on *Leishmania (Viannia) braziliensis* on*in vitro* and *in vivo* model.

2 Methods

Drug-susceptibility was assessed on promastigotes and intracellular amastigotes after 3 and 5 days of drug incubation respectively. Drug interaction was determined using the fixed ratio isobologram method. Fractional inhibitory concentrations (FICs), sum FICs (∑FICs) and an overall mean FICs (∑FIC) were calculated for each combination at the IC_{50} and IC_{90} level. The nature of the interactions was classified as synergistic ∑FIC ≤0.5, indifferent ∑FIC between 0.5 and 4.0 and antagonistic ∑FIC ≥4.0. *Leishmania (V.) braziliensis* -infected BALB/c mice were treated topically with 4 different semi-solid combined formulations (CINTROP_020-024) for 3 weeks. Control mice were treated with a formulation without drug. Changes in lesions and histopathological analysis were performed.

3 Results

The combinations of KET with PM, PMD and azithromycin were synergistic with a mean of ∑FIC from 0.43 and 0.53 at IC_{50}. In intracellular amastigotes, a combination of KET/PM was synergistic at IC_{90} level (mean ∑FIC 0.47). The other interactions were classified as indifferent with mean of ∑FIC from 0.6 to 1.2. No antagonistic interaction was observed. No significant interaction was observed in infected BALB/c. However, a less increase in nodule (3.3 versus 45.7 mm^2) and ulcer (8.5 versus 12.2 mm^2) size compared with untreated mice were observed after CINTROP-21 formulation treatment. Mice treated with CINTROP-22 reduced the ulcer size but not the nodule.

4 Conclusions

In vitro synergistic drug interaction was observed between some antileishmanial drugs treatment. A design of new types of formulations contained two synergistic drugs transporting efficiently drugs through the skin lesions are in course.

C1080 VERNONIA BRASILIANA LEAVES HARBOUR COMPONENTS WITH POTENTIAL LEISHMANICIDAL ACTION

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1 Background

For decades, Brazilians from rural areas have used plants of the genus *Vernonia* for the treatment of many diseases, which leaded the Brazilian National Health System to include this genus in a list of 71 plants that should be evaluated in order to verify their therapeutical potential, with the purpose to promote and recognize the popular and traditional practices of use of herbal and home remedies. The vast Brazilian biodiversity, associated with the endemicity of Leishmaniasis in the state of Maranhão, allowed the creation of several research networks to evaluate the leishmanicidal effect of plants of diverse biomes. Thus, it is very important to draw experiments using natural products in order to disclose new compounds, with a lower toxicity and easy to administrate. Based on this, this study aimed to evaluate the leishmanicidal and cytotoxic potential of *Vernonia brasiliana* under the parasite *Leishmania amazonensis*.

2 Methods

Phytochemical analysis was performed by gas chromatography and mass spectrometry. Subsequently, *in vitro* cytotoxic assay was carried out to determine cytotoxic concentration via MTT colorimetric assay; followed by *in vitro* antileishmanial screening and determination of the 50% inhibitory capacity of *L. amazonensis* promastigote forms under the effect of crude ethanolic extracts, oil and fractions of *V. brasiliana*; and ultrastructural analysis through electronic microscopy.

3 Results

Phytochemical screening showed that *V. brasiliana* major compounds are sesquiterpenes, notably caryophyllenes. The crude extract, its fraction and essential oil were not cytotoxic in leishmanicidal concentrations, with the selective ratio higher in the parasite than in the host cell. Electron microscopy showed that significant damage was observed in the parasite cell membranes, cytoplasm and flagellar pocket after treatment with *V. brasiliana*, demonstrating that this species is a potential leishmanicidal agent.

4 Conclusions

The compounds extracted from *V. brasiliana* leaves, especially the essential oil and chloroform fraction, are potential leishmanicidal agents in non-cytotoxic concentrations, highly selective to the parasite.

C1082 ANTI-LEISHMANIA SP ACTIVITY AND CITOTOXICITY EVALUATION OF NEW MESOIONICS COMPOUNDS

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1 Background

The World Health Organization considers leishmaniases one of the most serious diseases worldwide caused by protozoan parasites of the genus *Leishmania.* However, the control of this disease remains a problem; the available antileishmanial drugs still rely on the highly toxic pentavalent antimonials, which cause serious side effects and require long-term treatment. There is an urgent need for the development of new, efficient, and safe drugs for the treatment. The mesoionic derivatives are a class of compounds with a wide range of biological activity, against a large number of pathogens and anti-inflammatory, analgesic, antibacterial, antifungal, and antitumor activities. In addition, other classes of mesoionic compounds is known to have nitric oxide (NO)-releasing properties. In this present work, new mesoionic (MIC) derivatives were synthetized by a green chemistry application with potential antileishmanial activity.

2 Methods

Five MIC derivatives, containing $-NO_2$, $-OCH_3$ or -Cl radicals were obtained, in good yields, and all were fully characterized by FT-IR, ^1H and ^{13}C NMR. The antileishmanial activity of these derivatives was assayed in *L. braziliensis* and *L. infantum* promastigotes forms. In parallel, the cytotoxicity of these compounds was evaluated using BALB/c and Swiss peritoneal macrophages cells. Parasites were cultured with and without the drugs in Schneider ?s medium at 25°C, using pentamidine isethionate as reference drug. After 24 hours of incubation, parasites and macrophages viabilities were determined using the Alamar Blue assay.

3 Results

The results showed that derivatives containing $-OCH_3$ radical were the most actives for the two species, with IC_{50} values 1.32±0.6 and 2.05±2.0 µg/mL, for *L. braziliensis* and *L. infantum* respectively. In addition, all these derivatives were less cytotoxic than pentamidine. These results reinforce the MIC derivatives as potential antileishmanial agents. Furthermore, other studies on intracellular amastigotes are being performed.
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C1142 DESING AND EVALUATION OF TOPICAL FORMULATIONS OF KETOCONAZOLE IN CUTANEUS LEISHMANIASIS

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1 Background

Ketoconazole (KTZ) is a broad spectrum antifungal agent, active against some types of parasites. It acts blocking the synthesis of ergosterol, causing alteration on cell membrane permeability. The main aim of this study was to design a formulation of KTZ for topical use in cutaneous leishmaniasis (CL) by *Leishmania (Viannia) braziliensis*.

2 Methods

Semi-solid and liposomal systems contained 0.4 to 2% KTZ (CINTROP-25-32) and without KTZ (CINTROP-25[a]-32[a]) were prepared and characterized and the antileishmanial activity was measuring on mice. *L. (V.) braziliensis*-infected BALB/c mice were treated topically with each system once day for two months. Nodule and ulcer area (mm^2) and dermal irritation were evaluated before and each week after treatment. Smears form lesions were fixed, Giemsa stained and microscopically tested.

3 Results

Any of the used KTZ systems presented parasite or clinical cure. A no increase of nodule and ulcer size (25.0 mm^2 and 6.6 mm^2) compared with the control were observed during the topical application of CINTROP-29-2% KTZ. No dermal skin irritation was observed on all treated mice.

4 Conclusions

No antileishmanial activities (parasitological or clinical) were induced by the different system contained KTZ used in this study.

C1158 NANOSTRUCTURED LIPID CARRIERS BASED GEL FOR PERCUTANEOUS ADMINISTRATION OF ORYZALIN: A STRATEGY IN CUTANEOUS LEISHMANIASIS TREATMENT

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1 Background

Oryzalin is a dinitroaniline that has been successfully tested for the treatment of visceral leishmaniasis [1]. Its use as an antiparasitic agent against cutaneous leishmanisis has never been tested. Lipid-based nanosystems have great potential for dermal and transdermal drug delivery [2]. Topical delivery of therapeutics constitutes an advantageous alternative to treat infected skin lesions as it allows a direct treatment of affected areas avoiding unwanted systemic effects and reducing the drug dosage. In this study, nanostructured lipid carriers (NLC) were used to incorporate Oryzalin, overcoming formulation difficulties related to its limited low water solubility and to enhance its percutaneous penetration.

[1] RM Lopes et al. J Biomed Nanotechnol 10 (12), 3647-3657. 12 2014.
[2] S Simões et al. Curr Pharm Des. 2016 Jun 30.

2 Methods

NLC were prepared by hot homogenization followed by ultrasonication technique, using glycerol distearate as solid lipid, caprylic/capric triglyceride as liquid lipid and tween 80 as surfactant. The Oryzalin-NLC was uniformly dispersed after suitably gelled by carbopol preparation. The developed system was characterized in terms of size, charge, rheological study, and permeation through newborn pig using Franz diffusion cells. Cell viability at different concentrations of the formulation was determined. At last, infection of human macrophage cells *in vitro* with *Leishmania major* was performed to assess the therapeutic efficacy of the formulation.

3 Results

Oryzalin-NLC had mean size of 120 nm and showed a good entrapment efficiency of 95.6 % ± 3. Permeation and penetration assays revealed that Oryzalin-NLC gel has an adequate profile to be used in a topical formulation since epidermal and dermal drug retention was achieved. Pseudoplastic flows with thixotropy were obtained. Cell viability tests proved this formulation was less cytotoxic than Oryzalin ethanolic solution in HaCaTcells. This formulation also presented leishmanicidal activity *in vitro* against *L. major* amastigote forms.

4 Conclusions

In conclusion, NLC-based gel could be a promising vehicle for topical delivery of Oryzalin and the developed system constitutes a new oportunity in the treatment of cutaneous leishmaniasis.

C1160 IN VITRO DRUG INTERACTIONS OF FOUR CURRENT ANTILEISHAMANIAL DRUGS AGAINST LEISHMANIA (VIANNIA) PANAMENSIS

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1 Background

Leishmaniasis treatment includes the use of pentavalent antimonials, miltefosine (MIL), pentamidine (PMD) and paromomycin (PM). They are not ideal because their specie-dependent effectiveness, possible therapeutic failure due to resistance, lack of adherence and adverse effects. Our objective was to determine the *in vitro* activity of MIL, PMD, PM and ketoconazole (KET) in promastigotes and axenic amastigotes of *L (V.) panamensis*. Additionally, a range of fixed-rate combinations of MIL-KET, MIL-PM, MI-PMD were evaluated to determine the type of pharmacological interaction that occurs in axenic amastigotes.

2 Methods

Axenic amastigotes were generated by transformation of *L (V.) panamensis* promastigotes after modifications of culture medium and temperature for 7 days. They were characterized by estimation the time of generation and differential parasites counts. The inhibitory concentration 50 (IC_{50}) was determined in promastigotes and axenic amastigotes after 3 days treatment with each drug. The type of interaction was defined through a modified fixed ratio isobologram method for each drug combinations. IC_{50} and fractional inhibitory concentrations (FIC) were calculated. The nature of the combination was considered as antagonistic: ΣFIC > 4, without interaction: ΣFIC between> 0.5 and ≤4 and synergic: Σ FICs is ≤0.5.

3 Results

The percentage of promastigotes transformed into amastigotes at day 7 was 89%. The generation time for promastigotes and axenic amastigotes was 0.18 and 0.078 days respectively. Morphologically the axenic amastigotes were similar to the intracellular amastigotes obtained by infection of THP-1 cells. The IC_{50} for promastigotes and axenic amastigotes was 0.06 µM and 15.59 µM for KET, 0.28 µM and 0.30 µM for PMD, 39.80 µM and 150.44 µM for PM, 12.67 µM and 6.87 µM for MIL, respectively. Any of the combinations were synergistic or antagonistic interactions, with values of ΣFICs between> 0.5 and ≤4

4 Conclusions

All tested drugs showed anti-*Leishmania* activity in promastigotes and axenic amastigotes of *L (V.) panamensis*. The combination of MIL and other drugs did not show synergistic interactions in the *in vitro* model evaluated.

C1167 DEVELOPMENT OF LEISHMANIA (LEISHMANIA) AMAZONENSIS EXPRESSING FLUORESCENT TOMATO RED PROTEIN FOR IN VITRO AND IN VIVO SCREENING OF ANTI-LEISHMANIAL DRUGS.

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1 Background

The evaluation of anti-leishmanial activity of novel drugs is based on methods which are laborious, time consuming, and do no support automation for the parasite load quantification. Recently, many reporter molecules such as green fluorescent protein, B-lactamase and luciferase have been devised for the quantification of parasites with different degrees of sensitivity. The objective of this work was to standardize and validate a method based on parasites expressing tomato red fluorescent protein, applicable not only for in vitro parasite quantification, but also for in vivo experiments.

2 Methods

The tomato gene was subcloned into pIR1SAT plasmid, and before the electroporation of *Leishmania (Leishmania) amazonensis,* it was linearized with SwaI. The plasmid replaces one copy of the SSU rRNA gene, and the integration into *Leishmania* genome was confirmed by PCR. Parasites were selected in presence of nourseothricin and cloned in blood agar plate. Fluorescence was measured in promastigotes (pm), intracellular amastigotes (am) in macrophage cultures as well as in am obtained from cutaneous lesions of BALB/c mice in a plate reader. In addition, the infectivity of fluorescent parasites was compared with the wild ones. The leishmanicidal activity of miltefosine (IC50) in vitro and the efficacy of meglumine antimoniate (120 mg Sb^v/kg/day) during 20 days were determined with the method standardized and compared with the conventional techniques.

3 Results

An excellent linear correlation was observed between the number of pm or am and the fluorescence emitted by the parasites (r2 = 0.98). In vitro, the values of IC50 of miltefosine against pm and am assessed either measuring the fluorescence or by counting the parasites with an optical microscope were similar between the methods. In vivo, the fluorescence of parasites was stable during the infection in mice and the treatment with meglumine antimoniate inhibited 48% to 55% of the load parasite in lesions.

4 Conclusions

The fluorescence emitted by the tomato red protein in transgenic parasites is a good indicator of parasites viability, useful for the screening of anti-leishmanial drugs in a simple and fast way both in vitro and in vivo model of *Leishmania (L.) amazonensis*.

C1178 TREATMENT FOR LEISHMANIA MEXICANA USED IN MEXICO BY MAYA INDIGENOUS PEOPLES

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1 Background

Leishmaniasis is endemic in the state of Tabasco, Mexico, where 400 new cases are reported every year. The most frecuent clinical form is cutaneous leishmanaisis caused by *Leishmania mexicana.* This parasite can cause both the localized as well as the diffuse cutaneous leishmaniasis, known as Chiclero's Ulcer. In Mexico, leishmanaisis is treated with glucantime, which has toxic side effects, and parasite resistance is increasing. We therefore analyzed a traditional treatment used by native Maya peoples living in the highly endemic region known as "La Chontalpa", Tabasco. These people traditionally use the plant *Cleoserrata serrata* (Cleomaceae family). Traditional healers "curanderos" rub the toasted and crushed leaves onto a previously washed ulcer and cover it with vaseline. This treatment is repeated during 5 to 10 days and leads to patient cure.

2 Methods

We therefore analyzed the biological effect of the dry leaf extract on *Leishmania mexicana* promastigotes and amastigotes, as well as on bacteria infecting cutanous ulcers. For the organic extract, leaves were macerated and thereafter extracted with dichloromethane/methanol (CH_2Cl_2/ETOH) (1:1).

3 Results

The phytochemical analysis by thin layer chromatography of the organic extract (CH_2Cl_2), showed the presence of terpenoids and flavonoids. The *Cleoserrata serrata* extract had leishmanicidal effects on both *Leishmania mexicana* promastigotes (80%), using 200 µg/ml, and amastigotes (56%) using 10 µg/ml. Additionally, the extract had an anti-bactrial effect against *S. aureus* and *P. aeruginosa*

4 Conclusions

We conclude that the plant contains interesting metabolites that not only kill *Leishmania mexicana* parasites but also the concomitant bacteria that colonize ulcers, thereby aggravating the clinical outcome. This plant represents an alterative treatment for cutanoues leishmaniasis caused by *L. mexicana* and needs to be tested against other *Leishmania* species causing cutaneous leishmaniasis.

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C1182 BIOLOGICAL CHARACTERIZATION OF LEISHMANIA (VIANNIA) BRAZILIENSIS RESISTANT TO PENTAMIDINE, KETOCONAZOLE AND PENTAMIDINA-KETOCONAZOLE COMBINATION

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1 Background

The transmission of cutaneous leishmaniasis caused by *L. (Viannia) braziliensis* in Latin-American has been urbanized and increased number of cases has been register in some endemic areas. Pentavalent antimonial and amphotericin B have been used like main treatments, but other less toxic drugs as pentamidine (PMD) and ketoconazole (KTZ), or combinations constitute a treatment options. The potential risk of resistance developent of current antileishmanial drugs exist and their biological characteristic most be study before their natural appearance. The aim of this work was to generate and to characterize *L. (V.) braziliensis* resistant strains to PMD, KTZ and PMD-KTZ.

2 Methods

Promastigotes resistant lines were generated by stepwise selection. Some biological features as parasite growth, % of infection on human monocytic THP-1 cell line, PMD and KTZ activities against promastigotes and intracellular amastigotes, miltefosine (MTF) and paromomycin (PM) cross-resistance and resistance stability after 1-2 month were determined on resistant (R) and wild type (WT) lines.

3 Results

PMD-KTZ-R lines were more difficult to generate than PMD-R and KTZ-R lines**.** Growth of the R-lines was similar to the WT-strain until five day, but death phase of KTZ-R and PMD-KTZ-R parasites were faster than WT-strain. The % of infectivity on THP-1 cells of WT and R lines were greater than 80% at all times evaluated. At the IC_{50} level, PMD and KTZ activities in the respective-R strain was up to ten times lower than the WT strain. No cross-resistance between PMD, KTZ, MTF and PM was observed. The resistance was not compromised removing the drug pressure.

4 Conclusions

L. (V) braziliensis R lines to PMD, KTZ and KTZ-PMD were generated and some biological characteristics were discussed.

C1220 PHOTODYNAMIC THERAPY FOR THE TREATMENT OF AMERICAN CUTANEOUS LEISHMANIASIS: EVALUATION OF ASSOCIATION OF THERAPIES IN MICE INFECTED WITH LEISHMANIA (LEISHMANIA) AMAZONENSIS

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1 Background

American tegumentary leishmaniasis (ALT) is considerated by The Health World Organization a public health problem. The research for compounds with potential against Leishmania has increased, one of these is photodynamic therapy. Methylene Blue has the required characteristics to be used in photodynamic therapy and it is already used to some diseases. The aim of this study is to investigate the antileishmanial activity of Methylene Blue (0,5%) alone and in association with references drugs as Glucantime and amphotericin b in photodynamic therapy (PDT) on promastigotes forms of *Leishmania (Leishmania) amazonensis*.

2 Methods

The Ethics Committee on animal use of the Universidade Estadual de Maringá (process number 158/2013) approved this project. BALB/c mice were infected in right footpad with $5x10^5$ promastigotes/mL. The treatment (intraperitoneal and topic treatment) was made in two circles, the first circle started 45 days post-infection (irradiance 2,63 x 10^3 µWatt /cm^2) and the second 105 days post-infection (irradiance 4,27 x 10^3 µWatt /cm^2). Animals were divided into six groups: Glucantime (100mg/kg/dia for 20 days five days a week) in association with PDT (30 min lightly with red LED (Light Emitting Diode) two days a week after the topic treatment with Methylene Blue 0,5%); amphotericin b (5 mg/kg/day for 20 days five days a week) in association with PDT; PDT; Glucantime; amphotericin b and positive control(infected and non-treated). The first three groups after 105 days of infection were submitted to the second treatment circle (60 min lightly 3 days a week). Footpad edema was monitored every two weeks using plethysmometer and pachymeter. Lymph node and spleen were removed, weighed and diluted in serial dilution. After 7, 14, 21 and 28 days of incubation at 25ºC, samples were analysed for the presence of promastigotes. Title was the last dilution with at least one parasite. Number of parasites/g in tissue was calculated and results were compared between treated and non-treated animals.

3 Results

There were no reduction significantly in treated footpad edema in comparison to the positive control group, but the treatments with Glucantime in association with PDT, Glucantime, PDT and amphotericin b decreased significantly the parasite load in lymph node.

4 Conclusions

Although treatments did not improve footpad edema, they reduced the parasite load in lymph node. This supports further investigation of Methylene Blue as antileishmanial drug.

C1224 SCREENING METHODS FOR TESTING THE EFFICACY OF NEW DRUGS AGAINST LEISHMANIA INFANTUM

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1 Background

The drugs used against visceral leishmaniasis are expensive and with many side effects. Moreover, they do not totally cleared the infection and relapses are not uncommon. Research of new anti-*Leismania* drugs is necessary with new methods to rapidly screen new drug candidates.

2 Methods

To evaluate effect of different molecules on *Leishmania infantum*, different tests have been performed: a cytotoxicity test (alamar blue THP1), a test on promastigote form and another one on amastigote form

3 Results

The activity obtained in each case is compared with a negative control (DMSO) and a positive control: amphotericin B active against both forms of *L. infantum*.

4 Conclusions

Finally, the combination of these 3 tests, allows a fast and efficient screening, with low cost, of new molecules against *leishmania* parasites.

C1249 TREATMENT OF CUTANEOUS LEISHMANIASIS WITH THERMOTHERAPY IN BRAZIL: AND EFFICACY AND SECURITY STUDY

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1 Background

Pentavalent antimonials remain as the standard drugs for cutaneous leishmaniasis in Brazil. Its high cost, hard administration, long treatment time, toxicity and increasing morbidity are factors that limit the use of these drugs. The search for better therapy options is still a challenge. For this reason, we described the response to radiofrequency heat therapy while treating localized cutaneous leishmaniasis, as well as we assessed its safety and tolerability.

2 Methods

We conducted a non-comparative open label clinical trial at Natan Portella Tropical Diseases Hospital, in Teresina Piaui, Brazil, from April to September 2015, among 15 patients parasitologically confirmed to have cutaneous leishmaniasis**.** Patients with secondary infections in the injuries were previously treated with antibiotics. We exluded from the study patients with lesions in the oral or nasal mucous tissues, with over ten lesions, with lesions in the nose, lips or eyes or adjacent to these sites and immunosuppressed patients. A single radiofrequency heat therapy session (ThermoMed device) at 50ºC for 30 seconds was applied in the lesion and its edges. In patients with more than one lesion, only the largest one was initially treated. If after 30 days there was no evidence of healing, the smaller lesion was also treated with heat therapy. Clinic cure was defined as visible healing until three months after the treatment application. The patients were accompanied for six months and there was no follow-up loss.

3 Results

23 lesions in 15 patients were supervised and only two healed without being treated. Complete healing was achieved by 13 (86.7%) patients. The main described side effects were itching, burning sensation and pain. Only one patient presented blisters at the site of application. The new epitelial tissue had an excellent aspect in over half (60%) the patients. Hyperchromic scars were observed in 33,3% of the lesions and only one patients with two lesions evolved to hypochromia at the site of the treatment.

4 Conclusions

Localized cutaneous leishmaniasis can be safely and effectively treated with a single session of radiofrequency heat therapy, resulting in a good cosmetic result and without the toxicity and side effects usually found under conventional treatment with pentavalent antimonials.

C1289 CHARACTERIZATION OF CUTANEOUS LESIONS PRODUCED BY LEISHMANIA STRAINS IN BALB/C MICE TREATED WITH A TOPIC FORMULATION

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1 Background

Leishmaniasis is a zoonotic disease caused by species of the protozoan parasite of genus *Leishmania*, distributed in countries of tropical and subtropical zones. Clinical manifestations are generated by the presence of amastigote forms in macrophages of the dermis and posterior formation of small nodules to large painless plaques and disfiguring ulcers with regional adenopathy. Histopathological findings reveal alterations in dermis and epidermis that depend primarily on the leishmanial strain but also on disease stage. Currently available drugs are not ideal due that have many side effects. Topical treatments have been proposed like a better alternative especially in early cases of cutaneous leishmaniasis (CL). The aim of this study was to evaluate the histopathological features in tissues of infected mice by American cutaneous leishmaniasis treated with a topic formulation.

2 Methods

BALB/c mice was infected with *L.(V.) panamensis* and *L.(V.) braziliensis* promastigotes and evolution of lesions was observed for eight weeks. Mice were treated with a topical formulation containing miltefosine (f-MIL) and vehicle. After 0, 7, 15 and 20 days, animals were then euthanized and skin, liver, spleen and nodes lymph were collected. Tissues were processed and stained with hematoxylin and eosin. Histopathological features were analyzed.

3 Results

Eight weeks post infection nodular and ulcerative lesions were observed in mice infected with *L. (V.) braziliensis* but not by *L. (V.) panamensis* ones were nodular lesion were observed**.** Treatment with f-Mil decreased the size of the lesion area from 32.0 to 6.1 mm^2 and 23.0 to 0.0mm^2 in *L. (V.) panamensis* and *L. (V.) braziliensis*, respectively. Compared to vehicle-treated mice there was an increase in the lesions 25.0 to 50.2 mm^2. In the histopathological analysis, an increasemoderate of megakaryocytes in the spleen of the f-Mil treated mice compared with treated with the vehicle was determined. In the lesion skin of mice treated with f-Mil, no intracellular amastigotes were observed while in the mice treated with the vehicle a high amount of parasites and hyperkeratosis were observed. At the same condition of infection and treatment in *L.(V.) braziliensis* showed greater inflammatory infiltrate of mononuclear cells than *L.(V.) panamensis*.

4 Conclusions

Variability in the histopathological features and skin lesion evolution was demonstrated at the beginning and end of topical treatment f-MIL and vehicle of BALB/c infected with *Leishmania* strains.

C1308 TREATMENT OF CUTANEOUS LEISHMANIASIS WITH FLUCONAZOLE IN BRAZIL: A RANDOMIZED AND CONTROLLED TRIAL.

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1 Background

The treatment of cutaneous leishmaniasis (CL) caused by *L. braziliensis* in Brazil by pentavalent antimony (Sb^v) is associated with up to 45% of cure failure. Additionally, Sb^v is only administered by parenteral route with important toxicity. An effective, safe and oral treatment for CL is required.

2 Methods

A randomized and controlled clinical trial to compare the efficacy and safety of oral Fluconazole in high dosage (6.5-8.0mg/kg for 28 days) versus standard Sb^v (20mg/kg/day for 20 days) in the treatment of CL in Bahia, Brazil.

3 Results

A total of 53 subjects were included in the trial. Of these, 26 were treated with Sb^v, and 27 were treated with Fluconazole.Intention to treat analysis showed the initial cure rate (2 months after treatment) of 22.2% (6/27) in Fluconazole group and 53.8% (14/26) in Sb^v group (P = 0.037). Six months after treatment, the final cure rate remained the same in both groups, without any relapses. The occurrence of side effects in the Sb^v group and Fluconazole group was similar, 34.6% versus 37% respectively. One patient treated with Fluconazole discontinued treatment due to malaise, headache and moderate dizziness.

4 Conclusions

Oral fluconazole at a dosage of 6.5- 8mg /kg/ day for 28 days should not be considered an effective treatment for CL caused by *L. braziliensis*.

C1319 IMPACT OF CUTANEOUS LEISHMANIASIS IN DAILY LIFE OF COLOMBIAN PATIENTS: A QUALITATIVE STUDY

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1 Background

Treating cutaneous leishmaniasis (CL) often involves the administration of parenteral antimonial drugs which may cause side effects and have a negative impact on daily activities. Additionally, access to treatment in rural and/or remote endemic areas remains a challenge due to multiple economic, social and geographic barriers. We aimed to explore patients' perceptions about impact of disease and treatment in everyday life, as this understanding might provide useful information to improve case management.

2 Methods

A qualitative study using semi-structured in-depth interviews was conducted with patients with confirmed CL at two sites in south-west Colombia (Cali and Tumaco), as part of an international multicentre project exploring disease experiences in CL patients. Participants were selected based on a purposive, maximum variation sampling approach along a defined patient spectrum covering e.g. *Leishmania* species, age and gender, and clinical presentation. All patients provided informed consent.
The interviews followed a collaboratively developed topic guide. Thematic analysis of translated transcripts was based on a coding framework, developed based on initial interviews, using the OSOP (one sheet of paper) method and NVivo® software. Analysis focussed on elements related to everyday life with CL and related topics, including treatment accessibility and impact of disease in salary, employment and relations with others.

3 Results

A total of ten patients at different status of treatment were enrolled (seeking treatment, during and after treatment). Reported antileishmanial treatments included miltefosine (3 patients) and meglumine antimoniate (6 patients). Ages ranged between 18 and 52 years, and patients were predominantly men (8/10). Patients identified important impacts on their lives related to the disease including work loss and cost for transportation to seek diagnosis and treatment, limitations on daily activities related to treatment side effects, and having to spend time away from home while receiving treatment.

4 Conclusions

This study points to the considerable impact of CL on patients' lives which is not only related to the clinical presentation but also to delivering a parenteral treatment. These perspectives should be taken into account when developing drugs and strategies for drug delivery.

C1322 LIPOSOMAL AMPHOTERICIN B (AMBISOMEÆ): NEW PROTOCOL OF TREATMENT FOR MEDITERRANEAN VISCERAL LEISHMANIASIS IN ADULTS BY 10 MG/KG FOR 2 CONSECUTIVE DAYS.

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1 Background

Visceral Leishhmaniasis (VL) due to *Leishmania infantum* is endemic in South of Europe. To date, the first line treatment is Liposomal amphotericin B (AmBisome®) (L-AmB). Several protocol have been described for this drug administration with a total dose of 18 – 21 mg/kg recommended. In children, 2 consecutive doses of L-AmB at 10 mg/kg each has been proven to be effective and cost saving to treat VL. Though, this drug has several adverse effects as nephrotoxicity, hypokalemia and myocarditis leading physicians to be very cautious in using high single dose in adult population.

2 Methods

Eleven patients (8 males, 3 females) with VL (9 patients) or Mucosal leishmaniasis (2 patients) due to *L. infantum* were treated by 10 mg/kg of L-AmB on 2 consecutive days. Among these patients, 3 were HIV positive and for the 8 remaining patients, no immunodeficiency was present at the time of diagnosis.

3 Results

For 9 patients (2 HIV positive and 7 non immunocompromised patients), this treatment protocol was effective without any adverse effect and relapse post treatment. One patient experienced renal failure after the first dose of 10mg/kg and treatment was changed for 3 mg/kg from the second to the fourth day of treatment. One HIV patient never restores his immune system despite HAART and was considered presenting active chronic visceral leishmaniasis. Thus, this patient was treated by several protocol of L-AmB (from 4 mg/kg to 10 mg/kg) each 15 days. Despite these regular cures, patient died 4 years later.

4 Conclusions

The protocol of treatment by 10 mg/kg of L-AmB on 2 consecutive days was effective for 9 out of the 11 patients treated for leishmaniasis. None of this patients relapsed. As already described, one patient experienced renal failure but same drug with lower dose was pursue with effectiveness. For one HIV patient with active chronic visceral leishmaniasis, both treatments HAART and anti *Leishmania* failed despite several repeated cures and good observance of HAART treatment.
Thus, two doses of 10 mg/kg of L-AmB on 2 consecutive days appear a good alternative in term of effectiveness and cost saving for leishmaniasis with the exception of patients with active chronic visceral leishmaniasis. For these patients treatment is challenging with a struggle between the immune system and the parasite. To our knowledge, difficulties remain to define a good treatment protocol to totally cleared patients in active chronic visceral leishmaniasis from the parasite.

C1332 ANTILEISHMANIAL ACTIVITY OF THE NITRIC OXIDE AND NITROXYL DONOR DEA/NO, NITROXYL DONOR WR014614 (R1-HNOH) AND WR167996 (R2-HNOH) IN-VITRO

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1 Background

Leishmaniasis is endemic in 92 countries, with 350 million people at risk of becoming infected. More than 10 million people are suffering from cutaneous leishmaniasis and over 1.5 million new cases are diagnosed annually. The life cycle of *Leishmania* protozoa involves two stages: the promastigote that exists in the vector sand fly, and the amastigote that reproduces in macrophages of the host. Curative effect of Nitric Oxide (NO) in therapy for cutaneous leishmaniasis has been proven *in vitro* and *in vivo.*

2 Methods

The aim of this investigational study was to investigate *in vitro* the antileishmanial activity of the Nitric Oxide donor Diethylammonium (Z)-1-(N,N-diethylamino)diazen-1-ium-1,2-diolate (DEA/NO, $C_4H_{11}N_3O_2 \cdot C_4H_{11}N$), WR014614, and WR167996. DEA/NO and WR014614 (R^1-HNOH) in pH 7.4 solution form NO and/or Nitroxyl (HNO). HNO is the reduced form of NO and similarly to NO it has potential applications in medicine. WR167996 (R^2-HNOH) is a 3-electron reduced form of NO. We investigated the effect of these compounds against two species of the *Leishmania* parasites: *L. major* (Old World) and *L. panamensis* (New World). The promastigotes were cultured in Schneider's Insect Medium supplemented with fetal bovine serum (FBS) at 22°C, and the amastigotes in macrophages were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with FBS at 37°C under 5% CO_2 atmosphere.

3 Results

The tested compounds killed promastigotes and amastigotes in a time- and dose-depended manner. DEA/NO (400µg/ml) completely suppressed parasitemia in freshly prepared solution of macrophages and promastigotes (1:10 ratio of macrophages to promastigotes). The IC_{50} for WR014614 and WR167996 against promastigotes was 200 and 40µg/ml, respectively, while the IC_{50} for WR014614 against amastigotes in macrophages was less than 1µg/ml.

4 Conclusions

This study identified two classes of chemicals with the potential to be used as treatment of cutaneous leishmaniasis.

C1344 MODULATION OF EXTRACELLULAR MATRIX PROTEIN AND CYTOKINE EXPRESSIONS BYMEGLUMINE ANTIMONIATE IN MURINE CUTANEOUS LEISHMANIASIS

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1 Background

Meglumine antimoniate (MA) is the first-line drug for the treatment of cutaneous leishmaniasis. While its antileishmanial activity and immunomodulation action have been extensively described, little data exists regarding the effect of MA on extracellular matrix proteins and tissue repair. The present study aimed to examine the modulation of the extracellular matrix proteins and cytokine expression in C57BL/6 mice infected with *Leishmania amazonensis* and treated for 30 days with MA at 20 mg/kg.

2 Methods

Four to six week old female C57BL/6 mice 4-6- were obtained from the Instituto de Ciência e TecnologiaemBiomodelos(the Institute of Biomodel Science and Technology) (ICTB/FIOCRUZ) and maintained under pathogen-free conditions, at a controlled temperature and with food and water *ad libitum*. Infection was carried out with 10^4 amastigote forms of *Leishmania amazonensis* subcutaneously injected into the right hind footpad of C57BL/6 mice. The treatment protocol was performed with 3 groups of 5 animals, as follows: MA group, mice infected with 10^4 amastigotes *L. amazonensis* and treated with meglumine antimoniate (donated from the Instituto Nacional de Infectologia Evandro Chagas (the Evandro Chagas National Infectology Institute), Fiocruz, Department of Health, Brazil) at 20mg/kg of pentavalent antimonial (Sb^{+5}) by intramuscular injection, twice a week; La group, mice infected with 10^4 amastigotes of *L. amazonensis* and untreated; and Control group (Ctrl), non-infected and non-treated mice. Treatment started 55 days after infection. After 30 days of treatment the animals were euthanized and blood, footpad, draining lymph nodes and liver fragments were collected for parasite load quantification by qPCR, histopathology, extracellular matrix protein and cytokines expression analysis by qPCR.

3 Results

MA treatment reduced the parasite load in the footpads and draining lymph node. Treatment allowed an enhancement in laminin and type I and III collagen expression in skin lesions, leading to a reorganization of collagen fibers. The lymph nodes of treated mice exhibited a significant over expression of TNF-α, IL-10 and IFN-γ, as well as a strong upregulation of IL-12 associated with a low parasite load.

4 Conclusions

It was concluded that MA, in addition to its leishmanicidal and immunodulatory activities, modulates extracellular matrix components of the skin contributing to effective tissue repair and the clinical remission of the lesion in murine cutaneous leishmaniasis.

C1369 LIPID-BASED DELIVERY SYSTEMS FOR DINITROANILINES IN THE TREATMENT OF L. MAJOR SKIN LESIONS

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1 Background

Cutaneous Leishmaniasis (CL) is considered the most serious protozoan skin infection in many developing countries for which therapy remains inadequate. Thus, the development of alternative treatments is very urgent. Dinitroanilines are tubulin-binding agents used as herbicides with reported anti-leishmanial properties due to their specific mechanism of action and low toxicity in mammals. Some unfavorable physicochemical properties have compromised their development as antiparasitic agents. Association of drugs with lipid-based delivery systems is a strategy used to reduce the undesirable effects and may also enhance efficacy. Another strategy is the chemical modification of the molecule to modulate its physical and chemical properties. The work reported discusses the use of lipid-based delivery systems for trifluralin (TFL) and a hemi-synthetic analogue (TFL-A) to secure their stabilization; enable the delivery of higher concentrations; provide sustained release and selective targeting to skin lesions caused by *L. major* parasites. Different routes of administration were also addressed.

2 Methods

Based on the properties of TFL or TFL-A and the route of administration; conventional liposomes (Lip), long circulating liposomes (LCL) and ethosomes (ETS) formulations were developed by appropriated methods. All formulations were characterized for vesicle size, surface charge, drug load and other relevant incorporation parameters. Their *in vitro* properties and behavior against *L. major* parasites were evaluated using appropriated assays. The *in vivo* anti-leishmanial activity was assessed in a murine model of CL.

3 Results

All lipid formulations were stable, drug loads were high and other incorporation parameters were as expected. The *in vitro* assays confirmed that formulations kept the anti-leishmanial activity of the free drug and hold the desired properties for administration by different routes. In the *in vivo* studies Lip and LCL formulations were administered by i.v., i.p. and s.c. routes while ETS were applied topically. All formulations demonstrated a reduction of *L. major* skin lesion sizes in all treated groups compared to the control.

4 Conclusions

Lipid-based delivery systems may contribute to the treatment of *L. major* skin lesion by enhancing the efficacy of the anti-leishmanial drugs. Less invasive routes of administration can create new opportunities for local treatment.

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C1385 SAFETY AND EFFECTIVENESS OF SODIUM STIBOGLUCONATE AND PAROMOMYCIN COMBINATION FOR THE TREATMENT OF VISCERAL LEISHMANIASIS IN EASTERN AFRICA: RESULTS FROM A PHARMACOVIGILANCE PROGRAM

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1 Background

In 2010, WHO recommended a new first line treatment for visceral leishmaniasis (VL) in eastern Africa. The new treatment, a combination of IV or IM sodium stibogluconate (SSG) and IM paromomycin (PM) was an improvement over SSG monotherapy, the previous first line VL treatment in the region. To monitor the new treatment's safety and effectiveness in routine clinical practice a pharmacovigilance (PV) program was developed.

2 Methods

A prospective PV cohort was developed. Regulatory approval was obtained in Sudan, Kenya, Uganda and Ethiopia. Twelve sentinel sites sponsored by Ministries of Health, Médecins Sans Frontières (MSF) and Drugs for Neglected Diseases *initiative* (DNDi) participated. VL Patients treated using the new treatment were consented and included in a common registry that collected demographics, baseline clinical characteristics, adverse events, serious adverse events and treatment outcomes. Six monthly periodic safety update reports (PSUR) were prepared and reviewed by a PV steering committee.

3 Results

Overall 3,126 patients were enrolled; 1,962 (62.7%) from Sudan, 652 (20.9%) in Kenya, 322 (10.3%) in Ethiopia and 190 (6.1%) from Uganda. Patients were mostly male children (68.1%, median age 11years) with primary VL (97.8%). SSG&PM initial cure rate was 95%, no geographical differences were noted. HIV&VL co-infected patients and patients older than 50 years had initial cure rate of 56% and 81.4% respectively while 1,063 (34%) patients had at least one adverse event (AE) during treatment while 1.92% (n=60) had a serious adverse event (SAE) with a mortality of 1.0% (n=32). There were no serious unexpected adverse drug reactions.

4 Conclusions

This first regional PV program in VL supports SSG&PM combination as first line treatment for primary VL in eastern Africa. SSG&PM was effective and safe except in HIV&VL co-infected or older patients. Active PV

surveillance of targeted safety, effectiveness and key VL outcomes such us VL relapse, PKDL and HIV/VL co-infection should continue and PV data integrated to national and WHO PV databases.

C1386 TAMOXIFEN AND MEGLUMINE ANTIMONIATE COMBINED THERAPY IN CUTANEOUS LEISHMANIASIS PATIENTS: A PILOT STUDY

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1Background

The first choice drug to treat cutaneous leishmaniasis caused by *Leishmania braziliensis* in Brazil is pentavalent antimony (Sb^V), which is associated with up to 45% failure rates. Combination therapy is now advocated for leishmaniasis treatment with the aim of increasing efficacy and reducing toxicity, cost and the chance of resistance selection. Tamoxifen, an oestrogen receptor modulator in use for the treatment of breast cancer, has been shown to possess antileishmanial activity against several *Leishmania* species, in vitro and in experimental models of leishmaniasis. The aim of this study was to evaluate the efficacy of the association of tamoxifen and meglumine antimoniate in a phase II pilot clinical test in localized cutaneous leishmaniasis patients.

2 Methods

A randomized controlled clinical trial was conducted to compare the efficacy and safety of oral (40 mg/day for 20 days) or topical tamoxifen (0.1% tamoxifen citrate) combined with meglumine antimoniate (20 mg Sb^V/kg/day for 20 days) *versus* a standard Sb^V protocol (20 mg/kg/day for 20 days) for the treatment of cutaneous leishmaniasis.

3 Results

A total of 30 subjects were included in the trial; 10 were treated with Sb^V, and 20 with the combination of tamoxifen and Sb^V. Of the patients treated with the combination scheme, 10 received tamoxifen orally and 10 were treated with topical tamoxifen. All patients have now been enrolled and treated. Tamoxifen administered by the oral or topical routes was well tolerated. The initial and final cure rates (2 and 6 months after treatment, respectively) are under evaluation and will be presented.

4 Conclusions

Results of this trial will determine whether tamoxifen is a viable drug candidate for leishmaniasis treatment.
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C1389 IN VITRO AND IN VIVO LEISHMANICIDAL ACTIVITY OF BACCHARISTOLA PHIL. AND DITERPENES DERIVATED

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1 Background

Baccharis tola Phil. is recognized in several countries of South America as a medicinal plant due to its antibacterial and anti-inflammatory properties. The *Ent*-beyerene diterpenoids has been studied as antimicrobial agents but antiparasite has not been yet evaluated.In the present work, we report the *in vitro* and *in vivo* antileishmanial effects of two isomeric *ent*-beyer-15-en-18-ol compounds (T1 y T2) as well as four derivatives.

2 Methods

Pure substances of the diterpenoid type were getting of the ethanolic extract of *Baccharis tola,* then the structural modification of these active compounds to improve their activity. *In vitro* leishmanicidal activity in intracellular amastigotes of *L. panamensis* was evaluated by flow cytometry and cytotoxicity in U937 cells was evaluated by the MTT method. Evaluation of *in vivo* toxicity and leishmanicidal activity carried out in the hamster model for cutaneous leishmaniasis.

3 Results

The compound showed high activity *in vitro* with EC_{50}<50µM, the structural modification did not improve the activity. The efficacy in hamsters resulted in notable reduction of the size of skin lesions obtained a 50% clinical cure and 50% clinical improvement with individuals treated with T1 compound. For the other diterpenoids a significant reduction of lesion size oscillating between 52-77% from the end of the study was observed.

4 Conclusions

Structural transformations in the double bound (hydrogenation, epoxidation) did not change the activity profile, but oxidation of the hydroxymethyl group practically abolished the leishmanicidal activity. According to this, a new pharmacophore could be derived from this natural product skeleton to obtain molecules to evaluation in the animal model.

C1401 DRESS SYNDROME TO GLUCANTIME: RARE EVENT

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1 Background

Pentavalent antimonials remain the first line therapy for treatment of visceral, cutaneous, or mucosal leishmaniasis in many areas of the world. Systemic administration of pentavalent antimonials has been associated with severe adverse events. To our knowledge, drug hypersensitivity syndrome, also referred to as drug reaction with eosinophilia and systemic symptoms (DRESS), was rarely associated with antimonial drugs. This the second case reported in the literature. Pentavalent antimonials remain the first line therapy for treatment of visceral, cutaneous, or mucosal leishmaniasis in many areas of the world. Systemic administration of pentavalent antimonials has been associated with severe adverse events. To our knowledge, drug hypersensitivity syndrome, also referred to as drug reaction with eosinophilia and systemic symptoms (DRESS), was rarely associated with antimonial drugs. This the second case reported in the literature.

2 Methods

We report a case of DRESS induced by parenteral meglumine antimoniate (Glucantime)

3 Results

A 67-year-old women who was treated for mucocutaneous leishmaniasis. Two weeks after starting therapy, the patient had joint pain, a cutaneous eruption, and hypereosinophilia (1,300 cells/mm3), without increase liver enzyme, pancytopenia, renal and hepatic failure . The cutaneous biopsy confirmed the diagnosis of medical skin toxicity.These symptoms were resolved after drug withdrawal. The Glucantime was replaced by mitronidazole. The pharmacologic policy and skin tests permormed later confirmed the imputability of Glucantime in this event. Pentavalent antimonials should be definitively withdrawn in patients with hypereosinophilia > 1,000 cells/mm3accompanied by systemic manifestations consistent with DRESS.

4 Conclusions

it is important to recognize such reactions because their appearance justifies immediate and definitive withdrawal of pentavalent antimonial drugs to prevent potentially severe visceral complications

C1407 IN VITRO ACTIVITY AGAINST LEISHMANIA AMAZONENSIS, CYTOTOXICITY AND CHEMICAL COMPOSITION OF ESSENTIAL OILS

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1 Background

The current chemotherapy for cutaneous leishmaniosis (CL) has a series of drug limitations such as toxic side effects, long duration, high costs and drug resistance, requiring the development of new drugs or effective alternatives to the CL treatment. Essential oils (EOs) are complex mixtures of secondary metabolites from various plants. It has been shown that several EOs, or their constituents, have inhibitory activity against protozoa. Thus, this study aims to evaluate the biological activity of different essential oils (EOs) on *Leishmania (L.) amazonensis* promastigotes forms, as well as their cytotoxicity on mammalian cells and chemical composition.

2 Methods

Six EOs were evaluated by mean of IC50/24h on *Leishmania (L.) amazonensis* promastigotes forms and cytotoxicity against L6 cells (CC50/24h), using Rezazurin assay. Only those EOs that presented better results for IC50/24h were submitted to GC–MS analysis to determine their chemical constitution.

3 Results

The EO of *Matricaria chamomilla*, *Myroxylon peruiferum*, *Salvia sclarea*, *Bulnesia sarmientoi*, *Ferula galbaniflua* and *Melissa officinalis* were active against *L. amazonensis* with IC50/24h ranging from 54.05 to 162.25 µg/mL. Evaluation of cytotoxicity showed that the least cytotoxic EO was that of *F. galbaniflua* (CC50/24h = 377.26 µg/mL), followed by *S. sclarea*, *M. officinalis*, *M. chamomilla*, *B. sarmientoi* and the most cytotoxic EO was of the one from *M. peruiferum* (160.80 µg/mL). The cytotoxicity against L6 cells and *L. amazonensis* were compared using the selectivity index and the EOs with higher selectivity indexes were those from *F. galbaniflua* (SI = 3.94), *M. chamomilla* (2.87) and *M. officinalis* (2.25). Analysis of EOs by GC–MS showed mainly the presence of β-farnesene (52.73 %) and bisabolol oxide (12.09 %) for *M. chamomilla*; α-copaene (13.41 %), safrole (8.35 %) and δ-cadinene (7.08 %) for *M. peruiferum*; linalool (28.80 %) and linalyl acetate (60.08 %) for *S. sclarea*; guaiol (48.29 %) and 2-undecanone (19.49 %) for *B. sarmientoi*; ethyl phthalate (13.09 %) and methyl-8-pimaren-18-oate (41.82 %) for *F. galbaniflua*; and neral (37.18 %) and citral (5.02 %) for *M. officinalis.*

4 Conclusions

The EO from *F. galbaniflua* proves to be effective against *L. amazonensis* promastigotes forms, and has low cytotoxic activity. Thus, it represents a strong candidate for future studies aiming its molecular activity on these pathogenic parasites.

C1439 ACUTE GENERALIZED EXANTHEMATOUS PUSTULOSIS DUE TO MEGLUMINE ANTIMONIATE (GLUCANTIME).

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1 Background

Cutaneous leishmaniasis is endemic in many rural areas in Tunisia. It can be caused by several *Leishmania* spp and is transmitted to human and animals by sandflies. Acute generalized exanthematous pustulosisis (AGEP) is a severe cutaneous adverse reaction characterized by the rapid development of non follicular sterile pustules caused in the majority of cases by drugs. AGEP induced by Glucantime is rarely reported in literature

2 Methods

we report, here, a case of treated cutaneous leishmaniasis complicated by glucantime-induced AGEP.

3 Results

A 52 year old female diagnosed with cutaneous leishmaniasis confirmed parasitologicaly by Giemsa staining (direct smear) and PCR. She was treated with progressive doses of Glucantime. Four days after, She presented a maculopapular rash with small, sterile pustules on an erythematous base in the trunk, and limbs. No mucosal lesions were found in the clinical examination. The laboratoy signs were normal and the viral serologies were negative. The diagnosis of an AGEP was suggested and confirmed by histological examination of a skin biopsy wich revealed an intracorneal, subcorneal and intraepidermal pustules located in the majority in the upper epidermis with papillary dermal edema containing neutrophilic, lymphocytic and eosinophilic infiltrates. Glucantime was stopped and the outcome was good.

4 Conclusions

The diagnosis of AGEP depends on clinical and histologic criteria. The main incriminated drugs are pristinamycin and aminopenicillins. To our knowledge, there was no reported case of a Glucantime-induced AGEP.

C1444 CLINICAL EFFICACY AND TOLERANCE OF MILTEFORAN® IN THE TREATMENT OF CANINE VISCERAL LEISHMANIASIS IN NATURALLY INFECTED DOGS WITH L. INFANTUM (SYN CHAGASI).

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1 Background

In 2007, miltefosine (MIL) was registered in Europe under the brand name of Milteforan® (Virbac, France) for the control of dogs with canine leishmaniasis. More recently, Milteforan® has been registered for its use in Brazil. The aim of this study was to assess the efficacy and tolerance profiles of Milteforan® in dogs with natural Visceral Leishmaniasis (VL) under controlled conditions, by analysing clinical and infection progress, and measuring haematological and biochemical parameters in dogs under treatment.

2 Methods

Dogs naturally infected with *L. infantum,* anddiagnosed as suffering from VL *w*ere included and housed in a kennel. All the dogs received the drug orally at the standard dose of 2mg/kg b.w. once a day for 28 days. During the 12-week follow-up, efficacy was assessed through the reduction of the Clinical Score (CS) and reduction in the parasite load measured by qPCR (at W0, W6, W12) in lymph nodes, bone marrow aspirates, and skin biopsies.Safety was assessed by evaluating the kidney function (BUN, Creatinine), liver function (AST, ALT, GGT), haemogram, total proteins, albumin, and albumin-globulin ratio.

3 Results

36 dogs were included (19 males and 17 females; various breeds; aged 2-5 years). Medical examination, weighing and blood sampling were performed before starting therapy (W0), and then every 14 days for the all duration of the trial (W: 2, 4, 6, 8, 10, 12). For each dog, a CS was established at each time. Over the follow-up period, the dogs showed a weight gain, and a statistically significant decrease of CS ($p<0.001$). In the animals showing a reduction in the CS (as early as W2 for some of them, or W4 for most of them), the average percent of reduction was 70% (44-100%). A correlation was observed between clinical improvement and parasite reduction in BM, LN aspirates, and in skin biopsies. There was a statistically significant increase in the number of dogs that became negative over time. There was no significant change in kidney parameters (within normal ranges), liver function (normal ranges), or proteinogram during the all observation period.

4 Conclusions

This study demonstrated a significant clinical improvement of the treated dogs, and a strong correlation between clinical improvement and reduction of parasite load (leishmanicidal activity of the drug). In parallel, Milteforan® was well tolerated.

C1446 EVALUATION OF THE POTENTIAL OF INFECTIVITY OF DOGS WITH VISCERAL LEISHMANIASIS AND TREATED WITH MILTEFOSINE BY USING A XENODIAGNOSIS

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1 Background

Various authors believe that the reduction in or the absence of clinical manifestations in leishmanian dogs could reduce the infectivity of phlebotominae (Travi et al., 2001; Courtenay, 2002). In this study, the clinical benefit of Milteforan® (Virbac, France) was compared with xenodiagnosis.

2 Methods

Dogs naturally infected with *Leishmania infantum,* anddiagnosed as suffering from Visceral Leishmaniasis(VL) were included in the study and maintained in a test kennel built with 3 screened divisions for phlebotomine entrance blocking. A CDC trap was placed in the kennels in order to observe any possible insect invasion. Visual inspection of the screens was also daily performed. All the dogs received Milteforan® orally at the standard dose regimen of 2mg/kg b.w. for 28 days. To avoid any interference in the xenodiagnostic exam, no ectoparasiticide was administered. Efficacy criterion was the decrease in transmission through the reduction or absence of the number of promastigote variants in the gut of the vector submitted to xenodiagnosis.

3 Results

36 dogs with VL were randomly subdivided into 3 groups of twelve, all receiving Milteforan®. At W0 (before treatment) and W12 (3 months after the end of treatment), the dogs were sedated and exposed to phlebotomines (*L. longipalpis phlebotominae* colony) for 60 minutes. Then, anesthetised *phebotomina*e were dissected for parasite count in the insect intestine. Quantity of females infected and not infected was determined and intensity of infection was categorized according to quantity of parasites (weak to strong infection). At W0, 51.4% of the dogs were infectious for the *phlebotominae* while 74.2% of them were negative at W12, amongst which 92% also showed a strong reduction in CS (average reduction 67%). Of the 9 dogs positive in the xenodiagnosis at W12, 89% of them had a high CS at W0, but all of them showed a reduction in CS at W12 (average 66%). The results observed in the xenodiagnosis were correlated to the CS and qPCR results in the NL, BM and skin samples.

4 Conclusions

This study allowed to demonstrate a significant clinical improvement of the treated dogs with Milteforan®, linked to a reduction of parasite load in the BM, NL and skin samples, highly correlated to the xenodiagnosis, making 74.2% of the animals non-infectious for the *phlebotominae*.

C1456 BIOASSAY GUIDED ISOLATION AND IDENTIFICATION OF ANTILEISHMANIAL COMPOUNDS FROM TUNISIAN ROSMARINUS OFFICINALIS EXTRACT

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1 Background

Since the past, Medicinal and Aromatic Plants (MAP) have been used in traditional medicine to treat several illnesses, and up to now they still the most importanthealth care source for several countries around the world. Extracts and essentials oils, obtained from those plants have been widely used in the pharmaceutical, foods and cosmetic industries. Recently, studies on the evaluation of the biological activities of some MAP species have confirmed their interesting pharmacological benefits. Rosemary plant (*Rosmarinus officinalis*) known for its diverse biological properties and its richness for active compounds was investigated, in the present study, for its activity against *Leishmania amazonensis.*

2 Methods

Hexanic extract was fractionated by different column chromatography on Sephadex LH-20 and/or Silica gel. The process was repeated for sub-fractions until active compounds were obtained. In vitro leishmanicidal activity was evaluated using the Alamar Blue method against two different genus of the parasite: *Leishmania amazonensis.*

3 Results

The hexanic fraction, obtained by liquid- liquid bipartition, was the most active extract against *Leishmania amazonensis*. Consequently, this fraction was submitted to a bio-guidedfractionation. The collected fractions from each column chromatography were combined on the basis of their TLC profiles and leishmanicidal activity. The bio-guided fractionation led to the isolation and identification of an active diterpene the *Carnosol.*

4 Conclusions

The MAP evaluated in this study could be used for the development of novel therapeutic approaches against *Leishmania* infections. Meanwhile, further studied are recommended to investigate the mode of action of this compound against the parasite tested.

C1468 EFFICACY OF DODAB NANOPARTICULES ON LEISHMANIA PROMASTIGOTES VIABILITY

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1 Background

Current treatment against *Leishmania* presents several problems specially related the high cost of drugs and also the emergence of drug resistant parasites. Thus, searching of new alternative chemotherapeutic methods have become imperative to control leishmaniasis. The main objective of this study was to evaluate the effect of DDAB nanoparticules on the viability and multiplication of stationary phase promastigotes of *Leishmania (L.) amazonensis*

2 Methods

The efficacy of DODAB on the viability of *Leishmania* promastigotes was performed by two recognized methods. First, samples were incubated with propinium iodate (PI) after treatment with different concentrations of DODAB. The incorporation of PI in dead parasites was visualized by time lapse on immunofluorescence microscopy (DMI6000) and quantified by the number of stained and unstained nuclei parasites. In the same procedure, MTT assay was performed to verify the efficacy of different concentrations of DDAB. *Leishmania* growth was quantified by the incubation of treated parasite in complete media on different days. Electronic microscopy was used to visualize parasite structures after treatment. To confirm the anti-leishmanial effect of DODAB, treated parasites were allowed to infect murine macrophage to quantify intracellular proliferation

3 Results

When *Leishmania* promastigotes are treated with DODAB 20 mM for 90 min, 50% of parasite are PI(+), obtaining more than 60% PI(+) at 120 min.Treatment ofDODAB at 20 mM kills 33% of *Leishmania* promastigotes reaching 92% at 200 mM by MTT assay. These experiments showed DODAB presenting an IC_{50} of 25 mM. At 20 mM of DODAB, few parasites can grow in complete media but at 200 mM, no viable *Leishmania* promastigote is detected on the media. Microscopic visualization showed loss of internal parasite structures at different drug concentrations, with complete destruction of typical structures (nuclei, kinetoplast and flagellum) when parasites are treated with 200 mM of DODAB. Finally, treatment with 20 mM of DODAB is sufficient to impair intracellular parasite replication in murine macrophages

4 Conclusions

DDAB treatment by itself was able to affect *Leishmania* promastigotes (survival and replication) and future experiments may be considered to explore this anti-*Leishmania* activity as an alternative low cost treatment for human population in conditions of poverty and to control dog infections in endemic areas

C1472 ANTI-LEISHMANIA ACTIVITY EVALUATION OF METALS NANOPARTICLES

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1 Background

Treatments against American Cutaneous Leishmaniasis (ACL), an endemic parasitic disease in several countries, uses drugs as pentavalent antimony or diamidines presenting efficiency varies according to the species of *Leishmania* involved in the infection, but have high toxicity, manifested in significant adverse effects with hepatic and renal impairment in some situations.Nanotechnology is enabling the use of new active principles for the treatment of parasitic diseases, from new approaches using antimony to the use of non-toxic metals as active principles of nanoformulations. The literature describes the effects of nanoparticles of silver, titanium, zinc and gold against *Leishmania.* The aim of this study was to evaluate the action of two metal nanoparticles (X and Y) against amastigotes of *L. amazonensis* in murine macrophages (*Mus musculus*), being identified in this study as NP-X and NP-Y.

2 Methods

Macrophages were obtained from peritoneal lavage and subsequent culture in RPMI medium (10% iFBS) and the infection was from the culture of promastigotes forms of *L. amazonensis*in stationary phase. The treatments were performed at three different concentrations (0.5, 0.25 and 0.125 mmol/mL), in addition to negative control (NC) and positive control (PC), saline and Glucantime® respectively, for 24 hours. 200 cells of each replicate were analyzed for a total of three replicates, and the mean number of parasite cells was titrated as Infection Rate (IR) for comparison purposes with the controls. After the treatment it was observed that in the highest concentration there was precipitation of the nanoparticles, which may explain the IR of 35.33% and 41.67% for NP-X and NP-Y in the highest concentration, respectively, while CN was 43.67% and CP 37.33% (higher concentration).

3 Results

The results were more promising in the intermediate concentration of CP (31.33%) and NP-Y (24.67%). It is important to observe that the two nanoparticles achieved similar results to Glucantime®, treatment recommended by the WHO, with the exception of NP-X at 0.5 mmol/mL, which was similar to CN, probably due to precipitation. In preliminary analysis, low toxicity of the nanoparticles was evidenced against healthy murine cells, but the values ??did not differ significantly in relation to Glucantime®.

4 Conclusions

The studies should be deepened, requiring monitoring for longer exposure times, as well as other methodologies for assessing leishmanicidal and cytotoxic activity.

C1473 DEVELOPMENT AND CHARACTERIZATION OF IRON OXIDE NANOPARTICLES FOR THE TREATMENT OF CUTANEUS LEISHMANIASIS FOR HYPERTHERMIA BY SHORT WAVES

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1 Background

Leishmaniasis is a pathology that affects around 12 million people worldwide, being prevalent in 5 continents and considered endemic in 98 countries. The cutaneous form is the most common and can be caused by approximately 20 species. In Brazil, the antimony drug is used as the first choice in the treatment of leishmaniasis. After intravenous administration, it is rapidly absorbed and almost 90% of the antimony is excreted within the first 48 hours by the kidneys. As a consequence, the administration of high doses is indispensable, which generates many side effects, including cardiorespiratory and renal alterations. In this sense, the general objective of this work was the development of pure maghemite nanoparticles and coated with glucuronic and fructose acid, shortwave, aiming to generate an innovative treatment protocol, via hyperthermia for the treatment of cutaneous leishmaniasis lesions.

2 Methods

In addition to the synthesis of nanoparticles, characterization tests were performed, including field emission gun microscopy (FEG); X-ray diffraction; Fourier-transformed infrared spectroscopy (FTIR); Möusbauer spectroscopy and magnetic measurements (ZFC). Short-wave parameter definition tests and *in vitro* cell viability assays, with and without the use of short waves, were also performed.

3 Results

The results of the FEG revealed a well ordered structure of nanoparticles, with an average diameter of 80nm. X-ray diffraction data, together with Möusbauer, indicated the oxidation state and crystalline phase of the iron oxide compatible with a pure sample of maghemite. The FTIR measures indicated adsorption of the binders to the nanoparticles, which gives them stability. The measurements of ZFC magnetization indicate a magnetic material, which when exposed to an alternating electromagnetic field will allow hyperthermia. Finally, the *in vitro* assays indicated a real leishmanicidal effect when the pure or coated nanoparticles were exposed to the alternating magnetic field of the short-wave for 30 minutes, which generated hyperthermia and consequent death of the pathogen.

4 Conclusions

The application of the proposed treatment method indicated a high potential of use for the treatment of cutaneous leishmaniasis via short-wave hyperthermia with nanoparticles of maghemite.

C1492 NOVEL OXABOROLE ANALOGS FOR TREATMENT OF CUTANEOUS LEISHMANIASIS

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1 Background

Cutaneous leishmaniasis (CL) is the most common form of leishmaniasis that affects humans, with over one million *new* cases per year and rapidly increasing to catastrophic proportions with the Syrian refugee crisis. CL is an area of interest for military medical research, given lost duty time, cost burden and required evacuations when units deploy into endemic areas. Although multiple treatment options exist, only one is FDA-approved, and all are systemic therapies with significant toxicities, leading to a risk-benefit mismatch for the treatment of CL. Safe, effective next generation treatment options for CL remain an unmet need.

2 Methods

Approximately 4,500 compounds from the Anacor boron-containing library were screened in a Leishmania major (L. major) intracellular amastigote assay. This screen produced more than 300 hits with IC50 values <250 nM. These hits were clustered into sub-classes based on chemical signature and three sub-classes were identified for lead optimization. A drug discovery algorithm was followed to assess compounds for progression into more labor intensive and clinically relevant animal models.

3 Results

Multi-parameter optimization of in vitro potency, pharmacokinetic properties and in vivo efficacy in murine models of CL through specifically designing, synthesizing, and evaluating analogs for three lead series produced several pre-clinical candidates for further evaluation. The following criteria: IC50 L. major amastigote IC50<150nM; known metabolic stability and a therapeutic index of >100 fold in HepG2 liver mammalian cell and RAW265 macrophage cytotoxicity assays were used to identify candidates for in vivo testing. Fifteen compounds exceeded these in vitro criteria and were advanced to select in vivo models: mouse leishmania suppression (MLS) and mouse leishmania lesion (MLL).

4 Conclusions

More than 300 compounds were identified with potent activity against L. major intracellular amastigotes. Lead optimization of these hits produced eight oxaboroles that reduce parasite load in an MLS model and two cured lesions in the

MLL model. The optimization of these hits produced late lead compounds with activity comparable to Amphotericin B in CL mouse models.

C1505 EFFECTS OF EUTERPE OLERACEA ON LEISHMANIA (LEISHMANIA) AMAZONENSIS AND DURING INTERACTION WITH MACROPHAGES

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1 Background

Leishmaniasis are neglected emerging diseases in 98 countries caused by several species of protozoa of the genus *Leishmania*. *Leishmania (Leishmania) amazonensis* is the species that causes the American cutaneous leishmaniasis (ACL), diseases that have high incidence in developing countries such as Brazil. The treatment used for ACL is limited due to the high cost, side effects and still require a long period of treatment. Currently, some natural products with immunomodulatory properties are considered important alternative source as leishmanicidal agent, which includes the *Euterpe oleracea* Martius, a plant commonly known as açai, frequently consumed in the Amazon region, with potent antioxidant, anti-inflammatory and anticonvulsant properties. Furthermore, the plant is used by indigenous people to treat ACL. However, the biological activity of açai juice on species *L. (L.) amazonensis* is unknown. Thus, this study aims to evaluate the leishmanicidal action of the clarified açai juice (EO) against *L. (L.) amazonensis*.

2 Methods

Promastigotes of *L. (L.) amazonensis* (MHOM/BR/26361) were treated with different doses of EO and subsequently analyzed by transmission electron microscopy, cytometry and fluorimetry assays. Macrophages obtained from peritoneal cavities of mice were used for anti-amastigote and viability assays (CEPAE/ICB/UFPA, grant number 7056260916- Committee of Ethics of Animal Experiments).

3 Results

We observed that EO (dilution 1: 12.5) promoted a reduction in the number of promastigotes, increase in the production of reactive oxygen species (ROS) and induced cell death phenotypes seems by apoptosis in the promastigotes of *L. (L.) amazonensis*. Ultrastructural analysis showed several morphological alterations in promastigotes treated with the dose 1:12.5 of EO for 72 hours. In addition, EO (1:12.5) also presented activity against amastigotes. There was a reduction in IL-17 cytokine levels and a decrease in the number of intracellular amastigotes in macrophages infected with *L.* (*L.) amazonensis* and treated with the dose 1:12.5 EO for 72 hours. In addition, no cytotoxic effect was observed in murine macrophages treated with different doses of EO for 72 hours.

4 Conclusions

Our results demonstrated that EO presented leishmanicidal activity against *L. (L.) amazonensis* and has no cytotoxic effects for the host cell.

C1509 LEISHMANIAL ACTIVITY OF INTRALESIONAL TREATMENT WITH ANACARDIUM OCCIDENTALE EXTRACT

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1 Background

Leishmaniasis represents a complex of diseases caused by protozoa of the genus Leishmania that affects millions of people worldwide. Different plant species are used empirically for the treatment of this disease, including Anacardium occidentale L. (Anacardiaceae). The objective of this study was to evaluate the potential leishmanicidal activity of an ethanol extract prepared from the flowers of A. occidentale (EAo), of individual substances, and of a Lanette formulation containing A. occidentale (FAo).

2 Methods

the in vitro cytotoxicity of EAo against forms Leishmania amazonensis promastigotes was evaluated. The effectiveness against amastigote forms was evaluated in RAW macrophage cultures infected in vitro with L. amazonensis. For in vivo assays, the footpad of Balb/c mice was infected with 105 L. amazonensis promastigotes. Four weeks after infection, the footpads were treated intralesionally or topically with four doses of EAo at concentrations of 5 and 50 mg/kg (EAo5 and EAo50) every 3 days or with FAo.

3 Results

EAo exerted cytotoxic activity against promastigote forms, with an IC50 of 26.9 µg/mL. Treatment with EAo reduced the number of parasites into the macrophages, with an activity similar to that of amphotericin B. The parasite burden in the spleen and proximal lymph nodes was always lower in animals treated with EAo. On the other hand, FAo exerted no leishmanicidal effect.

4 Conclusions

Intralesional treatment with EAo, but not with FAo, was effective in reducing the local and systemic parasite burden, an effect frequently associated with a better prognosis of L. amazonensis infection.

C1524 COMPUTER GUIDED DRUG REPOSITIONING FOR THE TREATMENT OF LEISHMANIASIS

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1 Background

There are about 12 million people infected with Leishmaniasis worldwide, and it is estimated that 30,000 deaths occur annually. The most frequently used drugs for the treatment of leishmaniasis are very toxic and display drug resistance issues, thus being a persisting need to find new drugs which overcome these limitations.
Polyamine metabolism is a relevant target for the development of novel medications. Polyamines can be either synthesized or taken up from the medium and are essential precursors of trypanothione, the major redox cosubstrate of trypanosomatids.

2 Methods

We have compiled a 268-compound database containing polyamine analogs with and without inhibitory effects on trypanosomatids. From this dataset, we have inferred computational models capable of identifying compounds that inhibit the polyamine metabolism. These models were applied in a virtual screening campaign on DrugBank and Sweetlead databases, to identify active compounds against trypanosomatids among already known drugs used for other therapeutic indications (i.e. computer-guided drug repositioning).
We have tested the biological activity of the candidates towards *Trypanosoma cruzi*, *Trypanosoma brucei* and *Leishmania infantum*, determining the % of inhibition at 5 μM and the half-maximal growth inhibition concentration (EC_{50}). Additionally, we have tested *in vitro* their inhibitory potential towards trypanothione synthetase (TryS).

3 Results

The *in silico* screening resulted in 45 hits predicted as possible inhibitors of the polyamine metabolism. Five of these candidates were evaluated in cellular models from *T. cruzi*, *T. brucei* and *L. infantum*. 3 drugs (triclabendazole, paroxetine and sertaconazole) displayed inhibitory effects on the proliferation of these trypanosomatids; one of them (paroxetine) showed an EC_{50} of 2.2 μM on *L. infantum* promastigotes. None of the compounds displayed activity on TryS, excluding this as major target of their biological activity.

4 Conclusions

The results highlight the importance of the applied *in silico* strategy, since three active compounds were found with a minimal time- and resource-investment. Computer – guided drug repositioning could efficiently provide solutions for neglected tropical diseases, such as Leishmaniasis.

C1525 REPURPOSING FDA-APPROVED DRUGS FOR THE TREATMENT OF METASTATIC LEISHMANIASIS USING A SEMI-AUTOMATED IMAGE-BASED HIGH-THROUGHPUT DRUG SCREENING APPROACH

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1 Background

Leishmania is a genus of obligate intracellular protozoan parasites, which are the causative agents of leishmaniasis. The metastatic types of the disease such as muco-cutaneous (MCL) and disseminated (DCL) leishmaniasis spread from the symptomatic or asymptomatic primary cutaneous lesions.The pathology results in severe destruction of the targeted tissues, mainly in the oral and nasopharyngeal areas. These metastatic forms predominantly occur in the New World, an area inhabited by species of the *Leishmania Viannia* subgenus. The discovery of a double-stranded RNA (dsRNA) virus, LRV1, in some *Viannia* species (*L. guyanensis* and *L. brazilensis*) partially elucidates to the association of metastatic leishmaniasis with these species of parasites. Our group reported that the host innate sensing of LRV1 within *L. guyanensis* through TLR-3, an endosomal innate sensor of dsRNA, renders mice more susceptible to infection. Furthermore, we and others demonstrate that there is an increased incidence of clinical relapse in LRV1-bearing *L. guyanensis* or *L. braziliensis* infected patients, who were previously treated with pentamidine or pentavalent antimonial, respectively. *Amphotericin* B and miltefosine are other currently available FDA-approved therapeutics against leishmaniasis. Despite effective parasiticidal activity, they are nephrotoxic, with miltefosine also being teratogenic. Further, there is an increase in parasite-resistance to orally available miltefosine, and Amphotericin B requires to be administered intravenously thus requiring the presence of professional health care workers. Therefore the search for new drugs is essential to continue the battle against leishmaniasis.

2 Methods

Here, we established a semi-automated image-based high-throughput drug screening (HTDS) protocol to measure parasiticidal activity of drugs in primary murine macrophages. We validated our screen using miltefosine and Amphotericin B. We then applied it to measure antiparasitic activity of 1280 FDA-approved drugsfrom the Prestwick chemical library in primary murine macrophages infected with LRV1-containing *L.guyanensis*.

3 Results

The two-independent screens generated 14 hit compounds with an effective sixty nine percent reduction in parasite growth compared to control.

4 Conclusions

Results of our screening illustrate a great potential of the established assay to search for novel, and repurpose, known therapeutics. This approach might be a key strategy to combat various forms of leishmaniasis.

C1527 THE EFFECT OF MIX EXTRACTS OF CURCUMINE AND PROPOLIS ON L. MAJOR INFECTION IN BALB/C MICE

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1 Background

Background: Leishmaniasis is a vector-borne disease caused by flagellated protozoan parasites of the genus *Leishmania.* The available drugs against the disease are toxic and resistance is reported.Objective: The goal of this study was to assess the effect of combination of propolis and curcumin ethanolic extracts on the lesions induced by *L. major* infection in Balb/c mice.

2 Methods

Methods:Balb/c mice were inoculated with 2 x 10^6 *L. major* promastigotes harvested at stationary phase at the base of the tail, after 3 weeks, the lesion appeared. The animals were divided into 6 groups and were treated with either mix ethanolic extracts solution or propolis or curcumin ethanolic extracts or ethanol alone two times a day, and two groups were kept as positive and negative controls. The lesion development was monitored weekly using kulis-vernieh for six weeks**.**

3 Results

Results:A significant difference was seen between the mean of lesion diameter before and after treatment in groups received propolis and curcumin with control group ($p<0.05$). Paired t test showed no significant difference between the mean of lesion diameter after treatment between the experimental group and the group which was treated with meglumine antimonite.

4 Conclusions

Conclusion: Ethanolic extract of propolis and curcumin showed therapeutic effect on *L. major* lesions on BALB/c mice, the extract worth for further studies.

C1544 TOPICAL TERBINA?NE IN THE TREATMENT OF CUTANEOUS LEISHMANIASIS: TRIPLE BLIND RANDOMIZED CLINICAL TRIAL

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1 Background

Leishmaniasis is a spectrum of disease condition with considerable health impacts, caused by different species of *Leishmania*. This disease is currently endemic in 98 countries and territories in the world. There are many treatment modalities for cutaneous leishmaniasis. The use of topical Terbinafine in the treatment of cutaneous leishmaniasis has recently been considered.

2 Methods

Eighty-eight participants more than two years old with proven acute CL by a positive direct smear were randomly allocated to one of the two study arms: One group received meglumine antimoniate (Glucantime) 20 mg/kg/day IM plus a placebo ointment (Mahan Vaseline) for 20 days. The other group received meglumine antimoniate (Glucantime) 20 mg/kg/day IM plus topical terbinafine 250 mg/day, for 20 days and were monitored closely by dermatologist during the course of the study

3 Results

Crude regression analysis showed that there was no significant difference between placebo and intervention group regarding partial or complete treatment (partial treatment: HR_{crude}= 1.1, CI 95%= 0.7 - 1.7; complete treatment: HR_{crude}= 1.1, CI 95%= 0.8-1.7).

4 Conclusions

Treatment rate was more effective in terbinafine received patients groups than the placebo group. However this rate depended on the type of lesions. As data indicated ulcerated nodules, papules, and plaque in experimrntalgroup have been completely improved two times faster than placebo group. Ulcerated nodules, nodules, and plaque were partially improved faster in those used tebinafine than placebo ointment.

C1564 IN VITRO ANTIMICROBIAL, ANTILEISHMANIAL STUDY, PHYTOCHEMICAL SCREENING AND HEAVY METALS TOXICITY OF DIFFERENT PARTS OF BALLOTA PSEUDODICTAMNUS

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1 Background

The study was done to assess the antimicrobial and antileishmanial activities of different parts of *Ballota pseudodictamnus*. These activities were then compared with the class of phytochemicals present in different parts of the plant.

2 Methods

Phytochemicals were investigated qualitatively through already established protocols. Antimicrobial evaluation was done by agar well diffusion and Disc diffusion methods. In cutaneous leishmaniasis the study was by counting amastigote and staining. The heavy metals toxicity in different parts of the plant was investigated by Atomic Absorption Spectrophotometry.

3 Results

The results indicate that in root and stem flavonoids, terpenes and phenols were present in ethanol, chloroform and ethyl acetate fractions. While in leaves flavonoids, terpenes and phenols were present in ethanol, chloroform and *n*-butanol fractions which were the most active inhibiting fractions against microbes in *in-vitro* study. In *in-vitro* antileishmanial results the ethanolic extract, chloroform and ethyl acetate fractions in roots of *Ballota pseudodictamnus* showed antileishmanial activity. The ethanol, *n*-butanol and ethyl acetate fraction in stem of the plant showed inhibition of amastigote form of leishmania. The ethanolic extract, chloroform and *n*-butanol fractions in leaves shows inhibition of leishmanial parasite. In heavy metals study Chromium was above permissible value in all parts of the plant except leaves. Nickel was observed above WHO limit in root. Cadmium and Lead were found above permissible value in all parts of the plant.

4 Conclusions

The Result concluded that different parts of the plant show different inhibition properties. So each part of plant should be checked for antimicrobial and antiprotozoal assay separately. It is clear from the results that the plant *Ballota pseudodictamnus* accumulates different phytochemicals and metals in different parts (root, stem and leaves) with miscellaneous concentration.

C1568 DNDI-0690: A NEW PROMISING DRUG CANDIDATE FOR THE TREATMENT OF VISCERAL LEISHMANIASIS

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1 Background

Leishmania donovani / infantum transmitted by sand flies is the causative pathogen responsible for visceral leishmaniasis (VL), a fatal disease present in Eastern Africa, India and Latin America. There are between 150,000 and 300,000 cases a year, 50% of which are children. Few treatment options are available and all drugs suffer from significant drawbacks, limiting their use in endemic countries. There is a clear need for novel drugs for VL that are efficacious, affordable, better tolerated and safer than current treatments.

2 Methods

DNDI-0690, a 7-substituted nitroimidazooxazine compound issued from a DND*i* collaborative lead optimization effort, is currently undergoing formal pre-clinical development before moving to first in human trials.

3 Results

DNDI-0690 is potent against a wide variety of *Leishmania* strains (IC_{50} 30-100nM; IC_{90} 60-560nM) including clinical isolates and cutaneous leishmaniasis (CL) strains *in vitro*. It is very efficacious in two *in vivo* models of the disease: mouse acute model (>95% parasite burden reduction following 6.25 kg/mg qd for 3 days) and hamster chronic model (>95% parasite burden reduction following 12.5 mg/kg bid for 5 days).
In all species tested, DNDI-0690 exposure following oral administration increased less than dose-proportionally and was limited by solubility. DNDI-0690 has showed a good overall safety profile so far: no cytotoxicity or significant effect on a panel of receptors, channels and enzymes except on human hERG, albeit at concentrations above efficacious exposure; negative in the AMES and *in vitro* micronucleus tests, predicting a low risk of genotoxicity; no obvious toxicity in a 14-day rat toxicity study; and a predicted low potential for drug-drug Interactions. Moreover, a scalable, robust and cost-effective synthesis route is achievable.

4 Conclusions

In order to further understand DNDI-0690 potential for VL (and possibly CL), additional studies related to deciphering its mechanism of action and identifying the metabolic pathways involved following its bio-activation are ongoing. These results indicate that DNDI-0690 has the properties of an oral drug candidate for VL.

C1571 DNDI-6148: A NEW POTENTIAL CLINICAL CANDIDATE TO TREAT VISCERAL LEISHMANIASIS

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1 Background

DND*i* (Drugs for Neglected Diseases *initiative*) is a collaborative, patients' needs-driven, not-for-profit organization whose mission is to develop new drugs for the most neglected tropical diseases, including both visceral (VL) and cutaneous (CL) leishmaniases. To address the need for new oral drugs to treat VL, a library of oxaboroles originating from Anacor Pharmaceuticals was screened against parasites that cause three diseases of the DND*i* discovery portfolio (human African trypanosomiasis, visceral leishmaniasis and Chagas disease). Starting from the screening hits, a focused medicinal chemistry program led to the discovery of a number of oxaborole analogs showing efficacy in animal models of the three kinetoplastid diseases.

2 Methods

DNDI-6148 (AN10705) was evaluated in a range of *in vitro* and *in vivo* studies and displays pharmacological and physicochemical properties consistent with DND*i*'s Target Product Profile for the development of new chemical entities to treat VL. DNDI-6148 is now entering formal preclinical development before moving to first in human trials.

3 Results

DNDI-6148 was shown to be effective in different VL *in vivo* models, acute and chronic, and *in vitro* against several *Leishmania* species including drug sensitive and drug resistant strains. An efficacious dose (leading to more than 98% reduction in parasite burden in main target organs: liver, spleen when applicable) of 25 mg/kg bid was demonstrated after treatment for five consecutive days in mice infected with *L. donovani* or *L. infantum* and in hamsters infected with *L. infantum*, and after 10 days of treatment in hamsters infected with *L. donovani*. DNDI-6148 was also discovered to be active in CL animal models.
Following salt screening, characterization and evaluation in PK studies, an arginine salt of DNDI-6148 was selected for further development. After oral administration to rats and cynomolgus monkeys, the compound displays very promising ADME properties (low clearance, high bioavailability).
A promising safety profile has been demonstrated, with absence of off-target activity including hERG channel and a NOAEL (No Observed Adverse Effect Level) found in rat during a 14-day toxicity study.
Human dose predictions suggest that bid dosing of 75 mg for a maximum of ten days will provide the required level of clinical efficacy.

4 Conclusions

In summary, DNDI-6148 shows promising properties to be developed as a clinical candidate for the treatment of visceral leishmaniasis.

C1586 MYCOBACTERIUM INDICUS PRANII MEDIATED IMMUNE RESTORATION OF LEISHMANIA DONOVANI INFECTED MACROPHAGES VIA RECIPROCAL REGULATION OF DUSP6 AND DUSP1

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1 Background

Visceral Leishmaniasis is caused by a pathogenic hemoflagellate that interferes with macrophage pro-inflammatory pathways through various means among which one major manipulative target of parasite is Dual Specificity Phosphatase (DUSPs), a type-I Cys based phosphatase that can dephosphorylate both Tyr and Ser/Thr within activated T-x-Y motif of MAPK. Leishmania infection leads to skewed Th2 response so only treatment with conventional chemotherapeutic agents is unable to cure completely. Antimonial (Stibogluconate), anti protozoan drug (Amphotericin B and Miltefosin) have shown severe toxicity at high dose. Therefore we have attempted to examine potential of a novel saprophytic non-pathogenic cultivable soil bacterium ***Mycobacterium indicus pranii (Mw)***, for restoring immune function of infected THP1 via reciprocal modulation of p38 and ERK through DUPSs. In this study *Mw* mediated modulation, as well as possible regulatory mechanism of DUSP activation by *Mw* were analysed. Mw has successful Immuno-modulator effect in HIV, Cancer and Tuberculosis, but study in LD infection is quite limited.

2 Methods

siRNA transfection with lipofectamine-2000, Geimsa stain for parasite burden count, RNA isolation with TriZOL followed by Reverse transcriptase PCR, Flow cytometry (FACS) Sandwitch ELISA, Nitric oxide assay with Griss Reagent, Co-immunoprecipitation followed by Western blot

3Results

Here we have screened 30 human phosphatases with siRNA in *L. donovani* infected THP1 and found 3 DUSPs to be anti and 5 DUSPs of pro parasite nature. 10^7cell/ml Mw treatment for 24h has been used in our study. Therefore we found that Mw can significantly up and down regulate DUSP6 (anti) and DUSP1(pro) respectively. Time kinetics study revealed that 4-6h post infection Mw causes marked upregulation of DUSP6 and downregulation of DUSP1 in infected macrophages through TLR4. Knock down of DUSP6 with siRNA caused abrogation of MW mediated p38 upregulation, and ERK1/2 downregulation . We also obtained heightened IL12, iNOS and lower IL10 and Arginase1 followed by Mw treatment. To come up with mediator between TLR and Mw, we screened PKCs with inhibitors and found that PKCβ is increased upon Mw treatment and PKCε gets dropped significantly. Co-IP study confirmed that Mw causes activation of DUSP6 through PKCβ and inactivates DUSP1 by diminishing its interaction with PKCε.

4 Conclusions

Our experiments opened an interesting drug-immune synergism that culminated in anti-leishmanial effect of *Mw.*

C1593 THE ROLE OF CPG ODN IN THE HEALING OF LESION AND THE SURVIVAL OF L.MAJOR-INFECTED BALB/C MICE.

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1 Background

Background: leishmanization is the most effective protective measure against Cutaneous Leishmaniasis (CL), CL lesion induced by leishmanization sometimes takes a long time to heal. Manipulation of leishmanization inoculums needed to induce a mild and acceptable CL lesion. The aim of this study was to explore if CpG ODN or liposomal form of CpG ODN (Cytosin phosphate Guanin Oligodeoxynucleotides) mixed with *Leishmania major* would induce a milder lesion size in Balb/c mice.

2 Methods

Methods: Different groups of BALB/c mice were subcutaneously (SC) inoculated with *L. major* mixed with liposomal form of CpG ODN, or *L. major* plus free CpG ODN, or *L. major* mixed with empty liposomes or *L. major* in PBS.

3 Results

Result: Footpad thickness was significantly ($P<0.01$) smaller, death rate was also significantly ($P<0.05$) lower in the mice received *L. major* mixed with liposomal CpG ODN or free CpG ODN than control groups received *L. major* in PBS or *L. major* plus liposomes, also mice which received *L. major* mixed with CpG ODN in soluble form showed a significantly ($P < 0.001$) smaller lesion size than control groups.

4 Conclusions

Conclusion: CpG ODN seems to be an appropriate immunopotentiator mixed with *Leishmania* stabilate in leishmanization.

C1615 SODIUM STIBOGLUCONATE AND PAROMOMYCIN FOR TREATING VISCERAL LEISHMANIASIS UNDER ROUTINE CONDITIONS IN EASTERN SUDAN

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1 Background

Medecins Sans Frontieres (MSF) started a vertical programme in January 2010 for diagnosis and treatment of visceral leishmaniasis (VL) in Gedaref state of eastern Sudan – one of the most endemic regions in the world. Combination therapy with sodium stibogluconate and paromomycin (SSG/PM) was introduced as first line treatment in July 2011.

2 Methods

Among patients with primary and relapse VL, we determined the proportion eligible for treatment with SSG/PM and, of these, their a) demographic and clinical characteristics, b) initial treatment outcomes including adverse effects requiring treatment discontinuation c) treatment outcomes at six months and d) risk factors associated with initial (slow responders) and late treatment failure (relapses and post kala azar dermal leishmaniasis-PKDL).

3 Results

Of 1252 individuals diagnosed with VL (1151 primary and 101 relapses), 809 (65%) were eligible for SSG/PM including 671(83%) children, almost half of them malnourished and anaemic.
Thirty-three (4%) individuals discontinued treatment due to side-effects, while 6 (0.7%) patients died during treatment. Initial cure was achieved in 719 (93%) of 774 primary cases and 27 (77%) of 35 relapse cases ($P<0.001$). There were 18 (2%) slow responders among primary cases and 6 (17%) among relapse cases ($P<0.001$). Among the 809 patients eligible for SSG/PM, 218 (27%) were lost to follow-up. Outcomes at six months among the 591 patients with available follow-up data were: definitive cure (n=506; 86%), relapse (n=38; 6%), treatment discontinuation (n=33; 6%), PKDL (n=7; 1%) and death (n=7; 1%). Among those completing a full course of SSG/PM, relapses and children under-five were at significantly higher risk of early and late treatment failure, respectively.

4 Conclusions

SSG/PM as a first-line regimen is highly effective and safe and means undeniable progress compared to SSG monotherapy. However, a proportion of VL patients was not eligible for SSG/PM or discontinued treatment due to adverse effects. We call for an accelerated development of new drugs and treatment regimens to further improve VL treatment in Sudan.

C1616 STUDY OF THERAPEUTIC EFFECT OF TOPICAL FLUCONAZOLE COMBINED WITH LOCAL INJECTION OF GLUCANTIME COMPARED WITH LOCAL INJECTION OF GLUCANTIME IN THE TREATMENT OF CUTANEOUS LEISHMANIASIS

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1 Background

Leishmaniasis is caused by infection with *Leishmania* parasites, which are spread by the bite of phlebotomine sand flies and is one of the most important endemic disease in Iran. Due to some of the problems observed in the treatment of the disease multiple medical treatment options are used throughout the world for cutaneous leishmaniasis (CL).

Regarding to similarity between the membrane of fungi and *Leishmania* cell wall and the same function of fluconazole to disturb them, a study was designed to evaluate the efficacy of fluconazole along with glucantime in the treatment of CL.

2 Methods

In a double blind prospective clinical trial 100 patients with confirmed CL were selected and divided randomly into two groups: one group were treated with topical fluconazole twice daily along with intralesion glucantime once weekly until complete healing of the ulcer for a maximum duration of 6 weeks. The other group were treated with topical placebo twice daily along with intralesion injection of glucantime once weekly in accordance with the above mentioned time. The patients were followed for 3 months and data were analyzed using statistical tests including chi – square.

3 Results

In the fluconazole along with glucantime treated group, 32 (64%) patients had complete cure whereas in the group treated with glucantime & topical placebo, 19 (38%) patients achieved complete cure in the course of treatment. This difference was significant statistically (p value <0.01).

4 Conclusions

The therapeutic effects of fluconazole along with glucantime is more than placebo plus glucantime in patients taking the two regimen drugs. Also fluconazole decrease the time of healing and the dose of glucantime. This can reduce the dosage and side effects of glucantime.

C1645 EVALUATION OF ANTI- LEISHMANIA ACTIVITY INDUCED BY ELLIPTICINE ANALOGUES

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1 Background

The Ellipticine is a DNA intercalating agent and a DNA topoisomerase II inhibitor. Is also a natural product, isolated in 1959 from the Australian evergreen tree of the Apocynaceae family. Studies of the activity of ellipticine have demonstrated the potential for the treatment of pathologies showing antimicrobial, anti-inflammatory and especially promising antitumor activity, thus considering the possibility of using ellipticine analogues in search of new treatments for cutaneous leishmaniasis. The objective of this study was to evaluate the antileishmanial activity of different ellipticine analogues against *Leishmania amazonensis* and *L. guyanensis.*

2 Methods

Chemical synthesis of the ellipticine analogues Elipt(HCl) and Elipt(NE) was held at the University Helsinki. At INPA (National Institute of Amazonian Research, BR), the analogues were filtered, dissolved in complete medium and the bioassay performed using microplates against promastigotes (10^6cells/mL) of *L. amazonensis* and *L. guyanensis* in different concentrations (125 – 7,81µg/mL) to determine EC_{50}. Glucantime® was used as positive control and DMSO as a negative control. The quantification of live promastigotes were counted in a Neubauer chamber, with Trypan Blue, and compared with the controls after incubation for 24, 48 and 72 hours at 25°C.

3 Results

The biological analysis showed that the Elipt(HCl) had better activity against *L. guyanensis* in 48 hours, with EC_{50}90,06 ± 0,08 in 24 hours, 6,38 ± 0,09 in 48 hours and 46,87 ± 0,10 µM/mL in 72 hours. The results for *L. amazonensis* the Elipt(HCl) showed activity in 48 hours, with EC_{50}85,84 ± 0,22 in 24 hours, 58,44 ± 0,23in 48 hours and 59,37 ± 0,27 µg/mL in 72 hours. The analogue Elipt(NE) do not show satisfactory results. Glucantime® presented EC_{50} less than 20 mg/mL in both species of *Leishmania*.

4 Conclusions

These data enabled us to select the promising analogue of ellipticine to continue future *in vitro* and *in vivo* studies in search of new therapies for cutaneous leishmaniasis.

C1648 EVALUATION OF ANTI-LEISHMANIA ACTIVITY INDUCED BY SILVER METAL COMPLEXES AGAINST LEISHMANIA (LEISHMANIA) AMAZONENSIS AND LEISHMANIA (VIANNIA) GUYANENSIS

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1 Background

Leishmaniasis is a disease caused by parasites of the genus *Leishmania* and transmitted by infected female sand flies. Infections can manifest in different ways, depending on the species. Pentavalent antimonials remain the drugs of first choice for treatment and, despite their effectiveness, have side effects. Inorganic Medicinal Chemistry has offered new possibilities for research, metals, which offer some advantages over drugs. In the present work the *in vitro* effect of two metal complexes based on silver (Ag_1 and Ag_2) against the promastigote and amastigote forms of *Leishmania amazonensis* and *L. guyanensis* were studied in 24, 48 and 72 hours.

2 Methods

The metal complexes were synthesized by the team of the ICIS, CNR (Institute of Inorganic Chemistry and Surfaces, National Research Council, Padova, IT) and forwarded to biological tests at INPA (National Institute of Amazonian Research, BR).

3 Results

The Ag2 substance had a better anti-leishmania activity at the concentration of 160 µM/mL, in the 72 h period, EC50 14.53 µM/mL in the counting method with neubauer chamber and testing with the MTT method, at 24 hour with EC50 12.69 µM/mL against *L. amazonensis*. In tests against the amastigote form, there was a reduced infection of macrophages, Ag1 presented 61% and Ag2 53% of the uninfected cells after 24h. Results of *L. guyanensis*, by the Neubauer chamber counting method, the Ag1 complex did not present satisfactory values, with EC50 >60 µM/mL, and Ag2 with a moderate activity in the 24h period, EC50 43,22 MM/mL. By the MTT method both substances had EC50 values >60 µM/mL.

4 Conclusions

The Ag2 complex presented satisfactory results in both species, with more promising results against *L. amazonensis*, indicating that in vivo studies with the Ag2 complex and with this species, tend to be promising.

C1657 ALTERNATIVE HEAT THERAPY FOR CUTANEOUS LEISHMANIASIS IN THE STATE OF AMAZONAS, BR

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1 Background

The American Tegumentary Leishmaniasis (ATL) in Brazil, is widely distributed, occurring in all states of the Federation. The northern region of Brazil in the State of Amazonas notified 8407 (46.12%) cases of the disease in 2013 (SINAN/SUS/MS). In the Amazon region, Cutaneous Leishmaniasis (CL) is caused by seven species of leishmanias (*Leishmania braziliensis, L. guyanensis, L. lainsoni, L. lindenbergi, L. amazonensis*). The Ministry of Health (MS/BR) recommends pentavalent antimonial treatment as the first choice drug and isethionate of pentamidine or an amphotericin B as a second option. Some of these drugs have been used to treat ATL for decades, they are toxic and require prolonged treatment duration. Moreover, parasites resistant to these drugs have emerged culminating in disease progression. Another fact is the difficulty in accessing the UBS (Basic Health Unit), the delay in seeking treatment and abandonment as a result of its side effects. Our purpose is to describe the efficacy and safety of thermotherapy for the treatment of CL in Amazonas State, BR.

2 Methods

Volunteers with parasitological positive diagnosis of CL were included. A single thermotherapy session involving the application of 50°C/30seconds at the center and active edge of each lesion. Was considered the inclusion and exclusion criteria to treat the patients (submitted to CONEPI/BR, No.118400/2016). Was performed diagnosis, laboratory evaluation and treatment with localized heat therapy (ThermoMed® model 1.8; ThermoSurgery Technologies, Inc, Phoenix, Ariz). All patients are being followed up to 30 days for complete re-epithelialization. The patients who did not respond to the treatment during this period is indicated conventional treatment. Patients will be followed for up to one year after local treatment for the presence of lesions.

3 Results

Systemic effect of heat therapy on cutaneous leishmaniasis lesion was observed in a patient with multiple lesions at days 15, 45 and 60 days. The re-epithelialization of the lesion with complete healing was observed. From day 15, the reduction in the size of the cutaneous lesions was observed.

4 Conclusions

Controled heat delivered as radio waves has been used in the treatment of cutaneous leishmaniasis in the Amazonas State, BR. This study is in progress.

C1665 DEVELOPMENT OF TOPICAL LIPOSOMAL FORMULATION OF AMPHOTERICIN B FOR THE TREATMENT OF CUTANEOUS LEISHMANIASIS

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1 Background

Cutaneous leishmaniasis (CL) is the most common form of leishmaniasis and a major health problem in some endemic areas. Treatment of CL depends on multiple injections of antimoniate derivatives which is painful and as such is not tolerated by most of the patients and is not always effective. The aim of this study was to develop delivery system for topical Amphotericin B (AmB) to treat CL.

2 Methods

Liposomes containing 0.1, 0.2 and 0.4% AmB were formulated using phosphatidylcholine and cholesterol under GMP condition with the support of DNDi and characterized for their colloidal properties. The penetration of AmB through and into the skin was evaluated *in vitro* using Franz diffusion. The efficacy of liposomes were determined by *in vitro* promastigote and amastigote assay both in *L. major* and *L. tropica* parasites using GFP (green fluorescent protein), and *in vivo* using BALB/c mice model of leishmaniasis. *Stability studies were carried out on Lip-AmB 0.4% according to ICH.*

3 Results

The liposome diameters were around 100 nm. The *in vitro* permeation data showed that almost 4% of the applied Lip-AmB penetrated across the mice skin and retained amount in the skin was around 60% for formulations. Minimum inhibitory concentrations (MIC) of Lip-AmB formulations and Fungizone™ against *L. major* promastigotes *in vitro* were 0.625 and 0.5 µg/ml, respectively and remained unchanged when stored at 4 ?C or room temperature for up to 20 months. The 50% effective doses of Lip-AmB 0.1, 0.2, 0.4% and Fungizone™ against *L. major* amastigotes in macrophages was 0.151, 0.151, 0.0856 and 0.063 µg/ml. The results indicated that Lip-AmB 0.4% inhibits the growth of *L. tropica* amastigotes in B10 macrophages and this inhibition was comparable with that of AmB USP reference standard. The In vivo results showed a smaller lesion size ($p < 0.001$) compared to the control groups received either empty liposomes or PBS. At week 8 post-infection, spleen parasite burden was significantly ($p < 0.001$) lower in the group of mice treated with Lip-AmB compared to the control groups, but at week 12 post-infection, parasite burden of the site of infection was significantly ($p < 0.001$) lower in group of mice treated with Lip-AmB 0.4% than all the other groups.

4 Conclusions

The results suggested that topical Lip-AmB 0.4% is stable at room temperature for 20 months and effective against *L. tropica in vitro* and *L. major* both *in vitro* and *in vivo* and may be a useful tool in the treatment of cutaneous leishmaniasis.

C1679 SAFETY AND EFFICACY OF MILTEFOSINE ALLOMETRIC DOSE FOR THE TREATMENT OF CHILDREN WITH PRIMARY VISCERAL LEISHMANIASIS IN EASTERN AFRICA

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1 Background

Miltefosine (MF) is the only oral drug currently available for treatment of Visceral Leishmaniasis (VL) and has been proven safe and efficacious in Asia; whereas in EA, when used as monotherapy in adults and children with the conventional dose of 2.5 mg/kg/day, it showed an average cure rate of only 72%. A sub-group analysis indicated an efficacy of 86% in adults >12y and 59% in children <12y. Pharmacokinetic (PK) analysis showed underexposure in children, which could partially explain poorer outcome in this age group. This study assessed whether drug exposure in children can be increased to equivalent adult exposure by using MF allometric dosing regimen and whether this dose is safe and exposure correlates with increased efficacy.

2 Methods

This Phase II, multicenter, non-comparative, open-label clinical trial to assess PK and safety of MF allometric regimen was conducted in Kenya and Uganda. Primary VL patients aged 4-12y and with weight <30 kg were given MF allometric dose BID for 28 days and were followed for 6 months. Efficacy was assessed as secondary endpoint at the end of treatment (Day 28) by parasitological examination and at Day 210 by clinical examination. Safety assessments included patient history, physical exam, haematology and biochemistry tests. Adverse events (AEs) were recorded throughout the study duration and assessed by temporality, causality, severity, relationship to MF, seriousness and outcome.

3 Results

158 subjects were screened, with 30 subjects (19%) enrolled in the trial. Efficacy was 96.7% (95% CI: 82.8-99.9%) at Day 28 and 90% (95% CI: 73.5-97.9%) at Day 210 (no lost to follow-up). Compliance to treatment was 100%, without interruption due to AEs. Two serious adverse events were reported (anaemia and transfusion reaction), neither related to MF. All 30 subjects experienced at least one treatment-emergent AE. 45% of AEs were related to MF, the most common being vomiting (17%) and neutropenia (20%). Neutropenia cases were transient (worsening of severity from baseline) and resolved spontaneously.

4 Conclusions

The efficacy observed with MF allometric regimen at 6 months follow-up (90%) was higher than the efficacy previously observed in children treated with the conventional MF dose (59%). Therefore, allometric dosing regimen seemed to improve efficacy in children, whereas maintaining a satisfactory safety profile, with no major safety concerns identified. PK results of this study are presented in a separate abstract.

C1680 EVALUATION OF VORICONAZOL (VFEND®) ACTIVITY AGAINST LEISHMANIA AMAZONENSIS AND LEISHMANIA GUYANENSIS

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1 Background

The treatment of American Cutaneous Leishmaniasis is basically with pentavalent antimonial, which has several adverse effects. In view of this, it is necessary more effective and safe treatment. Among the alternatives are the drugs already used for other therapeutics, thus favoring a cheaper, safer treatment, besides taking lesstime for approval for the release of the drug, since the physicochemical and pharmacokinetic characteristics are already known. Voriconazole is one of the second azoles generation, originated from the idea to obtain a drug with a broader action spectrum than fluconazole. In this study was evaluate the *in vitro* activity of voriconazole against promastigotes of *Leishmania amazonensis* and *L. guyanensis*.

2 Methods

For the bioassay, *Leishmania* spp. were cultured in RPMI medium with 10% FBS at 25°C. The 50% inhibitory concentration (IC50) of the voriconazole solution was evaluated by inhibition of growth and mortality of the promastigote forms of *Leishmania* species. Promastigotes were centrifuged at 4,400 rpm/15 min and adjusted to 10^6cells/mL. The antifungal solutions were diluted to the following concentrations: voriconazole from 200 µg/mL to 6.25 µg/mL and fluconazole from 400 µg/mL to 25 µg/mL Positive control of the test was done using Pentacarinat (6 µg/mL) and the tests performed after incubation of 24, 48 and 72 hours at 25°C, counting in a Neubauer chamber under an optical microscope.

3 Results

Were observed statistical significant differences regarding the concentrations of live parasites in the presence of 200µg/mL of voriconazole in relation to the negative control ($P < 0.001$) at 24 /48 and 72 hours in the bioassays of the activity against *L. amazonensis.* Differences were observed between the maximum concentration and the remaining concentrations below 50 mg/mL, 48 hours after incubation of *L. guyanensis*. In 72 hours of incubation, no statistical differences were detected with 200 µg/mL and the positive control. The IC50 value of voriconazole in *L. guyanensis* was 5.66 µg / mL and 5,34µg/mL to *L. amazonensis*.

4 Conclusions

In this work it was possible to conclude that the *in vitro* test with voriconazole was considered promising against the promastigote forms of *Leishmania amazonensis* and *L. guyanensis*. With IC50 considered active, demonstrating that it is a drug that has a good chance to be introduced in the treatment of cutaneous leishmaniasis, but needs more *in vitro* and *in vivo* assays.

C1711 SYNERGISTIC EFFECT OF GLUCANTIM WITH SHARK CARTILAGE EXTRACT IN THERAPY OF VISCERAL LEISHMANIASIS IN BALB/C MICE

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1 Background

Visceral leishmaniasis, is the acute form of leishmaniasis which is always fatal if left untreated. In visceral leishmaniasis treatment, there are reports about failure treatment in some countries. Thus, using an immunomedulator such as shark cartilage extract might be solve this problem. In this study we used glucantim alone and combined with shark cartilage extract for treatment of visceral leishmaniasis in murine model for the first time.

2 Methods

Glucantim and shark cartilage extract was given parentral and oral forms respectively. Parasite loads in the spleen and liver were defined by homogenizing and culthering the parasite and were compared with those in the untreated mice. At the end, INF-γ and IL-4 cytokines levels were assayed by ELISA in spleen cell culture supernatants. During treatment the survival rate were measured. In vivo evaluation in the L. infantum BALB/c mice model determined that treatments with at doses of 20 mg/kg/ and 0.5mg/kg/ respectively for 21 consecutive days.

3 Results

parasite burden in the spleen and liver were reduced in combined form of drugs ($p < 0.001$) against to the single drug treatments given at the similar dosages. The survival rates of mice during treatment showed, no death in any mouse from each of the target drug groups throughout the experimental period and this concluded a highly significant survival rate in test groups against to the control group ($p < 0.001$). The results of lymphocyte proliferation in a three- week treatment of mice indicated a significant increase of IFNγ and IL-4 ($P<0.05$) after the treatment. But IFNγ increase more than Il-4. In this study also, shark cartilage extract in the test group had shown increase of IFNγ, so showed significant difference compared to control group($P<0.05$). The IFN-γ in glucantim-shark cartilage extract treated group were higher than those of other groups($P<0.05$).

4 Conclusions

In conclusion, we have demonstrated the positive advantage and possible application of glucantim/shark cartilage extract drug combination in the safe and effective treatment of VL. These results seem to account for the remarkable efficacy of the glucantim/shark cartilage extract in the treatment of visceral leishmaniasis due to L. infantum.

C1765 TREATMENT OF NATURALLY INFECTED L. INFANTUM DOGS WITH A NOVEL FORMULATION OF AMPHOTERICIN B

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1 Background

Visceral leishmaniasis (VL) remains a fatal neglected disease caused by protozoan parasites of the genus *Leishmania*. Dogs are the main reservoir host for zoonotic VL, a sand fly-borne disease caused by *Leishmania infantum*. In endemic areas, dogs can either suffer from a severe disease or remain subclinically infected for years or lifelong. Amphotericin B (AmB) is highly effective in the treatment of VL but its use is limited by the nephrotoxic, refractive cost and thermal instability of the liposomal formulation. Here we present a novel cost-effective poly-aggregated AmB albumin microsphere formulation (AmB-AME) for the treatment naturally infected dogs with *L. infantum*.

2 Methods

Six dogs from Asunción, Paraguay (endemic area of VL) were recruited with consent of their owners and approval of the local Ethical Committee. Infected dogs received a rapid infusion with 3 doses of AmB-AME(5 mg/kg) every 2 weeks. Leishmania DNA from popliteal lymph node aspirates were analysed by real-time PCR at baseline and every month after treatment. In each assessment, clinical signs, symptoms of disease and hematological and biochemical parameters such as albumin content or hematocrit were evaluated.

3 Results

PCR confirmed infection of all animals with *L. infantum* apart from one that was also co-infected with *L. braziliensis*. In this preliminary report, the results until the fourth post-treatment month are presented. According to the clinical evaluation, all dogs showed an improvement in their clinical profile, such as weight gain, decreased in lymph node size, and improvements in the muscle, mucosa, gastrointestinal tract and skin. Before treatment, all animals had anemia and altered liver and kidney enzymatic values. A normalization of the hematological and biochemical parameters occurred one month post-treatment; except for the albumin/globulin ratio, where an improvement of these values was observed but did not reached the standard values. Two of the six treated animals (33%) gave negative results to *L. infantum* by PCR and ELISA one month post treatment and this result was maintained up to four months, indicating elimination of the parasites and no recidivism.

4 Conclusions

Results so far indicate that three doses of our cost-effective AmB-AME (5 mg/kg) have shown promising results in the elimination of *Leishmania sp* parasites in 33% of naturally infected dogs (33%) combined with improvement in clinical profile and biochemical markers in 100% of dogs.

C1767 A PHASE IB/II CLINICAL STUDY TO EVALUATE THE SAFETY AND EFFICACY OF TOPICAL 3% AMPHOTERICIN B CREAM (ANFOLEISH) FOR THE TREATMENT OF UNCOMPLICATED CUTANEOUS LEISHMANIASIS IN COLOMBIA

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1 Background

Despite their toxicity, difficulties to administrate and high cost, pentavalent antimonials continue to be the first-choice drug for the treatment of cutaneous leishmaniasis (CL) worldwide. The use of Amphotericin B for treatment of CL has been limited due to its toxicity, need of hospitalization and limited experience. The use of liposomal amphotericin B, although less toxic, its high cost have limited its use. Anfoleish is a topical formulation containing 3% of AmB developed by PECET which showed . encouraging efficacy results both *in vitro* and *in vivo* studies. Herein, we report the results of a recently completed phase 1b / 2, randomized trial in Colombia to assess the safety and efficacy of topical Anfoleish.

2 Methods

An open, randomized, phase Ib/II clinical trial was performed in patients with parasitological diagnosis of CL. Volunteers were randomized to receive either two (BID) or three (TID) applications of Anfoleish for 28 days. All subjects were followed for 6 months from initiation of therapy.

3 Results

Subjects in both treatment groups were comparable in terms of age, sex, and lesion's characteristics. All the 80 subjects but one completed their 28 days of treatment. In the per-protocol analysis, final cure (100% re-epithelization of any lesion present at baseline at Day 90 and no relapse at Day 180 follow-up visit) was achieved by 39.4% (24.7 – 56.3% IC 95%) and 35.3% (21.5 – 52.1) of subjects in the BID and TID group respectively. In the intention to treat analysis, final cure was achieved in 32.5% (20.1 – 48 IC 95%) and 30% (18.1 – 45.5) in the BID and TID group respectively. The cream was well tolerated and in no cases, the treatment was interrupted due to adverse events.

4 Conclusions

Overall efficacy of Anfoleish with either regimen was lower than expected. Additional studies to improve its current formulation are needed before planning any additional clinical study

C1770 PHOTODYNAMIC ACTIVITY OF ALUMINUM PHTHALOCYANINE TETRASULFONATE CHLORIDE AGAINST PROMASTIGOTES OF LEISHMANIA (VIANNIA) PERUVIANA, L. (V.) BRAZILIENSIS AND L. (LEISHMANIA) AMAZONENSIS

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1 Background

The search for alternative treatments for tegumentary Leishmaniasis is necesary for improving efficiency, with less toxicity and comparable results to conventional treatment

Photodynamic Therapy (PDT) with phthalocyanines has received particular attention because it contains a variety of metal ions, which confer greater light absorption, affinity for white tissue, selectivity for cellular compartments and greater excretion that minimizes effects by prolonged photosensitivity. Most studies have been carried out with strains of Leishmania major, L. donovani or L.tropica, which are common in Europe. While few studies have been done with circulating strains in South America, there are not studies in Peru.

The aim of the present study was determine the effect of photodynamic therapy *in vitro* using aluminum phthalocyanine tetrasulfonate chloride (AlS4Pc-Cl) on promastigote of *Leishmania (Viannia) peruviana*, *L. (V.) braziliensis, L. (Leishmania) amazonensis* and evaluate the toxicity on the J774 cell line.

2 Methods

Promastigotes of *L. (V.) peruviana, L. (V.) braziliensis, L. (L.) amazonensis*, and J774 cells were treated with (AlS4Pc-Cl) and irradiated only once with red LED light at 30 J/s*m^2 at a distance of 10 cm for 35 minutes. For the inhibition control, sodium stibogluconate at 20 mg/ml was used. The evaluation was done every 24 hours for 4 days in triplicate. The morphological changes of the parasite were determined by Giemsa staining; the viability were evaluated by the quantitative colorimetric MTT test.

3 Results

PDT-AlS4Pc-Cl had a dose-dependent phototoxic effect on the Leishmania promastigote with an inhibitory concentration (IC 50) of 100 uM for Leishmania peruviana and 200 uM for L. braziliensis and L. amazonensis. Promastigotes had a growth recovery after 24 hours. On the other hand, the J774 cell was photosensitive to treatment with CC50 activity to 200 uM. Both treatment alone with LED irradiation and AlPcClS4 treatment without irradiation, did not have a toxic effect on the J774 cell and promastigote at any of the concentrations.

4 Conclusions

The results of the PDT showed an inhibition of promastigote growth more than 50% as from 100 uM of the compound and at an irradiation of 30J/s*m2. Besides, it was observe morphological changes in comparisson to the control, being L. (V.) peruviana and L. (V.) braziliensis more susceptible than L.(L.) amazonensis. On the other hand, the AlS4Pc-Cl treatment was toxic for both the cell line and parasites, showing a low selectivity index (IS).

C1780 INHIBITION OF ARGINASE FROM LEISHMANIA MEXICANA BY BENZIMIDAZOLE DERIVATIVES.

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1 Background

Leishmaniasis is a parasitic disease that is transmitted to the host by the phlebotomine sandfly bite, this disease can involve the skin, mucous membranes and viscera.The drugs that are currently used for the treatment of leishmaniasis are inadequate due to the toxicity, high cost, severe adverse reactions and the emerging drug resistance which limits their use. Therefore it is important to looking for new drugs against leishmaniasis. Polyamines are molecules important for the survival of both the intracellular and extracellular forms of the parasite. Arginase, is the first enzyme in polyamine biosynthesis and catalyzes the hydrolysis of L-arginine to L-ornithine and urea. Here we used this enzyme as target to search inhibitors trough a virtual screening strategy.

2 Methods

Virtual screening, having the enzyme active site as the binding pocket, was made with Glide software (www.schrodinger.com), using the crystal structure of arginase from *Leishmania mexicana* (LmARG, PDB: 4IU0) and an *in house* library of approximately 450 benzimidazole derivatives. The one hundred molecules with the highest binding energy were selected for inhibition studies. Arginase activity was measured in a coupled assay system. Compounds were evaluated at 200 µM. The concentration that causes 50% enzyme inhibition (I_{50}) was determined by curves at different inhibitor concentrations. Furthermore, Drug-like descriptors and prediction of some toxicological properties of the compound were evaluated.

3 Results

From the one hundred molecules tested *in vitro*, compound **JOULZ-01** showed 100% inhibition of LmARG. Additional characterization showed that **JOULZ-01** had an I_{50} 88 µM. Sigmoid behavior suggests that more than one molecule of the compound **JOULZ-01** is required to inhibit LmARG. The compound **JOULZ-01** made pi-pi interactions with His154 and His139, a hydrogen bond interaction with Thr257 and a cation - pi interaction with the Mn^{+2}. Predicted drug like and toxicological properties from this molecule was in the range to be considered as potential drug.

4 Conclusions

The molecule reported here could serve as starting point in the search of a new leishmanicidal therapy.

C1789 ANTIINFLAMMATORY EFFECT OF PARAMOMYCIN: IS IT BEYOND ITS EFFICACY IN THE TOPICAL TREATMENT OF CUTANEOUS LEISHMANIASIS?

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1 Background

In spite of its low in vitro antileishmanial activity, nowadays paramomycin (PM) is the only topical treatment effective against localized cutaneous leishmaniasis. A good dermal accumulation (where infected macrophages are localized) of this aminoglycoside have been confirmed in treated patients and it is due to PM high aqueous solubility that allow the preparation of topical formulations with high drug concentration and permeability alterations of skin lesions that favor the penetration of more hydrophilic drugs. The immunomodulatory profile of PM remains poorly explored: although immunostimulatory effect of antileishmanial agents is currently regarded as positive in order to counteract the immune deactivation produced by the parasite in macrophages, antileishmanial drugs with antiinflamatory effect locally applied could be more suitable for the healing of the lesions without scarring. The aim of this work was to evaluate the immunomodulatory profile of PM.

2 Methods

Bone marrow derived-macrophages (BMDM) were polarized with different stimuli (IFNg, IL-4, LPS, imiquimod (IMQ) either alone or in combination). 24 h later BMDM were infected with *L. major* or *L. braziliensis.* After removal of non-internalized parasites, BMDM received the same stimuli with or without PM. Cytokines expression levels were measured by real-time PCR 48 h later. The same procedure was performed with non-infected BMDM. *In vivo,* the immunomodulatory effect of PM was analyzed in an IMQ-induced inflammation mice model and also in *L. major* infected BALB/c mice after topical application of PM ointment for 30 days.

3 Results

PM treatment modulated macrophage polarization and down-regulated expression of M1 (IL-1b, TNF-a, iNOs, IL-12) and M2 markers (Arg-1). TGF-b and IL-10 expression was differently modulated in the presence of IFN-g or IL-4 and suggested different immunomodulatory effect of PM in function of the cytokines environment. *In vivo* the topical application of PM was significantly able to prevent the local inflammation induced by IMQ administration and the infiltration of neutrophils, macrophages and T cells. The expression of IL-1b, IL-6, IL17, COX-2, CCL-3 and TNF-a was also significantly down-regulated by PM. In infected mice, PM treatment also decreased the inflammatory infiltrate, although in the context it seems be mainly produced by the leishmanicidal effect of the drug that decreased the parasite load.

4 Conclusions

We have some evidences of PM has an anti-inflammatory effect.

C1796 SUPPORTING POLICY CHANGE BY CONDUCTING A PHASE IV EFFECTIVENESS AND SAFETY STUDY IN LIMITED-RESOURCE SETTINGS IN BIHAR

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1 Background

It is easier to conduct Good Clinical Practice (GCP) compliant trials in a controlled set up with a specialized team at an institution with adequate funding, but results not necessarily generalizable to what can be expected to be seen in less resourced real life conditions. However, due to the challenges in implementing clinical trials to assess treatment effectiveness, very few studies have been conducted in the government sector at the district or primary health care level under real-life conditions within national program settings. A study aiming at informing policy treatment for VL was conducted in Bihar.

2 Methods

A Phase IV clinical trial for new Visceral Leishmaniasis (VL) treatment regimens was conducted at public health facilities in two VL endemic districts (Vaishali and Saran) in Bihar state, India and at a kala-azar referral hospital in Patna between 2012 to 2015: single dose Ambisome (SDA) and the combinations miltefosine with paromomycin (M+P) and Ambisome with miltefosine (A+M) all recently developed and showing high efficacy in RCTs were tested. DND*i* (sponsor) worked in partnership with Bihar State Health Society, Rajendra Memorial Research Institute of Medical Sciences and Médecins Sans Frontières. Significant training was provided prior and during the trial.
Regulatory and ethical approvals were obtained, laboratories and health facilities were upgraded, and research ethics/GCP/GCLP training was provided to health professionals.

3 Results

A total of 1,761 patients were treated and were followed up for at least 6 months to determine treatment outcome; a cohort was followed for 12 months.
The regimens showed high effectiveness and good safety profiles. As a result, the national program revised its treatment policy in August 2014, recommending SDA as the 1st option and M+P as the 2nd option. Currently, up to 24 month follow-up data are being collected from the cohort to provide long-term assessment of late relapse risk and PKDL incidence.
This study demonstrates that strengthening infrastructures and human resources in endemic sites facilitates the conduct of GCP-compliant clinical trials. This increase in capacity and knowledge in our countries enables the conduct of research that addresses our public health needs.

4 Conclusions

This study shows that it is possible to implement clinical research in real life settings, generating evidence to support policy change. This can be translated into research for other neglected tropical diseases in similar settings.

C1797 STRUCTURE-BASED DESIGN OF NEW INHIBITORS OF LEISHMANIA INFANTUM TRYPANOTHIONE REDUCTASE

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1 Background

The thioether derivatives of our in-house library have been evaluated in whole-cell screening assays in order to determine their *in vitro* activity against *Leishmania* protozoan. Among them, promising results have been achieved with compound RDS 777 (6-(sec-butoxy)-2-((3-chlorophenyl)thio)pyrimidin-4-amine) (IC_{50} = 29.43 μM), which is able to impair the mechanism of the parasite defense against the reactive oxygen species by inhibiting the trypanothione reductase (TR) with high efficiency (K_i 0.25 ± 0.18 μM)(Saccoliti et al., J Enzyme Inhib Med Chem, *in press*). The X-ray structure allowed the identification of the structural features responsible for the RDS 777 binding to TR. In particular, due to the presence of several nonpolar aminoacids within the catalytic site, RDS 777 interacts with the hydrophobic Val53 and Val58 and, furthermore, it is hydrogen bound to the residues more involved in the catalysis namely, His461', Glu466', Cys57, Cys52 and Glu466' and with the acidic residue Glu467'. The identification of the residues responsible for the binding allowed the structure-based synthesis of a new series of diaryl sulfide compounds to test on *Leishmania*.

2 Methods

Ten additional thioeter derivatives (RDS) have been tested on *L.infantum* strain (MHOM/TN/80/IPT1) promastigote stage. To estimate the 50% inhibitory concentration (IC_{50}), the MTT (3-[4.5-dimethylthiazol-2-yl]-2.5-diphenyltetrazoliumbromide) micromethod (Sereno & Lemesre, 1997, Parasitol Res) was performed and Amphotericine B was used as control.

3 Results

Five thioeters display an activity higher than RDS 777 on *L.infantum*: RDS 562 (IC_{50}=10.79±2.32 μM); RDS 802 (IC_{50}=17.74±1.27 μM); RDS 832 (IC_{50}=15.11±1.22 μM); RDS 939 (IC_{50}=16.66±1.36 μM);L.infantum and RDS 615 (IC_{50}=30.60 ±1.66μM).

4 Conclusions

To confirm this encouraging results the selected compounds will be tested on *L.infantum* TR enzyme in order to understand their mechanism of action.

C1802 UNDERSTANDING THE IMMUNOLOGICAL MECHANISM OF DIETARY NUCLEOTIDES AND ACTIVE HEXOSE CORRELATED COMPOUND (AHCC) ON LYMPHOPROLIFERATION AND IMPROVEMENT OF CANINE LEISHMANIASIS

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1 Background

It is well known the close relationship between the development of leishmaniasis and the interaction of the immune system in canine leishmaniasis (CanL). Orally administered dietary nucleotides (NT) and AHCC have shown to play a key role within the immune system enhancing the Th1 response and influencing the lipid metabolism, immunity and tissue growth, development and repair. Co-administration of NT and AHCC with anti-leishmanial drugs has been associated with a better prognosis of the disease (Segarra et al. SEVC Granada. 2016).

The aim of this work was to identify *in vitro* the effect of NT and AHCC on the cytokines produced by lymphocytes in order to understand how the administration of these compounds lead to an enhancement of the Th1 response in infected dogs.

2 Methods

The effect of NT and AHCC on the proliferation of lymphocytes was determined by utilizing cell suspension obtained from spleens of naïve mice co-stimulated with 5 µg/ml of either lipopolysaccharide (LPS) or 25 µg/ml of soluble leishmanial antigen (SLA) of *L.infantum in vitro*. Lymphoproliferation was measured by the resazurine assay. Th1/Th2 profile was analyzed by determining the cytokines levels in the splenocyte culture supernatants of several combinations of NT and AHCC in the presence or not of SLA or LPS. Quantibody mouse cytokine array kit was used.

3 Results

No differences were observed in lymphoproliferation when NT or AHCC were used (either by themselves or in combination) on non-stimulated lymphocytes. However, significant differences were observed in lymphorproliferation when NT were added on SLA stimulated lymphocytes (being higher than on LPS stimulated lymphocytes). Regarding the cytoquine profile, AHCC increased significantly the levels of IFN-g, IL-1b, IL-2, IL-5, IL-12 but also IL-9, IL-10, compared to non-stimulated lymphocytes used as control. Interestingly, IL-10 values were reduced to basal levels when NT were co-cultured with SLA. The combined effect of NT:AHCC (1:1, w/w) also led to production of IFN-g and reduction of IL-10.

4 Conclusions

IL-10 is a cytokine that modulates the immune system towards an anti-inflammatory Th2 response inhibiting macrophage activation, antigenic presentation, clonal expansion and release of pro-inflammatory cytokines. The immunomodulatory effect of NT and AHCC on the immune system (enhancing the Th1 response) may be related mainly to with their efficacy to reduce the IL-10 levels and then limiting the parasite dissemination.

C1804 EVALUATION OF THE LEISHMANIASIS VISCERAL THERAPEUTIC RESPONSE ACCORDING TO THE DRUGS USED IN THE CONTROL PROGRAM OF VISCERAL LEISHMANIASIS IN THE STATE OF SÃO PAULO, BRAZIL.

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1 Background

Visceral Leishmaniasis (VL) is a severe systemic disease, with a worldwide distribution and Brazil being one of the six countries with highest prevalence of the disease. It implies significant morbidity and mortality, especially if not treated early and appropriately. In the state of São Paulo, in the southeast region of Brazil, the first autochthonous human cases were recorded in 1999. The treatment for VL, in the state, is mainly carried out with three drugs: pentavalent antimony, amphotericin B deoxycholate and amphotericin B liposomal. In Latin America, there are few studies that compare the effectiveness of these drugs. In this study has aimed to describe the clinical and epidemiological characteristics of patients with VL diagnosis reported in the state of São Paulo between 2007 and 2015, comparing the cure rates of these patients treated with the three different therapeutic regimens used, besides evaluate whether there is a relationship between higher lethality, according to drug used and age.

2 Methods

Observational study, descriptive, from retrospective cohort. Carried out a descriptive analysis of all confirmed cases of VL reported in the São Paulo state from between 2007 to 2015. We analyzed only patients who had a diagnosis of visceral leishmaniasis, confirmed by laboratory clinical criteria and who were HIV negative.

3 Results

In this period, we found 2121 confirmed cases of VL. Most of them were male, living in urban area. The age group most affected by VL was 20 to 59 years of age. About treatment, 402 patients were treated with pentavalent antimony, 105 used amphotericin B deoxycholate and 906 with amphotericin B liposomal. The lethality by VL was 7.8%, with an overall lethality of 9.62%. Analyzing these rates for each drug used, both were significantly higher in the group that used amphotericin B deoxycholate, with a cure rate lower than the other drugs. There were not significant differences between those who used pentavalent antimony and amphotericin B liposomal. Lethality was higher in patients over 60 years old, with statistically more important in that used amphotericin B deoxycholate.

4 Conclusions

The results of this study showed a higher lethality and lower cure rate of amphotericin B deoxycholate compared to pentavalent antimony and amphotericin B liposomal, especially in age group of over 60 years. However, further studies are required to maintain on causal relationships between the use of certain medication and increase lethality by VL.

C1810 CODELIVERY OF ANTIMONIALS AND BERBERINE IN LIPOSOMES WITH M1-POLARIZATION EFFECT IN MACROPHAGES FOR THE TREATMENT OF VISCERAL LEISHMANIASIS (VL)

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1 Background

Antimonials remain one of the most important therapeutic options for the treatment of VL. Their co-administration with other antileishmanial drugs and their selective targeted delivery to infected macrophages could decrease the effective dose and the risk of resistance and therapeutic failures. The aim of this study was to find a drug with synergistic effect in combination with Glucantime and their co-delivery in liposomes with M1-polarization features. The efficacy of antimonials has been associated with the immunological status of the patients; thus, carriers able to reverse the macrophage de-activation induced by the parasite could benefit the resolution of the infection. Cholesterol, saturated or cationic lipids have been associated with the induction of pro-inflammatory macrophage polarization and then, suitable for the preparation of liposomes with indirect antileishmanial activities (macrophage-mediated).

2 Methods

Several compounds were combined with Glucantime and their activity was tested in vitro in *L. donovani* infected macrophages. A combination is defined as synergistic when its fractional inhibitory concentration (FICI) is below 0.5. Liposomes were prepared by the film method, containing Lecithin, Cholesterol and Dimethyldioctadecylammonium bromide (DDAB), Dihexadecyl phosphate (DAP) or D-α-Tocopherol succinate (TS) at 75:40:5 mM. Size and homogeneity were adjusted by sonication. Bone-marrow derived macrophages (BMDM) were incubated with the different liposomes formulations for 24 h. Nitric oxide was measured in the supernatant with Griess Reagent. M1 or M2 polarization markers were also measured by real-time PCR.

3 Results

The combination of glucantime and berberine chloride showed a synergistic effect against *L. donovani* amastigotes-infected macrophages (FICI = 0.16). Liposomes had a mean size of 150-200 nm and polydispersity index below 0.3. The loading of each drug was around 10.29 nmol Berberine/μmol lipids and 37.79 nmol Glucantime/μmol lipids. Although the presence of cholesterol enhanced the encapsulation of drugs, M2- instead of M1-polarization effect was observed: the PPAR-γ expression was up-regulation and the liposomes inhibited LPS induced pro-inflammatory effect.

4 Conclusions

Liposomes can be more than inert carriers for drugs. Their composition should be carefully selected in order to achieve maximal therapeutic benefit. In the context of VL, we are looking for liposomes composition with M1-polarization effect in macrophages.

C1827 LIPOSOMES FOR THE TOPICAL TREATMENT OF CUTANEOUS LEISHMANIASIS

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1 Background

Cutaneous Leishmaniasis is the most prevalent form of Leishmaniasis in Latin America. Topical treatment would minimize drugs side effects and enhance patient compliance. We have tested ultra-flexible liposomes carrying different candidate drugs for flexibility, skin penetration and effect on amastigotes viability in *Leishmania (L.) amazonensis* - infected macrophages.

2 Methods

Four different drugs encapsulated in ultra-flexible liposomes were tested separately: AmphotericinB (AmB), Indole (Ind), Imiquimod (Imiq) and Miltefosine (Milt). Liposomes flexibility was studied by extrusion. Skin penetration was assayed with abdominal human skin in Franz cells in non-occlusive conditions and quantified by validated HPLC methods. Fluid liposomes were used as references.
In vitro assays were performed in Raw 264.7 – murine macrophages infected with L. amazonensis parasites in a ratio of 20 promastigotes per cell. Non adhered parasites were removed after five hours of incubation. Formulations were added in different quantities, and the leishmanicidal effect was measured after 48 hs by microscopy. Diff Quick coloration was used, and selectivity indexes were calculated (% infected macrophages X number of intracellular amastigotes) for comparative purposes.

3 Results

The incorporation of Im did not produce significant changes in the flexibility of liposomes (ANOVA, $p>0.05$). An increase in flexibility was observed in Ind- containing liposomes. Formulations containing AmB could not be extruded at the working pressure, possibly due to a decrease in liposomes elasticity.
From the liposomes applied on the skin surface, Ind penetrated the most, reaching even the receptor medium, followed by Im and AmB, which penetrated in smaller percentages into the epidermis and dermis. Drugs incorporated into ultra-flexible liposomes penetrated more than drugs in fluid liposomes.
We found that each drug had its own most effective concentration in reducing amastigotes charge in vitro. AmB reduced 96% of amastigotes respect to control infected cells, while Im reduced 66%, Milt in 62% and Ind in 59%.

4 Conclusions

There is a strong correlation between liposomes flexibility and drug skin penetration, regardless of the drug molecular weight. All drugs tested reached the dermis when incorporated in ultra-flexible liposomes, although in different amounts. Regarding leishmanicidal ability against infected macrophages, the AmB formulation was the most effective, followed by Im, Milt and Ind.

C1829 EVALUATION OF METABOLOME PROFILE OF LEISHMANIA MAJOR (FREDLIN STRAIN) AMASTIGOTES AFTER TREATED WITH ANTI-LEISHMANIAL XANTHIUM STRUMARIUM LEAF EXTRACT.

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1 Background

Cutaneous leishmaniasis (CL) is one of the most important parasitic diseases in humans, in which one of the responsible organisms is *Leishmania major*, transmitted by sand fly vector. The drugs used for the treatment of cutaneous leishmaniasis are administered parenterally and are associated with a high frequency of adverse effects such as musculoskeletal pain, nausea, vomiting, diarrhea, abdominal pain, headache, anorexia, asthenia, fatigue, fever, exanthema, erythema, and urticaria.The genus *Xanthium* is used as traditional herbal medicines, Xanthium *Strumarium* is source of sesquiterpene lactone with Xanthatin (0.033%), xanthinin (0.04%), xanthinosin (0.002%), 4-epiisoxanthanol (0.025%), 4-epixanthanol (0.03%) and 4-oxobedfordia (0.01%) as constituents, this species shows cytotoxic, anti-malaric, anti-leishmanial and fungicidal activities. In our study, we have examined the leishmanicidal activity and the effect of this plant extract on *Leishmania major* amastigote's metabolome profile.

2 Methods

Xanthium Strumarium methanolic leaf extract was prepared and phenolic activity was assayed. Parasites strain *L major Fredlin* (MRHO/IR/75/ER) and macrophages J774 was used for *in vitro* culture in this study. Cell extracted samples were analyzed on a Bruker AV 500 NMR spectrometer. One-dimensional 1H NMR spectra were obtained using 1D NOESY. NMR spectra were converted to matrix by the help of ProMatab code in MATLAB environment. Outliers were selected after applying PLS-DA classification chemometric method. Metabolites were identified with LeishCyc, a biochemical pathways database for *Leishmania major*. Identified metabolites were applied in MetaboAnalyst (V.3) online analysis package and pathways were worked out

3 Results

IC50 for *Xanthium Strumarium* was determined as 35 µg/ml . Among the most affected pathways were the, Phenylalanine, tyrosine and tryptophan biosynthesis (p value 0.151), valine, leucine and isoleucine biosynthesis (p value 0.218), tyrosine metabolism (p value 0.219) and glutathione metabolism (p value 0.311), Pantothenate and CoA biosynthesis (p value 0.339), Alanine, aspartate and glutamate metabolism (p value 0.506) and glycine, serine and threonine metabolism (p value 0.546).

4 Conclusions

This study, demonstrated that *xanthium Strumarium* has active leishmanicidal activity and could alter the amino acids pathways which is the backbone of amastigotes energy sources.

C1862 ANTILEISHMANIAL ACTIVITY OF CINNAMALDEHYDE ON AMASTIGOTES FORMS OF LEISHMANIA (LEISHMANIA) AMAZONENSIS

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1 Background

American tegumentary leishmaniasis (ALT) is considerated by The Health World Organization a public health problem. The available treatments with meglumine antimoniate (Glucantime) and amphotericin B in Brazil have side effects and this in association with their administration complexity led to a large number of therapeutic withdrawals. Based on this, studies has been done with the purpose to obtain alternative therapy. Cinnamaldehyde, a major component of the oil extracted from the bark of cinnamon, is a compound widely used in the cosmetic, pharmaceutical and food industry, with antimicrobial effects already known in the literature and has not been evaluated to species of *Leishmania*. The aim of this study is to investigate the antileishmanial activity of cinnamaldehyde on macrophages J774A.1 infected with promastigotes forms of *Leishmania (Leishmania) amazonensis (L.L.a).*

2 Methods

Macrophages J774A.1 infected with promastigotes forms of *Leishmania (Leishmania) amazonensis* (10 promastigotes for macrophage) were exposed to cinnamaldehyde at the concentrations of 200µM and 400µM for 24 hours at 37^0C in atmosphere with 5% CO_2. After that, coverslips were stained with Giemsa and evaluated by microscopy. The supernatant was used to indirect determination of nitrite production.

3 Results

The percentage of inhibition of infection on concentrations of 400 µM and 200 µM in relation to the uninfected control was 53.40% and 22.12%, respectively. There was no significant variation in the production of nitric oxide (p> 0.05).

4 Conclusions

Cinnamaldehyde can be a promising compound in the treatment of ALT because of their antileishmanial activity.

C1866 CUTANEOUS LEISHMANIASISIN TUNISIA: THE PATIENTS´ PERSPECTIVES.

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1 Background

Although non fatal and mostly self-healing in the case of *Leishmania major* (*L major*), Cutaneous Leishmaniasis (CL) is treated to improve lesion healing time and quality of scars, especially in cosmetic sites which can dramatically affect patients' wellbeing. Whilst there have been some advances in treatment methods currently available, there remains a lack of importance attached to the psychological needs of CL patients when considering case management options. As part of this work, we explored patients' perspectives about socio economic psychological burden of disease (lesion and scar) and treatment in everyday life, as this understanding might provide useful information to better implement effectiveness of treatments and the follow up of scarring in a country where access to treatment remains a challenge due to multiple economic, social and geographic barriers.

2 Methods

Individual semi-structured in-depth interviews were conducted with confirmed CL patients at two *L. major* endemic sites in the south of Tunisia (SidiBouzid and Gafsa) in their mother tongue, after obtaining their consent. Participants were selected based on a purposive, maximum variation sampling approach along a defined patient spectrum covering e.g. *Leishmania* species, age, gender, and clinical presentation.

3 Results

We enrolled eight patients (4 men and 4 women) aged 27 to 65years. Three were treated at the primary health care center of SidiBouzid, four had more severe lesions and were treated in the regional hospital of Gafsa and one patient sought care at the private sector.
The impact of CL was considerable. Most patients were not happy with the treatment performance and case management. They expected the time to heal to be shorter and better accessibility (psychological, geographical and organizational) from the health system.
Tolerance of the burden of disease was variable and ranged from acceptance of hidden scars to suicidal thoughts resulting from the fear to become handicapped and the stress from the close relatives. Some patients believe that CL is a form of skin cancer. This was unexpected and shows the big gap between the medical perspective and the patient perspective of this disease.

4 Conclusions

The interviews provide valuable information for new drug developers and public health professionals that will guide future case management and drug development strategies for CL and emphasized the importance of improving communication with patients.

C1880 MARBOFLOXACIN ACTION IN PROMASTIGOTES OF LEISHMANIA INFANTUM

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1 Background

Background: Canine Visceral Leishmaniasis (CVL) is a worldwide anthropozoonosis caused by an intracellular protozoan *Leismania (Leishmania) infantum* and transmitted by *Lutzomya longipalpis*. Marbofloxacin is a fluorquinolone third generation with leishmanicide action. In the present study was evaluated the marfloxacin effect in promastigotes of *L infantum* IOC/LO579 (MHOM/BR/1974/PP75).

2 Methods

Methods: The inoculum was prepared in quadruplicate and cultivated in LIT medium at concentration of $3x10^5$ promastigotes/mL at different culture times to observe the effect of the drug on the development of the agent (0, 12, 24, 48 an 72 hours) against three concentrations of the antiotic (100, 500 and 750 mg/mL using 1 mL of the LIT medium containing $3x10^5$ promastigotes and 1 mL of the respective antibiotic concentration. In order to differentiate viable promastigote forms the vital blue trypan dye at the concentration 0,2% was used. The promastigotes were evaluated in a Newbauer chamber under 40 x magnification optical microscope in 5 fields according the formula: Number of promastigotes/mL divided by 5, multiplied by the running factor of the chamber and again by 10,000.

3 Results

Results: The value of the medium abtained from the four replicated test was used for the counting of viable promastigotes in the moments and concentrations. The results were transformed in log 10 and calculating the standart error at each time and as well the value of p for the two conditions. With 24 hours of exposure to the drug there was statistical difference with $p<0.05$ only in the concentration 750 mg/mL. The comparison between the moments for each concentration revealed that at 100 and 500 mg/mL there was a significant difference between moments 0 and other moments, between 12 and 72 and between 24 and 72 hours ($p<0.05$). For the concentration 750 mg/mL there was a statistical difference ($p<0.05$), and also difference between moments 12 and 72 hours. The comparison between the concentrations at the various moments revealed that at 12 hours there was no statistical difference. On the other hand, with 24 hours, there was difference between only the concentrations of 100 and 750 mg/mL ($p<0.05$). With 48 hours there was no difference between the other concentrations. With 72 hours did not detect difference in the various concentrations used.

4 Conclusions

Conclusions: All concentrations were effective in reducing number of promastigotes. The concentration of 750 in 24 hours showed a lower count.

C1907 EFFECT OF GLYCINE ON LEISHMANIA MEXICANA INFECTIONS OF BALB/C MICE

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1 Background

Cutanenous leishmaniasis caused by *L. mexicana* is the most frequent clinical form in Mexico. In the murine model, it has been observed that a Th1 cellular response mediated by cytokines such as TNF-α and IFN-γ is associated with protection. In murine models, susceptibility towards *Leishmania* has been associated with a Th2 response. In *L. amazonensis* infections the host diet was to change the profile of cytokines (Blok *et al.,* 2002). Intracellular *Leishmania* amastigotes acquire the nutrients needed for their replication from the phagocytic cells. One of the nutrients that it obtains is glycine, which is involved in the synthesis of 5,10-CH2-THF through the glycine decarboxylase complex. This is related to the growth and virulence of the parasite, since mutants in the subunit of the complex are less virulent and grow slower. On the other hand, glycine at high concentrations can also have toxic effects inhibiting the growth of bacteria such as *Helicobacter pylori*, *Enterococcus faecalis*, *Lactococcus lactis* (Cruz-Rodz *et al.,* 1990; Minami *et al.,* 2004), yet little is known of its effect on *L. mexicana.* Objective: We here analyzed the effect of glycine on the *L. mexicana* growth.

2 Methods

The effect of different concentrations of glycine (10-6000 µg/ml) on *L. mexicana* promastigotes and amastigotes was analzed *in vitro*. Furthermore, the effect was also analyzed *in vivo*, on *L. mexicana* infected mice. Infected mice were given different concentrations of glycine in their drinking water (25mM-1M) during 8 weeks. The survival of promastigotes and intracellular amastigotes was quantified. For *in vivo* studies the parasite load and the size of the footpad swelling were quantified.

3 Results

No effect on promastigote growth was observed with different doses of glycine. However, glycine significantly inhibited ($p<0.05$) the growth of intracellular amastigotes and also reduced the survival of the parasite within infected macrophages. *In vivo* studies should that the oral ingestion of glycine in drinking water reduced the parasitic load and the size of the lesion caused by the parasite infection.

4 Conclusions

Glycine not only inhibits the growth of intracellular amastigotes and decreases their survival within infecting macrophages, but it also reduces the parasite load and lesion size of infected animals. With these results we propose the use of glycine as a nutritional supplement for patients infected with *Leishmania mexicana*. Financed by PAPIIT IN217515 and CONACyT 221405.

Early drug discovery. Animal models and genetic susceptibility. Drug resistance mechanism

C0186 ANTILEISHMANIAL POTENTIAL OF LEMONGRASS OIL – AN IN VITRO AND IN SILICO STUDY

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1 Background

Leishmaniasis is one of the most dangerous neglected topical disease. What makes it even worse, is its elusive mechanism of survival in the host macrophages and lack of preventive therapeutics. In order to overcome these bottlenecks and circumvent the escalating drug resistance, we used metabolic network based approach for prioritizing new drug targets. This study describes the use of lemongrass oil as antileishmanial agent and discovery of possible new lead compounds which may be used as inhibitors against proteins responsible for the growth and survival of *Leishmania*.

2 Methods

The antileishmanial activity of lemongrass oil was analysed *in vitro* in growth kinetics and reversibility assay and 50% inhibitory concentration (IC_{50}) determined. Cellular and nuclear morphology by fluorescence microscopy and DNA fragmentation by TUNEL assay using flow cytometry were elucidated. 50% cytotoxic concentration (CC_{50}) on RAW 264.7 macrophages was evaluated. For *in silico* studies, the phytoconstituents of lemongrass oil were analyzed by GC-MS and a 3D ligand library created. Metabolic networks were analyzed for choke points that were selected on the basis of the highest metabolic load routing through them. The 3D crystal structures of the selected proteins were obtained from PDB and docking simulation was performed using Schrodinger Suite. The ADME profiles for components of lemongrass oil were also analyzed.

3 Results

In vitro assays confirmed the cytocidal effect of lemongrass oil with IC_{50} of 6.75 and 2.5 mg/ml against the promastigotes and intramacrophagic amastigotes, respectively with negligible side effects on RAW cells. The treated promastigotes appeared to be oval, shrunken, non-motile and aflagellated with increased volume of mitochondrial kinetoplast. A large number of cells were found to be TUNEL positive indicating DNA fragmentation, a hallmark of apoptosis. In total, five targets were selected based on the highest metabolic load routing through them (choke points). Most of the lemongrass oil components showed significant docking scores, confirming their inhibitory potential for these targets. ADMETOX profiles for these components were found to be within safe therapeutic limits.

4 Conclusions

We report a new approach for discovery of lead compounds and docking new targets, which may not only help in developing more effective and safe drugs but also alleviate the menace of drug resistance. Lemongrass oil was found to be an efficient and safe therapeutic lead against leishmaniasis.

C0196 ANTILEISHMANIA ACTIVITY AND VECTORIZATION OF SUGIOL DITERPENE USING YEAST CELL WALL PARTICLE OBTAINED FROM SACCHAROMYCES CEREVISIAE

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1 Background

Incidence of leishmaniasis has been increasing in the last years, mainly at war regions, emphasizing the need to develop new approaches to treat neglected diseases. In previous studies sugiol diterpene showed remarkable *in vitro* activity on *Leishmania amazonensis*, but this molecule presented poor solubility in aqueous medium, hindering *in vivo* studies. Using microcarriers to deliver poor solubility compounds to a specific cell is an interesting strategy to reach intracellular parasites. β-1,3 glucan is found in abundance in the cell wall of fungi, such as *Saccharomyces cerevisiae*, also known as "baker powder". Macrophages, fibroblasts and dendritic cells present dectin-1 receptor on cell membrane surface allowing the recognition of these particles and, consequently, their phagocytosis by the host cells. Moreover, some studies suggested that yeast cell wall particles (YCWP) accumulate in the liver, the main organ affected by visceral leishmaniasis, caused by *Leishmania infantum*.

2 Methods

YCWP were obtained from basic and hot extraction of intracellular material, ensuring the wall cell protein degradation and conservation of β-1,3 glucan. Acetone sugiol solution was added to YCWP and incubated for 2 h at -20 °C. *L. infantum* promastigotes were treated with free sugiol, sugiol within YCWP and empty YCWP. Peritoneal macrophages were harvested from BALB/c mice, and macrophages were infected with *L. infantum* promastigote, during 4 h. Next the cells were treated according to described above. In order to evaluate the cytotoxicity, the same treatments were used on RAW 264.7 macrophages.

3 Results

Antileishmania activity of free sugiol was demonstrate by IC_{50} values of 4.1±0.2 and 4.9±1.2μg/mL for promastigote and amastigote, respectively, and did not produce toxic effects on macrophages (CC_{50}=173.2±12.5 μg/mL). YCWP also did not produce toxic effects even in high concentration (20,000 μg/mL). YCWP containing sugiol were not active against *L. infantum* promastigote but showed IC_{50}=6.8±0.15 μg/mL for amastigotes.

4 Conclusions

Indeed, the poor hydrophilic nature of sugiol does not allow the release in aqueous medium. This was confirmed since there was no activity on promastige forms.These results also revealed a promising profile of sugiol against *L. infantum* and the YCWP specificity for intracellular forms. This strategy seems to be appropriate to administrate *in vivo* drugs with low solubility, such as sugiol.

C0221 BISABOLOL IS EFFECTIVE AGAINST EXPERIMENTAL CUTANEOUS LEISHMANIASIS THROUGH THE INDUCTION OF MITOCHONDRIAL-DEPENDENT APOPTOSIS

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1 Background

The sesquiterpene (-)-α-bisabolol has been proved effective against experimental visceral leishmaniasis, but its mechanism of action remains elusive. The aim of the present work was to evaluate the antileishmanial effectiveness and safety of (-)-α-bisabolol in a novel topical formulation on a cutaneous leishmaniasis model involving *Leishmania tropica*-infected Syrian hamsters; and assessing its antileishmanial mode of action

2 Methods

The compound was evaluated on *L. tropica* intracellular amastigotes using bone marrow-derived macrophages. Its cytotoxicity was assessed on L929 fibroblasts. Reactive oxygen species generation was detected using a fluorescent probe. Mitochondrial depolarization was assessed evaluating the fluorescence due to rhodamine 123 in a flow cytometer. Apoptosis was investigated by measuring annexin V/propidium iodide fluorescence in in a flow cytometer. The ultrastructure of treated parasites was analyzed through transmission electron microscopy. Hamsters were infected with 30 million promastigotes in the footpad. After 7 weeks, they were treated with an ointment containing the compound at different concentrations or intralesional meglumine antimoniate for 3 weeks. The parasite loads were determined through quantitative PCR. This project was funded by the Ministry of Economy and Competitiveness and FEDER funds (Pi14-01024).

3 Results

(-)-α-Bisabolol was active against *L. tropica* intracellular amastigotes displaying an inhibitory concentration 50 % of 25.2 μM and showing low cytotoxicity. This compound induced time and dose-dependent oxidative stress, mitochondrial depolarization and phosphatidilserine exposure. Treated parasites showed mitochondrial disruption and chromatin condensation. Topical treatment with this compound reduced lesion thickness to 56% at 2.5%, showing a higher efficacy than the reference control. The skin parasite load decreased after the experiment in all treatment groups, particularly in those animals treated with the 2.5% formulation (83.2%). Treatment with (-)-α-bisabolol was safe.

4 Conclusions

Topical (-)-α-bisabolol was more effective than meglumine antimoniate without showing toxicity effects on the hamsters, in accordance to the efficacy results obtained in a murine visceral leishmaniasis model through oral route. Our results suggest that this natural compound induces oxidative stress and mitochondrial-dependent apoptosis on *Leishmania* without disturbing the plasma membrane.

C0262 ADDITION OF A LEISHMANIA STRAIN PANEL TO THE HIT DISCOVERY SCREENING CASCADE FOR VISCERAL LEISHMANIASIS IMPROVES TRANSLATION TO IN VIVO EFFICACY STUDIES.

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2 Background

Visceral Leishmaniasis (VL) is caused by the protozoan parasites *Leishmania donovani* and *Leishmania infantum* which are transmitted by the bite of infected female phlebotomine sandlflies. VL is fatal if untreated with an estimated 310 million people at risk of infection and >50,000 deaths annually. 90% of VL cases are recorded in Bangladesh, Brazil, Ethiopia, India, South Sudan and Sudan. The current treatments have issues with toxicity, cost and are difficult to administer, therefore, new drugs for VL are urgently required.We have an *in vitro* screening cascade to identify new phenotypic hits against *L. donovani* which could progress to new clinical candidates for VL. This cascade starts with an axenic amastigote luminescence assay, followed by a high-content image-based intracellular assay which uses *L. donovani* LdBob strain and PMA differentiated THP1 host cells. However, to identify compounds that are effective against clinically relevant strains from different geographical locations development of a *L. donovani/ L. infantum* strain panel was undertaken and incorporated into our VL screening cascade.

2 Methods

A species-confirmed *L. donovani/ L. infantum* panel of strains isolated from patients in India, East Africa and Morocco was established. Low passage metacyclic parasites were used to infect CD14+ M-CSF differentiated human peripheral blood monocytes (PBMC) which were subsequently washed and dispensed onto compound containing 96 well plates for 96h. These are fixed and stained with Sytox green and imaged by the Operetta high content imaging system to generate potency and host cell toxicity data. (Human buffy coat was obtained ethically and its research use was in accordance with the terms of donor informed consent).

3 Results

High levels of PBMC infection was observed for all *Leishmania* strains. However, replication rates varied between strains, with the highest rate of replication observed for Sudanese strain Suka001. Strain-specific variation in potency profiles was also reported for some compounds. In addition, the potency data generated in this assay was a better predictor of the pharmacokinetic/ pharmacodynamic relationships reported in our *in vivo* animal model relative to our THP-1/ LdBob assay.

4 Conclusions

Incorporation of a *Leishmania* strain panel using primary macrophages and clinically relevant strains has proved essential in building confidence in hits coming through our VL screening cascade and has improved predictability of *in vivo* efficacy results.

C0292 BIOCHEMICAL ANALYSIS OF A LEISHMANIAL THERAPEUTIC TARGET AND SELECTION OF SPECIFIC INHIBITORS

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1 Background

Leishmaniases are Neglected Tropical Diseases (NTD) caused by protozoan parasites belonging to the genus *Leishmania*, and transmitted by an insect vector: the phlebotomine sandfly. Currently, 310 million people in 98 countries are threatened by these diseases with 2 million new cases each year. The existing treatments are limited and present main issues of toxicity, drug resistance emergence, and cost. In this context, the design and synthesis of inhibitors specifically directed against a target in the parasite is an attractive strategy for the development of new antileishmanial drugs. The GDP-Mannose Pyrophosphorylase (GDP-MP) is a prominent target to develop new antileishmanial agents due to its essential role in parasite survival and its involvement in the synthesis of diverse glycoconjugates necessary for host cell recognition.

2 Methods

In this work, we produced, purified and compared the enzymatic properties of recombinant GDP-MPs from *L. mexicana*, *L. donovani* and human. From the design of potential inhibitors based on GDP-MP molecular models, 100 compounds were evaluated on purified GDP-MP and on parasites *in vitro*.

3 Results

All GDP-MPs showed high substrate and cofactor specificities and exhibited sequential random mechanism of reaction. Four compounds were identified as promising specific inhibitors of leishmanial GDP-MPs, with selective antileishmanial activities.

4 Conclusions

These compounds could be further used as pharmacological tools for the development of new specific antileishmanial agents.

C0309 PHENOTYPIC SCREENING TO IDENTIFY SMALL MOLECULE INHIBITORS OF VISCERAL LEISHMANIASIS AND CHAGAS DISEASE

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1 Background

Leishmaniasis and Chagas disease affect millions of people worldwide and impart a heavy burden against human health. Due to the limitations of the existing treatments, an urgent need exists to develop novel therapies to treat, prevent and eliminate these diseases. Despite renewed efforts, the pipeline remains relatively sparse. Using the Calibr chemical collection and the tools developed in GSK the final aim of the project is to identify at least one, novel hit-to-lead series for each parasite.

2 Methods

To screen Calibr's library we used *Leishmania donovani* axenic amastigotes assay as primary screen, then InMac (Intra-Macrophage assay) to determine efficacy and macrophages toxicity of the hits. For *Trypanosoma cruzi* screen we employed β-gal assay (*T. cruzi* expresing β- Galactosidase) as primary and then Imaging assay (Intracelular amastigotes in H9c2 cells) to determine efficacy and toxicity in H9c2 cells, and Trypomastigotes assay in order to progress cidal compounds.

3 Results

The 150k compounds from Calibr library were screened against *L. donovani* in axenic amastigotes assay at single concentration rendering 1.2k compounds that were progressed to confirmation assays (axenic amastigotes and InMac) at single concentration by duplicate. InMac actives (500 compounds) were progressed to dose response assays: axenic, InMac, and HepG2 (cytotoxicity) assays.
The same library was screened against *T. cruzi* in β-gal assay at single concentration rendering 3.5k compounds that were progressed to confirmation assays (β-gal, Imaging and Trypomastigotes) at single concentration by duplicate. Trypomastigotes actives (1k compounds) were progressed to dose response assays (including HepG2).

4 Conclusions

For *Leishmania donovani*, 4 families and 5 singletons have been identified and will be progressed to further characterise series. For *Trypanosoma cruzi*, 5 families and 3 singletons have been selected. Among these compounds we have identified one panactive series. Next steps include kinetic assays and physicochemical profiling of the series.

C0312 SUBCUTANEOUS INFECTION MODEL FOR VACCINE STUDIES OF VISCERAL LEISHMANIOSIS: MIMICKING NATURAL CONDITIONS

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1 Background

BALB/c mice are among the most widely used experimental models of visceral leishmaniosis (VL) using different doses and routes of infection. In Spain, the zoonotic VL due to *Leishmania infantum* occurs when the infected *Phlebotomus perniciosus* sandfly deposits parasites alongside saliva at the site of bite in the skin. It is well known that a combination vaccine that includes a parasite antigen and a vector salivary protein has the potential to improve vaccine effectiveness. Interestingly, under experimental conditions of infection the induction of a protective *Leishmania*-specific immune response in the host requires the use of the subcutaneous (s.c.) administration of salivary gland homogenate (SGH) plus *Leishmania* parasites. In this context, we intend to develop a murine model of VL using the s.c. route of infection in the footpad.

2 Methods

BALB/c mice were infected subcutaneously into the footpad with $15x10^6$ stationary-phase promastigotes of *L. infantum* (M/CAN/ES/96/BCN150, zymodeme MON-1). Groups of infected mice were euthanized at weeks 4 and 10 post- infection (p.i.). Draining lymph nodes, spleens and livers were removed in order to determine parasite burdens and other immunological parameters.

3 Results

Our techniques for parasite determination by limiting dilution assay and detection of *Leishmania*-specific IgG antibodies by ELISA, revealed that infection was successfully established in all mice. Indeed, the parasite burdens in the draining lymph-node and the spleen persisted at high levels throughout the time period of the experiment (10 weeks) while the parasite burdens in the liver significantly reduced. Production of nitrites and arginase activity remained constant, compatible with the sustained parasite burden in the spleens of infected mice. However, TNF-α levels were increased at 10 weeks p.i., indicating its possible contribution to parasite clearance in the liver.

4 Conclusions

In this study, wedeveloped a model of VL that may better reflect the immunologic context associated with natural transmission conditions. Our data revealed that a fine immunological balance determined parasite-specific immune responses that promoted parasite clearance in the liver and those that promote the development of chronic disease in the spleen, at the end of the experiment. Consequently, this murine model of VL using the s.c. route of infection in the footpad could be relevant for testing antileishmanial vaccines.

C0317 ASTERACEAE ARTEMISIA CAMPESTRIS AND ARTEMISIA HERBA ALBA ESSENTIAL OILS TRIGGER APOPTOSIS AND CELL CYCLE ARREST IN LEISHMANIA INFANTUM PROMASTIGOTES

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1 Background

Leishmaniases constitute a group of neglected, vector-borne tropical diseases that are widespread and considered as major health problems. Due to toxicity and costs of current therapeutical formulations, the development of new and non toxic affordable drugs, which can improve current therapies, constitutes a research priority for the treatment of leishmaniasis. Plant secondary metabolites constitute promising candidates recognized as low-cost starting materials rich in bioactive compounds. The aim of this study was to investigate the chemical compositions and antileishmanial activities of hydro-distilled essential oils (EOs) from *Artemisia herba alba* Asso. and *Artemisia campestris* L. species collected in Tunisia.

2 Methods

Chemical compositions of the essential oils were determined by GC-MS. The anti-*Leishmania* activity of these two natural products was evaluated against *Leishmania infantum* promastigotes using resazurin assay and against intracellular amastigotes in experimentally-infected peritoneal macrophages. Apoptosis was assessed using Annexin-V/7-AAD staining and the cell cycle was examined with Propidium Iodide.

3 Results

Both EOs possess significant anti-leishmanial activity against *Leishmania infantum* promastigotes with IC_{50} values of 68 μg/mL and 44 μg/mL for *A. herba-alba* and *A. campestris*, respectively. Both inhibitory doses were able to reduce the intracellular amastigote rates without cytotoxic effect against mammalian macrophages. The anti-leishmanial activity was mediated via apoptosis, as evidenced by externalization of phosphatidylserine and cell cycle arrest at the sub-G0/G1 phase. By using LDH release assay, we confirmed that even after 72 h exposure to both EOs the cell membrane of the promastigotes remains intact and the parasites die with a programmed cell death mechanism.

4 Conclusions

These results showed that EOs from *A. herba alba* and *A. campestris* plants are promising candidates as anti-*Leishmania* medicinal products which trigger *L. infantum* apoptosis. Our study expands the scope for future design of novel chemotherapeutic strategies against leishmaniasis.

Key words: *Artemisia herba alba*, *Artemisia campestris*, essential oil, anti-leishmanial activity, *Leishmania infantum*, apoptosis, cell cycle arrest.

C0333 ENERGY BLACKOUT AGAINST GLYCOLYTIC PARASITES

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1 Background

Leishmaniases, trypanosomiases and malaria are parasitic diseases that constitute a major global health problem. The increasing number of drug-resistances to current treatments, toxicity cases, and the limited access to medicines and preventive tools in the lowest socioeconomic environments where they are principally spread, make it urgently necessary the developing of new affordable therapies, easy to apply and resistant to harsh storage conditions.These diseases share similar metabolic requirements with much better studied disorders. Here we chose drug repurposing as a potentially effective approach against them. Five different compounds used in anti-tumoral research —Dichloroacetic acid (DCA), 3-bromopyruvic acid (3BP), 2-deoxy-D-glucose (2DG), lonidamine (LND), and metformin (MET)— were selected according to their ability to modulate metabolic routes involved in energy production, also essential for parasite activity. The aim of this study was to assess the effectiveness of these compounds in *in vitro* models of these diseases.

2 Methods

Non-cytotoxic concentrations of these compounds to host cells were assayed on *in vitro* models of these parasitic diseases for 48–72h, depending on each parasite requirements for cycle completion. The number of parasites was then assessed, and IC50 values were determined by non-linear regression analysis (GraphPad Prism Software v5).

3 Results

L. infantum promastigotes did not result susceptible to these compounds, whereas *L. infantum* intracellular amastigote growth was dose-dependently reduced by 3BP (IC50 = 24.88 µM) and DCA (IC50 = 700 µM). In case of *T. brucei*, 3BP (IC50 = 76.09 µm), DCA (IC50 = 1.25 mM), LND (IC50 = 27.05 µM) and MET (IC50 = 17.47 mM) affected parasite survival; whereas for *T. cruzi* and *P. falciparum in vitro* models, both resulted susceptible toDCA (IC50 = 25.82 mM and 5.4 mM), 2DG (IC50 = 7.26 mM and 4.19 mM), LND (IC50 = 82.3 µM and 209.13 µM), and MET (IC50 = 18.48 mM and 1.23 mM) respectively.

4 Conclusions

Our results reinforce the hypothesis that drugs with proven efficacy in the treatment of cancer by interfering with energy production might be useful in treating threatening parasitic diseases, and provide new opportunities for their repurposing.

C0341 IN VITRO EVALUATION OF TRADITIONALLY USED SURINAMESE MEDICINAL PLANTS FOR THEIR POTENTIAL ANTI-LEISHMANIAL EFFICACY

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1 Background

The unique plant biodiversity from the jungle of Suriname provides ample raw material to be processed into ethnopharmacological preparations for treating cutaneous leismaniasis (CL). However, the scientific evidence to support the therapeutic usefulness of these substances is scant. If efficacious, (some of) them may represent lead compounds for the development of novel drugs for treating CL. For this reason, a number of plants that are used in Suriname against this disease were evaluated for their activity against cultured promastigotes and amastigotes from various *Leishmania* species, including *L. (V.) guyanensis*.

2 Methods

Twenty-five medicinal plant extracts from Suriname were evaluated for their efficacy against leishmaniasis. For this, different plant parts were collected on the basis of traditional knowledge and extracted according to traditional methods, concentrated, and evaluated for their effect on the viability of *L. (V.) guyanensis* AMC, *L. (L.) major* NADIM5, and *L. (L.) donovani* GEDII promastigotes, as well as intracellular amastigotes of *L. (L.) donovani* BHU814 in infected THP-1 cells. Selectivity was assessed by cytotoxicity against THP-1 cells.

3 Results

The wolfapple *Solanum lycocarpum* A.St.-Hil. (Solanaceae) showed potentially meaningful anti-leishmanial activity with mean IC_{50} values of 51, 61, and < 16 µg/mL against the promastigotes of *L. (V.) guyanensis*, *L. (L.) major* and *L. (L.) don*ovani, respectively; 374 µg/mL against the *L. (L.) donovani* amastigotes; and > 500 µg/mL against the THP-1 cells. Extracts from the amargo *Quassia amara* L. (Simaroubaceae), the guavo *Inga alba* (Sw.)Willd (Fabaceae), and the mother of thousands *Bryophyllum pinnatum* (Lam.) Oken (Crassulaceae) displayed low to moderate IC_{50} values against promastigotes and/or amastigotes (51 to > 500 µg/mL) but were relatively toxic to the THP-1 cells (IC_{50} values of <16 to 42 µg/mL). The remaining plant extracts exhibited high IC_{50} values, above of 500 µg/mL against promastigotes, amastigotes, and THP-1 cells.

4 Conclusions

S. lycocarpum preparations may be useful for treating CL and may cause relatively low toxicity to the normal tissues. In light of the lack of efficacious forms of CL treatment, this observation makes this plant extract an interesting candidate for new drug development programs. At this moment, preparations are being made to identify the pharmacologically active principle(s) in the *S. lycocarpum* extract applying bioassay-guided purification.

C0356 ESTABLISHMENT OF AN ANIMAL MODEL FOR DESERT-TYPE KALA-AZAR

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1 Background

It has been difficult to isolate Leishmania of desert-type kala-azar, resulting in part from the lack of a suitable animal model. Leishmania isolates were used to infect BALB/c mice, *Phodopus sungoris sungoris* and *Lagurus.lagurus*, in attempt to establish an animal model for human leishmaniasis.

2 Methods

BALB/c mice, *P.sungoris* and *L.lagurus* were each inoculated intraperitoneally with leishmania from the desert-type kala-azar at 2.6×10^5 per animal.We examined parasites, splenomegaly, spleen coefficients (100%×spleen quality/body quality), infection rate and rK39-positive antibodies in the animals after inoculating for 29, 46, 90, 152 and 217 days.

3 Results

The mice were found not susceptible to the leishmania. Leishmania infection of *P.sungoris* and *L.lagurus* became notable 29 and 46 days after the infection, reaching 80% and 100% on day 90. Splenomegaly began to rise on day 46 and 152 days for *P.sungoris* and *L.lagurus* respectively, reaching a value 30 times greater than normal spleen quality of *L.lagurus*. The antibody positive rate of *L.lagurus* reached 70% and 100% on day 60 and 90 respectively, whereas the rate of *P.sungoris* was only 12.5% on 217 day after inoculation.

4 Conclusions

BALB/c mice are not susceptible to the Leishmania via intraperitoneal inoculation. The same strain infected *P.sungoris* as noted after 46 days, causing splenomegaly after 152 days and antibody positivity after 217 days. Typical clinical symptoms of leishmaniasis were manifested after infecting *P.sungoris* for 217 days. In infected *L.lagurus*, all clinical parameter appeared 90 days after infeciton, including splenomegaly, 100% of infection rate, high splenomegaly and antibody positive rates. *L.lagurus* is thus a suitable animal model for visceral leishmaniasis.

C0363 TWO ASSAYS TO DETERMINE POTENTIAL EFFICACY OF NEW ANTI-LEISHMANIA COMPOUNDS

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1 Background

Currently available drugs for treatment of Leishmania infections are highly toxic and drug resistance to first line therapies has been observed. Therefore there is an urgent need for new, safe and effective drugs. Critical to the search for new anti-leishmanial drugs is the availability of (preferably high-throughput screening, HTS) methods to test chemical compounds against the relevant stage for disease pathogenesis, the intracellular amastigotes. However, a simple and efficient test for measuring drug activity against Leishmania clinical isolates is lacking. Here we present two methods that could aid drug developmen

2 Methods

Two assays have been developed in our laboratory. The first method is a quantitative colorimetric assay, whereby the activity of a Leishmania native enzyme (disulphide-trypanothione) is used to assess parasite viability. The second is a duplex quantitative Reverse-Transcriptase PCR (qRT-PCR), which simultaneously quantifies Leishmania 18S ribosomal RNA and the human b2-microglobulin (b-2M) mRNA, used for monitoring drug cytotoxicity and test performance

3 Results

Pharmacological validation of the enzymatic assay was performed against the conventional AlamarBlue® method for promastigotes or standard microscopy for intracellular amastigotes. The activity of a selected-compound panel, including several anti-leishmanial reference drugs, demonstrated high consistency between the newly developed assay and the reference method, and corroborated with previously published data. Quality assessment with standard measures confirmed the robustness and reproducibility of the assay, which performed in compliance with HTS requirements. Furthermore, accurate determination of parasite viability by the newly developed qRT-PCR was confirmed by parallel assessment of compound performance against standard microscopy. Highly reproducible anti-leishmanial activities were obtained with a set of structurally- and pharmacologically-diverse compounds, whose toxicity against host cells correlated with a low b-2M amplification.

4 Conclusions

The simple and rapid enzymatic assay provides a reliable, accurate method for screening anti-leishmanial agents, at high-throughput. The basic equipment and manipulation required to perform the assay makes it easy to implement even in more resource limited settings. The duplex qRT-PCR has a very high sensitivity and offers a valuable tool for assessment of drug activities against Leishmania amastigotes and their host cells.

C0373 THE ROLE OF GENES INVOLVED IN THIOL METABOLISM IN DRUG RESISTANCE OF CLINICAL ISOLATES OF LEISHMANIA TROPICA

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1 Background

In human leishmaniasis management, drug resistance developing under natural conditions, is a great concern. Studies on laboratory induced resistant mutants of *Leishmania* spp. have shown that efflux transporters and thiol enzymes contributed to resistance to antimonials but their role in resistance of clinical isolates of *Leishmania* is still unknown. The present study was designed to elucidate the role of genes involved in thiol metabolism in natural antimony resistance in *L. tropica* clinical isolates.

2 Methods

Parasites samples were isolated from lesions of patients with cutaneous leishmaniasis (CL).The identity of the parasites was determined using ITS-1-PCR method. Specimens were cultured on NNN medium and promastigotes were grown using RPMI1640 + 10% FCS. A macrophage-*L. tropica* amastigotes co-culture system with serial concentrations (from 3.75 to 100 µg/mL) of meglumine antimoniate (Glucantime®) was used for drug sensitivity assay. Two efflux pumps inhibitors, verapamil (4 µM) and lovastatin (5 µM) were used. Inhibitory concentration of 50% (IC_{50}) was determined, then amastigotes were subjected to RNA extraction followed by real-time RT-PCR for gene expression assay of TRYR, ODC, *gsh*1, *mdr*1, *pgp*A genes.

3 Results

Twenty CL patients with different backgrounds and clinical resistance profile were included. Changes in the expression levels were seen in *mdr*1 efflux transporter and some of thiol metabolism enzymes genes. Further details of the results will be presented at the Congress.

4 Conclusions

Results suggest that changes in the expression levels of genes of thiol metabolism enzymes and efflux transporters are involved in Glucantime® resistance of clinical isolates of *L. tropica* amastigotes.

C0406 EVALUATION OF IMMUNE RESPONSE TO ZOONOTIC CUTANEOUS LEISHMANIASIS AGENT ISOLATED FROM NO CLINICAL IMPROVEMENT PATIENTS

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1 Background

Leishmaniasis is caused by an obligate intracellular parasite inoculated into the skin by the bite of a sand fly and lives and multiplies in monocyte phagocytic cells of vertebrates. Activation of macrophage is an initial mechanism to eliminate the *Leishmania* parasite presumably mediated by toxic metabolites also secretion of cytokines that are effective in promoting TH1 responses such as interleukin 18 and 27. IL-18 is known as Interferon-Gamma Inducing Factor or IGIF. IL-27 can act as a bridge between innate and adaptive immunity and it seems to play a role in the differentiation of Th1 and eventually overcome and effective recovery of leishmaniasis. The main objective of the study is evaluating the immune response and gene expression of two mentioned interleukins against patient's isolates who have no good response to standard medicine, Glucantime, at the clinic compared with the standard species.

2 Methods

Experimental study was conducted in 2014. After isolation of Leishmania from patients' lesion that had at least one course of failure treatment with Glucantime in clinic, an in vitro and in vivo immune response and increasing the IL-18 and IL-27 gene expression in both untreated and treated Glucantime groups were evaluated. Murine macrophage cell line J774 for the in vitro, and Blab/c mouse for in vivo was used. Gene expression measured Using Real time PCR method. The results were analysed using covariance method.

3 Results

L.major is isolated from 10 lesion patients using nested-PCR. Mean gene expression of IL-18 and IL-27 in vitro also 2 and 3 weeks treatment in vivo stages in Glucantime group treatment showed significantly increasing compared to untreated group in both isolated patients and standard species. There was no significant difference between the patients' isolates and standard species.

4 Conclusions

In this study the interaction between patients isolates with no clinical improvement history to Glucantime and immune system was evaluated. According to the obtained results it can be concluded that glucantime was able to promote the immune system even in the presence of species with clinical resistance. So it seems unsuccessful treatment might have been caused by other factors, such as lack of appropriate drug delivery to target tissues and cells that can be used in other projects.

C0418 THERAPEUTIC EFFECT OF GLUCANTIME WITH RESPECT TO ENCODING OF P GLYCOPROTEIN A, AQUAPORIN 1 & GLUTAMYLCYSTEINE SYNTHETASE GENES OF ISOLATES FROM PHENOTYPIC RESISTANT ZOONOTIC CUTANEOUS LEISHMANIASIS CASES

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1 Background

Cutaneous leishmaniasis (CL) refers to chronic skin lesions cause by some leishmania species.Pentavalent antimonial (Sbv) compounds, have been the first-line treatment. Variation in therapeutic responses and resistance to Sbv have complicated the role of the first-line drugs.Treatment failure could be due to over expression of drug resistance genes. Here, we selected P glycoprotein A (PGPA: involve in efflux and sequestration the drug), Gamma-glutamylcysteine synthetase(GSH1: inducing reduction environment) and Aquaporin 1AQP1:as drug transporter) for comparative expression analysis in phenotypic resistance ZCl and L. major (MRHO/IR/75/ER)(as sensitive strain) before and after treat with Glucantime by Q-RT-real time PCR, in two section, in vitro and in vivo. Analysis and comparison of relative gene expression data were done by Using the 2 -ΔΔ CT method. Till by using the results of this study, about untreated patients, better decisions to be taken.

2 Methods

Parasites were isolated from non- healing patients lesion. It's species was identified by Nested-PCR. In vitro exams was performed by amastigote-macrophage model and expose with glucatime. For in vivo, inbred Balb/c mice were infected with mentioned samples. The rate of expression of mentioned genes were determined by triplicate quantitative RT-Real time PCR method prior exposure to glucantime and after that and compared.

3 Results

Nested-PCR showed all samples to be L. major. By comparison of expression genes level between phenotypic non healing samples and L.major (MRHO/IR/75/ER), weren't seen any statistically significant difference, before and after threat both in vitro and in vivo.

4 Conclusions

In the non-healing phenotypic samples weren't seen molecular resistance but it is important to say that large-scale misuse of the drug and disregard to treatment protocol can be induce untreated cases and gradually drug resistance.

C0456 DDD853651, A PRE-CLINICAL CANDIDATE FOR VISCERAL LEISHMANIASIS, ACTS THROUGH INHIBITION OF CYCLIN-DEPENDENT KINASE 12

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1 Background

Visceral leishmaniasis causes significant mortality and morbidity in many parts of the world. Current drugs are inadequate and there is an urgent need for the development of new, effective oral treatments for this disease. In the current study, we report the discovery of an exciting new anti-leishmanial compound (DDD853651), which inhibits a novel molecular target within these parasites.

2 Methods

Phenotypic screening, followed by medicinal chemistry, led to the development of a novel anti-leishmanial chemical series based around a pyrazolopyrimidine scaffold. Further optimisation identified the lead compound DDD853651.To determine the mode of action and specific molecular target(s) of this compound series, a number of complementary methodologies in the fields of genomics and chemical proteomics were employed. Specific details will be presented.

3 Results

DDD853651 has potent efficacy in a mouse model of visceral leishmaniasis and has suitable physicochemical, pharmacokinetic and toxicological properties for further development. Compounds from this series have a profound effect on the parasite cell-cycle inducing arrest at both G1/S and G2/M. Comprehensive mode of action studies utilising orthogonal approaches such as genomics and chemical proteomics indicate that compounds from this series act principally by inhibiting cyclin-dependent kinase 12 (CRK12) of *Leishmania* parasites.

4 Conclusions

DDD853651 shows real potential to become a much needed oral treatment for visceral leishmaniasis and has recently progressed into advanced pre-clinical development, with the aim of entering into human clinical trials. Our comprehensive mechanism of action studies confirm that the principal target of DDD853651 is CRK12. This cyclin dependent kinase, whose endogenous function remains to be determined, represents a novel, druggable target within *Leishmania*. The discovery of CRK12 as a viable anti-leishmanial drug target opens up new possibilities for drug discovery.

C0463 EFFECT OF ?/?3-PEPTIDE FOLDAMERS WITH CELL-PENETRATING CAPACITY AS DIMERIZATION INHIBITORS OF LEISHMANIA INFANTUM TRYPANOTHIONE REDUCTASE

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1 Background

Leishmania infantum trypanothione reductase (Li-TryR) is an essential enzyme for parasite viability. Given that the biologically active form of Li-TryR is a homodimer, we designed a library of peptides derived from a selected α-helix at the Li-TryR dimerization interface to try and inhibit the enzyme by disrupting the homodimer as an alternative to the classical approach of blocking the active site. This strategy led us to the discovery of the TRL35 prototype, which behaves as the most active member of the series in both enzymatic and dimerization assays. However, further optimization was necessary because this peptide was not active against *L. infantum* parasites, presumably due to its sensitivity to proteases and its inability to be taken up by the parasites.

2 Methods

The effect of the peptides on Li-TryR enzymatic activity was evaluated by monitoring the absorbance at 412 nm caused by reduction of Ellman's reagent (5,5'-dithiobis(2-nitrobenzoic acid, DTNB) during one hour. Homodimer disruption was evaluated by using an in-house ELISA. Peptide stability was studied after incubation with the broad-spectrum serine protease proteinase K and subsequent analysis of the digestion products by HPLC. Leishmanicidal and cytotoxic effects were evaluated by flow cytometry using the iodide propidium exclusion method.

3 Results

The designed α/β^3-peptide foldamers presented here mimickedthe Li-TryR homodimer disrupting activity of our TRL35 prototype and displayed improved proteolytic stability but they were still inactive against *L. infantum* parasites. Their leishmanicidal effects were finally enhanced when they were conjugated to an $(Arg)_9$ cell-penetrating peptide.

4 Conclusions

Using our previously reported TRL35 prototype as a lead, we designed and optimized a library of α/β^3-peptide foldamers that not only inhibit Li-TryR dimerization and enzymatic activity but are also endowed with improved proteolytic stability and leishmanicidal effects.

C0511 EFFECTS OF MILTEFOSINE ON LEISHMANIA CHAGASI CELLULAR OXIDATIVE STRESS DYNAMICS: UNCOVERING NATURAL MILTEFOSINE RESISTANCE PROCESS

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1 Background

Caused by *Leishmania donovani* complex parasites, visceral leishmaniasis (VL) is a serious disease with a challenging treatment plan, requiring the prolonged and painful application of poorly tolerated toxic drugs. Therefore, a major breakthrough in the leishmaniasis therapy was the identification of an effective and safe oral drug: miltefosine. However, its mechanism of action and resistance in *Leishmania* ssp. are only partly known.

2 Methods

Promastigotes of *L. chagasi* reference strain PP75 were cultured under different miltefosine concentrations and time length in order to assess the effects of the drug on the dynamic of reactive oxygen species (ROS) generation, cell cycle and phosphatidylserine exposure (PS). The effects were analysed by flow cytometer using H_2DCFDA and MitoSOX probes, Annexin-V and propidium iodide. The ability to resist to those miltefosine effects was then verified in four *L. chagasi* isolates from Brazilian VL patients with different miltefosine treatment outcomes. Parasite susceptibility was measured by Alamar Blue® assay.

3 Results

Miltefosine treatment of *L. chagasi* results in significant increase of ROS, PS exposure as well as disturbance on cell cycle profile in a time- and dose-dependent manner. Moreover, ROS generation (H_2O_2, HO•, ROO• and $ONOO^-$) and cell cycle perturbation overlap with miltefosine cytotoxicity effect. The first effects observed in cell cycle were G0-G1 decrease and G2-M increase, followed by accumulation of multinuclear cells. Interestingly, mitochondrial superoxide ($•O_2^-$), and DNA degradation (cell<2n DNA) were observed only under high miltefosine concentration, when cell viability is reduced by ~90%. This indicates that mitochondrion and DNA instability are likely the result of miltefosine action in secondary targets. Further analysis showed that two *L. chagasi* isolates from cured and one isolate from relapsed patients exhibited similar low capacity to resist to cell oxidation stress induced by miltefosine, while one isolate from relapsed patient exhibited greater ability to control redox homeostasis.

4 Conclusions

These data indicate that miltefosine induces cellular oxidative stress in a time and dose-dependent manner, although some of these events do not overlap with drug cytotoxicity effect. Our findings also revealed that the ability to control redox homeostasis under drug pressure is one of the potential natural miltefosine resistance mechanisms, but this process is likely complex and multifactorial.

C0556 ANTIMONY(V)-PORPHYRIN PRESENT HIGH ANTILEISHMANIAL ACTIVITY BY INHIBITING STEROL 24-C-METHYLTRANSFERASE AND RESENSITIZES RESISTANT MUTANTS

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1 Background

The treatment of leishmaniasis has several drawbacks including high cost, side effects and resistance development, requiring urgent antileishmanial drug alternatives. We have previously explored the antileishmanial potential of metalloporphyrins.

2 Methods

Here we present the screening of an antimony(^{V}) porphyrin ($Sb^{V}P$) against: (I) promastigote forms of *L. major*, *L. infantum*, *L. amazonensis*, *L. guyanensis* and *L. braziliensis*; (II) amastigote forms of *L. infantum* and *L. braziliensis*. The cytotoxic effect was evaluated on human-derived THP-1 macrophages and murine fibroblasts from L929 cell line. Lipid extracts from $Sb^{V}P$ treated parasites were evaluated under gas chromatography coupled to mass spectrometry (GC/MS)

3 Results

$Sb^{V}P$ was highly active amongst all tested evolutive forms, presenting IC_{50} values at nanomolar concentrations ranging from 0.016-0.94 µM which is 28-125 times selective towards *L. infantum* intracellular amastigotes. Promastigote forms are less sensitive to the pro-drug Sb^{V} compared to its active form Sb^{III}. We curiously observed, however, that $Sb^{V}P$ was either highly active against *Leishmania* spp. promastigotes. GC/MS revealed an accumulation of cholesta-5,7,24,trien-3b-ol, substrate of sterol 24-C-methyltransferase of the ergosterol biosynthesis pathway on trypanosomatids. Cells exposed to the porphyrin alone (ligand) or to a bismuth(^{III}) porphyrin ($Bi^{III}P$) do not accumulate this lipid precursor, indicating a specific antileishmanial activity of $Sb^{V}P$ through ergosterol biosynthesis inhibition. *In silico* docking analysis will be performed to corroborate the functional observations. $Sb^{V}P$ presented no cross-resistance against Sb-resistant *Leishmania* spp., confirming that different pathways are involved on its mechanism of action. Indeed, $Sb^{V}P$ was able to resensitize these mutants. Murine acute toxicity was assessed and $Sb^{V}P$ is safety in a dose up to 200 mg/kg/day.

4 Conclusions

These findings make $Sb^{V}P$ a promising candidate for *in vivo* efficacy evaluation of a drug that act by inhibiting parasite's ergosterol biosynthesis pathway and could be applied even against leishmaniasis caused by Sb-resistant mutants.

C0557 CHARACTERIZATION OF HITS IDENTIFIED FROM PHENOTYPIC SCREENING: POTENTIAL NEW LEADS FOR VISCERAL LEISHMANIASIS.

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1 Background

Leishmaniasis, caused by the trypanosomatid protozoan parasite, *Leishmania,* is endemic in 98 countries with more than 350 million people at risk of acquiring the disease. The life cycle of *Leishmania* parasite exists between the sand fly (promastigote form) and the mammalian host (amastigote form).
According to the clinical manifestations, the disease can be characterized as cutaneous, muco-cutaneous or visceral leishmaniasis; the latter being fatal. Current treatment regimens often have poor toxicity profiles and resistance has begun to emerge against the standard of care therapies. The therapeutic efficacy varies depending upon the disease pattern, species and geographical distribution of the parasite.
The drug discovery pipeline for neglected trypanosomatid diseases remains sparse. In particular, the field of leishmaniasis drug discovery has had limited success in translating potential drug candidates into viable therapies.

2 Methods

To identify compounds active against *Leishmania donovani* (MHOM/IN/80/DD8) parasites, a primary screen of 5560 structurally diverse synthetic compounds was undertaken. Screening incorporated a promastigote viability assay (extracellular form) and a high-content intracellular amastigote imaging assay. Confirmation of activity was performed together with cytotoxicity studies against both THP-1 (host cell) and HEK-293 cell lines. A comparative analysis against old world *Leishmania infantum* (MHOM/MA/67/ITMAP263) and new world *Leishmania chagasi* (MHOM/BR/1972/BH46) intracellular species was undertaken.

3 Results

Potent and selective anti-leishmanial compounds were identified, including compound BZ-1 with IC_{50} values of 0.59 ± 0.13 and IC_{50} of 2.37 ± 0.85 μM against the intracellular and extracellular forms of *L. donovani* DD8 parasites, respectively. Compound BZ-2 exhibited an IC_{50} of 0.57 ± 0.17 μM against the intracellular form of the parasite and IC_{50} of 0.60 ± 1.12 μM against the extracellular form. Both compounds showed activity against intracellular forms of *L. infantum* (IC_{50}: 1.56 ± 0.42 and 1.19 ± 1.01 μM) and *L. chagasi* (IC_{50}: 6.30 ± 0.14 and 5.45 ±1.06 μM).

4 Conclusions

From a library of 5560 compounds, two compounds were identified as active against both the old world and new world species causing Visceral Leishmaniasis. These hit compounds may provide urgently needed starting points for the development of novel lead series for future anti-leishmanial therapeutics.

C0619 DEVELOPMENT OF A METHODOLOGICAL PLATFORM FOR THE IN VITRO AND IN VIVO IDENTIFICATION OF ANTI- LEISHMANIA PANAMENSIS AGENTS

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1 Background

Background: Leishmaniasis is a tropical disease with a significant global health burden that is caused by *Leishmania* flagellate protozoa. Leishmaniasis has a wide spectrum of clinical manifestations depending on the *Leishmania* species and the immunological status of the host. These include localized and diffused cutaneous leishmaniasis, mucocutaneous form and visceral disease. *L. panamensis* from the subgenus Viannia is the strain responsible for most of the cases in Panama, Costa Rica and Colombia. Current treatments for leishmaniasis are lengthy and have high toxicity and serious side effects.Furthermore, resistance to some of these drugs has been reported for a diversity of *Leishmania* strains. Due to the differences in Leishmania species sensitivity to drugs, the search of species-specific antileishmania drugs has been encouraged. Hence, we have developed an *in vitro* and *in vivo* model for the search of new molecules as potential anti-*L. panamensis* agents.

2 Methods

Methods: In this study we evaluated the effects on intracellular amastigotes and promastigotes of *L. panamensis* of three families of bi- and tricyclic *N*-heterocycles: tetrahydrooxazinoindoles (TOIs), quinolines, and indoles. The promastigotes inhibition assay was based on quantitation of the ATP and the evaluation of compounds against *L. panamensis* intracellular amastigotes was performed by the Giemsa staining method.We have also developed an animal model of *L. panamensis* infection in Balb/c mice. For this, animals are infected in the ear and parasitemia, size and histopathology of the lesson are evaluated at different time points of the infection.

3 Results

Results: We have identified some TOIs and quinolines as competent antileishmanial agents *in vitro* while substituted indoles are inactive. Interestingly, we also found that one quinoline compound inhibits the production of IL-10 by macrophages infected with *L. panamensis,* suggesting that the antileishmanial activity of this compound might be, at least in part, mediated by the modulation of the macrophage activation.Our results *in vivo* have shown that Balb/c animals are susceptible to the infection with *L. panamensis*, presenting large lessons, with an inflammatory infiltrated and persistence of amastigotes along the infection.

4 Conclusions

Conclusions:Our results indicate that this animal model is suitable for testing *in vivo* the hits identified *in vitro*. The activity of TOI compounds opens an avenue for the search of structurally novel anti-*L. panamensis* agents.

C0661 ANTI-LEISHMANIAL ACTIVITY OF NATURALLY INSPIRED CHALCONES

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1 Background

Leishmaniasis is a phlebotomine-transmitted infection caused by protozoan belonging to the genus *Leishmania*. Infections can manifest as tegumentary or visceral leishmaniasis, the latter being fatal if untreated. Drugs for the treatment of leishmaniasis are toxic and expensive, indicating the urgent need to develop effective and safe molecules. Several natural products (NPs) or synthetic NP-inspired molecules have shown promising pharmacological activity as anti-leishmania agents; among these are the chalcones, precursors of flavonoids. A peculiar feature of this class of molecules is the *trans*-α,β-unsaturated carbonyl function, able to act as a nucleophile, establishing covalent linkages with the cysteine residues of various target proteins. In this contest, a small library of chalcones was designed, synthesized and tested to assess the anti-leishmanial potential evaluating the ability to affect parasite growth and to inhibit the protozoan enzyme trypanothione reductase (TR), an essential enzyme for *Leishmania* metabolism, that is not present in human cells.

2 Methods

The newly synthesized chalcones were obtained through the classic base-catalyzed Claisen-Schmidt procedure. Drug susceptibility assays were tested on promastigotes of a reference strain of *Leishmania donovani*. Citotoxicity was analyzed on mammalian cell lines. The ability of the compounds to bind TR (Kd) was assessed by Surface Plasmon Resonance studies whereas the ability to inhibit TR was assessed using spectrophotometric TR assays by following the NADPH oxidation at 340 nm.

3 Results

Screening of 33 newly synthesized chalcones on *L.donovani* promatigotes revealed that two compounds effectively inhibited the *in vitro* growth of the parasites with a $IC_{50} \leq 15$ μM and exhibited a good selectivity window (selectivity index range 150 – 19). Evaluation of inhibition of the intracellular form of *Leishmania* confirmed the low micromolar trend (IC_{50} = 23 μM and 6.2 μM, respectively). These two molecules also showed good affinity for TR and were able to efficiently inhibit TR (low-micromolar values).

4 Conclusions

These results indicate that two newly synthesized chalcones fulfilled lead selection criteria for anti-leishmania drug candidates, suggesting these promising compounds worth to be evaluated for treatment of leishmaniasis.The selective enzymatic inhibition of TR, an essential enzyme for *Leishmania* metabolism, indicate TR as a specific target for the two chalcones.

C0673 LEISHMANIA KILLICKI: GROWTH AND HISTOLOGICAL PATTERNS OF NEW CUTANEOUS LEISHMANIASIS SPECIE IN ANIMAL MODEL BALB/C MICE IN COMPARISON TO LEISHMANIA MAJOR

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1 Background

Several animal models have been established to study cutaneous leishmaniasis (CL). BALB/c mice experimental model is the most widely used. In this work, we expose the mouse model infected with new Algerian CL specie: *L.killicki* found in 2005 in south Algeria. To establish a model of skin infection with *Leishmania killicki* with low doses of parasites, we compared to a good known model of infection with *L. major*

2 Methods

The ear dermis of BALB/c mice were infected with 10^3 of purified metacyclic promastigotes of *L. major* or *L. killicki*. Following inoculation, parasite expansion in the ear dermis, draining lymph node and spleen were followed by PCR and by culture method 20 week post infection. At the inoculation site we also measure the development of the lesion induration as well as the parasite load. Finally, we compare the clinical evolution of *L. killicki* and *L. major* lesions, parasite load, and histopathology under antimonial treatment.

3 Results

Lesions induced by *L. killicki* were nonulcerative in their aspect, whereas those caused by *L. major* were highly ulcerative and necrotic, which matches well with the lesion phenotype reported in humans for *L. killicki* and *L. major*, respectively. The treatment of *L. killicki* lesions by injection of Glucantime® significantly reduced the lesion thickness and parasite burden

4 Conclusions

BALBc mice represent a valuable model in which physiopathological aspects of cutaneous lesions caused by *L. killicki* as well as immunological effectors of infection can be further studied. This new model of *L. killicki* animal infection can also be of some help for *in vivo* screening of new antileishmanial compounds.

C0677 BACTERIOCINS AS LOW-COST ANTI-LEISHMANIAL AGENTS. THE ENTEROCIN AS-48 AS A PROOF-OF-MECHANISM

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1 Background

Until recently, peptide-based antimicrobial therapies were despised due to their low bioavailability and high cost when compared with low molecular drugs, despite their low-induction of resistance induction and broad spectra of susceptible microorganisms. The feasibility of this approach on *Leishmania* was obtained for eukaryotic antibiotic peptides but not for bacteriocins, despite their advantages such as gene manipulation, broad structural diversity and low-cost.
In this work we assayed AS-48 a circular cationic bacteriocin from *Enterococcus faecalis* with low immunogenicity and highly structural stability, as a proof–of-mechanism for a leishmanicidal bacteriocin.

2 Methods

AS-48 was isolated from the fermentation broth of *E. faecalis*, and purified to homogeneity after two chromatographic steps. Leishmanicidal activity was assayed on *Leishmania donovani* promastigotes, and *L. pifanoi* axenic and intracellular amastigotes. Plasma membrane permeation was assessed by entrance of the vital dye SYTOX green, decrease of ATP, and electron microscopy. Monofluoresceinated AS-48 was used to assess its intracellular location by confocal microscopy, and cellular accumulation by cytofluorometry.

3 Results

IC_{50}s (μM) of AS-48 *L. donovani* promastigotes and *L. pifanoi* axenic amastigotes were 3.9 ± 1.1,10.2 ± 1.2. AS-48 at 7 μM reduced intracellular parasitation by 90%.
AS-48 induces the decrease of ATP, and entrance of SYTOX green under an all-or-none permeation pattern, but at lower rate than a pure membrane permeation process. This also applies to intracellular amastigotes. AS-48 was spotted inside parasites at concentrations under the threshold for membrane permeation, with decrease of the electrochemical potential of mitochondrion and increase of ROS production.

4 Conclusions

To the best of our knowledge AS-48 was the first bacteriocin assayed successfully as antileishmanial agent, acting at low micromolar concentrations. Its dual lethal effect encompasses membrane permeation and additional intracellular targets, evidenced the feasibility of broad spectrum bacteriocins as a source for new leishmanicidal agents, likely amenable for the treatment of non-disseminated cutaneous leishmaniasis.
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C0722 THE ROLE OF NEUTROPHILS IN THE OUTCOME OF DRUG TREATMENT AGAINST LEISHMANIA (VIANNIA) PANAMENSIS

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1 Background

Leishmania (Viannia) panamensis (L. (V.) p.) is the main causative agent of cutaneous leishmaniasis in Colombia. This disease is usually treated with either meglumine antimoniate (MA) or miltefosine (MIL). In recent years, there has been an increasing amount of evidence for the emergence of drug-resistance against these drugs. Data recently obtained show gene expression signatures in lesion biopsies that suggest enhanced neutrophilrecruitment to lesions of patients that do not respond to anti-leishmanial treatment when compared to those who respond to treatment. Neutrophils are known to play an important role in immunity against *Leishmania*. They are the cells first recruited upon infection and are also found in chronic lesions. However, their role in the outcome of anti-leishmanial treatment has not been investigated yet.

2 Methods

Neutrophils from naïve mice or human blood neutrophils from healthy volunteers were challenged in vitro with laboratory lines and clinicalstrains of *L. (V.) p.* that are drug-susceptible or resistant to either MA or MIL. Neutrophil extracellular trap (NET) formation was analyzed and quantified by confocal microscopy analysis and by measuring the amount of extracellular dsDNA. The impact of the different *L (V) p* strains on neutrophil effector functions upon in vitro infection were further assessed by the analysis of surface activation markers and production of reactive oxygen species (ROS).

3 Results

MA and MIL resistant *L. (V.) p.* strains were shown to elicit significant increased NET formation in murine and human neutrophils when compared to drug susceptible *L. (V.) p*. strains. Furthermore, we will present recent data on the impact of both laboratory lines and clinical drug susceptible or resistant *L. (V.) p.* strains on neutrophil activation phenotypes.

4 Conclusions

These results suggest that parasite drug susceptibility may influence neutrophil activation and function. Since neutrophils participate in the onset of infection as well as in the chronic phase, they should be considered as potential determinants of outcome of anti-leishmanial drug treatment.

C0728 MECHANISM OF ASCARIDOLE ACTIVATION IN LEISHMANIA

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1 Background

Leishmaniasis, a vector-borne infection caused by protozoal parasites, leads to destruction of mucous membranes, inner organs or even death. Due to global warming and resistance to standard first-line treatment with antimonials, the potential threat by this disease is increasing. This gives rise to a big need for new therapeutic options. The endoperoxide ascaridole (Asc) was more effective than Glucantime® to treat cutaneous leishmaniasis in a model of L. amazonensis and BALB/c mice. To elucidate its pharmacological mechanism we studied Asc activation in leishmania.

2 Methods

Activation of Asc was studied by electron spin resonance (EPR) spectroscopy in combination with spin-trapping in comparison to the endoperoxide artemisinin (Art) and the non-endoperoxide 1,4-cineole (Cin). Experiments were carried out in cell-free buffers, Leishmania tarentolae promastigotes (LtP) and J774 macrophages. Decomposition of Asc was studied by GC/FID and NMR in cell-free systems and LtP. The metal content of cells was assessed by ICP-OES and low-temperature EPR, their viability was measured by a resazurin assay.

3 Results

We identified secondary alkyl radical intermediates arising from reduction of Asc by Fe^{2+}/heme in LtP and cell-free systems by EPR spin-trapping. GC/NMR analysis confirmed these findings and showed the formation of further degradation products. Analysis of redox-active metals, activating Asc in LtP and J774, elucidated twice as high total Fe concentrations in LtP as in J774 macrophages. Low-temperature EPR spectroscopy showed that a significant part of the total Fe in LtP belongs to the labile iron pool (LIP) and that Asc shifts the redox state of the LIP to Fe^{3+}. Viability assays revealed IC_{50} values for Asc around 20 µM in LtP and above 200 µM in J774, whereas Cin showed high IC_{50} values both in LtP and J774 cells. In LtP the IC_{50} value for Asc was significantly increased in the presence of the radical scavenger DMPO.

4 Conclusions

Asc is effective against different leishmania species in vitro as well as in vivo. For the first time the radical intermediate, which is essential for Asc actions, was identified in leishmania. Activation of Asc in leishmania depends on iron, preferably from the LIP, but also heme may play a supportive role. The high availability of iron in leishmania compared to macrophages partly explains its selectivity. Understanding of these mechanisms will contribute to the development of endoperoxide derivatives with increased activity and selectivity.

C0771 DETECTION OF SAG-RESISTANT STRAINS OF LEISHMANIA DONOVANI BY GOLD NANOPARTICLES

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1 Background

Resistance emergence against anti-leishmanial drugs, particularly Sodium Antimony Gluconate (SAG) has severely hampered the therapeutic strategy against visceral leishmaniasis. More than 70% cases in the state of Bihar, India show SAG-resistance. Most of the resistance strains are associated with single nucleotides polymorphisms (SNPs) and altered expression of genes such as PCNA, CLrP, AQP1 etc. Gold nanoparticles (AuNPs) were earlier used for detection of resistant strains of *Mycobacterium tuberculosis*. We have established a method to detect SAG- resistant clinical strains of *Leishmania donovani* by AuNPs.

2 Methods

AuNPs were synthesised by Turkevich method and characterized by transmission electron microscope (TEM), dynamic light scattering (DLS) as well as UV-Visible spectrometry. This visual detection is dependent on salt-mediated aggregation of AuNPs in presence of an added oligonucleotide which is specific for complementary DNA/RNA sequence present or absent in resistant strain. Salt and detection probe concentration was optimized for detection. Multiplexing PCR was also performed to detect all possible SNP domains at a time. Assay was done to detect SNP in resistant strain by amplifying the SNP containing domains as a PCR product or by using a thiolated detection probe to detect SNP from the genomic DNA. Genomic DNA isolated from patient's samples was further used for confirmation of leishmania infection using AuNP.

3 Results

In this study we have used 3 SAG resistant clinical isolates and have found 3 different SNPs in these strains. We have also developed a method to detect and quantify the altered expression of genes such as PCNA, CLrP by these AuNPs. By detecting the level of mRNA we can differentiate resistance vs. sensitive strain. Therefore, both DNA and RNA based detection method can be applied to confirm the presence of SAG-resistance in the clinical isolate. Further, we have extended this study to analyze patient's serum to detect presence of *leishmania* and SAG-resistance, if any, using AuNP based detection method.

4 Conclusions

We have developed an easy, low-cost and quick detection method for SAG- resistant clinical isolates of *Leishmania donovani* using AuNPs. This method measures a distinct colour change (from red to blue and vice versa) based on differential hybridization of oligonucleotide probes directed against SNP region of sensitive/resistant strain as well as for mRNA quantification of specific gene.

C0774 LIPOSOMES CONTAINING 17AAG, A HEAT SHOCK PROTEIN 90 INHIBITOR, HAVE HIGH ANTILEISHMANIAL POTENCY UNDER LOW CONCENTRATIONS

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1 Background

Leishmaniasis remains a major health problem worldwide. Current long-term treatment cause severe side effects and an increasing number of resistance cases. The compound 17-allylamino-17-demethoxygeldanamycin (17AAG), a less toxic analog of the known heat shock protein 90 (HSP90)-inhibitor geldanamycin, has shown to present a strong antileishmanial effect in vitro. We have previewsly shown that 17AAG can kill *L. amazonensis* promastigotes with an IC50 of 65 nM and intracellular amastigote at concentrations as low as 125 nM.

2 Methods

In an attempt to increase 17AAG water solubility and potency, while reducing its possible side effects for *in vivo* use, novel formulations were prepared: Hydroxypropyl-β-cyclodextrin 17AAG inclusion complex (HP-β-CD:17AAG) and liposomes (LP)-containing HP-β-CD-17AAG (LP-HP-β-CD:17AAG). The liposomes were prepared by the method of hydrating the dry lipid film followed by ultra-sonication. The formulation was evaluated for compound encapsulation efficiency (EE%) by ultrafiltration/centrifugation, as well as by determining the particle size, polydispersity index, and pH variation. Parasite and macrophage viability were evaluated by AlamarBlue assay and intracelular parasite viability were evaluated by direct count of live parasites after treatment with different formulations.

3 Results

Stability study revealed that liposomes showed high stability in formulations smaller than 200 nm and 99% of EE. HP-β-CD:17AAG and LP-HP-β-CD:17AAG activity against *L. amazonensis* promastigotes was calculated as IC_{50} of 0.006 and 0.001 nM, respectively, while control empty LP were only toxic at doses 83 times higer. Toxicity of these formulations against macrophages were also evaluated and were toxic to macrophages only at concentrations higer than 1 nM. We also evaluated the efficiency of HP-β-CD-17AAG and LP-HP-β-CD:17-AAG against intracellular amastigotes at early (6h) and late (72h) times of infection. Treatment of infected-macrophages with 0.006 nM of HP-β-CD:17AAG or LP-HP-β-CD:17AAG almost completely cleared parasites inside macrophages after 48h.

4 Conclusions

Our results showed that nanotechnology and drug delivery systems can be used to increase drug efficiency and potency of 17AAG and reduce its toxicity. The very low dose needed to obtain parasite clearance in the *in vitro* systems indicates that this formulations may become an important therapeutic strategy against leishmaniasis, especially in cases of parasite resistance.

C0805 PERIFOSINE MECHANISMS OF ACTION IN LEISHMANIA SPP.

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1 Background

The current available leishmanicidal treatments include pentavalent antimonials, amphotericin B, miltefosine, paramomicine and pentamidine. These drugs are normally highly toxic to the patient even at low doses. Nevertheless, the appearance of resistant strains to these active compounds is raising as a main problem in the current therapeutic measures against these parasites. With no immediate prospect of vaccines, there is a great need to develop new leishmanicidal agents with an acceptable efficacy and safety profile. Although there is already data about alkylphospholipids against *Leishmania*, the scientific community still continues the contribution to this field. We propose perifosine (P) to deep in its study due to previous results obtained in our laboratory.

2 Methods

Leishmania amazonensis and *Leishmania donovani* were used to develop the experiments. IC_{50} was determined in both promastigote and intracellular amastigote stage using a colorimetric assay with the alamarBlue® reagent. Assays to measure the mitochondrial membrane potential using JC-1, or the phosphatidylserine (PS) externalization using annexine v, were performed in order to determine the type of induced cell death after incubation with P. Also we evaluate the membrane permeability and the ATP level after treatment. In order to deep in the mechanism of action of P, we perform an array to study the phosporilation of Akt.

3 Results

P showed IC_{50} ranging from 1.98 to 3.59 µM for the promastigote stage of both strains, and around 2.82 µM for the intramacrophage stage. Moreover, P induced an externalization of PS as well as a decrease in the mitochondrial membrane potential in both strains of *Leishmania*. The ATP level of the parasites undergo a decrease after the treatment with P, however the cytoplasmic membrane did not experience any alteration. After performing the array we observed a strong decrease of phosphorylated Akt when *L. donovani* was incubated with P.

4 Conclusions

In conclusion, P induce an apoptosis-*like* process on *L. amazonensis* and *L. donovani* at very low doses, avoiding an unnecessary immune response, and considering perifosine as a good compound for further studies.

C0812 LETHAL ACTION STUDIES OF THE ANTIDEPRESSANT ESCITALOPRAM AGAINST LEISHMANIA (L.) INFANTUM: AN IN VITRO APPROACH

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1 Background

Chemotherapy in leishmaniasis is stigmatized by scarce number of drugs with severe side effects and increased resistance. In the search for alternatives, drug repositioning has been reducing time and costs of research to provide new treatment options by reallocation of FDA-approved drugs. Antidepressants have been demonstrating *in vitro* and *in vivo* efficacy against *Leishmania* spp. In this work, the antileishmanial activity, mammalian cytotoxicity and the mechanism of action (MoA) of the antidepressant escitalopram was investigated in *Leishmania (L) infantum.*

2 Methods

The 50% Cytotoxic Concentration (CC_{50}) of escitalopram was determined in L929 cells, and its 50% Effective Concentration (EC_{50}) was determined in *Leishmania* (L.) *infantum* promastigotes by MTT. The EC_{50} was also determined in intracellular amastigotes using light microscopy counting. The effects of escitalopram in *Leishmania* were studied using spectrofluorimetry and flow cytometry with focus in plasma membrane, intracellular Ca^{+2} and mitochondria, using different fluorescent probes.

3 Results

Escitalopram demonstrated activity against promastigotes and intracellular amastigotes, with EC_{50} values of 13 and 27 µM, respectively. The drug also demonstrated toxicity to mammalian cells with a CC_{50} value of 125 µM. The MoA studies demonstrated that escitalopram caused a rapid increase in cytosolic Ca^{+2} levels, as determined by Fura2AM. By using Rd123, escitalopram-treated promastigotes showed decreased fluorescence levels, similar to the positive control FCCP, suggesting the depolarization of the mitochondrial membrane potential. The drug showed neither interference in the plasma membrane potential nor in the plasma membrane permeability, as determined by Sytox Green and bisoxonol.

4 Conclusions

This work demonstrated for the first time the potential *in vitro* antileishmanial effect of escitolopram. The drug was selective against the *L. infantum* intracellular amastigotes and preliminary results in promastigotes, pointed out that an alteration of Ca^{+2} homeostasis and the loss of the mitochondrial membrane potential, probably contributed to the leishmanicidal effect of the drug. This second generation FDA-approved antidepressant may be useful as a candidate for future experimental studies against *L. infantum.*

C0831 EFFECT OF XANTHOHUMOL ON LEISHMANIAL MITOCHONDRIA

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1 Background

Leishmaniasis is a disease caused by protozoal parasites which threatens millions of people in (sub)-tropical regions. A large canine reservoir in the Mediterranean area and the spread of the sand fly vector due to global warming threatens also previously non-endemic areas. Current antileishmanial drugs are frequently toxic, partially costly and subject of resistance development. These problems trigger the search for natural drug candidates with antileishmanial activity. Mitochondria are essential for survival of both leishmania parasites and macrophage host cells. Therefore, on the first view mitochondria seem not to be an ideal target. However, established antileishmanial drugs, such as pentamidine, were reported to have effects on mitochondrial functions in leishmania. These observations underline that mitochondria in leishmania are essential for their survival. In the current study we analyzed the antileishmanial effect of xanthohumol (Xan), an ingredient of hop plants and constituent of human nutrition, on leishmania and their mitochondria in comparison to effects in macrophage host cells.

2 Methods

Viability of *Leishmania amazonensis* promastigotes/amastigotes (LaP/LaA), and *Leishmania tarentolae* promastigotes (LtP) was studied in the presence and absence of Xan in comparison to the cytotoxic activity of Xan in peritoneal macrophages from mouse (PMM) and J774 macrophages. Mechanistic studies were carried out in LtP and mitochondrial fractions isolated from LtP. In these cells, mitochondrial complex II and III activity, mitochondrial membrane potential (JC1), superoxide radical formation (EPR) and oxygen consumption were studied.

3 Results

Xan demonstrated antileishmanial activity in LaA (IC_{50}: Xan 7 µM); while it had less influence on the viability of PMM (IC_{50}: Xan 70 µM). Xan strongly inhibited oxygen consumption in LtP but not in J774 cells. This was based on the inhibition of the mitochondrial electron transfer complexes II/III by Xan. However, Xan did not increase mitochondrial superoxide release in LtP. Xan decreased the mitochondrial membrane potential in LtP. Bioenergetic studies showed that LtP mitochondria have no spare respiratory capacity in contrast to mitochondria in J774 cells.

4 Conclusions

These data demonstrate that Xan in spite of its interaction with both mammalian and leishmanial mitochondria has antileishmanial activity. This is based on the fact that leishmania can much less adapt to stress by mitochondrial inhibitors, such as Xan, than macrophages.

C0833 DESERT-TYPE KALA-AZAR LEISHMANIA ATTENUATED BY PROLONGING IN VITRO CULTIVATION

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1 Background

#771 was a Leishmania strain isolated from *Phlebotomus wui* on the endemic area of desert-type kala-azar in Bachu County of Xinjiang, China in 1977,which has been unable to infect animals due to long-term subculture in vitro.-Other Leishmania, isolated in 2009 from *P.wui* in the same area, can infect *Lagurus lagurus.* To obtain the attenuated strains, we used an animal model for visceral leishmaniasis in *Laguruslagurus* toestablish Leishmania attenuatedstrain.

2 Methods

Leishmania were recovered from these experimentally infected animals, and subcultured for 7, 30, 36, 44, 60, 90 and 150 days. They were used to infect *L.lagurus* at 2.6×10^5/animal. All inoculated animals were infected after inoculation for 60 days. Based on splenomegaly (100%×spleen weight /body weight), infection rateand anti-Leishmania antibody positive rate.

3 Results

The infection rate and the antibody positivity decreased in the inoculated animals from 0 at 60 days. Splenomegaly is correlated with the anti-Leishmania antibody rate of infected *L.lagurus*, peaking at day 30 and declined to normal after 90 days.

4 Conclusions

The isolated Leishmania loses infectivity to *L.lagurus* increasingly with the period of its in vitro cultivation, causing splenomegaly after day 60 and becoming totally avirulent after day 90. These attenuated parasites may be of value for vaccination.

C0851 SCREENING FOR DRUGS AGAINST VISCERAL LEISHMANIASIS USING EX-VIVO SPLENIC EXPLANT CULTURES AND BIOIMAGING.

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1 Background

Leishmaniasis is a Neglected Tropical Disease causing different pathological symptoms. No effective vaccines exist and most of treatments are outdated, toxic, and with adverse effects. Hence, several different strategies have been made to develop High Throughput target-based and target-free Screenings platforms (HTS). However, resistance to leishmaniasis has an important immunological component, that it is omitted in all of those assays.

2 Methods

The global objective of this project was to implement and validate an *ex vivo* model of Visceral Leishmaniais based on splenocytes explants from mice infected with genetically modified *Leishmania* using infrared protein (iRFP) and evaluate if this system can better predict the *in vivo* efficacy of selected antileishmanial compounds, in order to prioritize compounds from GSK Leishmania Box. This system involved several steps, from HTS in *L.infantum* and *L.donovani* infected macrophages (inMac) and *L.infantum*-iRFP *ex vivo* splenic explants to chemical optimization of the lead compounds and finally testing the infection curing ability in the *in vivo* infrared *L.infantum* infection in mice by bioimaging.

3 Results

We carried out an initial screening of 7065 compounds in *L.infantum*-iRFP infected macrophages, previously selected based on inMac and axenic activity in *L. donovani*, in Single Shot (SS) at 5 µM. From the resulting data, 1038 available compounds were screened in SS in the *ex vivo* assay. Of those, 96 compounds were selected to be progressed to Dose Responses assays by applying a cut-off of 70% inhibition to the average value of the copies and low or null cytotoxicity in this or previous cytotoxic assays. Interestingly, many of the compounds having good IC50 and low toxicity did not show activity in previous *in vitro* assays based on inMac.

4 Conclusions

Huge efforts have been placed on massive HTS in extracellular, axenic and intracellular platforms looking for small molecules active against *Leishmania* parasites. However, none of those HTS took into account the possible influence of the cellular milieu and the immune response to the anti-leishmanicidal activity of the potential drug candidate. The model described here has the advantage of mimicking more closely *in vivo* situations since in the explants and the physiological and immunological milieu is similar to that of the *in vivo* infection. Thus, new HTS tools mimicking more closely *in vivo* situations are needed to select the most promising candidate(s).

C0890 USE OF CLINICAL AND PARACLINAL TOOLS IN THE EVALUATION OF INFECTION BY L. INFANTUM IN THE HAMSTER (MESOCRICETUS AURATUS) MODEL AND RESPONSE TO TREATMENT.

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1 Background

The visceral leishmaniasis (VL) model in the hamster (Mesocricetos auratus) presents clinical and pathological signs similar to those observed during human disease such as cachexia, organomegaly, pancytopenia and death in the absence of treatment. However, one aspect that should be improved for clinical follow-up of the infection is the sacrifice of some of the animals to confirm the infection and to assume that the other animals are equally infected. This procedure requires not only a high number of animals per group, but also leads to treating animals that may be healthy, leading to false therapeutic success. Therefore, it is necessary the evaluation of different clinical and paraclinical tools that allow detection and monitoring of infection without the need of the sacrifice of the animals.

2 Methods

Golden hamsters, both genders at 4 weeks of age, were used. Animals were experimentally infected with $5x10^8$ promastigotes, intraperitoneally. The infection was monitored by ultrasonography, blood gases, hematology, blood chemistry and qRT-PCR. When infection was established, animals were treated during four weeks with Amphotericine B and *Artemisia annua* and followed during 10 weeks. At the end of the study animals were euthanized and sampled for pathological, molecular and blood studies.

3 Results

At week 9 post ifection, ultrasonographic signs of hepatic and splenic congestion were identified; lactate was increased and leukogram parameters decreased, suggesting establishment of infection that was confirmed by qRT-PCR in blood and liver samples. No weight loss was observed. Animals At the end of the study (week 24) the ultrasound signs of congestion persisted, lactate decreased and progressive leucopenia and mild weight loss were observed. Parasite burden was quantified in blood, spleen and liver; the histopathology study evidenced periportal hepatitis, splenitis and glomerulonephritis. None of the compounds evaluated led to clearance of infection, although some parameters were restablished to normal values.

4 Conclusions

Paraclinic tools such as ultrasound and blood gases helped in the early detection of infection and evolution of the infection after treatment which reduces the number of animals needed per group. This work contributes to the optimization of hamster model of VL

C0893 ALTERATION OF MAPK SIGNALING BY OCTYL-B-D-GALACTOFURANOSIDE IS ASSOCIATED WITH PARASITIC CLEARANCE IN HUMAN MACROPHAGES INFECTED WITH LEISHMANIA DONOVANI

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1 Background

The recent emergence of resistance during leishmaniasis treatment in developing countries may become a problem in a near future. In this context, the development of new drugs is necessary. For several years, our team has been developing furanosides analogues, initially designed to target metabolism pathways of furanosides synthesis, which are absent in mammalian cells. Furanosidic derivatives have showed to reduce intramacrophagic *Leishmania donovani* amastigote multiplication by half. Interestingly, this anti-parasitic effect was associated with immunomodulatory properties.

2 Methods

In order to better understand the mechanisms involved, we examined the impact of a specific furanoside derivative; octyl-β-D-galactofuranoside (Gal*f*-oct), on the dysregulation of cell signaling pathways by *Leishmania donovani,* notably on the mitogen-activated protein kinases (MAPK) signaling. An *in-vitro* approach was designed, by treating with Gal*f*-oct, peripheral blood mononuclear cell (PBMC)-derived-human macrophages infected with *L. donovani*.

3 Results

A significant alteration of MAPK has been observed in macrophages with a specific dephosphorylation of p42/Extracellular signal-regulated kinase 2 (ERK2), and an activation of stress-activated protein kinases (SAPK)/jun amino-terminal kinases (JNK) after 48h of treatment with Gal*f*-oct. By comparison with a lipopolysaccharide (LPS)-stimulation control, this treatment did not induce an alteration on MAPK signaling during the 2 first hours suggesting that the alteration observed is probably independent of an early event such as a receptor activation by the compound. This signaling modification correlated with a significant decrease of the percentage of infected macrophages and of the mean number of parasites per cell.

4 Conclusions

Whether these changes of MAPK signaling is accompanied by activation of the nuclear factor-kappa B (NF-κB) pathway remains to be determined, this could explain the immunostimulation properties of Gal*f*-oct.

C0912 IN SILICO APPROACH REVEALS HIGHER AFFINITY OF 17AAG TO LEISHMANIA HSP90 THAN HUMAN HSP90

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1 Background

Leishmaniasis treatment is mainly based on the use of pentavalent antimonials. Such drugs exhibit high toxicity, require a long period of treatment and cause severe side effects. Thus, it is mandatory the development of new chemotherapy agents. We have previously shown that treatment with the compound 17-N-allylamino-17-demethoxygeldanamycin (17AAG), an inhibitor of Heat Shock Protein 90 (HSP90), was able to kill intracellular *Leishmania amazonensis* while not being toxic to the macrophage. HSP90 inhibition is known to lead to the formation of misfolded proteins that are ultimately degraded via proteasome, interfering with cellular viability. This makes HSP90 an ideal target for anticancer, antifungal and antiparasitic drugs. The fact that 17AAG is more toxic to *Leishmania* than macrophage has not yet been investigated. We hypothesized that 17AAG has a higher affinity for the parasite HSP90 compared to the HSP90 affinity of the host. The present work aims to identify the possible differences in the interaction of 17AAG with host and *Leishmania* HSP90.

2 Methods

We performed an *in silico* approach using a molecular docking tool. The targets (human and *L. amazonensis* HSP90) were selected from the Protein Data Bank. Initially, the flexibility of 17AAG was analyzed, as well as other known HSP90 inhibitors, such as geldanamycin (GA) and 17-Dimethylaminoethylamino-17-demethoxygeldanamycin (17DMAG), using the Ballon program. Molecular docking was performed by Gold program. To confirm the *in silico* data, the IC50 of the inhibitors in axenic promastigotes of *L. amazonensis* was determined.

3 Results

17AAG showed higher *in silico* affinity to *Leishmania* HSP90 than to human HSP90, with a docking score of 70.72 and 56.02, respectively. The same was observed for other inhibitors tested. When evaluating the affinities of each of the compounds to the *Leishmania* HSP90, 17-AAG had the highest score (70.72), when compared to GA (61.41) and 17DMAG (65.97). Also, of the three inhibitors tested, 17AAG and 17DMAG had the lowest IC50 values (94.70 nM and 71.15 nM, respectively) when compared to the IC50 value for GA (177.5 nM).

4 Conclusions

17AAG exhibits low IC50 nanomolar level and higher in silico affinity to *L.amazonensis* HSP90 than human HSP90, revealing its potential as an anti-leishmania compound. This could explain the differential susceptibility of parasite and host to 17AAG. However, in vitro studies with human macrophages should be performed to corroborate the data obtained *in silico.*

C0968 GENE SPECIFIC SEX EFFECTS ON EOSINOPHIL INFILTRATION IN LEISHMANIASIS.

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1 Background

Sex influences susceptibility to many infectious diseases, including some manifestations of leishmaniasis. The disease is caused by parasites that enter skin and can spread to lymph nodes, spleen, liver, bone marrow, and sometimes lungs. Parasites induce host defenses including cell infiltration, leading to protective or ineffective inflammation. These responses are often influenced by host genotype and sex. We analyzed the role of sex in the impact of specific gene-loci on eosinophil infiltration and its functional relevance.

2 Methods

We studied the genetic control of infiltration of eosinophils into inguinal lymph nodes after 8 weeks of *L. major* infection using mouse strains BALB/c, STS and recombinant congenic strains CcS-1,-3,-4,-5,-7,-9,-11,-12,-15,-16,-18, and -20, each of which contains a different random set of 12.5% genes from the parental "donor" strain STS and 87.5% genes from the "background" strain BALB/c. Numbers of eosinophils were counted in hematoxylin-eosin stained sections of inguinal lymph nodes under a light microscope. Parasite load was determined using PCR-ELISA.

3 Results

Lymph nodes of resistant STS and susceptible BALB/c mice contained very low and intermediate numbers of eosinophils, respectively. Unexpectedly, eosinophil infiltration in strain CcS-9 exceeded that in BALB/c and STS, and was higher in males than in females. We searched for genes controlling high eosinophil infiltration in CcS-9 mice by linkage analysis in F_2 hybrids between BALB/c and CcS-9 and detected fourloci controlling eosinophil numbers. *Lmr14* (chromosome 2) and *Lmr25*(chromosome 5)operate independently from other genes (main effects). *Lmr14* functions only in males, the effect of *Lmr25* is sex-independent. *Lmr15* (chromosome 11) and *Lmr26* (chromosome 9) operate in cooperation (non-additive interaction) with each other. This interaction was significant in males only, but sex - marker interaction was not significant. Eosinophil infiltration was positively correlated with parasite load in lymph nodes of F_2 hybrids in males, but not females.

5 Conclusions

We demonstrated a strong influence of sex on numbers of eosinophils in lymph nodes after *L. major* infection and present the first identification of sex-dependent autosomal loci controlling eosinophilic infiltration. The positive correlation between eosinophil infiltration and parasite load in males suggests that this sex-dependent eosinophilic infiltration reflects ineffective inflammation.

C0985 HIGH THIOL LEVELS CONFER DRUG RESISTANCE IN CLINICAL ISOLATES OF LEISHMANIA INFANTUM FROM BRAZILIAN ANTIMONY REFRACTORY PATIENTS

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1 Background

Visceral leishmaniasis (VL) is a serious infectious disease caused by parasites of the genus *Leishmania*. Antimonial drugs are the first-line therapy, however refractory to antimony treatment is increasing and few studies have been performed examining the drug sensitivity of clinical isolates and their ability to modulate immune response. Recently, data from our group showed that *L. infantum* clinical isolates from antimony refractory patients were *in vitro* resistant to trivalent antimony and microbicides mechanisms of macrophage. Thereby, this study aims to evaluate the presence of drug resistance mechanisms in *L. infantum* clinical isolates from antimony refractory patients.

2 Methods

Four clinical isolates were selected: two from antimony relapse patients and two from responsive patients. First, we evaluated promastigotes ultrastructure after antimony exposition by Transmission Electron Microscopy (TEM). To assess if the resistance profile was associated with MDR1 pumps activity we used a specific channel blocker, verapamil hydrochloride. To investigate if the resistance was associated with thiols up-regulation, we performed pathway inhibition with L-buthionine sulfoximine (BSO).

3 Results

In TEM analysis, we observed similar ultrastructure in both isolates. After drug exposition, the two isolates from antimony refractory patients showed more conserved than isolates from antimony responsive patients. In the evaluation of the resistance profile, we not observed a direct action of MDR1 efflux pumps in the resistance phenotype. Interestingly, after the exposure to antimony in presence of BSO, only clinical isolates from antimony refractory patients showed decreased parasite viability and increased reactive oxygen species production, these effects demonstrate essential role of thiols as resistance mechanism.

4 Conclusions

At the first time, the findings of this study show that increased production of thiols by *L. infantum* parasites increases antimony resistance in clinical isolates from Brazil.

C0992 DIFFERENTIAL SUSCEPTIBILITY TO MILTEFOSINE IN BRAZILIAN CLINICAL ISOLATES OF LEISHMANIA (VIANNIA) BRAZILIENSIS: STUDY OF THE MOLECULAR BASES RELATED TO REDUCED DRUG SUSCEPTIBILITY

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1 Background

The first choice drug for cutaneous leishmaniasis treatment in Brazil, where *L. (V.) braziliensis* is the most prevalent etiological agent, is meglumine antimoniate, administered intravenously for 20 days. Success rates for antimonial therapy can be as low as 50% in some areas. Miltefosine is the only oral drug in use for leishmaniasis treatment and, although two clinical trials conducted in Brazil in cutaneous leishmaniasis patients indicated efficacy superior to antimonials, miltefosine is not yet approved in the country. In a previous study, we described variability in the susceptibility to miltefosine among Brazilian clinical isolates of *L. (V.) braziliensis*, with half maximal effective concentrations (EC_{50}) varying by a factor of 6 to 15 for promastigotes and amastigotes, respectively. Interestingly, patients had not been exposed to miltefosine or other anti-leishmanial drugs before parasite isolation. To understand the molecular bases of the intrinsic variation in susceptibility to miltefosine observed in these isolates, we chose the most and the least susceptible isolates for further characterization.

2 Methods

The uptake of labelled phosphocholine and miltefosine in promastigotes of the three selected isolates was evaluated by flow cytometry and confocal microscopy. We also determined the nucleotide sequence of the two copies of the miltefosine transporter (*MT*), and *Ros3* genes, both of which have been associated with miltefosine resistance.

3 Results

Evaluation of phosphocholine and miltefosine uptake revealed a direct correlation between the amount of lipid/drug accumulated inside the parasite and its susceptibility to miltefosine. Sequencing of both *MT* genes and *Ros3* of theses isolates revealed the presence of 25 SNPs in *MT* and 44 SNPs in its paralogue, most of them non-synonymous substitutions. Only 5 SNPs were present in *Ros3*, two of them non-synonymous. Nevertheless, none of the SNPs could be strongly correlated with the differential susceptibility and drug uptake phenotypes observed among the clinical isolates.

4 Conclusions

The differential susceptibility found among the isolates correlates with drug accumulation, suggesting differences in features such as binding, metabolism, influx or efflux. We did not find polymorphisms in *MT* and *Ros3* genes that could explain the differential susceptibility and uptake. Phospholipid and sterol contents in these isolates, as well as miltefosine efflux rates are under evaluation.

Supported by FAPESP and CNPq.

C1026 EFFECTIVENESS OF THE O-ALKYL HYDROXAMATE MTC-305 AGAINST CANINE LEISHMANIASIS: A PILOT CLINICAL TRIAL

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1 Background

O-alkyl hydroxamates are a new family of inhibitors of the *Leishmania* histone deacetylase. We have previously shown the activity of the compound MTC-305 on a murine visceral leishmaniasis model due to *L. infantum.* The main aim of the present study was to assess the potential effectiveness and safety of this compound in a pilot clinical trial involving dogs with naturally acquired canine leishmaniasis

2 Methods

Eighteen dogs with naturally acquired canine leishmaniasis were selected upon clinical and parasitological eligibility criteria. They were treated with MTC-305 (3.75 mg/kg subcutaneously). Glucantime® treatment was used as a control reference drug (100 mg/kg subcutaneously). A combination of MTC-305 (1.5 mg/kg) and Glucantime® was also tested. Two series of treatment were administered during 28 days separated by a 30 days interval. Analyses were carried out before starting the treatment, once each series of the treatment was administered and 4 months after the last treatment. Their clinical state was evaluated by means of the clinical score; their immunological profile was evaluated through the determination of IgG subtypes titres by IFAT and by the quantitation of their cytokine expression level using reverse transcriptase quantitative PCR. Parasite loads were evaluated in bone marrow, lymph node, peripheral blood and hair through quantitative PCR. Efficacy criteria was established based on the ability of the treatment to decrease the parasite load in those organs. This project was funded by CEI-Biotic and Research Results Transfer Office (University of Granada)

3 Results

Treatment with MTC-305 in monotherapy was considered effective in the six dogs treated reducing bone marrow, lymph node and blood parasite loads in at least 50% after the second treatment series, whereas meglumine antimoniate was effective in two out of the six dogs. Therefore, the treatment with MTC-305 was more effective than the reference treatment without showing toxicity. The combination was effective in 5 out of 6 dogs, thus not improving MTC-305 treatment in monotherapy, and no relevant side effects were observed on any of the dogs. MTC-305 did not seem to influence the immune response in the treated animals

4 Conclusions

MTC-305 was effective against canine leishmaniasis, a disease lacking a suitable treatment. The successful results in this stringent model make this compound a potential candidate for the treatment of canine and human visceral leishmaniasis

C1028 PARASITE BURDEN AND HISTOLOGICAL ASPECTS IN DIFFERENT REGIONS 1 OF THE SPLEEN OF DOGS WITH VISCERAL LEISHMANIASIS

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1 Background

The protozoa of the Leishmania donovani complex can cause from subclinical infection to severe disease in humans and dogs. From the dermal inoculation site, protozoa disseminate to organs rich in mononuclear phagocytic cells, affecting mainly the spleen, liver, bone marrow and lymph nodes. Although the parasitic load distribution and histological alterations may not be homogeneous in the organs affected in naturally infected individuals, the studies on visceral leishmaniasis usually disregard tissue sampling.

2 Methods

Methods: In the current work, the parasite load and histological changes in the spleen of 6 dogs with visceral leishmaniasis were evaluated by sampling each third section of the organ.

3 Results

The parasite load showed a considerable degree of distribution variation in the spleen of the same animal. Histological changes (white pulp disorganization, granulomas, perisplenitis, plasma cell, and polymorphonuclear infiltration) displayed variation in frequency (granulomas) or intensity (perisplenitis) in the spleen of 2 out of the 6 dogs.

4 Conclusions

The data shown in the current work suggest that distribution of parasite burden and histological changes in spleen of dogs with visceral leishmaniasis may present some heterogeneity.

C1041 PREDICTED COMPARATIVE STRUCTURAL DIFFERENCES CORROBORATE POTENTIAL OF LEISHMANIA INFANTUM AND TRYPANOSOMA BRUCEI METHYLTHIOADENOSINE PHOSPHORYLASE AS DRUG TARGETS FOR TRYPANOSOMIASIS AND LEISHMANIASIS.

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1 Background

Trypanosoma and *Leishmania* parasites lack *de novo* purine pathways and thus rely on the salvage pathways. The 5'-deoxy-5'-methylthioadenosine phosphorylase (MTAP) is a rate limiting enzyme and the only one that recycles the 5'-deoxy-5'-methylthioadenosine (MTA), a toxic by-product of the polyamine pathway, into adenine and 5'-methylthioribose-1-phosphate, respectively incorporated into the salvage pathways of purine and methionine. It is a drug target against cancer and trypanosomiasis. In spite of homologies, inhibitors specific to *Trypanosoma brucei* (*Tb*) MTAP (vs human cells) have been already described. We here hypothesize MTAP could also constitute a drug target in *Leishmania*. Our aim is to bring structural arguments through comparison of homology based 3D models of *Leishmania infantum* (*Li*) and *Tb* MTAPs to human (*hu*) MTAP crystal structures.

2 Methods

We first generated 3D models of *Li*MTAP and *Tb*MTAP using I-TASSER server. Solvent accessible surface areas and Electrostatic potentials (EPs) were calculated on parasitic structure models and *hu*MTAP structure. Then, SOM-BSfinder was used to identify potential binding sites (BSs) in general, and the active site (AS) in particular, on all three proteins. MTA and known inhibitors were docked into ASs. Structural analysis lead to the selection of a specific *Li*MTAP peptide and its respective polyclonal antibody was produced for specificity evaluation by western blot on crude extracts of human cells and *in vitro L. infantum* promastigotes.

3 Results

The 3D structural analysis of the 3 MTAP proteins has shown similarities on the whole protein topology. However, in spite of 36% identity of *Li*MTAP and *Tb*MTAP with the human protein, significant features were revealed; involving occurrence of additional a-helices, contrasted EP distributions, different shapes, volumes and residues of the ASs. Unique BSs to each of the parasitic proteins were identified. Binding modes of MTA and two known inhibitors were strikingly different between the 3 proteins. One structural specificity at the C-terminus of *Li*MTAP allowed design and validation of a *Li* specific polyclonal antibody.

4 Conclusions

Structural comparison of *Li*, *Tb* and *hu* MTAPs revealed a similar 3D folding, common to all nucleoside phosphorylase-I family members. Nevertheless, we conclude to significant structural peculiarities and differences to corroborate that *Li*MTAP could constitute a drug target. The *Li*-specific antibody will facilitate study of *LiM*TAP.

C1043 EFFECTIVENESS AND SAFETY OF THE O-ALKYL HYDROXAMATE MDG ON EXPERIMENTAL VISCERAL LEISHMANIASIS AND CUTANEOUS LEISHMANIASIS

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1 Background

Leishmaniasis is desperately in need for novel drugs: an ideal candidate should be effective, safe and affordable. We have previously demonstrated the effectiveness of *O*-alkyl hydroxamates as novel histone deacetylase inhibitors with potential to treat visceral leishmaniasis. This study aims at evaluating a promising candidate compound, MDG, against *Leishmania* spp. *in vitro* and *in vivo* on experimental visceral and cutaneous leishmaniasis models

2 Methods

In vitro activity was studied against *Leishmania* spp. promastigotes and intracellular amastigotes while cytotoxicity was assessed on L929 fibroblasts. *In vivo* effectiveness was evaluated in a murine visceral leishmaniasis model due to *L. infantum* using spleen and liver parasite loads as primary outcome. MDG was administered intraperitoneally at 10 and 25 mg/kg, or at 10 mg/kg in combination with meglumine antimoniate, the reference control. Efficacy against cutaneous leishmaniasis was evaluated in *L. tropica*-infected hamsters. MDG was administered using two different ointments at two concentrations. The parasite load and lesion sized were used to assess the treatment outcome. Safety was assessed by evaluating body weight, organ weight and biochemical parameters in serum. The mutagenicity, potential cardiotoxicity and cytochrome P450 profile of MGD were investigated as well. This study was funded by the Ministry of Economy and Competitiveness (Spain) and FEDER funds (Pi14-01024)

3 Results

MDG showed very active *in vitro* against intracellular amastigotes (IC_{50} =0.69µM)without showing cytotoxicity (CC_{50} >100 µM), whereas it was not active on the promastigote stage. MDG was effective at decreasing spleen and liver parasite load at 25 mg/kg (90 and 99% reduction) in the murine visceral leishmaniasis model without causing toxicity, showing more effective that the anti-leishmanial reference control. In the cutaneous leishmaniasis model, MDG reduced lesion thickness and parasite load (up to 75% for the 2.5% ointment) without showing safety issues in hamsters, and its activity was greater than that showed by the antimonial reference. MDG did not show mutagenic or cardiotoxic activity and it did not influence the cytochrome P450 enzymes investigated

4 Conclusions

MDG is a safe and effective compound that has proven more active than meglumine antimoniate at decreasing parasite load in spleen, liver and skin.These results makes MDG a promising candidate for the treatment of visceral and cutaneous leishmaniasis

C1056 SEARCH FOR LEISHMANIA RIBOSE 5-PHOSPHATE ISOMERASE B SPECIFIC INHIBITORS

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1 Background

The ribose 5-phosphate isomerase B (RpiB) is involved in the non-oxidative branch of the pentose phosphate pathway catalysing the inter-conversion of D-ribose 5-phosphate and D-ribulose 5-phosphate. This enzyme has been genetically validated in *Leishmania infantum* and *Trypanosoma brucei* as essential, and therefore identified as a possible drug target candidate. To find LiRpiB specific inhibitors a fragment library was tested for RpiB interactions using thermal shift (TS) assay and subsequently validated the fragments as possible hits by combining enzymatic assays with *in vitro* anti-parasitic activity.

2 Methods

A fragment library was screened for interaction with LiRpiB using the TS assay. The fragments that originated significant variations in the protein Tm were tested for their capacity to inhibit the *Li*RpiB enzymatic activity, as well as their effect on *L. infantum* growth. Both Wt and RpiB sKo parasites were used to further address the RpiB specificity of the hits. The determination of the RpiB X-ray structures was also performed.

3 Results

RpiB structure could be solved at high-resolution, 1.6Å. The protein is predicted to be a tetramer composed by two dimers. Active sites are located in clefts between the two subunits of a dimer. Key residues of the active sites were also identified. The TS assay identified 15 fragments that induced significant changes in the LiRpiB Tm. From these 15 fragments, 7 presented significant inhibitory activity against the LiRpiB isomerase activity. From the latter, 3 presented significant activity at 10 µM against *L. infantum* Wt promastigotes and importantly, being more active against the sKo than the Wt strain. Noteworthy, 2 of these compounds were active against intra-macrophagic amastigotes at 10 µM.

4 Conclusions

The TS assay detected relevant fragments that are able to inhibit RpiB enzymatic reaction and have anti-*Leishmania* activity demonstrating that this approach enables the detection of novel hits. Moreover, the enzymatic reactions presented sufficient predictive value for the screening of RpiB specific compounds. Ultimately the fragments 338 and 540 represent examples of the feasibility of this approach being interesting scaffolds for the development of novel anti-leishmanial drugs. The crystal structures will enable structure–activity relationship drug design and subsequent optimization studies. Altogether this data further strengthens RpiB as an attractive target for drug development against leishmaniasis.

C1078 DEVELOPMENT OF NOVEL ANTI-LEISHMANIA CHEMOTHERAPEUTICS: HIGH-THROUGHPUT SCREENING AND INVESTIGATION OF STRUCTURE-ACTIVITY RELATIONSHIPS OF HETEROAROMATIC SCAFFOLDS.

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1 Background

The high toxicity associated with the current anti-leishmanial therapies, rampant increase of resistance, and the absence of a vaccine that is safe and effective in humans, demand the identification of new anti-leishmanials. We utilized fluorometric high-throughput screening (HTS) of small molecule libraries followed by combinatorial chemistry to identify new lead scaffolds to address this need.

2 Methods

Primary screening of 10,000 compounds from the DIVERset-EXP library (Chembridge), was performed against axenic *L. donovani* amastigotes constitutively expressing the red fluorescent protein mCherry. Active compounds moved on to a secondary screening against intracellular *L. donovani* and *L. major* amastigotes in the human THP-1 macrophage cell line, and finally an *in vivo* murine model of cutaneous leishmaniasis (CL) was used to identify lead scaffolds.

3 Results

One hundred and sixteen compounds had an effectiveness greater than 90% of the 50μM miltefosine response; 61 exhibited effectiveness equal to that of 50μM miltefosine. Three scaffolds were represented multiple times in the 'hit' compounds from the preliminary screen, one such scaffold was the 2,4-diaminoquinazoline (2,4-DAQ). Active compounds that exhibited EC_{50}s in the less than 10mM range, were selected for secondary screening. Twenty-six (26) of the 2,4-DAQs assessed in secondary screening exhibited EC_{50}s between 1-10mM against axenic *L. donovani* amastigotes with selectivity indices ranging from 1-122. One of two 2,4-DAQ compounds tested in our *in vivo* imaging CL model was as effective as antimony tartate.

4 Conclusions

Comprehensive analyses of SAR of all active scaffolds is ongoing utilizing medicinal chemistry for enhanced efficacy and selectivity as well as optimal adsorption, distribution, metabolic and excretion (ADME) profiles. Utilization of this robust high-throughput platform allows for identification of novel anti-leishmanial compounds. These compounds are both potent and selective, with efficacy in an *in vivo* CL model. Future studies will test activity of these compounds in a visceral leishmaniasis model.

C1084 GLUCANTIME® ACTION ON MICE MACROPHAGE.

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1 Background

Glucantime® is a pentavalent antimonial used as first choice to treat leishmaniasis. However its mechanism is not fully understood. On intracellular amastigotes of *Leishmania sp*, Glucantime® induce damage to the DNA of the parasite. Studies suggest that Glucantime® enhances phagocytosis and TNF-α production by phagocytes. The aim of this study is to evaluate the modulation of macrophages by Glucantime®.

2 Methods

Glucantime's toxicity was tested on peritoneal macrophages from BALB/c mice, by treating the monolayers *in vitro* for 48 hours and was evaluated by MTT method. The capacity of Glucantime of modulate macrophages was evaluated by treating the monolayers of peritoneal macrophages for 24 hours before the infection with *Leishmania braziliensis*. After 48 hours of incubation with culture medium the infection index was evaluated by counting. Before and after the infection were analyzed the production of nitric oxide (NO) by Griess method, reactive oxygen species (ROS) by fluorimetry using the H2DCFDA dye and cytokines by ELISA and kit CBA. To evaluate if Glucantime could modulate macrophages *in vivo*, Swiss Webster mice were treated for 5 consecutive days with 8 mg Glucantime by intraperitoneal route. Peritoneal macrophages were evaluated about its capacity of control the *in vitro* infection with *L. braziliensis* and cytokines production.

3 Results

Results showed that until the concentration of 10 mg/ml, Glucantime did not alter the macrophages viability in vitro. The pre-treatment of macrophages with Glucantime at 0.1mg/mL, 1mg/mL and 10mg/mL was able to reduce the infection index in 49%, 74% and 85%, respectively. On non-infected macrophages the NO production was increased at 10mg/ml of Glucantime. The treatment with 1 and 10 mg/ml de Glucantime was able to significantly increase the ROS production ($p<0,05$ and $p<0.01$, respectively). The treatment with Glucantime was able to significantly increase IL-12, TNF and IL-6 production, however the IL-10 production was not altered. There were no significant changes of these parameters for comparing to control after the *L. braziliensis* infection except for IL-6, IL-10 that decrease and TNF that increase.

4 Conclusions

The macrophages from treated mice were capable of reduce the infection index by *L. braziliensis* and had the TNF production increased. These results suggest that Glucantime is capable of activate macrophages *in vitro* and *in vivo* and this effect could contribute to the mechanism of action of this drug.

C1087 CHARACTERIZATION OF A VISCERAL LEISHMANIASIS MURINE MODEL INDUCED BY TWO LEISHMANIA INFANTUM STRAINS

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1 Background

Visceral leishmaniasis is a severe disease caused by *Leishmania* protozoa. The urgency for more selective and less toxic drugs has led us to search for others chemicals therapies. However, the murine model using BALB/c mice as host is not the ideal for chemotherapeutic researches because this mouse is very sensitive to *Leishmania* sp infection. In our previous work, we found out that *L. infantum* of the New World (*Li*NW), and Old World (*Li*OW) presents some biological differences. In accordance to our observations, the aim of this work was to study these two *L. infantum* strains infections in different murine models as well as the macrophages infectivity index and the sensitivity to drugs *in vitro*.

2 Methods

BALB/c and Swiss mice were infected through intraperitoneally route by *Li*NW or *Li*OW using $1x10^8$ parasites. At 30 and 60 days post infection mice were euthanized and the parasitic load in spleen and liver were analyzed. The urea and nitric oxide (NO) production were assayed in these tissues culture. The isolated parasites from both hosts were tested *in vitro* to evaluate the infectivity and the sensitivity to Miltefosine and Pentamidine.

3 Results

Both parasite strains were able to infect the BALB/c and the Swiss mice. However, the *Li*OW demonstrated more severe disease in both mice in comparison with *Li*NW infection concerning the highest parasitic load since 30 days post-infection. NO production from spleen and liver cells demonstrated a significant increase in the supernatant at 30 and 60 days of infection on both infections. Furthermore, the *Li*NW-infected BALB/c mice revealed the lowest value in arginase activity in spleen cells and in animals infected with *Li*OW there was no significant change from control without infection.The *Li*OW strain isolated from Swiss mice demonstrated the higher infection index *in vitro* compared to the *Li*NW strain in 24h and 72h post-infection. The highest IC_{50} to Pentamidine and Miltefosine were showed by *Li*OW parasites suggesting lower sensitivity in comparison to *Li*NW.

4 Conclusions

Thus, the data obtained in this study suggest that the choice of an adequate animal model may be crucial for the results of a new therapeutic compound to treat visceral leishmaniasis. Additionally, the use of an animal lineage such as Swiss mice, less sensitive but not resistant to *L. infantum* infection could be determinant for new therapeutic strategies choices.

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C1104 LEISHMANIZATION IN DOGS INDUCES LONG-TERM SPECIFIC CELL-MEDIATED IMMUNE RESPONSE

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1 Background

Dogs have been experimentally inoculated with Leishmania infantum by several different protocols aiming to the development of models for the study of canine visceral leishmaniasis (CVL). However, a protocol to induce strong cellular immune response has not been described yet.

2 Methods

In this study, six mongrel dogs intradermically inoculated with com 1 x10E8 L. infantum promastigotas and 6 negative control animals were followed for longer than 2 year.

3 Results

Dogs injected with Leishmania developed a self-healing skin lesion and showed low level of specific antibodies, lymphoproliferative response, interferon gamma synthesis and absence of IL-10 production as detected by a modified quantiFERON assay, and low splenic parasite load.

4 Conclusions

The current findings suggest that leishmanization induce strong specific celular imune response in dogs. The model described herein is potentially useful for antigen screening for vaccine development and to the study of resistance to CVL. Modified quantiFERON assay is useful for evaluation of canine immune responses.

C1148 IN VITRO INDUCED TOLERANCE TO AMPHOTERICIN B IN LEISHMANIA (VIANNIA) BRAZILIENSIS AND LEISHMANIA (VIANNIA) PANAMENSIS REFERENCE STRAIN DERIVED CLONES

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1 Background

Amphotericin B (AmB) is a second line of treatment in cases of visceral leishmaniasis or therapeutic failure in other clinical presentations. AmB resistance has not been reported in America, although there have been reports of therapeutic failure in India and it has been shown that it is possible to induce *in vitro* resistance in *Leishmania (Leishmania) donovani* and *L. (L.) mexicana* promastigotes. It is not known if it is possible to induce an *in vitro* tolerance to the drug for *L. (Viannia) panamensis* and *L. (V.) braziliensis* species.

2 Methods

Limiting dilution cloning was used in this study to generate *L. (V.) braziliensis* (MHOM/BR/00/M2903) and *L. (V.) panamensis* (MHOM/PA/71/LS94) reference strains derived clones. Two clones of each species were selected and exposed to increasing concentrations of AmB deoxycholate (Sigma), starting at 0.01 µg/ml. The AmB was maintained until its growth and viability that were equal to that of the control culture without drug. The concentration of AmB was successively doubled until exceed the EC_{50} reported for the reference strains.

3 Results

Five clones of the reference strain of *L. (V.) panamensis* and 7 clones of *L. (V.) braziliensis* strain were obtained. Clones that presented a growth phenotype similar to the reference strains were selected to be exposed to AmB. After five rounds of culture with increasing AmB to reach concentrations of 0.4 µg/ml, which is equivalent to 10 times the EC50 reported for these strains, cultures with a growth similar to the control strains without AmB were obtained.

4 Conclusions

The results suggest that it is possible to induce *in vitro* tolerance to AmB in clones derived from *L. (V.) panamensis* and *L. (V.) braziliensis* promastigotes by the selection pressure exerted by gradually increasing AmB concentrations. The findings of this study could be correlated with eventual cases of treatment failure with AmB treatment when the drug is used in suboptimal doses.

C1172 CAST, PIGMENTATION AND MITOCHONDRIAL HAPLOGROUP VARIATION IN ENDEMIC REGION OF VISCERAL LEISHMANIASIS IN INDIA

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c/o Prof. Heui-Soo Kim Busan Republic of Korea

1 Background

Visceral Leishmaniasis (kala-azar) is an endemic disease in central-east region of India. Bihar states is one of the oldest population habitat in central-east region. The progress of kala-azar involves many epidemiological risk factors, which may be associated with significant clinical diversity of the patients. Recent advancement in the population genetics by using mtDNA sequence analysis was able to infer the genetic diversity of modern human. Our data shed light on maternal and pigmentation variation in endemic region populations and show correlation of kala-azar with specific cast populations.

2 Methods

In the present study, we explored pigmentation (Melanin Index) diversity in 2736.333 km^2 regions of Bihar states of India. The study design was based on household surveys for skin pigment (Melanin Index; MeI) and cast variation (endogamy cast populations) in 360 kala-azar affected individuals. Population genetic study was conducted based on mitochondrial DNA analysis of 12 cast populations (115 subjects) belonging to General, Other backward class and Schedule cast categories. We did Sanger sequencing of the mitochondrial DNA of 115 healthy subjects and manually annotated the haplogroup on the basis of variations observed. Dermaspectro-photometer, Advanced GPS (Tungustene) and SPSS software were used for study.

3 Results

We found that darker people (higher value of MeI) belonging to specific cast populations were more affected by kala-azar. Anova test shows that density of Kala-azar in different cast group is highly significant (p value 0.018). Skin pigment (MeI) is found to be highly significant in human body (p value 0.000). On consideration of major MT-haplogroup, we observed 4 major haplogroup with >10 subjects; (a) L haplogroup (b) M haplogroup, (c) R haplogroup, and (d) U haplogroup.

4 Conclusions

We found high pigmentation diversity and wide presence of major mitochondrial haplogroups in endemic region of central east part of India. Most importantly, presence of major mitochondrial haplogroup M, L, R, U and T, in populations, indicated that central-east regions were center of various genetically distinct populations. Our data confirm strong clustering and suggest that identification of genetically resistant and susceptible population were useful in effective management of kala-azar control programmes.

C1190 IN VITRO EVALUATION OF LEISMANICIDAL ACTIVITY OF THIAZOL-DERIVATIVES ON LEISHMANIA INFANTUM

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1 Background

Visceral leishmaniasis (LV), caused by *Leishmania infantum,* is a severe and potentially fatal zoonotic disease. The current chemotherapy against VL is based on the use of pentavalent antimonials. However, these drugs presented severe side effects and the appearance of resistance is commonly reported. In the search of new agents against VL, thiazoles compounds have been pointed as promising, due to their trypanocidal and leishmanicidal effects. This work aimed to evaluate the effects of novel thiazoles derivatives on *L. infantum.*

2 Methods

The effects of five thiazoacetylpyridines (TAPs-01; 04; 05; 06; 09) and five thiazopyridines (TPs-01; 04; 05; 06; 09) on *L. infantum* promastigote growth were determined by cell counting. The concentration that inhibits 50% of the parasite growth (IC_{50}) was evaluated by regression analysis. The cytotoxic potential of drugs on peritoneal macrophages was assessed by using the CellTiter-Glo® Luminescence Cell Viability and MTT assays. The 50% cytotoxic concentration (CC_{50}) was determined as mentioned above. The selectivity index (SeI) was determined as the ratio of CC_{50} for the macrophages to IC_{50} for the protozoa. In order to investigate the effects of drugs on amastigotes, infected peritoneal macrophages were treated or not with the drugs and the concentration able to inhibit 50% of macrophages infection (IC_{50}) and the survival index of amastigotes were determined by direct counting of infected cells stained with Giemsa.

3 Results

All the compounds are able to inhibit significantly the promastigote growth. The estimated IC_{50} ranged from 0.13 to 6.72 µg/mL. The CC_{50} for peritoneal macrophages ranged from 0.419 to 32.69 µg/mL. All the compounds were more selective for the parasite than for mammalian cells. Among the ten compounds, the TAP-01, TAP-04 and TAP-06 presented highest selectivity for promastigote (ISe = 12.73, 18.94 and 7.79, respectively) For amastigote forms, compounds TAP-01, TAP-04, and TAP-06 presented IC_{50} values of 0.29, 0.16 and 0.19 µg / mL, respectively. TAP-04 presented the highest SeI (137.37) and was also the most effective in inhibiting the survival of amastigote inside the macrophages.

4 Conclusions

The low toxicity to mammalian cells and the effective activity against promastigotes and amastigotes, point to the use of thiazoles derivatives as a promising agent for the treatment of visceral leishmaniasis, being the TAP 4 the most effective against amastigote forms and the less toxic for mammalian cell.

C1195 EVALUATION IN VITRO AND INDUCED CELL DEATH CHARACTERIZATION OF QUINOXALINE 1,4-DI-N-OXIDE DERIVATIVES ON LEISHMANIA (L.) MEXICANA

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1 Background

In Mexico, cutaneous leishmaniasis caused by *Leishmania (L.) mexicana* is the most common type. Currently, drugs available have multiple adverse effects, they are expensive and frequently ineffective, so it is necessary to find new therapeutic alternatives. Several research have been demonstrating that quinoxaline derivatives have good antiprotozoal activity. Quinoxalines are heterocyclic compounds formed by a benzene ring and a pyrazine ring; the oxidation of both nitrogen increases biological activity. The aim of this study was to evaluate the *in vitro* biological activity of 29 quinoxaline 1,4 di-*N*-oxide derivatives (Q1,4dNO) on *L. mexicana* promastigotes and to characterize the mechanism of induced cell death.

2 Methods

The *in vitro* leshmanicidal effect of Q1,4dNO derivatives was assayed against *L. mexicana* (MNYC-BZ/62/M379/ATCC)promastigotes in stationary phase and the cytotoxic effect was determined on the J774A.1 macrophages cell line; both evaluations were performed by the alamar blue assay; after this, the selectivity index (IS) was calculated. Compounds with the best activity were selected and various cell death markers were evaluated by flow cytometry; data acquisition and analysis were realized in a BD FACSCalibur flow cytometer using the CellQuestPro software. Additionally, analysis by light and transmission electron microscopy (TEM) was performed. Amphotericin B was used as the reference drug.

3 Results

Seven compounds with an inhibitory concentration 50 (IC_{50})≤5μg/mL were selected: **T-069, T-070, T-072, T-073, T-085, T-086** and **T-116.** The leader compound was **T-071** IC_{50}=0.32±0.11μg/ml), however, **T-073** was the compound with the best IS with a 3.75 value. The selected compounds showed a dose-dependent effect. All caused decrease in cell size, loss of plasma membrane integrity, loss of mitochondrial membrane potential, phosphatidylserine exposure, DNA fragmentation, and early production of reactive oxygen species. Drastic morphological alterations were observed by microscopy and the mitochondria was the most sensitive organelle.

4 Conclusions

Compounds with the best leishmanicidal activity were derivatives of 7-isopropyl carboxylate quinoxaline 1,4-di-*N*-oxide, all with a trifluorocarbon substituent. The evaluated compounds induced a necrotic-like cell death inducing cellular damage via reactive oxygen species production.

C1207 IN VITRO, IN VIVO AND CELL DEATH CHARACTERIZATION OF LEISHMANICIDAL ACTIVITY OF CINNAMOMUM VERUM ESSENTIAL OIL AGAISNT LEISHMANIA (L.) MEXICANA.

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1 Background

Leishmaniasis is a vector borne affliction and a tropical disease. The search of alternative drugs for leishmaniasis treatment continues until now because the basic treatment for leishmaniasis promotes different and numerous side effects. Essential oils (EOs) is a highly complex and volatile substance, a mixture of secondary metabolites produced by aromatic plants. The present study determines the *in vitro* leishmanicidal activity of the EO of *C. verum* and their constituents against *Leishmania mexicana* (MNYC/BZ/62/M379). Additionally, an i*n vivo* biological evaluation was carried out.

2 Methods

Cinnamon cortex pulverized (*C. verum*) was brought from Chihuahua, México. The extraction of EO was carried out by hydrodistillation; to determine the components present, the EO was analyzed with a Gas Chromatograph and TurboMass Gold Spectrometer. The half maximal inhibitory concentration (CI_{50}) against promastigotes of *L. mexicana* was evaluated through the of Alamar Blue technique. Different cell death markers (propidium iodide, AnnexinV-PE, Rhodamine 123) were evaluated by flow cytometry (FACSCalibur). For each determination, a total of 10,000 events were recorded. For the *in vivo* evaluation, 12 female BALB/c mice were infected in the dorsum with $10x10^6$ promastigotes in stationary phase. Three groups with four mice each were established: Control group (PBS 1X), Treated group with cinnamaldehyde and treated group with EO. Treatments were administrated daily from day 25 to day 38 post-infection (30 mg/kg body weight) in a volume of 40 µL orally.

3 Results

The EO showed an IC_{50} of 22.61 ppm while Cinnamaldehyde presented an IC50 of 8.66 ppm; minor constituents such as eugenol and β-caryophyllene presented activities of 25.32 and 199.23 ppm respectivelly. In particular, EO and cinnamaldehyde decreased the mitochondrial membrane potential without altering the plasma membrane integrity of promatigotes when evaluated at low concentrations, while concentration close to the IC_{90}, provoked membrane integrity lost. *In vivo* model demonstrated that EO and cinnamaldehyde were able to reduce the dermal lesion produced by *Leishmania mexicana* at 30 days of infection.

4 Conclusions

C. verum EO showed leishmanicidal activity both in vitro and in vivo attributed to its mayor component, the cinnamaldehyde.The present study is a contribution in the search of natural products with leishmanicidal activity.

C1208 DFT, QSAR AND DOCKING MOLECULAR OF ISOPROPYL QUINOXALINE-7-CARBOXYLATE 1,4-DI-N-OXIDE DERIVATIVES ON LEISHMANIA (L.) MEXICANA.

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1 Background

Leishmaniasis is a neglected tropical disease caused by the parasite Leishmania spp.Treatments available have severe side effects, so research is currently focused on more active and less toxic compounds. In this context, quinoxalines have been described as promising alternatives. Here, seventeen new isopropyl quinoxaline-7-carboxylate 1,4-di-N-oxide derivatives were synthetized and evaluated as potential leishmanicidals agents. The effect on cellular metabolism of L. mexicana (MNYC/BZ/62/M379) promastigotes was determined.

2 Methods

Seventeen new isopropyl-7-carboxylate-Q1,4dNO derivatives were synthetized. The effect on cellular metabolism caused for each particular compound were evaluated by the Alamar Blue reduction assay. Several computational methods were used. Firstly, the designed compounds were built to molecular systems with the Spartan 03 software. The minimum energy conformation was selected to obtain the geometry optimization through an in silico calculation by Density Functional Theory. Quantitative structure-activity relationship (QSAR) and quantitative property-activity relationship (QPAR) studies were carried out using genetic algorithms. A Docking Molecular study was carried out on the Trypanothionine Reductase from *L. mexicana*.

3 Results

T-071, T-085 and T-116 were the most active derivatives with IC_{50} values < 1 ppm, while T-064, T-067, T-098, T-107, T-108, T-117 and T-118, showed low activity. The study of multiple linear regressions demonstrated the importance of trifluoromethyl group in the biological activity of the isopropyl-7-carboxylate-Q1,4dNO. The QPAR model considers the Unipolarity, the Balaban centric index and the Total absolute charge as molecular properties that contribute directly to the biological activity. The Docking Molecular study demonstrated that the compounds preferred a binding location near to trypanothione active site, and is interacting with critical residues of Trypanothionine Reductase.

4 Conclusions

The present study is a contribution to the structural, molecular and biological properties of Q1,4dNO derivatives helpful for the rational design of new derivatives with leishmanicidal activity.

C1209 SEASONAL VARIATION OF THE CHEMICAL COMPOSITION AND LEISHMANICIDAL ACTIVITY OF LIGUSTICUM PORTERI COULTER & ROSE ESSENTIAL OIL AGAISNT LEISHMANIA MEXICANA

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1 Background

Natural products play a significant role in the drug discovery; phytochemicals may also offer an effective and cheaper alternative treatment for many microbial infections. The rhizome of *Ligusticum porteri* is representative of a complex of medicinal plants. The roots and leaves of *L. porteri* have been used traditionally by the Raramuri and Zuni communities in Northern Mexico and Southern United for many treatments, including tuberculosis and others pulmonary diseases.

2 Methods

L. porteri plant was recollected in the La Alta Babícora region in the Gómez Farías Township of the Sierra Tarahumara in Chihuahua State. The recollect was performed in April, July, October months of 2015 and January 2016. The extraction of essential oil (EO) of roots of *L. porteri* was carried out by hydrodistillation, to determine the components present, the EO were analyzed with a Perkin Elmer AUTOSYSTEM XL Gas Chromatograph and TurboMass Gold Spectrometer (USA). The half maximal inhibitory concentration (CI_{50}) against promastigotes of *L. mexicana* and half maximal cytotoxical concentration (CC_{50}) against Murine macrophage cell line J774.A1 were evaluated through the of Alamar Blue technique in a 96 well-plates.

3 Results

The results show differences in the chemical composition of essential oils of *L. porteri*, demonstrating a greater presence of components in the EO extracted in autumn. All EOs presented to z-ligustilide as the major component. The evaluation on promastigotes showed that the EO extracted in autumn has greater activity with an IC_{50} of 17.96 ppm and a selectivity index of 8.24.

4 Conclusions

The present work demonstrates the leishmanicidal activity of *Ligusticum porteri* essential oil. The perspectives of the work are based on the evaluation of the constituents of the essential oil, as well as studies that allow to elucidate its mechanism of action.

C1212 EVALUATION OF TREATMENT IN C57BL/6 WITH N-METHYL GLUCAMINE ANTIMONATE ASSOCIATED TO PHOTODYNAMIC THERAPY WITH LIPOSOMAL PHTHALOCYANINE CHLOROALUMINUM, IN CUTANEOUS LEISHMANIASIS CAUSED BY L.(L.)AMAZONENSIS

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1 Background

Cutaneous Leishmaniasis (CL) is an endemic and reemerging disease in world whose first choice treatment is pentavalent antimonial (SbV) without much success. Association of drugs that involves oral and topical use such as Photodynamic Therapy is desired (PDT). Liposomal phthalocyanine chloroaluminum (LPC) was effective *in vitro*. This study aims to compare effectiveness and toxicity of N-methylglucamine (NMG) with PDT with LPC, isolated and associated.

2 Methods

In vivo experimental study. C57BL/6 mice were used in 9 groups of female mice: 8 were inoculated with metacyclic *L.(L.)amazonensis* and 1 remained healthy without infection and without treatment - Negative Control (NC). Of the 8 infected groups 1 remained untreated – Positive Control (PC) - and the other 7 groups received: SbV20mg, SbV10mg, SbV20mg+PDT with LPC, SbV10mg+PDT with LPC, PDT with LPC, LPC, Laser. All groups were treated for 10 alternate days. Effectiveness and toxicity were analyzed after treatment using 2 mice from each group, evaluated according to the diameter paws and parasitological measurements (amastigotes search, culture in NNN medium and limiting dilution).

3 Results

The mean difference of the groups was significant for p-value less than 0.05. SbV20mg was different from all other groups, showing effectiveness. SbV10mg and PDT showed a significant difference from the others, but did not differentiate between them, which demonstrates that the use of PDT was effective with a reduced dose of NMG. SbV20mg+PDT and SbV10mg+PDT were different from the others and among them, both groups being more effective in the treatment, demonstrating that the NMG dose also influences the result. LPC group showed no difference with Laser, demonstrating that there was no action on the parasites. Laser did not present difference of the LPC and PC, which shows that the isolated action of the Laser does not influence the death of parasites. NC was different from the others. PC did not have significant difference with the Laser. N-Methyl20mg+PDT and N-Methyl10mg+PDT groups had different results from the other groups, being the ones closest to Negative Control, which demonstrates their greater effectiveness.

4 Conclusions

20mgSbV associated with PDT with LPC was the most effective treatment in CL caused by *L. (L.) amazonensis*. There were no hepatotoxicity and nephrotoxicity in the tests performed. Toxicity analysis is suggested with larger groups of animals.

C1274 IN VITRO ACTIVITY OF 1H-PHENALEN-1-ONE DERIVATIVES AGAINST LEISHMANIA SPP. AND ITS MECHANISMS OF CELL DEATH.

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1 Background

The current available leishmanicidal treatments include pentavalent antimonials, amphotericin B, miltefosine, paramomicine and pentamidine. These drugs are normally highly toxic to the patient even at low doses. Nevertheless, the appearance of resistant strains to these active compounds is raising as a main problem in the current therapeutic measures against these parasites. With no immediate prospect of vaccines, there is a great need to develop new leishmanicidal agents with an acceptable efficacy and safety profile. 1*H*-Phenalen-1-one (PH) containning compounds have been isolated from plants and fungi, where they play a crucial role in the defense mechanism of plants. Natural as well as substituted PHs exhibit a diverse range of biological activities against fungi (*Fusarium oxysporum*, *Mycosphaerella fijiensis*), protozoan parasites (*Leishmania donovani*, *L. amazonensis, L. infantum*, *Plasmodium falciparum*) or human cancer cells. New substituted PHs have been tested in this study.

2 Methods

The sensitivity and activity of the PH was tested *in vitro* against the promastigote and the intramacrophage amastigote stage of *L. amazonensis* using a colorimetric assay based on Alamar Blue® reagent. For the evaluation of drug-induced cytotoxicity effects was used a commercial kit based on the measurement of lactate dehydrogenase (LDH) activity released to the media. Assays to measure the mitochondrial membrane potential using JC-1, or the phosphatidylserine (PS) externalization using annexine v, were performed in order to determine the type of induced cell death after incubation with P. Also we evaluate the membrane permeability and the ATP level after treatment.

3 Results

These compounds showed a significant dose-dependent inhibition effect on the proliferation of the parasites, with IC_{50} values up to 1 µM for the promastigote stage of both strains, and 0.6 µM for the intramacrophage stage. The majority of the PHs showed low cytotoxic effect and high Selectivity Index. Moreover, PH derivatives induced externalization of PS as well as a decrease in the mitochondrial membrane potential in both strains of *Leishmania*. The ATP level of the parasites were not altered after the treatment with some PHs, in addition the cytoplasmic membrane did not experience strong alterations.

4 Conclusions

In conclusion, substituted PHs induce an apoptosis-*like* process on *Leishmania* at very low doses, avoiding an unnecessary immune response, and considering PHs as a good compounds for further studies

C1293 THE 6-PHOSPHOGLUCONATE DEHYDROGENASE ENZYME FROM KINETOPLASTIDS. STRUCTURAL VARIABILITY IN THE ACTIVE SITE

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1 Background

The 6-phosphogluconate dehydrogenase enzyme(6PGDH) is a homo-dimeric and a key enzyme to protect kinetoplastids parasites, such as *Trypanosoma brucei*, *T. cruzi* and *Leishmania spp.*, against oxidative stress. Inter sub-units salt bridges are important in the maintenance of the quaternary structure of the enzyme; in *T. brucei* enzyme there are five of these with the following amino acids interactions: 1. $R^{459} \rightarrow E^{134}$; 2. $E^{477} \rightarrow K^{139}$; 3. $E^{463} \rightarrow R^{259}$; 4. $R^{464} \rightarrow D^{246}$; and 5. $D^{466} \rightarrow H^{255}$. Kinetic instability in the native enzyme is found in *T. cruzi* and *Leishmania spp*. For instance two salt bridges (3 and 4) in *T. cruzi* and one (2) in *L. (L.) mexicana* are missing in the native enzyme 6-PGDH; site-directed mutagenesis experiments in *T. cruzi*, to recovery the bridges, suggested that the instability observed could be due to weak associations between both sub-units of the enzyme.

2 Methods

In order to known the role that the salt bridges interactions play in the structure of the enzyme, we have carried out comparative molecular dynamics studies of 5 ns for the native enzymes of these kinetoplastids, *T. brucei*, *T. cruzi* and *L. (L.) mexicana*.

3 Results

The distances between residues of mentioned ionic pairs over time were analyzed; the resulting trajectories reveal that pairs ionic 3 and 4 in *L. (L.) mexicana* are not salt bridges (distances around 5?6 Å). *In silico* mutations on *L. (L.) mexicana* and *T. cruzi* enzymes reestablished interactions and they helped to interpret the role of studied ionic pairs. Ionic pair 2, located in the entrance of the active site, appears to be essential to enzymatic activity because it could act like a zipper to this cavity.

4 Conclusions

All of our results with wild type and mutated enzymes suggest that intersubunits salt bridges located at the homodimer interface near the active site are keys to shaping the active site cavity and could be essential in the positioning of the substrate. In addition, the differences found with the human enzyme suggested that 6-PGDH can be further study as a potential target for the developing new drugs to improve existing chemotherapy treatments.

C1313 NEW SMALL HETEROCYCLIC SCAFFOLDS WITH LEISHMANICIDAL ACTIVITY

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1 Background

Current major chemotherapeutic treatments for leishmaniasis are increasingly ineffective, expensive and/or have severe side effects. Thus, there is an unfulfilled need to discover new chemotherapeutic agents. To this goal, screening of chemical libraries is a fruitful approach. In this case, we have tested 28 compounds from our in-house chemical library for their leishmanicidal activities. Those with the highest activity were tested as inhibitors of the bioenergetic metabolism of *Leishmania* due to its essential role for survival of the parasite and prior proved druggability.

2 Methods

The leishmanicidal activity of 28 heterocyclic compounds (MW<500) was tested against *L. pifanoi* axenic amastigotes, *L. infantum* promastigotes, and toxicity against murine macrophages. The bioenergetic collapse produced by the best ones was assessed by *in vivo* decrease of ATP levels (luciferase assay), mitochondrial depolarization (accumulation of rhodamine 123), respiration rate (Clark electrode), ROS production (MitoSOX Red fluorescence), apoptosis (cell cycle analysis) and electron microscopy.

3 Results

Five different heterocyclic compounds (thiophene, benzothiazole, thiadiazolidinone, quinoline and thiazole) were selected according to their leishmanicidal activity, IC_{50} for promastigotes (4.5 - 50 μM) and axenic amastigotes (2.2 - 14.4 μM) to assess their effects on the bioenergetic metabolism of the parasite. The most appealing compound (thiophene AM-94) decreased the intracellular ATP levels, depolarized mitochondrial electrochemical potential, decreased the respiration rate mapped at the level of Complex III (cytochrome c reductase), and induced apoptotic death of the parasites.

4 Conclusions

We have demonstrated the potential of our chemical library to discover new hits. By using this approach we have endorsed the thiophene scaffold for its druggability on the single mitochondrion of *Leishmania*. This is a new candidate to be further developed as its action on complex III, a crossroad from the contributions from glycerophosphate dehydrogenase and complex II, enhances the potential of this compound.

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C1333 A DUAL SCREENING PIPELINE FOR THE DISCOVERY OF NOVEL ANTI-LEISHMANIALS WITH HOST CELL-DEPENDENT MECHANISM OF ACTION

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1 Background

Human leishmaniases lead to severe morbidity and mortality worldwide. The emergence of drug resistant parasites and the side effects of current treatment call for the identification of new therapeutic targets. We present here a multi-stage screening procedure to determine new drug candidates that target essential pathways of host/*Leishmania* interaction.

2 Methods

We used a physiologically relevant phenotypic assay that relies on primary macrophages hosting virulent amastigotes of *L. amazonensis or L. donovani*. Fluorescent readouts were used to quantify parasite load by assessing the presence of parasite-containing phagosomes or the parasite themselves. Additionally, we performed viability assays on promastigotes and axenic amastigotes of both *Leishmania* species using a dye reduction assay and a FACS-based vital stain assay to reveal compounds with host cell-dependent mode of action. Finally, anti-leishmanial activity of selected hits is validated in a hamster model of visceral leishmaniasis.

3 Results

We tested compounds from two highly selective, dedicated libraries of potential inhibitors specific for kinases (>3000 compounds) and epigenetic regulators (>500 compounds). Combining our biochemical viability and phenotypic HCA assays we identified 27 hits able to kill host cell-free parasites and intracellular amastigotes without affecting host cell viability. A wide range of compounds belonging to different chemical scaffolds were identified and classified according to their mechanism of action into compounds targeting both extra- and intracellular parasites or compounds that show a strictly host-cell dependent activity on only intracellular amastigotes. Compounds of the latter group can act as pro-drugs, or can target pathways of host/parasite interaction, e.g. parasite-released ecto-molecules or host molecules that are exploited for intracellular parasite survival. Ongoing metabolomics, RNAseq and RNA interference experiments will allow us to distinguishing between these two possibilities. Finally, we validated an experimental hamster model for visceral leishmaniasis to assess the *in vivo* anti-leishmanial activity of confirmed hits.

4 Conclusions

Our drug discovery procedure allows the selection of hits active against intra-macrophagic amastigotes and/or extracellular parasite stages and highlights the discovery of unique anti-leishmanial scaffolds with host-cell dependent mode of action opening new avenues for drug discovery less prone to the generation of resistant parasites.

C1340 IN VITRO AND IN VIVO ANTILEISHMANIAL EFFECT OF STYRYL-QUINOLINES COMPOUNDS

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1 Background

Quinolines are present in many pharmacologically active natural compounds. The combination of quinoline ring and styryl moiety resulted in a small and powerful molecule to which several biological properties, including antiseptic and trypanocidal activities have been attributed. In the search of new molecules with potential to become a drug, we evaluated the *in vitro* antileishmanial and cytotoxic activity and the therapeutic response of some styryl-quinolines in the experimental model of cutaneous leishmaniasis in hamster

2 Methods

Styryl-quinolines were synthesized by Perkin reaction. The *in vitro* cytotoxicity was evaluated in the human U937 macrophages according to viability percentage by MTT method and the Lethal Concentration (LC_{50}) was determined. The antileishmanial activity of each compound was determined in intracellular amastigotes of *L. (V). braziliensis*-EGFP, based on the percentage of infected cells and parasitic load by flow cytometry and the Effective concentration (EC_{50}) and the Index of Selectivity were determined. The *in vivo* therapeutic response was tested in the hamster (*Mesocricetus auratus*) model for CL. Treatment was administered daily for 20 days. The effectiveness of each treatment was assessed comparing the lesion sizes prior to and after treatments and by quantification of parasite load using qPCR. The toxicity was evaluated according to hepatic and renal functions by blood chemistry and histopathological findings in liver and kidneys.

3 Results

Six styryl-quinolines compounds showed activity to intracellular amastigotes of *L. braziliensis* with a EC_{50} < 2.5 µg/mL and the Index of Selectivity was >5. Six formulations (cream) resulted in cure or clinical improvement. Compounds RQ-2, RQ6, RQ-7 showed the highest improve and cure rate. The parasite burden of lesions was lower in all animals after treatment, especially in those who cured or improved. No side effects or abnormalities in histopathological findings were observed.

4 Conclusions

The styryl-quinolines evaluated here have potential as lead compounds to development of an antileishmanial drug

C1352 STRUCTURAL STUDIES OF THE PLECKSTRIN HOMOLOGY DOMAIN (PHD) OF THE AKT-LIKE ENZYME OF LEISHMANIA PANAMENSIS.

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1 Background

Although new therapeutic approaches for the Leishmaniasis have been launched in recent years, classical treatments remain the choice in many endemic countries because of the limitations of the new chemotherapies. Therefore there is a need for the discovery of new antileishmanial compounds. Rational drug design seeks for the development of drugs based in the knowledge of the cellular target. We have studied the effects of the inhibition of the Protein Kinase B (PKB) of *Leishmania panamensis*, known also as Akt-Like. The Akt-like enzyme presents a predicted N-terminal Pleckstrin Homology domain (PHd), which binds to phosphatidylinositide 3'-OH kinase (PI3K) second messengers mediating several important cellular events making it an attractive drug target. We performed a series of biophysical and structural studies over the PHd of the Akt-like of L. panamensis aiming to the discovery and development of new antileishmanial molecules targeting the Akt-like.

2 Methods

Three versions of the predicted N-terminal PHd were isolated by sequential deletion of the C-terminal part of the Akt-like cloned into a pET-M11 vector. The 15N, 13C labeled versions of the recombinant PHd were produced in a prokaryotic system growing in minimal media containing 13C-Glucose and 15N-$(NH_4)_2SO_4$. The labeled proteins were purified by chromatographic techniques and a series of solution NMR experiments were performed for the backbone assignment and calculation of through-bond distances. NOE NMR spectra were used for the calculation of through-space distances. The NMR derived information was used for the calculation of the solution model of the PHd.

3 Results

We successfully produced a recombinant labeled version of the PHd of the Akt-like of *L. panamesis*. The protein yield is outstanding suggesting a high stability of the recombinant protein. However, the shortest version (1-100 residues) of the protein showed to be more stable according to the NMR studies in agreement with reported studies. The PHd of the Akt-like is more structured than predicted in silico, besides its fold differs from that of the reported structure from humans.

4 Conclusions

This work represents, to the best of our knowledge, the first structural evidence of Pleckstrin domains in in tripanosomatids. The solution structure reported here and the spectral assignment represent the substrate for the rational design of new Akt-like targeting compounds, which can lead to the development of new antileishmanial drugs.

C1354 ANTILEISHMANIAL ACTIVITY OF SELECTED PLANTS USED IN TRADITIONAL MEDICINE IN FRENCH GUIANA

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1 Background

Leishmaniasis is regarded as a major public health problem worldwide and in the absence of a vaccine, there is an urgent need for effective drugs to replace/supplement those in current use. The Amazonian rain forest represents a potential reservoir of biodiversity and an important source of bioactive molecules.
French Guiana is located northeast of the South America continent and its population is formed by a mosaic of ethnic groups. These populations settled in French Guiana bringing their respective traditional knowledge and practices with reference to the use of medicinal plants (from Asia, Europe, Africa and America). Exchanges between those communities were observed during several years and still occur among them. In French Guiana, cutaneous leishmaniasis due to *Leishmania guyanensis* is the predominant form of the disease where it is locally known as *pianbois* or *kalasapau*.
Here we present an inventory of medicinal plants used to treat leishmaniasis or wound in French Guiana and we evaluated the leishmanial activity of nine of them.

2 Methods

Nine plants among 120 listed were selected. One plant whose leishmanicidal activity has been demonstrated beforehand was also selected as control : *Lantana camara* (Verbenaceae). Pentamidine was chosen as reference drug.
Crude ethanolic extracts of nine plant species belonging to seven families were screened for their leishmanicidal activity on *Leishmania guyanensis* promastigotes. Viability tests were performed in triplicate using the Cell Counting Kit-8 assay (sigma®). Cytotoxicity assay were performed *in vitro* on human fibroblastes.

3 Results

An Araceae extract provided an higher leishmanicidal activity than pentaminide. *Lantana camara* and *Spondias mombin* ((Anacardiaceae) exhibited high antimicrobial level confirming previous finding on *L. Infantum (=L. chagasi*). Two more plants belonging to *Verbenaceae* genera provided also interesting results, *Stachytarpheta cayennensis* and *Priva lappulaceae*. For *Piper marginatum, Jatropha curcas Crescentia cujete* theIC50 values were higher demonstrating a lower activity. *Senna alata* extract (Caesalpinaceae) was considered as inactive against *L. guyanensis*.

4 Conclusions

We demonstrated the presence of leishmanicidal compounds in several plants used to treat leishmaniasis in French Guiana and highlight the particular importance of Verbenaceae family. The results obtained support the view that traditional medicinal plants still represent a valuable source of new drugs.

C1361 A MULTI-SPECIES HIGH CONTENT SCREENING ASSAY FOR THE DISCOVERY OF BROAD SPECTRUM ANTILEISHMANIAL DRUGS

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1 Background

High Content Screening (HCS) has been at the forefront of drug discovery for *Leishmaniasis* in recent years. Despite the advantages, HCS assays for *Leishmania* still have limitations such as low ratio of hits, comparing to other disease models, as well as high number of failure when compounds are translated to *in vivo* models. Additionally, HCS assays have so far been made available for a limited number of *Leishmania* species, mostly *L. donovani,* although different *Leishmania* species (and strains within species) may differ dramatically in regard to drug susceptibility.Therefore, there is a need to expand the portfolio of *in vitro* assays in order to better understand compound and drug candidate activity against different species and strains.

2 Methods

Based on previously reported HCS assays, we developed a robust multi-specie high content assay, to evaluate the activity of compounds against intracellular amastigotes in THP-1 cells for *L. infantum, L.donovani, L.amazonensis* and *L.braziliensis*. For each specie, we focused on both the biological system (cells and parasites conditions, infection, period of drug exposure) and the adjustment of commercial software to provide an appropriate image analysis. The assay was validated using a commercial library containing FDA-approved and experimental drugs.

3 Results

During the assay validation, a robust ratio of infection (60 – 75%) was observed across different days, there was a consistent window between negative and positive controls, resulting in high values of Z'-factor (0.66 – 0.83). The mean value of correlation index was 0.89 ± 0.02, indicating the assay has a high rate of reproducibility. The reference compound Amphotericin B showed constant activity (EC_{50} = 0.8 - 2μM) against all the species. Furthermore, the screening of FDA-approved and experimental drug library resulted in the identification of compounds active against some of the *Leishmania* species with high efficacy and relatively moderate potency. However, only one compound presented a broad-spectrum activity against species.

4 Conclusions

We developed the first multi-species high content assay, to evaluate the activity of compounds against clinically relevant *Leishmania* species. It provides a profile on compounds activity against different species, which could be determinant for an appropriate selection of candidates as starting points for drug discovery and contribute to the development of novel broad spectrum antileishmanial. This project has received funds from the New Medicines for Trypanosomatidic Infections (NMTrypI), Grant Agreement no. 603240 (http://www.nmtrypi.eu/)and from DNDi.

C1373 REVERSION OF RESISTANCE TO MEGLUMINE ANTIMONIATE IN LEISHMANIA (V) PANAMENSIS BY CURCUMIN

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1 Background

Control of cutaneous leishmaniasis in the Americas relies on chemotherapy. However, high rates of drug resistance to first line pentavalent antimonials and miltefosine contributes to failure of treatment. Our group and others have described the role of macrophages in the activity of antileishmanials, and show that differential expression and function of macrophage *ABC* transporters and metal metabolism molecules impact intracellular parasite survival during exposure to antimonials. We explored the potential of host cell efflux pump inhibitors curcumin, elacridar and apigenin, in combination with meglumine antimoniate (MA), in the intracellular killing of drug-resistant *Leishmania Viannia panamensis*.

2 Methods

PMA-differentiated THP-1 cells and primary human macrophages were infected with antimony (Sb) resistant and Sb-susceptible *L.(V) panamensis* lines and clinical strains. The effect of apigenin, elacridar and curcumin (inhibitors of *P-gp* and *MDR1*) and their combination with MA, on intracellular parasite survival was evaluated. Parasite burden was determined by RT-PCR amplification and quantification of *Leishmania 7SLRN*A gene transcripts. Intracellular antimony was measured by ICP-MS.

3 Results

The combination of MA with 10μM apigenin or 2.5μM elacridar did not modify intracellular parasite killing. In contrast, pre-exposure of THP-1 macrophages to 10μM curcumin followed by exposure to 32μg/mL MA, significantly reduced over 50% survival of Sb-resistant *L.(V) panamensis* ($p<0.05$). Enhanced killing was not associated with increased intracellular accumulation of antimony in curcumin-exposed and infected cells. To explore potential mechanisms by which curcumin promotes killing of Sb-resistant parasites, we profiled the expression of a panel of immune-related genes, drug transporters and metabolic enzymes in primary human macrophages during infections with Sb-resistant and Sb-susceptible *L.(V) panamensis*. Among 17 genes evaluated, expression of *CXCL3* and *IL-1β* was significantly different among groups: infection with Sb-resistant strains induced significantly higher levels of macrophage *CXCL3* ($p<0.05$), while repressed *IL-1β* expression ($p=0.01$). Inhibition of *IL-1β* by drug-resistant parasites could result in enhanced parasite survival by impaired inflammasome activation in host macrophages.

4 Conclusions

These findings provide evidence of host-directed strategies towards control of drug resistant parasite populations.

C1376 GENE EXPRESSION ANALYSIS OF ANTIMONY RESISTANCE IN LEISHMANIA TROPICA USING REAL-TIME RT-PCR: FOCUSED ON GENES INVOLVED IN TRYPANOTHIONE METABOLISM

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1 Background

Pentavalent antimonials remain the treatment of choice for anthroponotic cutaneous leishmaniasis (ACL) in Iran; and increasing rates of antimony resistance is becoming a serious health problem in some endemic regions. Accordingly, unraveling molecular markers for monitoring of drug resistant parasites is crucial. Different studies have been suggested the importance of genes involved in trypanothione metabolism. In this regard, current study was designed to investigate the RNA expression level of gamma glutamyl cysteine synthetase (GCS), Spermidine synthase (SpS) and Trypanothione synthetase (TRYS) genes in sensitive and resistant Leishmania tropica isolates.

2 Methods

The clinical isolates, including 10 resistant and 10 sensitive samples, were collected from ACL patients. Drug sensitivity test was performed on the samples as well as reference strains; afterwards, gene expression analysis was performed on clinical isolates by quantitative real-time PCR.

3 Results

Drug susceptibility tests revealed that the average IC50 value for resistant isolates was about 4.5 times higher than the sensitive ones. The average expression level of GCS (2.2) and TRYS (3.1) was up regulated in resistant isolates compared to sensitive ones whereas the expression of SpS gene wasn't different significantly.

4 Conclusions

Our result suggested that over expression of genes involved in trypanothione metabolism as a defense mechanism could possibly protect Leishmania from metal-induced oxidative stress. Moreover, GCS and TRYS genes can be considered as potential molecular markers for screening of antimony resistance in clinical isolates.

C1394 SEARCHING FOR NEW ANTILEISHMANIALS IN ENDEMIC AREAS: HIT-TO-LEAD ACTIVITIES WITHIN DNDI'S LEAD OPTIMIZATION LATIN AMERICA CONSORTIUM

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1 Background

Visceral leishmaniasis (VL) is a systemic disease caused by the protozoan parasites *Leishmania donovani* and *L. infantum*. VL occurs mainly in Asia, Africa, Latin America, and the Middle East, with estimated 200–400,000 new cases per year. Available treatments for VL lack safety and/or efficacy, and hence new treatments are highly desirable. In this view, DNDi created decentralized early-research consortia, combining the expertise and research capacity in academia, industry, and contract research organizations. The consortia focus on providing high quality chemical leads that could be ultimately developed into short-course oral treatments, aligned with DNDi's target product profile. The Lead Optimization Latin America (LOLA) consortium, based in Brazil, is the youngest collaborative research network and represents a step towards capacity building objectives in endemic areas. This abstract summarizes the VL-driven hit-to-lead efforts performed within LOLA.

2 Methods

L. infantum MHOM/MA(BE)/67 strain was used. Primary peritoneal mouse macrophages were used as host cell. Compounds were screened at 5-10 concentrations. Amphotericin B and miltefosine were used as reference drugs. Parasite burdens were microscopically assessed after 5 days and the results expressed as IC_{50} (50% inhibitory concentrations). In parallel, cell viability was assessed fluorimetrically (resazurin assay – CC_{50} as 50% cytotoxic concentrations), and *in vitro* ADME assays were conducted to support hit prioritization.

3 Results

A total of 266 compounds, covering four chemical series (azaindoles, benzimidazoles, cyanopyridines, and pyridylpyrimidines), have been synthesized and screened. Considering pre-established cut-offs on potency and selectivity (IC_{50}<10μM; CC_{50}>32μM), 19 compounds were regarded as promising (mostly benzimidazoles), however, still presenting physicochemical (high lipophilicity) and metabolic (high clearance) liabilities.

4 Conclusions

Despite the early stage of the campaign, initial results provided relevant data on structure-activity/properties relationships, and guided the design of improved analogs that are currently under evaluation. We strongly believe supporting the development of research capacities in endemic areas can increase the delivery of high quality compounds in the long term, which hopefully will progress in the pipeline for neglected tropical diseases.

C1405 IMPLICATIONS OF HORSE SERUM REPLICATIVE ASSAY IN VISCERAL LEISHMANIASIS DRUG DISCOVERY CASCADE.

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1 Background

The protozoan parasite *Leishmania donovani* is the causative agent of visceral leishmaniasis, a disease potentially fatal if not treated. Current available treatments have major limitations, and new and safer drugs are urgently needed. Several assays have been implemented in the last years focussing in phenotypic screening. Horse serum replicative assay is one of the recent assays included in the progression cascade for leishmniasis drug discovery.

2 Methods

Horse serum (HS) inclusion in intramacrophague assay suposse a litle change in the process that permits to mantain the troughput and quality of the assays but implicates important differences in the parasite replication and permit to avoid the reinfeccction issues. Kinetic readout have been included to evaluate the speed of action of the different drugs tested.
"The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents"

3 Results

This communication reports the differences observed in different compounds behaviour in presence of HS in the assay, and permits see differences in the speed of action of the compounds that could be linked to different modes of action.

4 Conclusions

HS intramacrophague assay is a replicative assay, without reinfection, which is included now in the progression cascade for leishmnania drug discovery programs. The kinetic readout allows to differentiate compounds in different Mode of action categories based in their rate of action. We have also observed that the HS assay predicts better in vivo activity, as all *in vivo* active compounds were active in HS assay.

C1437 ANTIPARASITIC POTENTIAL OF EXTRACELLULAR METABOLITES FROM MARINE ACTINOMYCETES AGAINST LEISHMANIA (VIANNIA) PERUVIANA AND LEISHMANIA (V.) BRAZILIENSIS

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1 Background

Actinomycetes are recognized as producers of pharmacological and industrial compounds. In the last decades, the discovery of new metabolites isolated from the terrestrial actinomycetes has decreased and the researches have focused on searching unexplored habitats, like the marine environment. The vast majority of compounds produced by actinomycetes are antimicrobial and few have antiparasitic activity. This study aimed at determining the antiparasitic potential of actinomycetal extracellular metabolites isolated from marine actinomycetes against *Leishmania* (*Viannia*) *peruviana* and *Leishmania* (*V.*) *braziliensis*.

2 Methods

Ferments obtained from the actinomycetes 13A1, EIIIA, EIIB and EIIIC were evaluated against *in vitro* cultures of *L.* (*V.*) *peruviana* and *L.* (*V.*) *braziliensis* promastigotes maintained in Schneider's medium. For inhibition control, sodium stibogluconate at the concentration of 20 mg/mL was used. The *in vitro* cultures were incubated at 28 °C. The evaluation and parasite count was performed every 24 hours for 7 days. The test was done in duplicate.

3 Results

The results of this test showed that EIIB strain had the higher inhibitory activity. The EIIB strain was identified as a member of the genus *Streptomyces* using rRNA method. The minimum inhibitory concentration of butanolic extract of EIIB strain who filed the anti-Leishmania activity was 15 000 µg/mL for *Leishmania* (*V.*) *peruviana* and *Leishmania* (*V.*) *braziliensis*. The preliminary chemical characterization of the extract confirmed the presence of coumarins.

4 Conclusions

In conclusion, the results demonstrated the production of metabolites from marine actinomycetes with antiparasitic activity, which have a great potential in the biomedical and pharmacological field.

C1455 ENZYMATIC CHARACTERIZATION OF A COMPOUND "C208", AN INHIBITOR OF LEIF, LEISHMANIA TRANSLATION INITIATION FACTOR 4A

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1 Background

LeIF, the *Leishmania* eukaryotic initiation factor eIF4A, constitutes a potential drug target. We used it in a screening of the CNRS chemical library on two binding sites (P1 and P2) identified on the LeIF 3D model (but not human eIF4A) to select for LeIF ATPase inhibitors. 300 compounds were tested against LeIF RNA dependent ATPase *in vitro*. Among the four different compounds retained, one (C208) was selected for further investigation. It presented biochemically relevant IC50 values on LeIF and $eIF4AI_{Mus}$. C208 docked virtually on P2, which comprises a motif involved in RNA binding of eIF4As. Here, we aim to study the actual mechanism of inhibition of C208.

2 Methods

Kinetic measurements of the inhibition of LeIF ATPase activity were done using increasing concentrations of C208. Michaelis–Menten (MM) kinetics used 800nM of LeIF with either increasing concentrations of ATP (and saturating whole yeast [RNA]), or increasing RNA concentrations (with excess of [ATP]). P2 being located in NH_2 domain (D1), we studied effect of addition of large excess of recombinant D1 or D2 (COOH) domains and BSA on the ATPase reaction.

3 Results

C208 had an IC50 of 150+/- 15 µM. According to the nature of the MM kinetics curves, C208 seems to compete for both ATP and RNA binding sites, P1 and P2. However, ATP enhances the affinity of the protein for RNA and vice versa. Consequently, reduced RNA binding would reduce ATP affinity. Thus, C208 could directly binds P2 and competes with RNA fixation and consequently affect the ATPase activity of the protein, as proposed in the *in silico* study. Adding increasing concentrations of D1 reduced the inhibition. It would be an effective quencher of the observed inhibition of the ATPase activity. Addition of D2 that contains the RNA binding motifs IV and V also reduced the inhibition of LeIF ATPase. Actually, a compound inhibiting RNA binding would effectively reduce the apparent affinity of the protein for ATP as well as the ATPase activity. Hence, C208 inhibits the ATPase activity by blocking or reducing the affinity for RNA. No competition was seen between LeIF and BSA for C208.

4 Conclusions

C208 interacts with RNA binding as expected from docking studies. It constitutes a novel route to search for inhibitors against LeIF. Preferential selectivity for LeIF is plausible as we demonstrated that rocaglamid, a mammalian eIF4A ligand, is 7x more active on eIF4A than on LeIF.

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C1463 HISTOPATHOLOGICAL ASPECTS OF HAMSTER LESION TISSUES INFECTED WITH LEISHMANIA AMAZONENSIS TREATED WITH ANTIMONY NANOPARTICLES NANOPARTICLES (SBNPS)

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1 Background

Nanoparticles (NPs) promise an alternative approach to new drugs in the treatment of infectious diseases, and are more effective than traditional therapiesThis study aimed to investigate the tissue effect of treatment with pentavalent antimony nanoparticles (SbNPs) on hamsters (*Mesocricetus auratus*) infected with *L. amazonensis*.

2 Methods

Hamsters (*Mesocricetus auratus*) infected with *L. amazonensis* were treated intralesionally with pentavalent antimony nanoparticles (SbNPs). After 26 days, the animals were sacrificed to remove tissue fragments (lesion) that were processed for histopathological analysis. The material was cut into sections of thickness 3-5 µm and arranged in histological slides, stained with HE and analyzed by light microscopy, with degenerative, inflammatory and hyperemic changes being determined.

4 Results

The histological findings of the lesions of the groups treated with SbNPs showed intense inflammatory infiltrate, with the presence of numerous macrophages and parasites, including cell clusters, forming giant giants, compromising both the dermosuperficial layer and the dermoprofound of the tissue.

5 Conclusions

More experimental investigations are necessary to elucidate better conclusion regarding the safety of nanoparticles. This study suggests that SbNPs are capable of causing significant changes in tissue. However, this issue will have to be considered more extensively in future studies.

C1481 EVALUATION OF THE ANTI-LEISHMANICIDAL ACTIVITY OF A MOLECULE OF ZERUMBONE

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1 Background

Zerumbone is a sesquiterpene phytochemical from a type of edible ginger known as "Zingiber Zerumbet Smith"grown in Southeast Asia or "Zingiber aromaticum". Zerumbone is currently being explored for its effects on cancer in general and leukemia in particular. In view of this the zerumbone has aroused great interest of the scientific community in the possibility of using this compound for treatment of cutaneous leishmaniasis. The objective is to evaluate the anti-leishmania activity of zerumbone compounds to the promastigote forms of *Leishmania (Viannia) guyanensis* and *Leishmania (Leishmania) amazonensis*.

2 Methods

For the evaluation of the activity of the substances, promastigote forms of the two *Leishmania* species were cultivated, and these parasites were exposed to three zerumbone (Z-TSC; Z-OXIME and Z-NH2) derivatives at concentrations of 500-31, 25 mg / mL, in order to evaluate the inhibition of growth and mortality of the promastigote forms of each species. The positive control used was Glucantime®.

3 Results

The results expressed in EC 50 for *L. guyanensis* as for *L. amazonensis*, it can be seen that only the Z-TSC compound was considered active, presenting promising activity when compared to the Glucantime® control.

4 Conclusions

From the results presented in this work, it is possible to conclude that one of Zerumbone compounds presented leishmanicidal activity against promastigote forms of *L. amazonensis* and *L. guyanensis*. However, it is a preliminar results and is necessary tests of cytotoxic activity in macrophages and the activity against intracellular amastigotes.

C1513 METAL-ORGANIC RU(II) COMPLEXES AS NEW EFFICIENT PHOTOSENSITIZERS AGAINST LEISHMANIA

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1 Background

Photodynamic therapy (PDT) eliminates pathogens by generation of the highly reactive singlet molecular oxygen ($^1O_2$) species from tissular O_2 and a photosensitizer agent (PS), under adequate illumination. PDT has been successfully tested on animal models and patients to treat cutaneous leishmaniasis (CL) using a variety of PS dyes. In this study we evaluated 12 Ru(II) coordination complexes (RCCs) with ligands such as 2,2'-bipyridine, 1,10-phenanthroline and dibenzo[h,j]dipyrido[3,2-a;2',3'-c]phenazine (and derivatives), with high $^1O_2$ generation quantum yields (Φ_D) and absorption in the blue-green region, as new PSs against *L. major* promastigotes and *L. pifanoi* amastigotes.

2 Methods

Leishmanicidal activity of RCCs on *L. donovani* promastigotes and *L. pifanoi* axenic amastigotes, and cytotoxicity on Balb/c peritoneal macrophages, were measured by inhibition of the MTT reduction. SAR studies on the RCCs were carried out by considering log P (absorbance at 447 nm), Φ_D($^1O_2$ emission decay curve at 1270 nm) and parasite binding (time-resolved phosphorescence). The most active photosensitizer, RD3-C, was also encapsulated in 20-nm carboxylated polystyrene nanoparticles (NPs), used on infected macrophages and visualized by confocal microscopy. RB3-P was conjugated to the cell penetrating peptide (CPP) Tat and its leishmanicidal activity was analyzed.

3 Results

Four of the RCCs were highly leishmanicidal. The IC_{50}s under blue light in promastigotes were 0.47-2.12 μM, whereas axenic amastigotes were even more susceptible (IC_{50}s 0.08-1.88 μM). Selectivity indexes were 5 to 50 in darkness. SAR studies evidenced a multiparameter dependence. Vehiculation of RD3-C in NPs, or conjugation of RB3-P to a CPP, boost their activity and specificity.

4 Conclusions

We have demonstrated the activity of metal-organic Ru(II) complexes as new leishmanicidal agents. RCCs were active at submicromolar concentrations, yet with poor specificity, improved by their vehiculation and rational modification of their chemical structure. RCCs represent a new PS scaffold for PDT of CL, with excellent efficacy, low toxicity and active in a non-classical spectral region to capitalize on the modern blue light-emitting diodes (LEDs).

Projects: RETICS FEDER RICET RD12/0018/0007 & PI12-02706; PIE 201620E038, CSIC; MINECO: CTQ2015-69278-C2-2-R & CTQ2015-72708-EXP;EU: H2020-MSCA-RISE-2014 (INPACT), MINECO, project no. SAF2015-65740-R.

C1551 DEVELOPMENT OF LEISHMANIA SECONDARY ASSAYS FOR DRUG DISCOVERY: IN VITRO-IN VIVO TRANSLATION USING HIGH CONTENT SCREENING

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1 Background

In the recent years, millions of compounds have been tested in an attempt to identify new antileishmanial agents. Despite such efforts, *Leishmania* drug discovery is associated with low ratios of hit identification as well as high number of failure when compounds are moved forward in *in vivo* models. Part of this failure can be due to lack of assays for identifying possible liabilities of hit and lead compounds. This issue could be tackled in part by the development and validation of secondary assays that can facilitate the translation of *in vitro* data to *in vivo* disease models providing the basis towards a solid go/no go decision matrix in leishmaniasis drug discovery process.

2 Methods

Based on our established High Content Screening (HCS) assay, we have developed an *in vitro* time-kill assay using intracellular amastigotes *Leishmania*.THP-1 cells are infected with late stationary-phase promastigotes. After 24 h of infection compounds are added to the plate. The cidal or static activity profile of reference compounds was evaluated against *L. braziliensis* and *L. amazonensis*, every 24 h, for 8 days.In parallel, a HCS of primary macrophages hosting amastigotes was developed using bone marrow-derived macrophages (BMDM) infected with late stationary-phase promastigotes. These assays have also been used to screen compounds discovered during a screening campaign of experimental and FDA-approved drugs.

3 Results

So far, we standardized the infection of BMDM in 384 wells plate and the software analysis. Time-kill preliminary results show both concentration and time-dependent activity for all tested compounds, and a susceptibility profile specific for each *Leishmania* species. The data also suggests that *Leishmania* exposure to compounds will likely require longer than 96 h to be able to detect unambiguous activity/efficacy for cidal compounds.

4 Conclusions

Time-kill is a useful tool for obtaining information about the dynamic interaction between the compound and the parasite. In addition, this method can be used to determine synergism or antagonism between drugs in combinations. The understanding of the correlation between compounds activity in THP-1 and primary cells can deepen our understading of in vitro and in vivo disease models, and contribute to improving the *in vitro/in vivo* translation process.

This project has received funds from DNDi.

C1566 LARGE-SCALE SCREENING EFFORT TO IDENTIFY NEW CHEMICAL STARTING POINTS FOR VISCERAL LEISHMANIASIS USING A NOVEL IN VITRO SCREENING CASCADE

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1 Background

A key issue in visceral leishmaniasis (VL) drug discovery is the difficulty finding new chemical starting points. Many assays have been developed, some using insect stage promastigotes or axenic amastigotes and others with intracellular amastigotes. Poor translation between the assays using free-living parasites and the "gold-standard" intracellular assays has often been observed. The work presented here shows how some of these issues can be overcome by redeveloping existing assays and combining assays in screening cascades. The screening cascade presented was used to screen a large number of compounds from various pharma and commercial sources.

2 Methods

We reconfigured our existing axenic amastigote assay to only detect compounds that are cytocidal. This was achieved by improving the detection limit of the assay and increasing the starting cell density. A screening cascade was developed using the "cidal" axenic amastigote assay as a primary screening platform, followed by potency and selectivity determination in the cidal axenic amastigote assay and a human counter-screen assay, followed finally by assessment in a high-content intracellular amastigote assay. This cascade was used for the screening of >700,000 compounds.

3 Results

Our results show that the cidal axenic amastigote assay is a better predictor of intracellular amastigote activity compared to its non-cidal format. As such it provides a valuable primary screening platform for large scale screens, especially since throughput limitations do not allow the use of the intracellular assay for this purpose. The screening cascade used here was successful in identifying several new hit series for VL drug discovery. Results from a single screening campaign will be presented

4 Conclusions

This work shows that there is value in using axenic *Leishmania* screening assays, and that the way these are configured, in particular with respect to the detection limit, has a major impact on the hits identified, and their translation to intracellular amastigote activity. Through the use of a rational and pragmatic screening cascade we have been able to identify much needed new chemical starting points for VL drug discovery.

C1570 TRANSLATIONAL CHALLENGES IN VISCERAL LEISHMANIASIS DRUG DEVELOPMENT: DIFFERENT MODELS, DIFFERENT DRUGS' MECHANISM OF ACTION, DIFFERENT PREDICTIVE VALUE: TOWARDS AN EMERGING ANSWER?

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1 Background

New safe, low cost and field-adapted drugs for visceral leishmaniasis (VL) are urgently needed. Despite substantial screening and lead optimization efforts during the last five years within various organizations, few new chemical entities have successfully entered into the clinic. Whether the right tools or the correct decision-making processes are being used to progress compounds should therefore be reviewed. E.g. VL animal models need to be standardized, and variability linked to the animal or *Leishmania* species used, as well as readouts, should be assessed in a careful and systematic way.

2 Methods

We have generated systematic *in vivo* efficacy data in both Balb/c mouse and the Syrian hamster model of VL infection. To assess the potential influence of the *Leishmania* species, as well as the mechanism of action of compounds tested, models of infection with both *L. donovani* and *L. infantum* were used and a range of known reference drugs (with clinical data whenavailable, allowing back-translation analysis) and representatives of experimental hit series were evaluated. Supplemented by pharmacokinetic data (exposure, AUC_{last}, C_{max}), attempts were made at preliminary pharmacokinetic/pharmacodynamic (PK/PD) relationship modelling for different new VL chemical series, such as oxaboroles and nitroimidazoles.

3 Results

Current data suggest that the *Leishmania* species used in animal models should not play a significant role in the efficacy assessment of compounds, if the latter distribute effectively to each target organ. Differences in parasite load and distribution are among the most important discriminators between the two animal models tested. DMPK characteristics of a compound, such as its local drug concentration, protein binding and potential accumulation in target compartments, could therefore differentially affect its outcome depending on the model used, e.g. Tafenoquine.

4 Conclusions

Together with adequate *in vitro* profiling of compounds, a better PK/PD understanding should allow us to revisit our current screening sequence for VL drug discovery and improve its effectiveness during lead optimization processes, possibly also reducing the number of animals used. Overall it should lead to an improved prediction model for human studies (right dose, dosing schedule) and increase the likelihood of successfully delivering new drugs for neglected patients. In the long run, only clinical trial data with new candidates will validate standardized animal models.

C1601 ONE STEP TOWARDS AN ORAL TREATMENT FOR CUTANEOUS LEISHMANIASIS: THE IN VIVO EFFICACY OF THREE CHEMICAL CLASSES UNDER PRECLINICAL DEVELOPMENT FOR VISCERAL LEISHMANIASIS

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1 Background

DND*i*'s long-term objective in respect to cutaneous leishmaniasis (CL) drug development is to deliver a safe, efficacious, affordable and short-course oral treatment.

2 Methods

Working with limited resources DND*i* has taken an opportunistic approach to address the identification and development of candidates to be considered for clinical progression for CL. To proceed a short list of molecules currently in preclinical development by DND*i* for visceral leishmaniasis (VL) have been evaluated for their respective efficacies in a panel of *in vitro* and *in vivo* CL assays.

3 Results

We disclose the remarkable *in vitro* and *in vivo* efficacies of a few representative compounds belonging to 3 chemical series in preclinical development for VL at DND*i*, namely nitroimidazoles, aminopyrazoles and oxaboroles. Concordant results independently generated by two research laboratories advocate for a further exploration of those 3 chemical series in respect to preclinical development for CL.

4 Conclusions

Outcome will be a VL to CL preclinical candidate repurposing approach, with very low impact on development cost and an accelerated access to patients.

C1626 PHARMACOLOGIC SCREENING OF BETULINIC ACID* AND CAFFEIC ACID** DERIVATIVES AGAINST NEW WORLD LEISHMANIA

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1 Background

Treatment failure associated with infection by refractory *Leishmania* strains, the spread of the vector to new geographic areas, the toxic adverse effects produced by the available anti-leishmanial drugs, and last but not least, the increasing incidence of HIV-leishmaniasis co-infected patients, impose the need for the development of more effective, rapid, specific and economical therapeutic strategies to treat the disease. The derivatives of betulin (lup-20 (29) -ene-3beta, 28-diol), a triterpene extracted from the bark of Betula (birch) trees as well as of cinnamic acid bornyl ester and caffeic acid derivatives from *Valeriana wallichii* constitute potential alternatives for the design of such lead compounds. Herein we evaluated the activity of 28 betulin and 13 caffeic acid derivatives on reference strains from New World *Leishmania*, and parasites isolated from patients suffering DCL, selected the main active compounds with optimal selectivity indexes, and began the identification of their mechanisms of action.

2 Methods

The *in vitro* susceptibility of promastigotes and the cytotoxicity on cell line macrophages were evaluated by fluorometric means. The activity of the compounds in intracellular parasites was evaluated by the amastigote-macrophage model. Parasite plasma ($\Delta\Psi p$) and mitochondrial ($\Delta\Psi m$) membrane potentials were evaluated by fluorometric means. Parasite glucose consumption, as well as the production of nitric oxide (NO) and superoxide ($O2^-$) by infected macrophages were evaluated by spectrophotometric means.

3 Results

Two betulinic acid (BT17 and BT18) and one caffeinic acid (CF13) showed the best performance with GI50 < 3 mM and selectivity indexes < 10 > 169. Mechanistically BT18 collapses surface and mitochondrial membrane potential probably inducing programmed cell death, while CF13 induces NO production in naive and infected macrophages. Our

4 Conclusions

Our results thus suggest that betulin and caffeic acid derivatives could potentially be useful as lead compounds against leishmaniasis.

C1638 IDENTIFICATION OF GENE EXPRESSION OF RNA POLYMERASE II LEISHMANIA MAJOR (MRHO/IR/75/ER) EXPOSED WITH ANTIMONIALES, AMPHOTERICIN B AND PAROMOMYCIN IN VITRO

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1 Background

Leishmaniasis is caused by protozoan of *Leishmania* parasite. The pentavalent antimonials compounds remain the first-line treatment. RNA polymerase II encoded by *RPOILS* involves in different gene expression in exposure to the drugs. Based on our knowledge, there is no report about the *RPOILS* gene expression from *L. major* exposed with different doses. Therefore, this study was assessed the *RPOILS* gene expression in exposure of different doses from antimoniales, paramomycin, and amphotericine B.

2 Methods

L. major (MRHO/IR/75/ER) promastigotes were distributed in groups for exposure with antimoniales with end concentrations of 5, 10, 15, and 20 mg/ml; amphotericin B in final concentrations of 0.005, 0.010, 0.020 mg/ml; and paramomycine with end concentrations of 25, 50, and 100 mg/ml for 72 hours. Then, RNA was extracted cDNA synthesis was performed. Gene expression of *RPOILS* was assessed using SYBR Green Real Time PCR by ΔΔCT analysis.

3 Results

Gene expression in all groups had not any differences. Therefore, gene expression of the mentioned gene was done in susceptible and resistance isolates that results showed any differences in gene expression among them, too

4 Conclusions

As shown in results section, gene expression of *RPOILS* in all groups exposed with different doses of antimoniales, amphotericine B, and paramomycin were same. Therefore, it seems that involved this gene in drug pressure (for Glucantime, paramomycine, and amphotericine B) for parasites are not under control of RNA polymerase II. In all kinetoplastida, the transcription performs by polycistronic system without any RNA polymerase II and other common elements.

C1731 EFFECT OF CYCLODEXTRINS FORMULATIONS OF NMT-H0080 AGAINST CHRONIC EXPERIMENTAL INFECTIONS BY LEISHMANIA INFANTUM IN HAMSTER

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1 Background

Among a series of recently synthesized flavonols, NMT-H0080 has shown a remarkable effect against trypanosomatids *in vitro*. The molecule has a long t1/2 after intravenous (IV) or oral administration (PO). However the compound displayed low water solubility and bioavailability. Cyclodextrins have bioavailability enhancing properties, they are frequently used (>30 human drugs) and have improved pharmacokinetics (PK) of the molecule in BALB/c mice. A proof of concept experiment was designed in a hamster model of visceral leishmaniasis (VL) to determine: tolerability and toxicity of hydroxypropyl-β-cyclodextrin (CDX), antileishmanial efficacy in target organs and biodistribution of NMT-H0080.

2 Methods

Hamsters were infected IV with 10^8 promastigotes/animal of *L.infantum*.. Fifteen weeks post inoculation infected animals were divided onto 7 comparable groups and treated PO. Groups G13 to G15 (6 animals each) received vehicles (G13: 8%DMSO; G14:30% CDX; G15:50% CDX). G16-G18 (7 animals each) were treated with free NMT-H0080 in 8% DMSO (G16); G17 was treated with NMT-H0080 with 30% CDX and G18 with NMT-H0080 with 50% CDX. G20 (8 animals) was treated with Miltefosine (MIL). Animals were treated for 10 days: 40 mg/kg/day with NMT-H0080 (G16-G18) and 20 mg/kg/day for MIL. Experiment included an uninfected control group (6 animals).Ten days after the last treatment all animals were euthanatized and the antileishmanial efficacy in target organs determined.

3 Results

C

CDX or NMT-H0080 did not induce any noticeable adverse effect whereas some animals treated with MIL displayed transient dizziness, nausea and light to mild distress. None of the treatments elicited notable alterations of hepatic and kidney markers (AST, ALT, AP, urea, creatinine). MIL elicited a 97.3±3.9% reduction of parasite burden in the spleen and 81.2±28.3% in the liver. CDX alone did not show any antileishmanial effect. Reduction of parasite burden in the spleen of animals treated with NMT-H0080 ranged from 35.7±7% with the highest CDX concentration to 25.8±24.4% when administered alone. Antileishmanial efficacy was slightly lower in the liver.

4 Conclusions

Results suggest that NMT-H0080, PO, only has a moderate activity against chronic VL in hamster. Relationship between observed antiparasitic efficacy, bioavailability and biodistribution of the molecule will be discussed.

This project has received funds from the New Medicines for Trypanosomatidic Infections (NMTrypI), Grant Agreement no. 603240 (http://www.nmtrypi.eu/)

C1824 IDENTIFICATION OF GENE EXPRESSION OF J-BINDING PROTEIN1, J-BINDING PROTEIN2 FROM LEISHMANIA MAJOR (MRHO/IR/75/ER) EXPOSED WITH ANTIMONIALES, AMPHOTRICIN B AND PAROMOMYCIN IN VITRO

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1 Background

Leishmaniasis is caused by protozoan of *Leishmania* parasite. The pentavalent antimonials compounds remain the first-line treatment. The most important unique proteins in kinetoplastida are J-binding proteins which have a special role in J bas synthesis. Base J is a modified base that has been seen just in kinetoplastida such as *Leishmania*. Based on our knowledge, there is no report about the *J-binding proteins* gene expression from *L. major* exposed with different doses. Therefore, this study was assessed the *j-binding proteins* gene expression in exposure of different doses from antimoniales, paramomycin, and amphotericine B.

2 Methods

L. major (MRHO/IR/75/ER) promastigotes were distributed in groups for exposure with antimoniales with end concentrations of 5, 10, 15, and 20 mg/ml; amphotericin B in final concentrations of 0.005, 0.010, 0.020 mg/ml; and paramomycine with end concentrations of 25, 50, and 100 mg/ml for 72 hours. Then, RNA was extracted cDNA synthesis was performed. Gene expression of *J-binding proteins* was assessed using SYBR Green Real Time PCR by ΔΔCT analysis.

3 Results

Gene expression in all groups had not any differences. Therefore, gene expression of the mentioned gene was done in susceptible and resistance isolates that results showed any differences in gene expression among them, too.

4 Conclusions

As shown in results section, gene expression of *J-binding proteins* in all groups exposed with different doses of antimoniales, amphotericine B, and paramomycin were same. Therefore, it seems that involved this genes in drug pressure (for Glucantime, paramomycine, and amphotericine B) for parasites are not under control of RNA Polymerase II. In all kinetoplastida, the transcription performs by polycistronic system without any RNA polymerase II and other common elements.

C1831 PATHOGEN HOPPING APPROACH: A SOURCE OF NEW STARTING POINTS FOR VISCERAL LEISHMANIASIS DRUG DISCOVERY

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1 Background

Despite the considerable disease burden caused by kinetoplastid parasites, current drug treatments are inadequate therefore new therapies are required. Using whole-cell phenotypic assays, we performed a high-throughput screen (HTS) with GSK´s diversity set of 1.8 million compounds against the three kinetoplastid parasites i.e. *Leishmania donovani*, *Trypanosoma cruzi* and *Trypanosoma brucei*.
Assays with macrophage-amastigotes models are considered closest to the pathophysiological conditions of leishmaniasis, and are therefore the most appropriate for *in vitro* screening. However finding new starting points for *leishmaniasis* is extremely difficult. The intra-macrophage (InMac) assay has low sensitivity (low hit rate and low potencies). Although axenic assays provide an alternative route to find hits, but the translation to intra-macrophage activity is poor.

2 Methods

The overall objective of the pathogen hopping approach is to identify new hits for drug discovery for leishmaniasis from active compounds against axenic *Leishmania* amastigotes assay (mimicing pH gradient), other parasite(s) (giving permeability readout) and with some evidence of cidality (i.e. desirable MoA for progression). We have considered *Trypanosoma cruzi* as the related kinetoplastid intracellular parasite within this presentation, since appears to be more sensitive *in vitro* compared to *leishmania*. We have started developing a new strategy to mine the large amount of existing axenic amastigote and *T.cruzi* data from our HTS results and select the best candidates as a source of new visceral leishmaniasis starting points. Improvement in *T.cruzi* activity for the identified hits has been argued as a way to guide InMac activity.

3 Results

This poster will further explain the rationale behind the pathogen hopping approach and present the preliminary results from smaller subset of compounds that have gone onto demonstrate leishmania *in vivo* activity within a mouse model.

4 Conclusions

Preliminary results from pathogen hopping approach suggest a new way of identifying robust hits for visceral leishmaniasis that can be accelerated rapidly.

C1872 CYTOTOXICITY PROFILING OF ALKYLPHOSPHOCHOLINES AGAINST LEISHMANIA DONOVANI PARASITES AS A TOOL TO AID ANTILEISHMANIAL DRUG DISCOVERY

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1 Background

Background: Drug resistance in *Leishmania donovani* to miltefosine (MIL) and paromomycin (PMM) has not yet been demonstrated in the clinics, but can be induced, even in existing antimony (Sb) resistant parasites; thus the risk for their selection in the clinics is now inevitable. MIL represents a therapeutic backup drug for the treatment of visceral leishmaniasis (VL), thus its integrity for continued use needs to be preserved.

2 Methods

Method: Efficacy and cross resistance to 13 alkylphosphocholines (APCs) with distinct physicochemical properties was assessed *in vitro* and *in vivo*. Studies used transgenic *L. donovani* parasites expressing luciferase and resistant to MIL or PMM, on Sb-sensitive or Sb resistant background respectively. Parent lines sensitive to MIL or PMM but Sb-sensitive and Sb resistant respectively was used as controls in experiments.

3 Results

Results: Systematic screening of *L. donovani* Sb-sensitive parasites revealed that amphiphilic APCs with a short saturated tail and zwitterionic choline head group were effective antileishmanials. In depth characterisation of three lead APCs designated C12, C14 and C16 against the four drug resistant and two parent cell lines showed life cycle stage-specific differences in efficacy. Amastigotes resistant to MIL on a Sb-resistant background were refractory to C12, C14 and C16 relative to their control cell lines while MIL resistant parasites on a Sb-sensitive background was only resistant to C16. In contrast, promastigotes resistant to MIL on a Sb-sensitive and Sb-resistant background were only resistant to C16. *In vivo* studies showed that C12 administered by the intravenous route at a dose of 80mg/kg, cleared the spleen of detectable parasites and caused 95% in liver and bone marrow compared to controls ($p<0.01$).

4 Conclusions

Conclusions: The use of resistant parasites provides a predictive tool to eliminate redundant drugs for VL and have identified C12 as an alternative to MIL. These luciferase-expressing resistant and parent parasites are now being used to investigate the mechanism underlying these resistance and evaluate if a differential uptake of the drug into the cell is responsible for the complex phenotype observed.

C1913 KDNA REPLICATION INITIATOR PROTEIN UMSBP REGULATES AMPHOTERICIN B RESISTANCE MECHANISM BY CONTROLLING MDR1 IN CLINICAL ISOLATES OF LEISHMANIA DONOVANI

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1 Background

Universal minicircle sequence binding protein (UMSBP) is a mitochondrial DNA (kDNA) replication initiator protein and is involved in parasite survival, virulence and apoptosis. The activity of multi-drug resistance gene (MDR1), reported to be involved in Amphotericin B (AmB) resistance in *Leishmania donovani*, is regulated by the kDNA encoding enzymes and therefore kDNA replication machinery may regulates the reported MDR1 mediated AmB resistance. Therefore, we aimed to explore the involvement of UMSBP in AmB resistance mechanism in *Leishmania donovani*.

2 Methods

The expression levels of thiol metabolic pathway enzymes, kDNA, MDR1 and cytochrome-b (cyt-b) as well as oxidation-reduction status of UMSBP in AmB-resistant and AmB-susceptible parasites were measured. Over-expression and knockout constructs of UMSBP were transfected in AmB-susceptible and AmB-resistant parasites respectively. The status of kDNA, expression level of MDR1 and Cyt-b, level of ATP generation, the rate of AmB efflux, concentrations of reactive oxygen species (ROS) and levels of apoptosis were examined in WT and transfected parasites, and the AmB susceptibility of the respective parasites were measured by determining the LD50 of AmB.

3 Results

The deletion of UMSBP allele showed a decreased level of kDNA, mRNA level of MDR1, Cyt.b, lower drug efflux and ATP generation, increased ROS concentration, apoptosis-like phenomenon and decreased LD50 of AmB in resistant parasites. In contrast, UMSBP over-expression in susceptible parasites reversed drug susceptibility producing a resistant phenotype. This was associated with increased LD50 of AmB along with increased kDNA level, expression levels of MDR1, Cyt.b, drug efflux, ATP generation and reduced concentrations of ROS, corresponding to decrease apoptosis of resistant to WT sensitive.

4 Conclusions

UMSBP is involved in controlling AmB resistance by regulating MDR1, level of Cyt-b and ATP generation and ROS concentration. So, UMSBP may be used as a new marker for resistance in visceral leishmaniasis.

C1921 ANTILEISHMANIAL ACTIVITY OF NATURAL PRODUCTS FROM TEMPERATE ZONE PLANTS

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1 Background

Whilst the search for plant-derived antileishmanial natural products has focussed on plants from tropical and subtropical zones, we report here a screen of a library of natural products isolated from temperate zone plants. This proprietary library, PhytoPure, encompasses a wide range of chemical classes, two thirds of which are novel, and the remaining third not commercially available. Critically, the compounds are pure, overcoming common issues with screening fractions of complex mixes, and have been selected to reflect potential development (high degree of functionality and physiochemical properties that match Lipinski's rule-of-5).

2 Methods

Activity was first screened using an Alamar Blue assay against axenic amastigotes of *Leishmania mexicana*, with hits validated against *L. donovani*. Toxicity screens of the best hits were carried out against HepG2, with THP-1 intramacrophage assays performed using a SyBr Green I assay. Resistant lines are being generated.

3 Results

Twenty-three compounds with IC50 <2μM (0.15-1.38μM) were identified. Four closely related triterpenes from The Noble Fir and Grand Fir provide IC50 between 0.21-0.5μM against *L. mexicana* and 0.1-0.27μM against *L. donovani*. Toxicity assays reveal a selective index of >100, with *in vitro* intramacrophage activity showing a 50-90% reduction in parasite count after three days exposure to 9xIC50 concentrations. Resistant lines have been generated, showing cross reactivity between the triterpenes, with three months selection with one, 700-022, providing a >30-fold increase in IC50.

4 Conclusions

Potent and selective antileishmanial activity of triterpenes isolated from temperate zone plants has been demonstrated from a novel source of natural products for antiparasitic discovery.

Mucocutaneous leishmaniasis, diffuse cutaneous leishmaniasis, post-kala-azar leishmaniasis

C0066 MUCOCUTANEOUS LEISHMANIASIS PRESENTING WITH NASAL SEPTAL PERFORATION AFTER ALMOST THIRTY YEARS: CASE REPORT AND LITERATURE REVIEW

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1 Background

Cutaneous leishmaniasis is considered an emerging and uncontrolled disease by the World Health Organization (WHO) (1). Approximately 35,000 cases of mucosal leishmaniasis occur annually (1). It is present in 88 countries, but 90% of cases occur in only seven countries, including Peru (2,3). In Peru, between 2008 and 2014 there were approximately 5,100-7,350 leishmaniasis cases per year (4).

2 Methods

The patient reported a history of cutaneous leishmaniasis at 12 years of age, presenting three lesions: a 4 cm ulcer on her right hand, a 2 cm ulcer on her left hand, and a 7 cm ulcer on her left calf. Diagnosis was made with a biopsy of one of the lesions. At the time, she received 20 days of treatment with sodium stibogluconate, after which her ulcers healed completely.At 42-year-old woman first presented to her local health post with a two-year history of episodic, small-volume epistaxis associated with blowing her nose. .
On examination, the ENT physician observed a septal perforation measuring 1.5 x 3 cm in Cottle's areas II and III. There was approximately 90% obstruction in both nasal fossae due to crusting, dark lesions. An additional lesion measuring 2 cm with a granulomatous appearance was observed on her hard palate.

3 Results

Molecular biological results
PCR of kinetoplast DNA determined presence of *Leishmania (Viannia) DNA* (Figure 3), and real-time PCR identified the *L. (V.) braziliensis* species (Figure 4,5). Plasma IgG against *leishmaniasis spp* was measured by indirect immunofluorescence (IFI) and was positive at a titer of 1:160.
Laboratory results 10 and 16 months after treatment
Normal CD4 and CD8 counts were obtained and demonstrated the patient's immunocompetency, and were consistent with the patient's affirmation of no significant past medical history. A second IFI to measure leishmaniasis IgG was done on the last day of treatment, and results were positive for a titer of 1:80.
Sixteen months after her treatment, the patient underwent a follow-up biopsy of the border of her nasal septum lesion. Tissue was tested for PCR-kDNA of *Leishmania Viannia*. A third IFI was done and was positive for a titer of 1/40. Currently, physicians are evaluating the patient for further treatment.

4 Conclusions

We report a case of a patient who developed MCL thirty years after cutaneous disease, despite completing a full course of treatment and having a competent immune system.

Mococutaneous Leishmaniasis, difuse cutaneous Leishmaniasis, post-kala-azar Leishmaniasis

C0136 DISSEMINATED CUTANEOUS LEISHMANIASIS AFTER BEE STINGS: A CASE REPORT

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1 Background

In 2014, according to the Pan American Health Organization (PAHO), Brazil recorded 20,418 cases of Cutaneous / Mucosal Leishmaniasis out of a total of 51,098 cases reported by a group of 16 endemic countries in the Americas. The Disseminated Cutaneous Leishmaniasis (DCL) is nowadays an emerging form of leishmaniasis, although it has recently been considered a rare form of the disease. It has been described especially in the North and Northeast regions of Brazil and the main etiological agent is *L. (V.) braziliensis.* The DCL is a great challenge to be faced due to management difficulties and often unsatisfactory therapeutic responses, resulting in great morbidity.

2 Methods

The case report of a Brazilian patient with DCL who started after bee stings is presented. The diagnostic, clinical and laboratory findings are described.

3 Results

A 37-year-old male gardener living in Brasilia-DF, Brazil, native of the rural area of Pernambuco (northeastern Brazilian region), previously healthy and immunocompetent, started a clinical picture with erythematous papules on the upper back after bee stings. In a few days, it evolved with multiple ulcers on the back, face (including nasal mucosa), thorax and upper limbs. One month later, after trauma in the left leg, he also presented a new ulceration lesion that did not heal, totaling 67 ulcers in the body in a period of six months of evolution. An ulcer biopsy was taken and leishmaniasis was confirmed. Treatment was started with meglumine antimoniate, with progressive improvement of the lesions, but the patient evolved with drug hepatitis and the therapy was replaced with liposomal amphotericin B.

4 Conclusions

The importance of this case report is based on the peculiarity of the onset of lesions after bee stings, as well as a review of the differences between the classic and disseminated cutaneous forms of leishmaniasis. Local trauma has been related to the precipitation of cutaneous lesions in patients infected by some species of Leishmania and the early recognition of DCL is related to the patient's prognosis.

C0251 POST KALA AZAR DERMAL LEISHMANIASIS - BLASTOMYCOSIS CO-INFECTION: A NEW DIAGNOSTIC DILEMMA IN BANGLADESH

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1 Background

Post Kala-azar Dermal Leishmaniasis (PKDL) is a curious phenomenon usually develops after treatment of Kala-azar, and sometimes without any prior history of VL, presenting with wide varieties of skin lesions. The PKDL patients serve as potential reservoir for anthropometric leishmanial transmission. The affected skin lesions contain parasites and are proven to be infectious to sandflies. Blastomycosis is caused by *Blastomyces dermatitidis*, which is predominantly a North American disease, but sporadic human cases have been reported from this region especially from India. It occurs mostly in people involved in outdoor activities and the growth of the organism is favored by most soil with decaying vegetation.

2 Methods

In this case study we showed 4 cases of PDKL-blastomycosis co-infection which were diagnosed incidentally using direct microscopic examination. All 4 patients discussed here, were treated with single or multiple cycles of liposomal amphotericin B. Each cycle consisted of 20 mg/kg-body weight of the drug divided in 4 equal doses, infused on alternative days. Each cycle was given with a gap of 30 days in between. All the patients were followed up each month for any presence of Leishmanial parasite or blastomyces.

3 Results

As liposomal amphotercin B is the drug of choice for treating both PKDL and blastomycosis, hence it was chosen over miltefosine, which is the first line treatment for PKDL patients. Each of the patients was free of blastomycosis in direct microscopic test after the first cycle, but it took single to multiple cycles of treatment before the patients could be declared to have no presence of LD (leishmania donovani) body, using direct microscopy and Real Time- Polymerase Chain Reaction of the skin. Haemopoetic status, Renal and hepatic function tests were normal before and after administration of each cycle of AmBisome. No undue adverse event occurred during the course of the treatment.

4 Conclusions

Where PKDL has already been a diagnostic dilemma, PKDL-blastomycosis co-infection has added further complexity for the physicians in diagnosing such skin lesions in the kala-azar endemic region.

C0357 LEISHMANIAL LOCALIZED LYMPHADENOPATHY (LLL) BY L. INFANTUM. EXPERIENCE OF FUENLABRADA OUTBREAK

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1 Background

Leishmanial localized lymphadenopathy (LLL) is an atypical form of clinical presentation of leishmaniasis. Recently, we published our experience about 17 cases of LLL during leishmaniasis outbreak in our city (*Horrillo L, et al.. Clinical Microbiology and Infection 21 (3), 269-273).* We reported that LLL seems a benign condition than could be treated with lower doses of liposomal B amphotericin than VL. Now we report new cases and longer evolution of our patients to support previous results

2 Methods

We included retrospectively LLL cases in our hospital since 1st January 2004 to 1st June 2009, and prospective LLL cases during the epidemic outbreak (1 June 1 2009 to 1st November 2016). From January 2013 LLL patients were recruited for PCR leishmania, serology (IFAT) and SLA-cell proliferation test before and after treatment.

This work has been supported by "Red de Investigación Cooperativa en Enfermedades Tropicales" (RICET + RD12/0018/0008), VI PN de I + D + I 2008–2011, ISCIII- Subdirección General de Redes y Centros de Investigación Cooperativa; and fondos FEDER

3 Results

29 cases of LLL were collected. 24% had cutaneous lesion associated. 55% were male, mean age 45, 21% not born in Spain. None had systemic symptoms. Only one patient had splenomegaly and altered hematological results. Only 33% patients were rK39 positive. 3 patients were cured without treatment (mean follow up 302 weeks). The rest received liposomal B amphotericin at different doses: 9 were treated with 10mg/kg (mean follow up 181 weeks), 7 with 15 mg/kg (mean follow up 302 weeks), 2 with 18 mg/kg (mean follow up 198 weeks) and 8 with 21 mg/kg (mean follow up 212 weeks). No patient has relapsed nor presented visceral disease during follow up (mean 232 weeks). SLA-cell proliferation test after treatment between these doses groups showed not differences

4 Conclusions

In our experience, LLL by *L. infantum* is a benign disease that could be safely treated with 10mg/kg liposomal B amphotericin, and even in selected cases can resolve spontaneously

C0415 MUCOCUTANEOUS LEISHMANIASIS MIMICKING ACNE: A CASE REPORT

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1 Background

The clinical manifestations of Cutaneous Leishmaniasis (CL) depend on some factors, such as the species of Leishmania involved and the relation of the parasite with its host. A wide spectrum of lesions may occur, which makes clinical diagnosis sometimes complicated. There is a high incidence of cases of CL caused by *L. brasiliensis* in Brazil, that can dertermine destructive, disfiguring and incapacitating lesions with great repercussion in the psychosocial field of the individual.

2 Methods

This is a case report of a patient with acneiform lesions on the face, trunk and, later, of the gingival mucosa. She was treated at a referral center for infectious diseases in Brasília, capital of Brazil. It was performed the skin test, indirect immunofluorescence and lesion biopsy for smear, culture, histopathology and polymerase chain reaction (PCR) for Leishmaniasis. She was treated according to the current guidelines of the Brazilian Ministry of Health.

3 Results

A 39-year-old female patient with acneiform lesions on the face and trunk, some with minor ulcerations during the four-month period, was treated for adult acne with doxycycline. She presented worsening of the lesions and was submitted to cutaneous biopsy, which revealed a pattern of exudative and granulomatous necrotic infiltrate, as well as amastigote forms in a moderate amount. Indirect immunofluorescence was positive for leishmaniasis and the polymerase chain reaction compatible with Leishmania braziliensis. Meglumine antimoniate specific treatment was performed for 20 days, with clinical improvement. After five months, she returned with involvement of the gingival mucosa and new acneiform lesions, confirming the first therapeutic failure. Subsequently, she had to undergo alternative treatments after successive therapeutic failures.

4 Conclusions

In endemic countries, such as Brazil, the therapeutic failure in the treatment of leishmaniasis has a significant social and financial impact due to scars caused by the lesions. Agreeding with the literature, this case report demonstrates the therapeutic difficulty for atypical leishmaniasis lesions, and portrays the need for further studies on this neglected disease.

C0483 IMPACTS IN VOCAL QUALITY FROM AMERICAN TEGUMENTARY LEISHMANIASIS

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1 Background

Background: Cutaneous leishmaniasis is a public health problem in four continents (Americas, Europe, Africa and Asia). It is considered by the World Health Organization (WHO) as one of the six most important infectious diseases due to its high detection coefficient and the capacity to produce deformities. Mucosal leishmaniasis (LM) can occur as a result of cutaneous leishmaniasis. Mucosal lesions, when left untreated, may leave sequelae, interfering in the swallowing process, breathing, voice and speech, requiring rehabilitation. The objective was to describe the anatomical characteristics and the impacts on the vocal quality of patients with LM.

2 Methods

Methods: This is a cross-sectional study in patients with LM, followed at the Leishmaniosis Surveillance Laboratory of the Evandro Chagas National Institute of Infectious Diseases-Fiocruz, from 2010 to 2013. The patients were evaluated by otorhinolaryngology with endoscopic examination of upper airways and digestive tracts and speech therapy through guided anamnesis, auditory perceptual analysis, phonation times and vocal acoustic analysis. The variables of interest were epidemiological (sex and age) and clinical (location of lesions, associated symptoms and vocal quality).

3 Results

Results: Twenty-six patients undergoing treatment for LM were studied, and 21 (81%) males and five (19%) females, aged 15-78 years (54.5 ± 15.0 years). The lesions were distributed in the following structures, 88.5% nasal, 38.5% oral, 34.6% pharyngeal and 19.2% laryngeal, and each patient may present lesion in more than one anatomical site. The main complaint was nasal obstruction (73.1%), followed by dysphonia (38.5%), odynophagia (30.8%) and dysphagia (26.9%). It was verified that 23 patients (84.6%) had altered vocal quality. Dysphonia was significantly associated with lesions in the larynx, pharynx and oral cavity.

4 Conclusions

Conclusion: We observed that vocal quality alterations are frequent in patients with mucosal leishmaniasis, even without the presence of laryngeal lesions, and are probably associated with alterations in resonance structures or even compensatory mechanisms.

C0538 TREATMENT OF POST KALA-AZAR DERMAL LEISHMANIASIS IN INDIAN SUBCONTINENT- A UPDATE

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1 Background

Treatment of Post Kala-Azar Dermal Leishmaniasis(PKDL) is an important and unresolved issue which need to be addressed for achievement of Visceral Leishmaniasis Elimination programme which aims to eliminate Visceral Leishmaniasis from the Indian Subcontinent . All PKDL cases serve as a reservoir of *leishmania* infection in the population and should be diagnosed and treated effectively to achieve the above goal. Treatment options are meagre for PKDL and that to associated with long regimens and toxic. Present review explores the treatment options for PKDL.

2 Methods

A literature search was done in Pubmed. Studies done on PKDL symptomalogy and treatment in Indian subcontinent with drugs used for visceral leishmaniasis like sodium sbibogluconate(SSG), Miltefosine, Amphotericin B(AmB), Liposomal AmB and Paramomycin were reviewed and analysed.

3 Results

In the Indian subcontinent, up to 20% patients of VL develop PKDL whereas the incidence is much less in India.Several treatment regimen have been used for the treatment of PKDL in India.. In India cure rates are 64–92% with Sodium stibogluconate(SSG) in doses of 20 mg/kg per day for 120 days. Similarly three 20 day infusions of amphotericin B for an interval of 20 days poses a real threat of nephrotoxicity. These long parenteral regimens invariably lead to either non-acceptance or poor compliance. Ambisome (liposomal Amphotericin B) in the dose of 2.5 mg/kg for 20 injections is another alternative with minimal side effects but considering its high cost makes it not a feasible option. Milefosine and paromomycin, have been approved for the treatment of VL in India has also been tried for PKDL treatment. Oral miltefosine has been successfully used in doses of 100mg/day for 12 weeks and 8weeks regimen and found to be an effective alternative for treatment of PKDL with cure rate of 93 and 81% respectively. However, Paramomycin has failed to show its efficacy when used in a doses of 11mg/kg for 45 days in Bihar, India.

4 Conclusions

As few cases of PKDL are enough to maintain the reservoir of infection its early diagnosis and proper treatment is necessary for achieving targets of its elimination programme. With ineffective and limited treatment option, the efficacy of combination therapy should be explored to decrease the treatment duration ,increase compliance and to curtail the infection in the population.

C0589 INCREASED MILTEFOSINE EXPOSURE IN PEDIATRIC POST-KALA-AZAR DERMAL LEISHMANIASIS PATIENTS IN BANGLADESH TREATED WITH AN ALLOMETRIC DOSING REGIMEN

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1 Background

A lower miltefosine exposure in pediatric compared to adult visceral leishmaniasis (VL) patients has previously been established after conventional 2.5 mg/kg dosing. A three month allometric miltefosine dosing regimen has been evaluated in pediatric post-kala-azar dermal leishmaniasis (PKDL) patients in Bangladesh, in which children with a lower fat-free mass (FFM) received a relatively higher mg/kg dose. This initial pharmacokinetic report describes the miltefosine exposure following this dosing regimen.

2 Methods

Eighty-four pediatric PKDL patients aged 4 to 17 yrs were treated 3 months with a median daily miltefosine dose of 2.8 mg/kg (range 1.8-3.9 mg/kg). Dried blood spot samples were collected at 0.5, 1 and 3 months (EOT, end of treatment) after first dose, and 1, 3, 6 and 9 months after EOT. Miltefosine concentrations were determined using a validated liquid-chromatography tandem-mass spectrometry method. A non-compartmental pharmacokinetic analysis was performed in R (version 3.3.1) with the package "ncappc". The area under the concentration-time curve was calculated up to day 28 (AUC_{0-28}) to allow for comparison with previous VL studies. All results are reported as mean±SD.

3 Results

Miltefosine steady state concentrations after 1 month of treatment were 29.5±6.6 µg/mL. Concentrations remained steady until EOT (28.2±8.4 µg/mL), though a concentration decrease was observed in patients aged 13 to 17 yrs (31.1 to 26.9 µg/mL, p=0.060, paired t-test). There were no significant differences in EOT concentration between age categories 4-9 yrs (29.3±8.6 µg/mL), 10-12 yrs (28.2±7.8 µg/mL) and 13-17 yrs old (26.9±9.3 µg/mL). Male patients, however, reached significantly higher EOT concentrations than female patients (30.9±8.6 µg/mL vs 26.0±7.7, p=0.0081, Welch two sample t-test). The AUC_{0-28} was 573±115 µg·day/mL, and higher than previously found after conventional dosing in pediatric VL patients (~500 µg·day/mL).

4 Conclusions

Compared to conventional 2.5 mg/kg miltefosine dosing, allometric dosing in this Bangladeshi pediatric PKDL population resulted in an AUC_{0-28} increase of approximately 15%, raising miltefosine exposure towards adult levels, as predicted. However, exposure was significantly higher in male compared to female pediatric patients, possibly warranting an adjustment of the allometric miltefosine dose in female patients.

C0663 TREATMENT OF WIDESPREAD NODULAR POST KALA-AZAR DERMAL LEISHMANIASIS WITH EXTENDED-DOSE LIPOSOMAL AMPHOTERICIN B: A SERIES OF FOUR CASES

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1 Background

Post Kala-azar Dermal Leishmaniasis (PKDL) is a sequela of Visceral Leishmaniasis (VL) and suggested as reservoirs of the parasite. Currently, Miltefosine (MF), Amphotericin B Deoxycholate (ABD) and Liposomal Amphotericin B, AmBisome® (LAmB) are recommended by World health Organization (WHO) as treatment options for PKDL. But single MF or LAmB often causes incomplete cure specially cases with an extensive lesion. To overcome this problem here, we describe four cases of PKDL who had widespread nodular and macular lesions and were treated with two cycles of LAmB doses with 20mg /kg body weight divided into four equal doses and administered every alternate day.

2 Methods

Patients admitted to the Surya kanta kala-azar research center (SKKRC) with extensive nodular, or mixed lesion was considered to treat with LAmB. Hospital physician collected patients' history and performed the physical examination. Suspected cases were confirmed by microscopic examination of slit skin smear. At first, LAmB treatment was initiated at the dose of 20mg/kg into four divided doses every alternate day. Before starting treatment, all hematological and biochemical markers were checked. The second cycle was initiated for those who displayed marked improvement but not complete recovery. Finally, a follow-up physical and microscopic examination were also done to evaluate the treatment outcome.

3 Results

Of them, two had previous VL history, and two had no previous VL history at all. The lesions started from chin with macular type, later distributed to other parts of the body with both macular and nodular type. The lesions were non-itching, touch sensitive and rk 39 strip test was positive. All of them were confirmed through microscopic examination of slit skin smear. Their hematological and biochemical markers were within normal limit. After the first cycle, all of them showed initial progress, and after second cycle all of them attained the complete cure. Fever with shivering was the only documented adverse event during treatment and was managed by paracetamol.

4 Conclusions

The cases highlighted in this report explore the success of utilizing extended LAmB dose schedule for treating a patient with widespread lesions. Moreover, all the cases had minimal or no side effect which should be considered as a unique achievement of this treatment schedule. The triumph of this alternate treatment schedule for PKDL needs to be supported with clinical trials that evaluate its efficacy and safety.

C0793 VIRAL COINFECTION-INDUCED TYPE-1 IFNS PROMOTES METASTASIS AND RELAPSE OF LEISHMANIASIS

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1 Background

The presence of *Leishmania* RNA virus (LRV) within *L. guyanensis* has been correlated with the development of disseminated or mucocutaneous leishmaniasis. Moreover, LRV has been associated with increased resistance to treatment and with disease relapses. The recognition of viral dsRNA by Toll-like receptor 3 promoted the production of high level of pro-inflammatory cytokines, including type-1 interferons (IFNs), which increased the pathology. The involvement of type-1 IFNs in *L. guyanensis* infection suggests that viral co-infection could play a role in the determination of the outcome of the disease. In this study, we investigated the disease exacerbatory role of type-1 IFNs not only in LRV-bearing *L. guyanensis* (*LgyLRV1+*) infection, but also in mice co-infected with LRV-cured *L. guyanensis* (*LgyLRV1-*) and *Lymphocytic choriomeningitis* virus (LCMV) or the sand fly transmitted *Toscana* virus (TOSV).

2 Methods

We investigated the role of type-1 IFNs by infecting wild type (WT) or type-1 IFN receptor deficient (IFNAR-/-) mice or bone marrow derived macrophages with *L. guyanensis* parasites or by injecting recombinant IFN-β. Further, we investigated the role of viral infection by co-infecting mice with *LgyLRV1-* and LCMV or TOSV. As readout we monitored lesion development and parasite burden *in vivo* and macrophages activation *in vitro*.

3 Results

Infection of IFNAR-/- mice showed that the disease exacerbatory role of LRV completely rely on type-1 IFNs signaling, as they developed significantly smaller lesion and lower parasite load compared to WT mice. The detrimental role of type-1 IFNs was confirmed with injection of recombinant IFN-β at early time post *LgyLRV1-* infection. To determine the impact of other viruses on leishmanial virulence, we co-infected mice with LCMV or TOSV. The type-1 IFN anti-viral response significantly increased the pathology of *LgyLRV1-* infection by diminishing macrophage's sensitivity to IFN-γ. Remarkably, LCMV promoted parasite metastasis and infectious relapse in mice normally resistant to the infection, overriding the protective adaptive immune response.

4 Conclusions

Our findings highlight a major role for type-1 IFN-inducing viral infection in determining the outcome of *Leishmania* infection and propose that viral co-infections could be responsible for metastasis and relapsing cases in human patients.

C0811 SEROPREVALENCE OF ANTI BETA- GAL ANTIBODIES ACCOMPANY ENHANCED EXPRESSION OF GAL-BETA-1,4-GLCNAC ON BLOOD CELLS OF PATIENTS WITH POST KALA AZAR DERMAL LEISHMANIASIS FROM ENDEMIC DISTRICTS OF WEST BENGAL, INDIA

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1 Background

Indian Post Kala azar Dermal Leishmaniasis (PKDL) is a dermal sequel of VL, where the duration of lesional healing is extended to even years. PKDL patients harbour *Leishmania donovani* as a silent and insidious pool in endemic districts of West Bengal. There is a gross increase in incidence rates from 4.3% to 33.5% during 2010-2014 across nine endemic districts, out of which South Dinajpur, Malda and Birbhum are the significantly affected ones. Current diagnosis of PKDL has several limitations. Previously anti-gal antibodies have been exploited as important tools for diagnosis of trypanosomiasis. However, their status in Indian PKDL remains unaddressed.This study for the first time investigates the seroprevalence of anti-gal antibodies in Indian PKDL patients.

2 Methods

Among 1259 suspected cases,71 confirmed PKDL patients were enrolled from field surveys under kala azar elimination programme of GOI during February to December, 2015.Flow cytometry and SDS PAGE was employed to determine the expressions of gal-beta-1,4-GlcNAc antigen on peripheral blood cells. Subsequently, antibody responses were determined by performing gal-beta-1,4-GlcNAc specific ELISA(beta-gal ELISA)from all consented patients along with endemic (n=27) and disease controls(n=28).

3 Results

Multicolor flow cytometry revealed enhanced expression of beta-gal(1-4)glcNAc bearing glycoconjugates on CD4 and CD8 T cells of PKDL patients as compared to controls. The results were further corroborated with beta-gal ELISA. SDS PAGE analysis of PBMC membrane revealed differential banding patterns of carbohydrate rich polypeptides in PKDL patients. Subsequently, two to three fold high anti beta-gal antibody titers were obtained in PKDL patients ($p<0.0001$) as compared to control groups. 59.12% enrolled PKDL patients were positive in beta-gal ELISA .Seroprevalence in age group 5-15 is 25.00% (15.54% to 36.60%:CI 95%), in age group 15-35 is 44.33%(34.24% to 54.77%:95% CI) whereas in age group 35-60 is 15.62%(7.76% to 26.86%:CI 95%).Around 90% is having VL history in the past 6-8 years and having treatment with SSG or miltefosine.

4 Conclusions

Taken together, it is demonstrated for the first time that beta-gal(1-4)glcNAc are overexpressed on blood cells of PKDL patients which could trigger anti beta-gal antibodies having immense diagnostic application. Malda and Dinajpur districts showed huge seroprevalence of anti beta-gal antibodies in human host as reservoirs.

C0869 IN-VITRO AND EX-VIVO ANALYSIS OF PERIPHERAL BLOOD MONONUCLEAR CELLS IN DIFFUSE CUTANEOUS LEISHMANIASIS: A CASE STUDY

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1 Background

Diffuse Cutaneous Leishmaniasis (DCL) is a rare disease presented by plaques and nodules. It is caused by *L. (L.) amazonensis*. It represents the anergic pole of American Tegumentary Leishmaniasis with a predominance of the humoral immune response.

2 Methods

IFS, M, 65 years resident of Brasilia-DF. He reports a patch in the nose 1 month after fishing in Amazonas-Brazil. Dermatological exam: erythematous plaque on the nose. Laboratorial exams: culture and smear positive. Leishmanin Skin Test (LST) and indirect immunofluorescence were negative. PCR identification: *L. (L.) amazonensis.* Biopsy showed vacuolated macrophages filled with amastigotes. HIV serology was negative. Treatment: N-methyl glucamine (NMG) 20mgsbV/kg/day. After 26.775g, improvements were not seen. After a 1-month break he returned the treatment with NMG combined to allopurinol 1200mg/day, interrupting the NMG after 44.625g because of intense myalgia, and returning 1 week later, until 53.55g. Then, NMG was suspended and allopurinol sustained until 11 months, when there has been total remission of injury. **Methods:** Peripheral blood was collected after a few months of treatment and peripheral blood mononuclear cells were analyzed ex-vivo and in vitro after a 48 hours stimulation with soluble *L. amazonensis* antigen (SLA). Cell death, surface markers, IFN-gama and granzyme B stained with fluorochromes conjugated antibodies were used to analyze cells by flow cytometry.

3 Results

Among all surface markers analyzed, we observed a higher expression of inhibitory molecule PDL-1 in the monocytes restimulated with SLA. Around 20% of CD4+ T cells did not show IFN-γ production upon stimulation with SLA. On the other hand, around 15% of CD8+ showed a decrease in both IFN-γ and granzyme B detection. No differences in the cell death markers were observed.

4 Conclusions

DCL has a difficult therapeutic management. The patient had good results with NMG and after its combination with allopurinol. The negativity of LST and the high parasite load reinforces the classification of DCL. The early diagnosis and treatment seem crucial to achieve a satisfactory outcome. In this way, comparing to concanavalin A (con-A) positive controls, we can conclude that, even in treatment and with no lesions in the time point of immunologic evaluation, the patient still showed a selective anergic state to Leishmania antigen, apparently through the induction of inhibitory co-stimulatory pathway PD-L1.

C0904 A CASE OF CUTANEOUS LEISHMANIASIS WITH MULTIPLE SKIN LESIONS IN A PATIENT WITH PSORIASIS AND ARTHRITIS RHEUMATOID TREATED WITH ANTI-TNF-ALPHA

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1 Background

BACKGROUND

Immunosuppressive therapy can be a very effective treatment for several immune-mediated diseases. Nevertheless, this therapy has been reported to increase the risk of opportunistic infections, such as *Leishmania*. Herein we report a case of multifocal cutaneous leishmaniasis in a patient with rheumatoid arthritis and psoriasis treated with liposomal amphotericin B from an endemic area (Majorca Island).

2 Methods

PATIENTS AND METHODS

A 55-year-old man affected of psoriasis and rheumatoid arthritis and treated with Prednisone and a TNF-antagonist Adalimumab. Skin examination revealed the presence of multiple ulcerated nodules lesions (n=12) distributed on face and legs. The samples obtained were serum and biopsy of lesions and skin scraping collected using filter paper. Diagnosis wasperformed by direct examination, culture and a quantitative real time *Leishmania* PCR (qPCR). *Leishmania* identification was achieved by a specific *L. infantum* qPCR. The presence of specific antibodies were determined by Western blot.

3 Results

RESULTS

Direct examination, culture and qPCR of skin biopsy and filter paper indicated the presence of *Leishmania* that was identified as *L. infantum*. Antibodies against 14 and 16 kDa antigen fractions and against medium and high molecular weights fractions were observed. After diagnosis of leishmaniasis, anti-TNF-α treatment was interrupted and treatment combining intralesional meglumine antimoniate and liposomal amphotericin B was administered. Ulcers were completely resolved after six doses of 400 mg/day of liposomal amphotericin B administered each of them with a time interval of 7 days.

4 Conclusions

CONCLUSIONS

The immunosuppressive treatment modulate the classical clinical expression of leishmaniasis. In this case, the presence of multiple lesions due to *L. infantum*, as well as the full-band pattern observed by Western blot indicated that is necessary to monitor this type of patient to rule out a possible relapse or to develop a visceral infection.

C1145 ETIOLOGY OF MUCOCUTANEOUS LEISHMANIASIS, MATO GROSSO, BRAZIL - VISCEROTROPIC SPECIES MAY ALSO BE RESPONSIBLE?

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1 Background

In Brazil, leishmaniasis is caused by 7 species: *L. (V.) braziliensis, L. (V.) guyanensis, L. (V.) naiffi, L. (V.) Shawi, L. (V.) lainsoni and L. (L.) amazonensis for cutaneous leishmaniasis* and *L. (L.) infantum* for visceral. In recent study to identify species by molecular methods in Mato Grosso, a LC case presented mixed infection by *L. (V.) braziliensis / L. (L.) infantum*. In addition to, we attended patients who presented both clinical signs, for visceral and tegumentary leishmaniasis in suspicion of strains with no defined tropism. Here is presented these cases

2 Methods

Report of 4 cases with clinical signs of both: (1) visceral disease and cutaneous/mucosal lesion or (2) cutaneous leishmaniasis by viscerotropic species. Species were identified by extracted DNA and sequenced the ITS1 and Hsp70-234 PCR products

3 Results

1. Nine-month-old child, fever 60days, hepatosplenomegaly, pancytopenia, *Leishmania* sp in bone marrow; treated AmBisome®, discharged in good conditions. But 10 days after presented a skin ulcer, positive for *Leishmania* (species on investigation). **2**. A 56 yo male driver, fever 90 days, hepatosplenomegaly, pancytopenia; skin lesion began as papule, then ulcer, along with nasal septum injury. *Leishmania* absent in bone marrow, but present in skin, mucosal and lymph node (species on investigation). Used AmBisome®, 2 cycles; improvement with regression of hepatosplenomegaly, skin and nasal reepithelization, plus good laboratory tests. **3**. Male seller, 36 yo, skin ulcer for 3 months, after fishing and camping in endemic area for LV and LC. Negative for *Leishmania* twice, direct examination and culture. Performed PCR, mixed infection by *L. (V.) braziliensis/ L. (L.) infantum*. Ulcer in slow regression, patient no longer attended, nor answered the calls. **4**. Male tinker, 30 yo, cutaneous lesion on face (nose tip), as a plaque, infiltrated and erythematous, and malar nodule, both 2 years of evolution; negative direct tests for *Leishmania*, histopathological with lymphoplasmacytic infiltrate, without etiologic agent. PCR was positive for *Leishmania* with high similarity to *L. (L) infantum*. Prescribed Glucantime® 20 days, good results.

4 Conclusions

Studies are necessary to clear the complexity relation of clinical manifestations and *Leishmania* species, even more in tropical climates, gorgeous contrasts, as in Mato Grosso that is embraced by Amazon forest, Brazilian savannah (cerrado) and marshlands (Pantanal).

C1150 DIFFERENTIAL EXPRESSION OF THE EICOSANOID PATHWAY IN PATIENTS WITH LOCALIZED OR MUCOSAL CUTANEOUS LEISHMANIASIS

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1 Background

Unfettered inflammation is thought to play critical role in the development of different clinical forms of tegumentary leishmaniasis. Eicosanoids are potent mediators of inflammation and tightly associated with modulation of immune responses. In this cross-sectional exploratory study, we addressed whether the eicosanoid biosynthetic pathway could highlight a distinct biosignature in patients with mucocutaneous leishmaniasis (MCL) or localized cutaneous leishmaniasis (LCL).

2 Methods

To characterize the eicosanoid signaling pathways, we evaluated systemic levels in plasma and performed a comprehensive analysis of targeted RNA transcripts expressed in situ isolated from skin biopsy specimens and from MCL and LCL patients. The chosen targets were as follows: PGES (Prostaglandin E synthase), PGDS (Prostaglandin D synthase), PGD 2R (Prostaglandin D 2 receptor), PTGS1 (Cicloxygenase -1), PTGS2 (Cicloxygenase -2), LXA4R (lipoxin A4 receptor),PTGER1 (E-prostanoid receptor 1 [EP1]), PTGR2 (EP2), PTGER3 (EP3), PTGER4 (EP4), and ALOX5 (arachidonate 5-lipoxygenase).

3 Results

Among all the genes examined, we found that PGES, PTGER3, PGDS, PTGS1, and ALOX5 expression were significantly lower, whereas LXA4R expression was higher in MCL cases than in individuals with LCL. We next measured eicosanoids in MCL and LCL patients plasma. We observed that Prostaglandin 2 (PGE2) levels were significantly higher in LCL cases, compared with individuals with MCL. Noteworthy, levels of Leukotriene B4(LTB4) were >1 log higher in patients with MCL than in LCL cases. Athough our data reveal that there were no statistically significant difference for Prostaglandin D (PGD), Prostaglandin F(PGF) and Resolvin D1(RvD1) plasma levels between patients with LCL and those with MCL, we noticed that PGD2, PGF, LTB4, and RvD1 levels were significantly lower in patients with LCL compared with healthy controls. Next we performed an unsupervised hierarchical cluster analysis in which plasma values of all the eicosanoids measured were inputted. Simultaneous assessment of PGE2 and LTB4 could successfully segregate the different clinical groups evaluated.

4 Conclusions

Together, these observations led us to hypothesize that differences in immunopathogenesis between MCL and LCL may result from an imbalance between prostaglandins and leukotrienes, which may serve as targets for future host-directed therapies. Therefore, systemic mediators observed in LCL and MCL may be useful as biomarkers of active disease.

C1194 MUCOSAL LEISHMANIASIS: A NEGLECTED DISEASE. DESCRIPTION AND SPECIES IDENTIFICATION IN 50 CASES

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2 Background

Mucosal Leishmaniasis (ML) affects mainly nasal tissue, runs a progressive course and may cause deformity and even mutilation. It is endemic in the american continent and is caused by *Leishmania* species that belong to the Viannia subgenus, specially *L. (V.) braziliensis*. We describe a case series of Mucosal Leishmaniasis from a reference center in Bogotá, Colombia.

3 Methods

Fifty patients with clinical diagnosis of ML and parasite identification by culture, histopathology or PCR were included. We describe clinical and demographic features and laboratory results obtained. Species tipification was made through PCR-RFLP miniexon and hsp70 and was confirmed by gene sequencing.

4 Results

Seventy six of the patients were men, the age range was 4-84 years (median 45 yrs), disease evolution was from one month to sixteen years (median 2.8 yrs). Relevant clinical findings were: mucosal infiltration 94%, cutaneous leishmaniasis scar 74%, total septum loss 24%, nasal deformity 22%, ulceration 38%. Main symptoms reported by patients were: dysphonia 28%, dysphagia 18%, epistaxis 72%, nasal obstruction 90%, nasal pruritus 34%, rhinorrea 72%. Histopathologic features showed a pattern compatible with leishmaniasis in 88% of biopsies, and amastigotes were identified in 14% of samples. PCR was positive in 100% of cases, Montenegro skin test was positive in 86%, Indirect immunofluorescence was positive in 84%, culture in Senekjie médium was positive in 8%. *L.(V.) braziliensis* was identified in 88% of samples (44/50), *L.(V).panamensis* in 4% (2/50). *L.(V) guyanensis* and *L.(L.) amazonensis* were the causal species in one case each.

5 Conclusions

Scientific literature on ML is scarce. On this series we found severe nasal disease with septum destruction and deformity in near one fourth of the cases, probably related to a late diagnosis. Ten percent of our cases were children in whom this disease is rare. Most of the species were *L.(V). braziliensis*, similar to literature reports. We found that any of the species from the Viannia subgenus can cause mucosal disease. This is the first report in Colombia of ML caused by *L.(l.) amazonensis*. PCR iminiexon and hsp70 gene is a very useful test to confirm diagnosis of Mucosal Leishmaniasis. and it allows species identification.

C1219 CLINICAL FEATURES AND TREATMENT RESPONSE OF CUTANEOUS LEISHMANIASIS IN NORTH-WEST ETHIOPIA

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1 Background

Cutaneous leishmaniasis (CL) affects close to a million people globally and more than 20,000 people in Ethiopia every year. However, it receives far less attention than visceral leishmaniasis. Besides, CL in Ethiopia is caused by a unique species (*L aethiopica*), which is known to cause severe forms such as diffuse (DCL) and mucocutaneous leishmaniasis (MCL). We report on the number and type of CL cases diagnosed, the clinical features, treatment and treatment outcomes in North-West Ethiopia.

2 Methods

This is a retrospective chart record analysis of CL patients treated at the Leishmania Research and Treatment Center of the University of Gondar, Ethiopia.

3 Results

From 178 CL patients seen between Jan 2014 and Dec 2015, a total of 154 chart records were retrieved. These included 80 localized CL (LCL), 67 MCL and 7 DCL. The median age was 23 years; 71.4% were male. Most (n=121, 78.6%) of the lesions were on the face. The median time since onset was 12 months (6-24 months) and 28.6% presented after a trial of traditional medicine. The treatment of all forms of CL mainly consisted of 30 days of intramuscular antimonial injections. Of these, 51/133 (38.3%) required treatment extension or change due to non-response. Three cases were treated with liposomal amphotericin B or miltefosine (two received the combination), of which two responded well.

4 Conclusions

CL was found to be complicated and difficult to treat. MCL was common, and patients presented after long delays. There is an urgent need to look for better treatment options for CL and improve access for care.

C1243 APPARENT CURE OF DIFFUSE CUTANEOUS LEISHMANIASIS WITH INTRANASAL PENTAVALENT ANTIMONIAL AFTER 30 YEARS OF DISEASE: CASE REPORT

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1 Background

Cutaneous leishmaniasis is caused by several species of *Leishmania* and can be self healing or achieve high cure rates with proper therapy. However, diffuse cutaneous leishmaniasis caused by *L. amazonensis* is a rare, and extremely difficult to cure. Here we report a case of cure with intranasal pentavalent antimonial after 30 years of evolution.

2 Methods

Case report.

3 Results

A 35 years old lady started her disease with a small inflatrative papular nodule in her left leg when she was five years old. After the initial treatment lesions expanded and disseminated to the trunk and upper limbs. The skin test was negative. HIV was absent. The species was identified as *L. amazonensis* and the diagnosis of diffuse cutaneous leishmaniasis was established. For years the patient has been treated with several cycles of pentavalent antimonial, pentamidine, paramomycin, gamma-interferon, and amphotericin B. In all cycles, she obtained partial healing followed by relapses but lesions expanded. After repeated and long drug therapies, in 2010, she started a progressive kidney failure. In this year, after her second pregnancy, she had a major worsening with generalized nodules and ulcerations with bacterial infections. After an international pledge via the network Leish-L, she was treated with miltefosine with impressive improvement with regression of lesions. After this period, relapses were milder and locally treated infiltration with pentavalent antimonial. The last manifestation was the reappearance of lesions in her nose mucosa, papules in the face and nodules in the knees. Because she had difficulties in breathing due to the bilateral nose involvement she was treated with pentavalent antimonial sprayed in the nose 2-3 times twice a day. After only one vial that lasted three weeks, she healed not only the nasal legions but also those in the face and knees. New skin biopsy and a PCR revealed no amastigotes. At the moment she free of lesions 18 months after the local treatment with nasal spray.

4 Conclusions

The prognosis of this form of leishmaniasis is considered dismal due to disease chronicity and frequent relapses. The use of intranasal pentavalent antimonial appears as a possible therapy that should be better studied aiming the definite healing of this apparently incurable diseas.

C1353 ONGOING OUTBREAK OF AMERICAN TEGUMENTARY LEISHMANIASIS (ATL) IN CORRIENTES, ARGENTINA

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1 Background

Both urban and rural American Tegumentary Leishmaniasis (ATL) is endemic in different parts of Argentina and has long been recognized in the Corrientes province.
Corrientes is located in the northeast of the Argentina (27° 15 ' N and 58° 50´W)
Outbreaks of ATL have been reported from several localities of the province from 1988 and the situation is complicated by the presence of several different species of *Leishmania* circulating in enzootics.
Despite being considered an endemic disease, in this study, a new outbreak of ATL in the Corrientes province in 2015 is presented.

2 Methods

The outbreak of disease was related in the localities of Corrientes, Empedrado and Bella Vista. Following registration, each patient was questioned regarding the probable locality in which the infection was acquired, the period of incubation, number of lesions, and their morphology and location on the body.
All patients were submitted to the following procedures: a) Intradermal Montenegro skin tests were performed with leishmanin antigen Montenegro (*Leishmania amazonensis* 40 µg/N) *(*40 µg/N Inst. Oswaldo Cruz, Belo Horizonte, Brazil) to evaluate the delayed hypersensitivity cellular response. b) Smears of exudates from the border of lesions, fixed in absolute methyl alcohol, and stained by Giemsa's method to confirm the presence of leishmanial amastigotes.

3 Results

A total of 12 cases were reported from the 29th week to the 44th week of 2015.
Eight of the 12 patients were males (67%).
The intradermal test was positive in all patients and the leishmanial etiology was confirmed in eight patients.
The youngest confirmed case was 3 years and the oldest 75 years
At the time of consultation, the lesions had been present for between 1 and 6 months in the majority of patients. Clinical lesions were ulcers with indurate border. Lesions varied in sizes from 1.5 to 5 cm in diameter.
Eight patients (67%) had only one lesion and the others 4 (33%) had two or three lesions. Legs were the commonness site of lesions (67%) lowest number of lesions was seen on hands and feet, chest and back.
In a man of 38 years the skin and mucus membranes specially the nose were affected.
The agent in the three localities was identified as *Leishmania (Viannia) braziliensis*. The probable vector was *Nyssomyia neivai*.

4 Conclusions

This LTA outbreak confirms the endemic nature and the high prevalence of the disease.
This outbreak was observed in the context of increasing rates of ATL in the Corrientes province.

C1503 PHENOTYPIC CHARACTERIZATION AND INFECTIVITY OF LEISHMANIA VISCEROTROPIC AND DERMOTROPIC STRAINS IN DISSEMINATED CUTANEOUS LEISHMANIASIS

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1 Background

Leishmaniasis is a zoonosis with worldwide distribution. In Brazil, the cutaneous form is represented mainly by *Leishmania braziliensis* while visceral by *Leishmania infantum*, which can lead to death if untreated. We therefore undertook a phenotypic and genotypic analysis of a dermotropic and viscerotropic *Leishmania* clinical isolates derived from the skin and bone marrow from the same patient to compare genomes of closely related *Leishmania* isolates that cause different human pathologies.

2 Methods

The viscerotropic isolate were obtained from the bone marrow and skin of the same patient diagnosed with disseminated leishmaniasis. We used genomic tools and also pulse field gel and RFLP to try to characterize the isolates. Lastly, BALB/c mice were infected both in the ear dermis and intravenously with stationary promastigotes from skin isolate (SKI) and bone marrow isolate (BMI). Amastigotes were isolated from the liver and spleen, and the parasite burden was determined by limiting dilution. Ear swelling was monitored by caliper measurement.

3 Results

The genomic analyzes and also the karyotyping and RFLP tecniques could not identify these isolates acoording to the Leishmanias already described, though, the karyotyping assay showed that they are identical. BALB/c mice infected with BMI showed high levels of parasite load in the spleen, while SKI was not able to survive in this organ in the 4th (weeks post infection) WPI and it was slightly detectable in the 6th WPI, although, both isolates exhibited comparable levels in the liver. Hepatic histological analyses revealed that both BMI and SKI induced inflammatory response in BALB/c mice, with cellular infiltrate and compartmentalized granulomas around infected cells in the 6th WPI. On the contrary, SI was able to induce ear swelling, with increased number of parasites in the ear and lymph nodes, while BMI did not.

4 Conclusions

Characterization of these *Leishmania* clinical isolates provides invaluable insight into the etiology of visceral and cutaneous leishmaniasis in Brazil and provides unique insight into the genetic basis of visceral leishmaniasis.

C1537 AN IMPORTED CASE OF CUTANEOUS LEISHMANIASIS FROM EGYPT TO XINJIANG, CHINA

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1 Background

Little attention has been paid to leishmaniasis imported to Xinjiang endemic to both visceral (VL) and cutaneous (CL) leishmaniasis. Anthroponotic and zoonotic VL are endemic in Kashi and oases surrounding the Taklamakan desert, respectively. CL occurs sporadically in the desert of Karamay city. We report here a case of CL, which differs from indigenous CL apparently imported from Egypt.

2 Methods

The patient is a 26 years old male resident of Yi-Ning city. He went to Cairo, Egypt to study for a year from 2011 to 2012. In 2013, a nodular lesion appeared in his upper left arm and increased in size during the next 10 months. In the fall of 2014, the lesion enlarged to 3-5 cm in diameter with raised erythematic margin and necrotic center. Rash and necrotic skin noted in both arms. Medical exams showed no other clinical signs and symptoms; HIV tested negative. CL was suspected and the case was referred to the Division of Parasitic Diseases, Xinjiang CDC.

3 Results

CL was diagnosed after re-examination of the biopsied lesion samples by microscopy and cultivation in 3N medium. Exuberant growth of promastigotes was noted with rosette formation. The subject was admitted as an in-patient for daily treatment with 2-courses of intramuscular injections of sodium stibogluconate, each for 6 days at 1.6 g and 1.9 g, respectively. Supportive therapies were given for the duration of the treatment for 14 days. Blood smear revealed no *Leishmania* when examined 6 days after the last treatment. The patient was declared cured and released from the hospital in January, 2015. No recurrence of CL was noted when checked during the subsequent 3 months.

4 Conclusions

This is considered as an imported case based on the clinical symptoms of the lesion, travel history and the ease of cultivation as promastigotes, all being very different from the indigenous CL in Karamay – a place the patient has never visited. Acquisition of the infection in Egypt is suggested by his visit to Cairo where *L tropica* or *L major* is known to cause CL. The successful treatment indicates that it is not antimony resistance. Surveillance of imported cases is recommended with the impending increase of population traffic with the expected economic development of Xinjiang.

C1671 VISCERAL LEISHMANIASIS (VL) BASELINE CLINICAL PRESENTATIONS AND ASSOCIATION WITH INITIAL TREATMENT OUTCOME AMONG EASTERN AFRICAN PATIENTS

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1 Background

Visceral leishmaniasis (VL) is among the most neglected of the tropical diseases, afflicting the poorest of the poor. It is characterized by irregular bouts of fever, weight loss, enlargement of the spleen and liver, and anaemia. It is highly endemic in the Indian subcontinent and in Eastern Africa. An estimated 200,000 to 400,000 new cases of VL occur worldwide each year. Follow up of baseline clinical symptoms is important in monitoring treatment response, and in identifying and reporting of adverse events in trials.

2 Methods

We reviewed data from five open-label randomized clinical trials conducted in the region by Drugs for Neglected Diseases *initiative* (DNDi) between 2004 and 2015. The trials included non-HIV primary VL patients who presented for treatment. The studies were conducted in Ethiopia, Kenya, Sudan and Uganda. Data was analyzed to estimate the proportion of different clinical symptoms at baseline and the association with initial treatment outcome. The analysis also included the distribution of multiple symptoms.

3 Results

A total of 1,426 patients were enrolled in the trials, 1,017 (72%) were male. The median age was 13 years with a range of 4-60 years. Overall, the most common symptoms in addition to fever (100%) were weight loss (62%), inguinal lymphadenopathy (62%) and mucosal pallor (61%); less common symptoms were cervical lymphadenopathy (31%), diarrhea (30%), jaundice (30%), petechial haemorrhages (30%).The symptoms differed by the age, sex and country of residence. The most common symptoms in Kenya were mucosal pallor (75%) and abdominal swelling (79%) while in Sudan common symptoms were weight loss (80%) and inguinal lymphadenopathy (80%). Muscle wasting (63%) was most common symptom in Ethiopia. Multiple symptoms varied by country with high occurrence in Ethiopia (median=7, iqr=4), followed by Sudan (median=5, iqr=2) then Kenya (median=3, iqr=2). Abdominal swelling, epistaxis, jaundice, cervical lymphadenopathy and petechial haemorrhages were among the common VL symptoms that were significantly associated with initial treatment failure (p-value <0.05). Fever was the only symptom that had disappeared at the end of treatment (Day 28-30) among those with treatment success outcome.

4 Conclusions

Fever, abdominal swelling, weight loss, inguinal lymphadenopathy and mucosal pallor were the most common symptoms. Symptoms varied among the countries and fever clearance remains a better indicator of treatment success.

C1681 THE DISTRIBUTION OF HUMAN CASES OF LEISHMANIASIS IN AUSTRALIA

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1 Background

Leishmaniasis, a vector-borne parasitic disease caused by a protozoa of the genus *Leishmania* usually transmitted by sandflies. Leishmaniasis have human and vertebrates as reservoirs of about 17 species. This review aimed to understand the distribution of human cases of Leishmaniasis in Australia.

2 Methods

This is a descriptive study about the distribution of human cases of Leishmaniasis in Australia. Peer-reviewed articles and grey literature were searched and reviewed from multidisciplinary databases such as Academic Search Complete, Expanded Academic ASAP, ProQuest Central Science Direct; Health Sciences Databases such as MEDLINE with full text, CINAHL Plus with full text, ProQuest Health & Medical Collection, PubMed, and Google Scholar. The search terms used are visceral leishmaniasis, cutaneous leishmaniasis, post kala azar leishmaniasis, all ages, all sex, Australia, any time, then till 2016.

3 Results

About 20 published articles and reports in relation to cases of Human Leishmaniasis in Australia were found. The articles were from year 1998 - 2016. Ten articles on cases of Leishmaniasis were finally kept for this review. No locally transmitted human cases of Leishmaniasis in Australia was found in literature search. There were 25 cases in total. The kind of human leishmaniasis imported to Australia are cutaneous, visceral and post azar dermal Leishmaniasis. The countries from which the Leishmaniasis was imported are South America, Greece, Afghanistan, Pakistan, East and Sub-Saharan Africa. The cases of Leishmaniasis imported into Australia were based in Adelaide and Western Australia. There was no documentation of importation into other states including Queensland.

4 Conclusions

Australia regularly encounters imported cases of human leishmaniasis. The diseases are not notifiable and known to be transmitted locally. Therefore, clinicians and public health professionals should have a high index of suspicion for leishmaniasis in regions densely populated by migrants thus leading to early detection and treatment of leishmaniasis.

C1726 CL MAPPER: AN APP-BASED TOOL FOR MONITORING CUTANEOUS LEISHMANIASIS LESIONS

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1 Background

We want to improve the management and knowledge of cutaneous leishmaniasis (CL) by gathering images of lesions taken with smartphones by patients or primary health care workers worldwide. In many endemic areas, CL victims often delay seeking professional medical advice. Also, treating physicians may not see the patients routinely, relying instead on their memory for assessing the history and timing of disease progression. Lastly, physicians typically have no established means with which to track lesion evolution after treatment is administered. We plan to provide patients or primary health care workers with the tools to document the progression of their own lesions or those under their care, using a simple iPhone/smartphone app.

2 Methods

The suggested CL Mapper app will be comparable to the existing MoleMapper, which was designed for documenting changes in mole shape or size to hint at development of a melanoma. Additional information on parasite genetics and patient immunology will be added by clinical labs for a subset of lesions. The app will be tested in low and limited resource endemic settings, and also in travel clinics in non-endemic countries.

3 Results

We expect that massive data gathering can vastly improve our knowledge on disease progression and epidemiology of CL. At the individual patient level, the treating physician will have a better understanding of disease history, and will be able to follow lesion cure in real-time without having to see the patient. The huge amount of data will also allow correlating lesion morphology with the type of parasite and lesion evolution, with risk of developing more severe disease, and with optimal treatment. In addition, disease endemicity can be monitored in real-time.

4 Conclusions

We anticipate that CL Mapper will be a tremendous benefit for our understanding of disease progression and epidemiology of CL on a global scale. In time, CL Mapper could be extended to other skin conditions and will aid in tele-diagnosis. This would be extremely helpful in low-resource setting where patients can be far away from health centers.

C1747 MILTEFOSINE FOR TREATMENT OF AN INTRACTABLE CASE OF DIFFUSE CUTANEOUS LEISHMANIASIS (DCL) IN BRAZIL. A HOPE.

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1 Background

Diffuse cutaneous leishmaniasis (DCL) is a rare chronic disease, resistant to treatments and with frequent reactivations. Miltefosine, not registered in Brazil, has been shown to be an option for visceral and cutaneous leishmaniasis (VL and CL), but not evaluated for DCL.

2 Methods

Report of a DCL case, resistant to treatments for more than 20 years, treated now with miltefosine.

3 Results

MAZ, 42 years old. In 1997 he presented to the current service, reporting to be illness for 6 years and having been treated with Glucantime®, pentamidine, amphotericin B isolated and with α-interferon, and serial use of the Montenegro antigen. Initial lesion was an ulcer in the ear, no response to Glucantime®, and dissemination with clinical features other than ulcer. He had multiple disseminated lesions on plaques and nodules, ulcer-vegetating. The diagnosis was of DCL, by the clinic, massive presence of parasites and absence of response in the intradermal test of Montenegro. Today, we have identified *Leishmania (L) amazonensis*. Over time, he was treated with antimonial, with little improvement and reactivation; pentamidine series, drug with the best response but frequent reactivations; amphotericin B deoxycholate and liposomal, with no response to both and worsening renal function. He remained poorly controlled with pentamidine, as a "secondary prophylaxis". In mid-2015, the disease was uncontrolled. Treatment was instituted with miltefosine* 150 mg/day or 2.03 mg/kg (74 kg weight), in 3 doses/day, for 28 days. On the 7th day, improvement of the lesions; adverse events, thoracic and epigastric pain, controlled with symptomatic. On the 21st day progressive improvement of the lesions, adverse events controlled. At the end, lesions improved by 80% and negative parasitological examination of the lesions, condition never seen before. No worsening of renal function, no vomiting, no diarrhea. Lesions in progressive improvement until 6 months after, but 2 more months, 2 lesions relapsed and we prescribed miltefosine, with similar posology and satisfactory response. Improvement was slower, the adverse events difficult to control, but having completed the scheme.

4 Conclusions

There is no effective treatment for DCL in our reality due no therapeutic response or insufficient response with relapses. Miltefosine has been shown to be a viable alternative for treatment of leishmaniasis in general and may be the drug of choice for DCL, depending on other outcomes and follow-up of this and other patients.

C1754 MILTEFOSINE AS AN ALTERNATIVE TREATMENT FOR TEGUMENTARY LEISHMANIASIS IN MATO GROSSO, BRAZIL

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1 Background

Miltefosine is the only oral drug proven to be effective for treating leishmaniasis. Initially it was used as an alternative for visceral leishmaniasis (VL) not responsive to conventional treatments and more recently some studies have shown its efficacy for cutaneous leishmaniasis (CL). Considering in Brazil the expressive number of cases of both, CL and mucosal leishmaniasis (ML), different therapeutic regimens are needed, sometimes to use in the same patient. Then, valid alternatives are always expected. Despite the miltefosine still in the registration process, we received a donation of medicine and we had the opportunity to use miltefosine in 8 patients, with different indications.

2 Methods

Report of the experience with miltefosine (Impavido™) to treat 8 patients with a range clinical forms of tegumentary leishmaniasis, and diverse indications, period of Sept-Dec, 2016, except one treated in 2015, in the reference service to leishmaniasis at the University Hospital Júlio Müller.

3 Results

All patients were male, aged 35-79 yo. CL was present in 5 patients, 3 localized forms, 1 cutaneomucous form and 1 diffuse anergic form. Four had mucosal lesions, 2 nasal and oral lesions, 1 nasal and cutaneous lesions, and 1 nasal lesion. The lowest evolution time was 12 months and the highest was 16 years. Everyone had previous treatment with other drugs. The dose used was 1.72 to 3.31 mg/kg/day, always for 28 days in total. The initial response was satisfactory in all, with complete or partial regression of the lesions, except for one, the oldest and with the highest evolution time. Side effects were present in 6 out 8 patients (75%), always digestive symptoms; one had to discontinue a few days due to abdominal pain. Some data are still being analyzed.

4 Conclusions

Miltefosine is a promising drug for the treatment of leishmaniasis, but it is unlikely to replace the other therapeutic options. Frequent relapses reported in the literature and high prevalence of digestive symptoms following treatment are limiting factors for the role of standard gold treatment. But in a poor universe of options, this oral drug is a valid alternative.

C1756 MUCOSAL LEISHMANIASIS IN TRAVELERS

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1 Background

Mucosal leishmaniasis (ML) is an important complication of New World cutaneous leishmaniasis (CL) caused mainly by *L. Viannia (V.) braziliensis*. Previous reports on ML among travelers to Latin America is scarce. The study main objective was to evaluate the various clinical presentations, as well as disease progression, risk factors, diagnosis, treatment protocols and the outcome of mucosal involvement in CL caused by *L. (V) braziliensis* in travelers.

2 Methods

A retrospective study was performed on cases of ML due to *L. (V) braziliensis* in a tertiary medical center in Israel. Demographic data was collected.

3 Results

Between 1993 and 2015 a total of 145 CL cases were seen. Among them were 17 patients (16 males and one female) who developed ML symptoms. All the ML cases were acquired in Bolivia and included four patients with concurrent CL at the time of diagnosis and thirteen patients that failed to cure. Of the latter group three patients received intra venous (IV) sodium stibogluconate treatment, three patients IV Liposomal Amphothericin B treatment, and in seven patients failure was due to non-adequate treatment. Nasopharyngeal symptoms developed 0–20 years after returning from Bolivia. Diagnosis of ML was delayed up to 5 years from the onset of symptoms. PCR was positive for *L. V braziliensis* in 14 cases and cultures were positive in two cases. Successful treatment and cure were obtained in using IV Liposomal Amphothericin B and IV Sodium Stibogluconate (SSG) for eight and seven patients, respectively. Two patients were treated with orally Miltefosine. Two out of seventeen patients had irreversible nasal-cartilage damage due to delayed diagnosis. CL skin lesions relapsed in only one patient following treatment to ML. Mean follow-up period was 7.5 years.

4 Conclusions

An increased incidence of ML due to *L (V) braziliensis* infection is seen in travelers, therefore physicians including dermatologists, ENT and ophthalmologists doctors should be acquainted with this disease. High index of suspicion is required for prompt diagnosis and optimal management to prevent irreversible damage.

C1806 IS POLIMARASE CHAIN REACTION BASED ON KDNA AND HSP 70 A GOOD MARKER FOR ASSESSING THERAPEUTIC RESPONSE IN MUCOSAL LEISHMANIASIS?

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1 Background

The recurrence of mucosal leishmaniasis is common and patients are constantly undergoing repetitive treatment. Clinical failure occurs in 42% and relapses have been documented in up to 19% of patients after 3 to 10 years of follow-up. Despite the regression of clinical otorhinolaryngological evidence of tissue lesions after treatment for LM, previous data demonstrate the absence of complete cure of the inflammatory infiltrate. Studies already confirm the possibility of determining the prognosis of the disease by analyzing inflammatory aspects of the lesion site, however, it is still necessary to do more studies about a fast and efficient detection of recurrent leishmaniasis cases. In this work we evaluated the efficacy of PCR in samples collected by noninvasive method (lesion swab) as a tool to evaluate therapeutic response in mucosal leishmaniasis.

2 Methods

Between August 2013 and July 2015, were selected 32 patients who underwent post treatment control for ML from the Leishmaniasis out clinic from the Institute of Infectious Diseases Emilio Ribas, from which samples of nasal mucosa were collected from the site of the previous lesion through swab. Samples were evaluated by polymerase chain reaction targeting the Leishmania kinetoplast (kDNA) DNA mini-circle for conventional PCR and the 70Kda thermal shock protein (Hsp70) gene for conventional PCR. PCR-kDNA amplification products were cleaved with restriction enzyme *HaeIII*, this technique has potential for differentiation of *L. (V.) braziliensis* due to the cleavage pattern in samples infected with this species

3 Results

Leishmania DNA detection in samples from patients already treated for mucosal leishmaniasis collected by swab was 86% for the kDNA target and 22% for Hsp70. Two samples did not show cleavage in DNA fragments when incubated with the enzyme HaeIII, suggesting that it was not L. (V.) braziliensis. When comparing the results of the two targets used in our work, we obtained agreement of the results in 26% of the samples.

4 Conclusions

Our results suggestthat the practical application of PCR from mucosal samples collected through swab for therapeutic monitoring of LM can be used to provide valuable additional information for cases of therapeutic failure and relapse of the disease. In addition, PCR-kDNA has been shown to be a very important tool in the follow-up of disease recurrence. However, PCR-HSP70 requires further studies to compare its use for this purpose.

C1811 MISDIAGNOSIS OF CUTANEOUS LEISHMANIASIS: A REPORT OF TWO CASES

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1 Background

Though Nepal is an endemic focus for visceral leishmaniasis, a minority present with cutaneous form of disease especially those of migrant workers. Nepal is also endemic for leprosy and cutaneous tuberculosis which at times can be confused with Cutaneous leishmaniasis (CL).

2 Methods

Evaluation of patients' detail history, clinical examination, laboratory investigations and treatment was done and recorded.

3 Results

Case 1: A 53 year old male presented with erythematous indurated centrally crusted plaque on mid face since 8 months. His travel history included a 7-months stay in India 5 months prior to illness. He was clinically diagnosed as Borderline Tuberculoid leprosy at other center and treated with 6 months of antileprosy treatment but didn't respond so referred to Anandaban Hospital for further management. Slit skin smear for lepra bacilli was negative. Histopathological examination of skin biopsy from the lesion suggested CL and the patient was treated with Amphotericin B after which he began to demonstrate signs of recovery. After the course of Amphotericin B, weekly intralesional Sodium stibogluconate was given for 4 weeks for the residual lesion and patient got better with few residual scars.
Case 2: A 40 year male presented with erythematous indurated centrally crusted plaque on mid face since 10 months. His travel history included frequent short term visit to India prior to illness. He was diagnosed as lupus vulgaris both clinically as well as histopathologically and got 6 months of antituberculosis medication but didn't improve so referred to our center to rule out leprosy. Slit skin smear for lepra bacilli was negative. Repeat histopathological examination of lesional skin biopsy suggested CL; and the patient was treated with miltefosine 50 mg twice daily plus weekly intralesional Sodium stibogluconate for 8 weeks at the end the skin lesion resolved completely.

4 Conclusions

CL, leprosy and cutaneous tuberculosis are linked to areas with combination of poor socioeconomic conditions. Although CL is less common form of leishmaniasis, it's clinical as well as histopathological findings can be confused with leprosy and lupus vulgaris. So before coming to the diagnosis detail discussion between dermatologist and pathologist should be done. Nowadays people are being more mobile so the history of travel to the endemic area is also important in guiding clinician in right way.

C1878 EPIDEMIOLOGICAL , CLINICAL AND THERAPEUTIC ASPECTS OF CUTANEOUS LEISHMANIASIS IN ALBANIA ADULTS

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1 Background

Cutaneous Leishmaniasis are endogenous gain. We should not be surprised by non-native format in the era of globalization of infectious diseases worldwide.The ratio of visceral leishmaniasis /cutaneous is 1:3. Statistics indicate otherwise as cutaneous leishmaniasis has not sufficiently identified and disclosed regularly.We want to show epidemiological, clinical and therapeutic aspects of cutaneous leishmaniasis, and it follow up in infectious diseases service of UHC 'Mother Theresa" Tirana .

2 Methods

We ranked epidemiological aspects based on gender, residential, age group; clinical aspects was based on the number of lesions,size,type, and their localization,as well as we study curative efficacy of glucantime by cycles:14 days, 21 days and 28 days.Included 22 cases,31-70 years old , during 1994-2007

3 Results

Based on gender,16 was women and 6 men. 31-40 yeras old 9; 41-50 yo 7, 51-60 yo 4, 61-70 yo 2 cases.From urban area 5 cases,rural 7.With 1 lesion 21, multiple 1. According on lesions size: 0.5 cm were 9 cases, 1 cm 8, 1.5 cm in 5 cases.According to the type of lesion dried 19, sero hemorrhagic 2, purulents 1. According on localization forehead 5,eylied 2,nouse 2,face 3, beard 1,ear 1,neck 1 case,body 1,arm 1,the forearm 1,hand 1, pulp1,foot 1case.Therapeutic aspects, with 14-days treatment with glucantime 1out of 5 cases was cured,on 21-day cycle of treatment 5 of 8 cases was healed,on 28-day cycle was healed 9 of 9 treated .The beginning of treatment effects from the day 6-7, 3 cases. 11 cases in day 9-11, 8 cases in day 14-16. Reduction the size of lesion over 50% in 5 cases on day 13-14, 7 cases on day 16-20, 10 cases on day 25-28. The healing of lesions was observed after therapy respectively: disappearance of the lesion in day 32-49 in 6 cases, 9 cases in day 52-65, 7 cases in day 70-85. Treatment with systemic route is used in these cases due to the localization of protozoosis on the delicate part of the body and because we have noted cases of purulent complications, or not aesthetic scar during treatment with other ways from different specialists.

4 Conclusions

Most affected were males 68.18%; age group 41-50 years old 40.91%.Predominated dry forms 86.36%, localization in the face 63.64%, treatment with 28-day cycle resulted efficient in 100% of cases. Beginning of effect was observed on the day 9-in 11,50% of cases. Significant reduction in the day 25-28 was 45.5% of cases, the lesion disappearance day 70-85 resulted in 40.91% of cases

Thursday, May 18th

Clinical and experimental immunopathology and pathogenesis

C0182 PROBING THE STRATEGIES OF ANTIMONY RESISTANT LEISHMANIA DONOVANI TO INDUCE AGGRESSIVE INFECTION

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1 Background

Kala azar patients harbouring antimony resistant *Leishmania donovani* (LD) parasites show higher parasite burden and greater IL-10 compared to those harbouring sensitive parasites. Also, there are reports of expression of autophagy proteins in infection. The study aims to understand the molecular mechanism how these two categories of parasites are being 'scrutinized' differently by the host cells to explain clinical disease outcome.

2 Methods

Mouse peritoneal macrophages (MØ), infected with antimony resistant (LD-R) or surface glycan knockdown antimony resistant LD or sensitive LD and the resulting cytokines were assayed. Important interactions were confirmed by the use of knockout mice (TLR2$^{-/-}$, IL-10$^{-/-}$, IL-12$^{-/-}$). MyD88-specific shRNA cassette containing self-inactivating lentiviral vector pCRI.LV was injected into spleen of mice for generating MyD88 knockdown (KD) mice. Autophagy and apoptosis were studied by TEM and by flowcytometry respectively.

3 Results

As compared to the sensitive counterpart, the LD-R displays unique surface glycans, induces autophagy markers in MØ, and gives rise to aggressive infection in experimental models. The glycan of LD-R interacts with TLR2 and increases IL-10/IL-12 in NFkB dependent manner. MyD88 acts downstream of most of the TLR dependent signaling. We now show that infection of MyD88$^{-/-}$MØ with LD-R results in higher intracellular parasite as compared to wild type MØ. Such attribute is not seen with sensitive parasites. LD-R up-regulates miR-466i, which binds to 3'-UTR leading to down regulation of MyD88 post-transcriptionally. We also show that upon infection with LD-R, MyD88$^{-/-}$ mice harbour higher organ parasites as compared to wild type mice. Infected MØ with Sb-R but not with sensitive counterpart show markers of autophagy at the early stage of infection followed by apoptosis.

4 Conclusions

Thus LD-R adopts multiple strategies like increasing the disease promoting cytokines and inducing a switch from autophagy to apoptosis in the infected host cell. Such switch may favour parasites to egress from apoptotic cells to infect adjacent normal cells in the organs. Thus LD-R may exploit a multitude of mechanisms to induce aggressive infection.

C0267 CLINICAL STUDY OF CUTANEOUS LEISHMANIASIS PATIENTS IN SRI LANKA THROUGH LONG TERM PATIENT FOLLOW-UP

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1 Background

Leishmania donovani is the aetiological agent of visceral leishmaniasis in the Indian subcontinent. However, it is known to cause cutaneous leishmaniasis (CL) in Sri Lanka. Visceralization potential of *L. donovani* in Sri Lanka is not yet known. The current study was aimed at investigating the disease sequelae of CL patients through long-term follow-up.

2 Methods

Patients (n=250) were recruited via passive case detection. Laboratory confirmation of the CL was done by light microscopy/culture/PCR. All diagnosed patients were referred to Dermatologists for treatment. Patients were treated with weekly intra-lesional injections of sodium stibogluconate (100mg/ml) up to 10 doses (IL-SSG). Patients were followed up weekly during the first 3 months and bi-annually thereafter, up to 4 years. Patients were examined by a Physician at each follow up visit, to assess healing and to detect any likely sign(s) of visceralization, such as anemia, weight loss and hepato-splenomegaly. Serum samples of all patients were tested for rk39 antibodies using the dip stick assay.

3 Results

The age range of patients was between 11 months to 70 years (median=40 years) with a male to female ratio of 3:1 (179:71). The most common clinical feature was a single lesion of an ulcerating nodule (n=80). Lesions were mostly observed on upper limbs and face (upper limbs=106, face=82) of otherwise healthy individuals. The delay before diagnosis varied from 1 month to 1 year, with a median of 6 months. No signs of systemic infection in CL patients were observed during the 4 year period. Similarly, there was no evidence for the presence of anti- *L. donovani* antibodies in any of these patients as evidenced by negative rk39 assays. Treatment success of SSG with standard 10 week treatment was 76.4% (191/250) and the remaining patients (23.6%) were cured following extra 4-9 doses of IL-SSG. Recurrence of lesions was observed in 3.6 % of patients on the exact same spot of the initial lesion or in the vicinity. Such patients were referred back to the Dermatologists for treatments.

4 Conclusions

Sri Lankan *L. donovani* appears to be dermotropic in nature in humans with no clinical evidence of visceralization. Host- parasite interaction may play a vital role in determining disease outcome, which is likely to explain the clinical picture seen in local patients. The use of animal models is being pursued to better understand the visceralizing potential of local *L. donovani*.

C0278 IMMUNOPATHOLOGICAL CHARACTERIZATION OF THE HUMAN CUTANEOUS LESION CAUSED BY LEISHMANIA (VIANNIA) PANAMENSIS

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1 Background

Leishmaniosis is considered a spectral disease with multiple clinical and immunopathogical features in humans depending on the host immune/genetic background and parasite specie. In Panama, the most prevalent specie of parasite causing human cutaneous infection is *Leishmania (Viannia) panamensis.* Due the lymphatic tropism of *L. (V.) panamensis,* the dissemination of infection can cause mucocutaneous involvement. The little knowledge about the pathogenesis of the disease caused by this species, in addition to the increasing incidence of human cases observed in many regions from America, led us to study the histopathological and immunopathological aspects of cutaneous lesion caused by *L. (V.) panamensis*.

2 Methods

Biopsies (n = 47) from Panamanian patients with localized cutaneous lesions were collected and processed by usual histological techniques. Paraffin sections stained by HE were used to characterize the histopathological changes. *L. (V.) panamensis* infection was proven by isolation and characterization of the parasites was performed by HSP70-RFLP. *In situ* cellular immune response was evaluated by immunohistochemistry using anti-macrophage (CD68), anti-lymphocytes (CD3, CD4, CD8, FoxP3) monoclonal antibodies, anti-NOS2 and anti-*Leishmania* polyclonal antibodies. Development of the reaction was performed using LSAB kit. The number of positive cells was assessed by quantitative morphometric analysis using a light microscope connected to a microcomputer.

3 Results

Histopathological changes were characterized by a mononuclear inflammatory infiltrate in the dermis composed by lymphocytes, macrophages and few plasma cells. Granulomatous reaction was present in 36% (17/47) of biopsies. Amastigotes were observed in 23% (11/47) by HE and 66% (31/47) by immunohistochemistry. The number of labeled cells/mm^2 was: CD3$^+$ cells 3228+166, CD8$^+$ 1831+99, CD4$^+$ 826+95, FoxP3$^+$ 418+22, CD68$^+$ 752+114, iNOS$^+$ 464+73 and amastigotes 618+180. A high number of CD68$^+$ and iNOS$^+$ cells was associated with a granulomatous reaction and with a low number of parasites; despite a similar number of T cells subsets between granulomatous and non-granulomatous reaction. There was a negative correlation between the number of parasites and FoxP3$^+$ cells.

4 Conclusions

The *in situ* cellular immune response was characterized by predominance of T cells mainly by cytotoxic lymphocytes which probably influence the activation of macrophages for parasite destruction.

C0281 VISCERALIZING POTENTIAL OF L.DONOVANI, THE CAUSATIVE AGENT OF CUTANEOUS LEISHMANIASIS IN SRI LANKA

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1 Background

Cutaneous leishmaniasis (CL) is a newly established disease in Sri Lanka, caused by a genetic variant of *L. donovani*, a usually visceralizing parasite. Detailed genetic characteristics and visceralization potential of *L. donovani* in Sri Lanka are not yet fully understood.

2 Methods

Patients were recruited via passive case detection and confirmed by light microscopy/culture/PCR. CL patients (n=250) treated with intra-lesional antimony up to 10 weekly doses were followed-up, every six months for 4 years to detect evidence of visceralization. Parasites isolated from 34 CL patients and 4 visceral (VL) patients were genotyped using kDNA minicircle sequences followed by phylogenetic analysis and comparative genomics using reference *Leishmania* species sequences. Five parasite isolates were injected to intra-dermally (ID) (n=30) and intra-venously (IV) (n=30) into BALB/c mice with wild-type *L. donovani (*positive control; n=6)) and normal saline (negative control; n=12). After 10 weeks, mice were euthanized, spleen and liver removed and investigated for evidence of infection.

3 Results

All lesions, except those of 59/250 (23.6%) patients, healed with standard therapy. No signs of systemic infection detected in any patient during the follow-up period. Genetic diversity of Sri Lankan isolates was moderate (H_E=0.4337) with strong inter-geographical genetic differentiation (F_{ST}> 0.25). Within Sri Lankan group, there were 3 distinct sub-clusters, viz., poor responders to antimony therapy, antimony-sensitive group and VL isolates. Skin lesions were observed in BALB/c mice (22/30) infected intradermally with local parasites. None of them showed systemic infection. However, visceralization was evident in mice (24/30) infected intra-venously with local isolates and those infected intra-dermally with wild type strain (6/6).

4 Conclusions

This study reveals for the first time the moderate levels of genetic diversity of *L. donovani* in Sri Lanka with a distinct association of genotypes with phenotypic characteristics of clinical significance. SriLankan *L. donovani* appears to have modified virulence with no clinical evidence of visceralization in patients. It has the capacity to establish systemic infection in BALB /c mice, following direct intra-venous inoculation. Route of infection appears to play a role in determining disease outcome. Whole genome sequencing and animal models are being pursued to better understand the genetic characteristics and visceralizing potential of Sri Lankan *L. donovani*.

C0378 HUMAN VISCERAL LEISHMANIASIS PATIENTS DISPLAY ENHANCED EXPRESSION OF CXC CHEMOKINES, BUT REDUCED EXPRESSION OF THEIR RECEPTORS AND DECREASED TRAFFICKING OF INFLAMMATORY CELLS IN BLOOD

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1 Background

Chemokines play an important role in determining cellular composition at inflammatory sites, and as such, influence disease outcome. In this study, we investigated the expression profile, cellular source and role of various inflammatory chemokines and their receptors in human visceral leishmaniasis (VL).

2 Methods

The expression of chemokines or their receptors was measured at the gene and protein level by employing real time qPCR and a cytometric bead array assay, respectively. In addition, the cellular source of chemokines and their receptors in the spleen was identified using gene expression analyses.

3 Results

We identified elevated CXCL10, CXCL9, CXCL8, and decreased CCL2 from VL patients. Furthermore, we found reduced expression of the chemokine receptors CXCR1, CXCR2, CXCR3 and CCR2, but increased expression of CCR7 on VL PBMC, compared to endemic controls. Additionally, splenic monocytes were found to be the major source of CXCL10, CXCL9 and CCR2, whereas T cells were the main source of CXCR3 and CCR7. We also report a strong association between plasma IFN-γ and CXCL-10, CXCL-9 levels. Enhanced parasite burden positively correlates with increased expression of CXCL10, CXCL9, IFN-γ and IL-10.

4 Conclusions

Overall our result indicates that VL PBMC has an elevated inflammatory chemokine milieu which correlated with disease severity. However, expression of their chemokine receptors was significantly impaired, which may have contributed to reduced levels of blood monocytes and neutrophils. In contrast, enhanced expression of CCR7 was associated with increased numbers of activated T cells in circulation. These findings highlight the importance of chemokines for recruitment of various cell populations in VL, and the knowledge gained may help in global understandings of the complex interaction between chemokines and pathological processes, and therefore will contribute towards the design of novel chemokine based immunological therapies against VL.

C0382 ACTIVE AND TREATED VISCERAL LEISHMANIASIS PATIENTS EXHIBIT DISTINCT GENE EXPRESSION FOR NEUTROPHIL FUNCTION, IFN-G AND IL-1B

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1 Background

Visceral Leishmaniasis (VL) can be asymptomatic or can manifest as a progressive disease characterized by hepatosplenomegaly, fever, weight loss, hyperglobulinemia and pancytopenia. Patients with active VL have negative Delayed-Type Hypersensitivity to *Leishmania* antigen and their peripheral blood mononuclear cells (PBMC) do not produce Interferon-g (IFN-g) upon specific antigen stimulation. In contrast, high levels of IFN-g are detected in serum. Following treatment, the capacity of IFN-g production by PBMC is restored and IFN-g serum levels reach those of uninfected individuals. Identifying the full range of changes before and after VL treatment is critical to understanding the mechanisms of recovery from infection.

2 Methods

Herein we compared by RNA-seq the transcriptome of paired PBMC from seven VL patients before and xx-days after treatment. Total RNA was extracted and quality assessed on a Bioanalyzer 2100. High quality samples were sent for TruSeq library preparation following deep sequencing in an Illumina HiSeq platform. A range of 19 to 25 million reads per sample was aligned to the human genome followed by Cufflinks in order to estimate transcript abundancies and test for differential expression between groups.

3 Results

Forty-nine genes markedly (2.5 fold-change [FC]) differed from active disease to post-treatment samples. Several of differently expressed genes were related to neutrophil function, such as elastase and MMP9, which appeared down-regulated post-treatment. Non-coding RNAs were also markedly distinct in pre vs. post treatment time points. Albeit below the 2.5 fold change threshold differences were noted in IFN-g and IL-1b expression, appearing slightly repressed (FC=-1.82) and activated (FC=1.90) respectively relative to the pre-treatment condition.

4 Conclusions

Whole transcriptome sequencing proved useful in delineating the expression changes before and after VL treatment. It is noteworthy that neutrophils previously implicated only in the initial steps of leishmanial infection may also be relevant in the pathogenesis of established infections.

C0446 PROGNOSTIC FACTORS FOR DEATH FROM VISCERAL LEISHMANIASIS IN CHILDREN ADMITTED IN KAIROUAN HOSPITAL, CENTRAL TUNISIA

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1 Background

Visceral leishmaniasis (VL) is a public health problem in Tunisia affecting mostly children under five years old. It is associated with high case fatality rate and significant costs. The governorate of Kairouan (central Tunisia) is the most affected focus in the country. The aim of this study was to identify risk factors for death from VL in children admitted in Kairouan hospital.

2 Methods

A retrospective cohort study was conducted in the pediatric department of Kairouan hospital and included all the VL cases admitted from 2004 to 2013. Visceral leishmaniasis diagnosis was established by at least two of the following biological tools : Bone marrow smears,Novy-MacNeal-Nicolle blood culture, ELISA serology, Real time-PCR conducted on DNA blood extracts. A standardized form was used by medically-trained investigators to collect data from the medical records. Factors associated with death from VL were identified using multivariate logistic regression. Statistical analysis was performed using STATA version 11.

3 Results

Two hundred and forty cases were recorded. Age ranged from 2 months to 13 years, the female/male sex ratio was 1.03. The most common clinical and biological symptoms were splenomegaly (97.9%), anemia (91.7%), thrombocytopenia (83.9%), fever (79.9%) and leucopenia (56.1%). There were 15 deaths resulting in a case fatality rate of 6.3%. Age of patients who died ranged from 3 months to 28 months (median, 13 months). Risk factors for death were: age less than 12 months (OR = 4.85, 95% CI : 1.20-19.62), bleeding (OR = 21.62, 95% CI : 4.25-109.85), associated infections (OR = 4.57, 95% CI : 1.21-17.27) and severe leucopenia (OR = 4.28, 95% CI : 1.03-17.83).

4 Conclusions

Low age, bleeding, bacterial infections and severe leucopenia should be considered as alarming signsin VL cases. Early identification ofchildren at higher risk of death might contribute to reduce mortality by insuring appropriate intensive and supportive care.

C0476 LEISHMANIA (V.) BRAZILIENSIS AND LEISHMANIA (L.) AMAZONENSIS DRIVE DERMAL DENDRITIC CELL FUNCTION FOR THE OPPOSITE T-CELL IMMUNE RESPONSE POLES IN AMERICAN CUTANEOUS LEISHMANIASIS

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1 Background

In Brazil, *Leishmania (V.) braziliensis* and *Leishmania (L.) amazonensis* are the main pathogenic *Leishmania* species causing American cutaneous leishmaniasis (ACL), being implicated with the most frequent clinical form, localized cutaneous leishmaniasis ($LCL^{DTH+/++}$), as well as the mucocutaneous leishmaniasis ($MCL^{DTH++++}$) and anergic diffuse cutaneous leishmaniasis ($ADCL^{DTH-}$), the most severe clinical forms. Between the central LCL and the two polar MCL and ADCL, these parasites can also give rise to a intermediary borderline disseminated cutaneous leishmaniasis ($BDCL^{DTH\pm}$). This study evaluated the dermal dendritic cell (dDC) expression within the clinical-immunological ACL spectrum; dDC (MHC^{+} class II[$Langerin^{-}$] and MHC^{+} class I [$Langerin^{+}$]) is regarded as the main presenting cell of species-specific parasite-antigens to the T-cell immune response, responsible for the activation of the innate and adaptive immune responses.

2 Methods

26 ACL patients were examined: ADCL and BDCL by *L. (L.) amazonensis* with negative $DTH^{(-)}$ (five cases of each); LCL by *L. (L.) amazonensis* divided into two groups: five cases with negative $DTH^{(-)}$ and three with positive $DTH^{(+)}$; and eight cases of LCL by *L. (V.) braziliensis*, all with positive $DTH^{(+)}$. Paraffin-embedded biopsies of cutaneous lesions were carried out for immunohistochemical analysis of immunostained cells ($CD11c^{+}$), using rabbit anti-human CD11c McAb (ab52632 Abcam). A Zeiss image analysis system was used to quantify $dDCs^{+}$ in 5-8 fields per histological section (400x). dDC expression was analyzed by Mann-Whitney test using Biostat 5.0 ($P< 0,05$).

3 Results

dDC^{+} cell-density showed an increased expression from the central $LCL^{(DTH+/++)}$ by *L. (V.) braziliensis* to the sub-polar $BDCL^{(DTH-)}$ and polar $ADCL^{(DTH-)}$by*L. (L.) amazonensis*: LCL-*L.b.*$^{(DTH+/++)}$= 358 cells/mm^2→LCL-*L.a.*$^{(DTH+/++)}$= 244 cells/mm^2→LCL-*L.a.*$^{(DTH-)}$= 310 cells/mm^2→BDCL-*L.a.*$^{(DTH-)}$= 517 cells/mm^2→ADCL-*L.a.*$^{(DTH-)}$ = 674 cells/mm^2, revealing a more significant expression ($P< 0,05$) in the BDCL and ADCL compared to those of LCL(s).

4 Conclusions

These results strongly suggest that, even though dDC is regarded as the main activator-cell of the innate and adaptive immune responses, the species-specific antigens of *L.* (*V.) braziliensis* and *L. (L.) amazonensis* are determinant for modulating the dichotomy in the T-cell immune response of ACL; i.e., the dDC role depends on either *Viannia* or *Leishmania*-antigenic environment the dDC interact.

C0537 AMERICAN CUTANEOUS LEISHMANIASIS TRIGGERED BY ELECTROCOAGULATION

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1 Background

Cutaneous leishmaniasis is an endemic infectious disease and its diagnosis is based on the history of sand fly bite and clinical manifestations. There are few reports that describe unusual parasite infection ways. Furthermore, it seems that the initial American tegumentary leishmaniasis (ATL) lesion can be also triggered by several situations, such as immunosuppression, parasite dissemination and local trauma.

2 Methods

We report a case of ATL triggered after skin lesion electrocoagulation.
A healthy 59 year-old female, from Brasilia/Brazil, who travels to ATL endemic areas, underwent an electrocoagulation procedure to remove two lesions clinically and dermoscopically diagnosed as seborrheic keratosis, one on the nose and the other on the tight. Two months later, erythematous nodules emerged on the surgical scars. The histopathology showed lymphohistiocytic infiltrate with granulomes and multinucleated cells. HIV serology was negative. *Leishmania* culture of skin fragment was positive and the parasite was identified as *L. (V.) braziliensis*by PCR and restriction enzyme analysis. She was treated with 2g of liposomal amphotericin B with complete healing.

3 Results

An immunocompetent patient with no previous sign of leishmaniasis after being submitted to a common dermatological procedure presented simultaneously cutaneous leishmaniasis lesions on both sites. This reinforces that electrocoagulation may have triggered ATL.
Previous studies showed that in Brazilian endemic areas 10% of the population has subclinical infection. There are some cases in literature of primary or secondary ATL lesions after local trauma. In addition, studies in murine models infected with *Leishmania* support the possibility of metastatic cutaneous lesions at sites of trauma.
Therefore, we may consider that trauma generates an inflammatory cascade that favors *Leishmania* dissemination and lesion formation in previously infected patients. This is supported by the local formation of immunosuppressive cytokine and transformation of growth factor-p that exacerbates lesion development. Studies in Balb-c mice infected with *L. (V). braziliensis* showed that these factors could reactive subclinical infection.

4 Conclusions

Dermatologists should be aware of the possibility of the onset of ATL lesion on the site of dermatological procedures in endemic areas.

C0541 ISOLATED CERVICAL LYMPHADENOPATHY: DO NOT FORGET ATYPICAL LEISHMANIASIS

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1 Background

Leishmaniasis is a vector-borne disease caused by *Leishmania* spp. Cases are reported from 98 countries where more than 350 million people live in areas with *Leishmania* transmission. The infection, caused by intracellular hemoflagellates of the genus *Leishmania,* may affect the skin, mucous membranes or viscera, leading to a wide range of clinical presentations. Three major clinical syndromes are usually recognized: cutaneous (CL), mucocutaneous (MCL), and visceral (VL) leishmaniasis. Isolated lymph node leishmaniasis (LNL) is exceptional.

2 Results

We reported the case of a 60 year-old female patient, native of Poland and inhabitant in Jendouba (Northwest of Tunisia), with a past history rheumatoid arthritis treated by steroids. She presented with a one year gradually increasing left sub-mandibular swelling. Physical examination was normal. Sub-mandibular lymph node was 3 cm large, firm, elastic, mobile and painless, without fistula or abscess. Routine laboratory findings showed elevated gamma-globuline, a mild anemia and normal platelet count on CBC. Electrolytes, renal and liver tests were within normal range. Chest X-ray was normal as well as thoracic and abdominal CT. ELISA was negative for HIV. The fine needle aspiration (FNA) smears revealed a polymorphic population of cells and macrophages infiltrated with *Leishmania* amastigotes.
Real Time Polymerase chain reaction (RT-PCR) was performed on lymph node biopsy and identified *Leishmania infantum*. However, it was negative on peripheral blood sample. Serology of leishmaniasis was highly positive by immunofluorescence assay (IFA) at the dilution of 1/1600. Systemic visceral leishmaniasis was eliminated and localized node leishmaniasis was the retained diagnosis. The patient was treated with amphotericin B deoxycholate at a dose of 1mg/kg/day. At day 10, she developed renal insufficiency and despite giving amphotericine B 1 day/2, she altered her renal function at day 18 and treatment was stopped. The clinical course was favorable. The lymph node size decreased quickly. She was found asymptomatic for 1 year of follow-up with a disappearance of lymphadenopathy.

3 Conclusions

Only a few cases of isolated cervical lymphadenopathy due to leishmaniasis had been reported. This case highlights the need of detailed examination and evaluation mainly in endemic areas of some infectious diseases such as leishmaniasis. The combination of parasitological, serological and molecular methods is the best approach for diagnosis confirmation.

C0578 COMPARISON OF IMMUNE REGULATORY FACTORS IN EARLY AND LATE LESIONS OF CUTANEOUS LEISHMANIASIS

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1 Background

Recent studies on mouse models of cutaneous leishmaniasis (CL) showed that the type of immune response generated at the site of infection and especially the balance between regulatory and effector T-cells determines the outcome of the disease. Not enough data on human leishmaniasis is available.

2 Methods

Skin biopsies were collected from parasitologically proven lesions of 36 CL patients due to *L. major*, referred to the Centre for Research in Skin Diseases and Leishmaniasis, Isfahan University of Medical Sciences, Iran, from May 2009 to February 2011. Patients were distributed into two groups of early lesions (EL, <4 months) and late lesions (LL, >6 months). Quantitative real-time RT- PCR was performed to assess mRNA expression of Interleukin (IL)-10, transforming growth factor (TGF)-b and Forkhead box P3 (Foxp3) as a marker of natural regulatory T (nTreg) cells and to assess parasite burden in 13 EL and 11 LL patients. Immunofluorescent (IF) staining of biopsies was done to assess the frequency of nTreg cells and cells positive for IL-10 and TGF-β in 10 EL and 10 LL patients. Mann-Whitney U test was used to determine the significance of differences between the two groups. The correlations were analyzed using Spearman's rho test.

3 Results

Mean relative gene expressions of IL-10 and TGF-β showed a non-significant increase in LL *vs.* EL cases. However percentage of IL-10 positive cells (EL: 0.15±0.02, LL: 0.51±0.10, p=0.035) and TGF-β positive cells (EL: 0.16±0.01and LL: 0.53±0.11, p=0.008) were significantly increased in LL cases. Mean relative expressions of Foxp3 mRNA were 0.53±0.07 and 1.26±0.35 (displayed in arbitrary units) in EL and LL cases respectively which was significantly higher in LL lesions. Similar results were obtained in samples stained for nTreg markers with IF method. Parasite burden showed a significant correlation with IL-10 (r= 0.66, p= 0.001) and TGF-β (r= 0.54, p= 0.027).

4 Conclusions

A higher gene expression and/or protein staining of IL-10, TGF-β and nTreg cell markers in LL biopsies might be an indication of a role for these regulatory elements in long lasting CL. Moderate correlation of parasite burden with IL-10 and TGF-β gene expression might be an implication of contribution of these cytokines to disease severity. These data support the effectiveness of immune regulatory based immunotherapy for treatment of ZCL particularly for persistent lesions.

C0605 HEMOPHAGOCYTOSIS IN EXPERIMENTAL VISCERAL LEISHMANIASIS

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1 Background

Hemophagocytosis is a phenomenon of macrophages or histiocytes phagocytosing blood cells. There are reports on up-regulated hemophagocytosis in patients with infectious diseases including typhoid fever, tuberculosis, influenza and visceral leishmaniasis (VL). However, mechanisms of infection-associated hemophagocytosis remained elusive due to lack of appropriate animal models. Here, we have established a mouse model of VL representing hemophagocytosis in order to elucidate mechanisms behind this phenomenon in VL.

2 Methods

BALB/cA mice were infected with 1 x 10^7 promasitotes of *Leishmania donovani*. At 24 weeks after infection, mice were sacrificed to collect blood and spleen followed by hematological analysis, histological observation and immunohistochemical analysis on a molecule involved in hemophagocytosis.

3 Results

After 24 weeks post infection, BALB/cA mice exhibited splenomegaly with an average tissue weight of 10 times as those of naïve mice and anemia with lower hematocrit, hemoglobin and red blood cell counts than the naïve mice. In the spleen, 28.6% of macrophages contained phagocytosed erythrocytes. All of hemophagocytosing macrophages were parasitized, and higher levels of hemophagocytosis were observed in heavily infected cells. Besides, more than half of these hemophagocytes had two or more nuclei per cell whereas only 15.0% of splenic macrophages were multi-nucleated. Immunohistochemical analyses of the spleen from infected mice demonstrated hemophagocytes in the tissue have lower expression level of signal regulatory protein (SIRP) α, one of the inhibitory receptors for self-phagocytosis, compared with non-hemophagocytic macrophages.

4 Conclusions

To our knowledge, this is the first report on hemophagocytosis in experimental *Leishmania* infections. Results in the current study suggest that direct infection by *L. donovani* causes hyper-activation of host macrophages to engulf blood cells through down-regulation of SIRP-α.

C0623 HISTOLOGICAL AND IMMUNOLOGICAL DIFFERENCES BETWEEN ZOONOTIC CUTANEOUS LEISHMANIASIS DUE TO LEISHMANIA MAJOR AND SPORADIC CUTANEOUS LEISHMANIASIS DUE TO LEISHMANIA INFANTUM

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1 Background

In Tunisia, three forms of cutaneous leishmaniasis (CL) were described: the zoonotic cutaneous leishmaniasis (ZCL), caused by *Leishmania* (*L). major*, endemic in the Southern and Central regionof Tunisia; the sporadic cutaneous leishmaniasis (SCL), caused by *L. infantum* in north Tunisia and the chronic cutaneous leishmaniasis (CCL) caused by *L. tropica* described first in South of Tunisia. In order to study the impact of *Leishmania* species on the lesion feature, we evaluated the histological and immunological differences between ZCL and SCL.

2 Methods

Skin biopsies specimens obtained from 20 patients with active ZCL and 32 patients with active SCL were used. Biopsies were divided into two parts: one was used for histological and immunohistochemical analysis and the second was used for the analysis of mRNA expression of IFN-γ, IL-10, and IL-13, IL-8 and MCP-1, granzyme B (GrB) and granulysine (Grly)using real-time quantitative polymerase chain reaction (RT-qPCR). Immunohistochemical staining was performed using anti-CD3, CD4, CD8, CD56, GrB and IFN-γ. Data analysis was performed using Graph-Pad Prism.

3 Results

Histological analysis showed a mild to moderate infiltrate within ZCL lesions. In contrast a massive infiltration of the dermis was observed within SCL lesions. Contrary to ZCL, infiltrates within SCL lesions were organized and showed granuloma composed of macrophages and lymphocytes. In addition, immunohistochemical analysis showed a predominance of $CD4^+$T cells within both of CL forms. Otherwise, IL-8 mRNA levels were significantly higher in ZCL lesions compared to SCL lesions. Conversely, IFN-γ mRNA levels were higher in SCL lesions.Levels of IL-10, MCP-1, GrB and Grly mRNA levels were comparables within SCL and ZCL lesions.

4 Conclusions

CL due to *L. infantum* (SCL) is characterized by a massive organized dermal infiltrate with a predominance of $CD4^+$ T cells and high levels of IFN-γ. However, in CL due to *L. major* (ZCL) the dermal infiltrate is moderate and lesions are characterized by an important density of neutrophils and high mRNA levels of pro-inflamatory chimiokine IL-8. Both lesions showed high levels of GrB and Grly mRNA, indicating a cytotoxic activity which might be involved in lesion establishment and killing of parasite.

C0651 GENE SIGNATURES OF CD4+ T CELL FROM VISCERAL LEISHMANIASIS PATIENTS

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1 Background

CD4+ T cells are important for parasite control. Subsets of CD4+ T cells play important roles in protection, as well as pathogenesis during visceral leishmaniasis (VL). In our study we aimed to establish gene signatures of CD4+ T cells from VL patients.

2 Methods

We analysed 130 gene transcripts from MACS purified CD4+ T cells from 28 paired VL patient (pre-treatment and 30 days post treatment) and 15 endemic controls using a Nanostring n-Counter ™ CodeSet Design (Customized), based on novel digital molecular barcoding technology. Targeted gene transcripts were associated with T cell activation, regulation, differentiation and exhaustion, and we validated the Nanostring gene transcript data at the protein level using FACS.

3 Results

ClueGO analysis on Cytoscape demonstrated VL was associated with T helper-17 cell and T helper-2 cell regulation of T cell differentiation. Ingenuity Pathway Analysis (IPA) showed the top canonical pathways associated with VL were T helper cell differentiation, as well as JAK1 and JAK3 cytokine signalling. Our results also indicated the top up regulated genes were associated with T cell regulatory functions and anergy. Top down regulated genes were associated with chemokine-mediated cell trafficking and antigen presentation, while IL-2 was found to be a major upstream regulator.

4 Conclusions

Our results define a molecular signature for CD4+ T cells from VL patients that will help to better understand VL pathogenesis, as well as target specific molecules to supress the VL pathogenesis and/or improve anti-parasitic immunity.

C0660 ROLE OF IRON HOMEOSTASIS IN VISCERAL LEISHMANIASIS PATHOGENESIS

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1 Background

Visceral leishmaniasis (VL) is associated with anemia and leukopenia which can be pathophysiologic determinant of dysregulation in immune cells functioning. This study aims to explore the role of iron and its deficiency in disease associated pathogenesis and its homeostatic machinery in visceral leishmaniasis (VL) infection.

2 Methods

The study was performed on confirmed VL cases who were positive with routine diagnosis of VL and peripheral blood mononuclear cells (PBMC) from cases pre and post-treatment and endemic healthy controls were obtained. The hemoglobin levels, total iron binding capacities (TIBC) and serum iron were assessed during the course of disease (pre-/post-treatment) and total WBC count was obtained. The iron homeostatic pathway was analyzed by looking at major iron transporters by 7500 REAL TIME PCR platform (ABI, Foster City CA, USA) using SYBR Green chemistry for divalent metal transporter, ferroportin, hepcidin and transferrin receptor.

3 Results

The hemoglobin level were regained after treatment following similar trend as WBC count with direct correlation between two parameters but there were no differences in the iron carrying capacities and serum iron level in healthy controls as compared to active cases. The expression of iron importers were significantly decreased during disease while exporters' levels were unchanged but its level was in turn regulated by hepcidin which significantly increased during the disease therefore, preventing the iron efflux.

4 Conclusions

VL is associated with decreased iron uptake by transferrin-dependent and independent pathways and perturbation in iron homeostasis in PBMC of VL cases can be the major cause of defect in the immune machinery. Therefore, these findings provide direct support for targeting iron metabolism as a therapeutic target for treatment of VL.

C0690 IN SITU CYTOKINE EXPRESSION IN CUTANEOUS LEISHMANIASIS DUE TO LEISHMANIA DONOVANI: ROLE OF PATIENT GENDER

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1 Background

Cutaneous leishmaniasis (CL) in Sri Lanka is caused by the usually visceralizing *Leishmania donovani*. Gender plays an important role in the immune response due to the anti-inflammatory and pro-inflammatory properties of androgens and oestrogens respectively. This study describes the influence of gender on clinical presentation and in situ cytokine expression in CL due to *L.donovani*.

2 Methods

Fifty eight patients with parasitologically or histopathologically confirmed CL were recruited, clinical features recorded and lesion punch biopsies obtained. Twenty five patients undergoing minor surgeries due to unrelated causes were recruited as healthy controls and skin tissue obtained from incision site. Skin biopsies were analyzed for local tissue expression of interleukin (IL)-12A, IL-4, IL-10, interferon (IFN)-gamma and tumor necrosis factor (TNF)-alpha. Cytokine mRNA was quantified by real-time RT- PCR using SYBR green. Relative copy numbers were calculated for each gene by $2^{-\Delta\Delta Ct}$ method using β-actin as the reference gene and healthy controls as the calibrator. Mann-Whitney U test was used to detect the association of gender with cytokine expression.

3 Results

The study group consisted of 37 males (63.8%) and 21 females (36.2%) with a mean age of 35 years (SD=12.05, range 18-66), and a mean lesion duration of 6.8 months (SD=9.10, range 1-48). Number of lesions varied from 1- 4 but majority (75.9%, 44/58) had only a single lesion. Lesions were seen mainly on the upper limb (51.7%, 30/58) and lower leg (34.5%, 20/58). Majority of lesions were nodular (44.8%, 26/58) in both males and females. Expression of IL-12 was significantly elevated in females (p=0.03). Males had higher mean levels of IFN-gamma, IL-4 and IL-10 although not statistically significant. Mean treatment duration was 3.2 (SD=2.1) and 2.7 (SD= 0.9) for male and female patients respectively.

4 Conclusions

Both male and female patients with CL presents with similar clinical features despite female patients showing a greater probability of developing a parasite clearing pro-inflammatory response in dermatropic *L.donovani* infections.

C0737 NON-ULCERATED ATYPICAL CUTANEOUS LEISHMANIOSIS IN CENTRAL AMERICA: IN SITU EXPRESSION OF FOXP3, TGF-BETA AND IL-10

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1 Background

In Honduras, visceral leishmaniosis (VL) and non-ulcerated or atypical cutaneous leishmaniosis (NUCL) have as etiological agent *L. (L.) infantum chagasi.* NUCL is the most common clinical form, affecting mainly children and adolescents. The lesions are defined as papule, asymptomatic nodule, non-ulcerative, erythematous or not.Microscopically, the lesions are characterized by mononuclear inflammatory infiltrate in the dermis composed mainly by lymphocytes, followed by macrophages and few plasma cells. The intensity of the inflammation is variable, but the parasitism is frequently discrete. The presence of granuloma is common and it is associate to intense inflammatory infiltrate and higher number of $CD8^+$ cells; while non-granulomatous reaction showed discrete and focal mixed mononuclear response. The aim of this study was to evaluate the immunoregulatory response *in situ* through the expression of FoxP3, IL10 and TGF-β in lesions of patients affected by NUCL according to the histopathological features.

2 Methods

Biopsies (n = 21) from human cutaneous non-ulcerative lesions were collected and processed by usual histological techniques. *In situ* regulatory immune response was evaluated by immunohistochemistry in paraffin sections using anti-human FoxP3, IL-10 and TGF-β antibodies. For development of the reaction, NOVOLINK kit was used. The immunostained cells were counted by using an image analysis system (Zeiss).

3 Results

$FoxP3^+$, TGF-β^+ and IL-10^+cells were observed in the dermal inflammatory infiltrate in all studied cases varying in the intensity according to histopathological features.Higher density of $FoxP3^+$ (267cells/mm^2) was associated with more intense inflammation and granulomatous reaction while lower number was related to discrete nonspecific inflammatory infiltrate (50cells/mm^2). The expression of TGF-β^+ cells was also higher in lesion characterized by granulomatous reaction (118 cells/mm^2) when compared to discrete inflammation (14 cells/mm^2). IL-10^+ cellular density was similar in granulomatous (76 cells/mm^2) and non-granulomatous reaction (62 cells/mm^2). Direct positive correlation was observed between $FoxP3^+$ cells and TGF-β^+ cells ($p = 0.0006$).

4 Conclusions

The data suggest that T regulatory $FoxP3^+$ cells and the immunoregulatory cytokines TGF-β and IL-10 play an important role in the immunopathogenesis of NUCL, modulating an efficient cellular immune response in the skin that maintain low tissue parasitism and control of lesion size.

C0759 MOLECULAR CHARACTERIZATION OF LEISHMANIA INFANTUM CIRCULATING IN HUMANS, DOGS, AND SAND FLIES IN THE EMILIA-ROMAGNA REGION, NORTHERN ITALY

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1 Background

The unusual increase in the number of visceral leishmaniasis (VL) cases observed since 2012 in central areas of the Emilia-Romagna region (E-R), northern Italy, led health authorities to focus the attention on this re-emergent parasitic disease. With the aim to provide a better knowledge of the parasite population circulating in the region, we investigated the genetic characteristics of *Leishmania infantum* strains from different hosts.

2 Methods

Molecular characterization was performed on a panel of 62 *L. infantum* strains obtained from humans, dogs and phlebotomine sand flies between 2013-2016 from different locations abroad the region, including areas involved in recent VL outbreaks. Isolates obtained from other endemic Italian regions were also included in the study for comparison. Species identification was performed by ITS-1 sequencing, and subsequent sub-species characterization was carried out by *k26*-PRC typing. Finally, strains were characterized by multilocus microsatellite typing (MLMT), targeting 15 highly polymorphic markers. Genotype data were analyzed by a Bayesian model-based clustering method.

3 Results

All strains were identified as *L. infantum* by sequencing of ITS1. Capillary electrophoresis revealed different *k26*-PCR product sizes; the amplicon of 626bp, typical of MON-1 zymodeme, was the most representative in both canine and extra-region human isolates. Conversely, all the human isolates from the E-R region shared a distinct, never reported amplicon size.
Overall, the MLMT detected 48 genotypes, with canine isolates showing a higher level of polymorphism. Bayesian analysis inferred two populations; one grouping canine strains together with extra-regional human strains, named Population A, whereas regional human strains and strains from sand flies were grouped in a second population, the Population B.

4 Conclusions

Overall, the study revealed genetic heterogeneity within *L. infantum* population from northern Italy. MLMT proved to be a suitable method for tracking genotypes among different hosts and areas. Despite canine population of the E-R region hosted zoonotic genotypes, recent outbreaks of VL were sustained by a distinct *L. infantum* population, actively circulating in the sand flies and so far undetected in sympatric dogs. Further studies are needed to elucidate the role of wild or domestic animal species in the epidemiology of VL in the region.

C0760 THE INFLAMMATORY CYTOKINE EFFECT OF A TLR2 AGONIST (PAM3CSK4) ALONE OR IN COMBINATION WITH LEISHMANIA INFANTUM ANTIGEN ON EX-VIVO WHOLE BLOOD FROM DOGS IN DIFFERENT STAGES OF INFECTION

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1 Background

Immune responses mounted by dogs at the time of *Leishmania* infection appear to be the most important factor in determining whether and when infection will progress from subclinical infection into clinical disease. Ibizian hounds are more resistant to disease than other dog breeds. In contrast, dogs that are predisposed to developing a disseminated infection and progressing towards clinical disease are considered susceptible. Recognition of pathogen associated molecules patterns by toll like receptors (TLRs) rapidly triggers a variety of anti-microbial immune responses through the induction of pro-inflammatory cytokines such TNF-α and IL-6 which may play an important role in controlling *Leishmania* infection.The main objective of this study was to investigate the effect of a TLR2 agonist (TLR2a) alone or in combination with *L. infantum* antigen on *ex vivo* whole blood cytokines from dogs in different states of infection.

2 Methods

Eleven healthy seronegative dogs from a non-endemic area; 16 sick seropositive dogs and 21 healthy seronegative Ibizian hounds from an endemic area were enrolled in the study. Whole blood was stimulated with medium alone (Ø), *L. infantum* soluble antigen (LSA), concanavalin A, medium with TLR2 (PAM3CSK4) receptor agonist (Ø+TLR2a) and medium with TLR2a and LSA (LSA+TLR2a) for 48 hours. Culture supernatants were harvested for measurement of canine TNF-α and IL-6 cytokines by ELISA.

3 Results

A statistical significant increase of TNF-α was found in the supernatants of stimulated blood from all groups (Ø+TLR2a and LSA+TLR2a) when compared with medium alone. A similar pattern was observed for IL-6. Interestingly, a significant increase of TNF-α production was only observed when stimulation with LSA+TLR2a was compared with TLR2a alone in Ibizian hounds. A significant increase of TNF-α production was observed with stimulation of LSA+TLR2a when compared with LSA in all groups. Moreover, significant higher concentrations of TNF-α and IL-6 were detected in Ibizian hounds, especially for the LSA, Ø+TLR2a and LSA+TLR2a treatments when compared with other groups

4 Conclusions

This study demonstrated that TLR2a alone enhances the production of the inflammatory cytokines TNF-α and IL-6 in a canine *ex-vivo* whole blood assay model. In addition, a combination of TLR2a+LSA promoted a synergistic pro-inflammatory effect with TNF-α in Ibizian hounds but not in seropositive sick dogs or seronegative healthy dogs

C0766 EXPRESSION OF CTLA-4 AND TIM-3 IN SPLEEN OF NATURALLY INFECTED DOGS WITH LEISHMANIA INFANTUM

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1 Background

The spleen is one of the main affected organs in canine visceral leishmaniasis (CVL). The disorganization of splenic white pulp (SWP), immunosuppression and decrease of cytokines and chemokines expression have been reported in CVL. These findings are compatible with cellular exhaustion phenomena. As the spleen is considered a persistence parasitic organ, could cell exhaustion play a role in failure in parasite replication control? This study aims to evaluate the *in situ* expression of exhaustion markers and its relation to histopathology and parasite load.

2 Methods

41 dogs were grouped according to SWP organization as: 1- organized to slightly disorganized (OR-SD, n = 11); 2- Moderate to intense disorganization (MD-ID; n=30). The parasite quantification through qPCR using ssRNA target, histopathology and immunohistochemistry were performed. $CD4^+$, $CD8^+$, IFN-γ^+, IL-10^+, TIM-3^+ and CTLA-4^+ cells were detected.

3 Results

The disorganization of SWP occurred with a gradual reduction of lymphoid follicles/mm^2 ($p < 0.05$), $CD4^+/mm^2$ ($p = 0.033$) and disease worsening ($p=0.024$). Animals from MD-ID group presenting high parasite load showed reduction of CTLA-4 cell number when compared to OR-SD animals with low parasite load ($p=0.033$). The TIM-3 expression were high, abundant and similar among groups (4.73 to 52.2%). There was a correlation between expression of TIM-3 and CTLA-4 ($r = 0.444$; $p = 0.004$).

4 Conclusions

The data suggest that: 1. T cells exhaustion can occur in the early stages of infection, since OR-SD animals showed TIM-3 and CTLA-4 cells; 2. The reduction in the number of lymphoid follicles and $CD4^+$ cells could be a result of exhaustion and cell death; 3. There was no change in TIM-3, probably because other cells might be expressing this molecule. In conclusion, the blockage of the exhaustion process as therapeutic strategy should consider TIM-3 pathway blocking, moreover, the results suggest a potential association between exhaustion and splenic disorganization, CD4 lymphocyte number reduction, failure of parasite control and the worsening of the disease.

C0783 LEISHMANIA AMAZONENSIS INFECTION: REGULATION OF EXTRACELLULAR MATRIX PROTEINS IN SUSCEPTIBLE AND RESISTANT MICE

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1 Background

Leishmaniasis is a complex of diseases caused by *Leishmania spp.* The presence of adhesion molecules may be involved in leishmaniasis pathogenesis and may explain the preference of certain species to skin, which is rich in extracellular matrix (ECM) components. Parasites of the *L. mexicana* complex present adhesion molecules on their surface which interact with the ECM during the early stages of infection. This molecules help the parasites migration through the ECM, to degrade the components of this matrix, increasing the chances of the parasite to access the lymphatic system and blood circulation and facilitates the activation and migration of macrophages.

2 Methods

In this work C57BL/10 and C3H.He mice were infected with *L. amazonensis* and extracellular matrix proteins expression were measured by qRT-PCR in footpad, draining lymph node, liver and spleen.

3 Results

Footpad qRT-PCR analyses showed an enhancement of fibronectin (FN) and laminin (LM) expressions in *L. amazonensis*-infected C57BL/10 mice, 30 days after infection. On the other hand, C3H.He mice showed only a transient increase of LM 30 days post-infection. Collagen IV mRNA expression was evident only 30 days after infection in both mouse strains, but in the C57BL/10 mice the levels were twice those of the C3H.He mice. The lymph node evaluation showed low levels of mRNA from ECM proteins at all times after infection in both mouse strains. Liver evaluation showed that FN, collagens I and III presented the same pattern of expression with an enhancement of mRNA production 120 days after infection in both mouse strains, although the increase was more pronounced in the C57BL/10 mice. LN presented reduced expression levels in both mouse strains in comparison to mock-infected mice throughout the experiment. The FN expression in the spleen was more evident 24h after infection in both mouse strains. In the late phase, all mice present reduced levels of this mRNA. Collagen III expression was more evident in the initial phase of infection, showing a reduction in the late phase. The highest collagen IV expression was seen 30 days after infection in both mouse strains.

4 Conclusions

Extracellular matrix proteins are important for pathogen invasion and tissue reconstitution in experimental leishmaniasis. Our work adds knowledge and contributes to a better understanding of leishmaniasis immunopathology.

C0789 EPIDEMIOLOGICAL AND CLINICAL ASPECTS OF FELINE CUTANEOUS LEISHMANIOSIS IN VENEZUELA

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1 Background

Venezuela is an endemic area for human and canine leishmaniosis due to *Leishmania infantum* and *Leishmania braziliensis* among other *Leishmania* species. Limited data is available on feline leishmaniosis (FeL) in this region. The aim of this study was to describe clinical and epidemiological aspects of FeL in Venezuela.

2 Methods

Thirty outdoors domestic short hair cats, thirteen females and seventeen males between eight months and sixteen years of age from urban areas of Lara state (Venezuela) were studied. Blood samples were collected and physical examination was performed in all cats. Diagnosis of *Leishmania* infection was made based on histopathological and immunohistochemistry of cutaneous lesions as well as with the assessment of *L. infantum* and *L. braziliensis* specific antibodies by enzyme-linked immunosorbent assay (ELISA) and *Leishmania* DNA from blood samples by quantitative real-time polymerase chain reaction (qPCR).

3 Results

Six cats had skin lesions that consisted of solitary or multiple nodular lesions, which were located on the nose (n=3), ears (n=1), ears and nose (n=1),nose, ears, tail and lower limbs (n=1), all of them without systemic clinical signs. Histologically lesions were characterized by diffuse pyogranulomatous inflammatory infiltrate with numerous intracellular and extracellular amastigotes, and immunohistochemistry was positive for *Leishmania* spp. Two sick cats yielded positive ELISA result to both *Leishmania* antigens but higher antibody levels were found with *L. braziliensis* antigen when compared with *L. infantum* antigen. One seropositive sick cat was also blood *Leishmania* qPCR positive while the other seropositive sick cat was qPCR negative. Another sick cat was blood *Leishmania* qPCR positive but seronegative to both *Leishmania* antigens.The rest of sick cats and twenty four clinically healthy cats were negative by serological and molecular tests

4 Conclusions

We concluded that leishmaniosis should be included in the differential list of nodular-ulcerative lesions in cats, mainly on the nose and ears. Most reliable diagnosis in sick cats is histopathological examination along with immunohistochemistry, since blood qPCR and serology might be negative. The present findings might indicate that clinically healthy cats are not carriers of *Leishmania* spp. present in Venezuela.

C0808 SPLENIC IMMUNE RESPONSE IN DOGS VACCINATED WITH LBSAP AND LBSAPSAL AFTER 885 DAYS OF EXPERIMENTAL CHALLENGE WITH LEISHMANIA CHAGASI

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1 Background

Canine visceral leishmaniasis (CVL) has become one of the most important tropical diseases considered neglected by the World Health Organization, causing severe inconvenience to public health. In this sense there is a consensus worldwide between the governmental organizations that immunoprophylaxis is the most important method of the disease control.

2 Methods

Thus, this study employs an *in vitro* assay to evaluate the immunophenotypic and cytokine profile in splenocytes from dogs vaccinated with *Leishmania braziliensis* antigens and saponin (LBSap) or *Leishmania braziliensis* antigens and saponin and sand fly saliva (LBSapSal), after 885 days of *L. chagasi*-intradermal challenge. The *in vitro* stimuli were performed using vaccine stimulated antigen (VSA) or soluble *L. chagasi* antigen (SLcA).

3 Results

LBSap group showed reduction ($P<0.05$) in $CD5^+$ T-cells for both VSA- and SLcA-stimulated splenocytes. Moreover, LBSap group displayed high levels of TNF-alpha after VSA stimulation and enhanced IL-10 SLcA-specific. Similarly, LBSapSal group showed increased ($P<0.05$) frequency in $CD8^+$ T-cells, after SLcA stimulation. Furthermore, LBSapSal vaccination induced increased levels in TNF-alpha and IL-12, after VSA stimulation, in addition to increased levels in IL-10 SLcA-specific.

4 Conclusions

Taken together, our data demonstrated a balanced immune response in splenocytes, 885 days post *L. chagasi* challenge, triggered by LBSap and LBSapSal vaccination.

C0855 AGE, GENDER, HIV INFECTION AND ACUTE MALNUTRITION CONTROL LEISHMANIA INFANTUM BURDEN, WHICH INFLUENCES THE CLINICAL PRESENTATION AND MORTALITY OF NEW WORLD KALA-AZAR

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1 Background

Blood parasite burden of *Leishmania infantum* is an estimation of total parasite present in an infected host. Therefore, its measurement is important to investigate factors involved with parasite control.

2 Methods

Quantitative polymerase chain reaction from the blood was performed in consecutive 625 patients with kala-azar admitted to a reference hospital in Teresina, Brazil. Primers amplified a segment of kDNA by using the Taqman technology. Non-parametric statistical tests were applied.

3 Results

Median parasite burden was 499.2 amastigote equivalents (AE)/mL. Children <1 year old had higher parasite burden (798.5 AE/mL), which dropped sharply after the first year (192.8, AE/mL at 1<2 yo) and remained lower, increasing after adolescence (1049.5 AE/mL at 20<25 yo), and then reaching a trend to progressive increase up to the elderly. (2,465.1 AE/mL at 45<50 yo, and 2,588.1 AE/mL at 70<75 yo). Men had higher parasite burden the women (641.2 AE/mL vs. 308.9 AE/mL). HIV infected patients showed a much higher burden than non-infected patients (1,278.6 AE/mL vs. 412.7 AE/mL). Finally, acutely moderately to severe malnourished (weight-for-age and body mass index z-scores <-2) children under 5 yo also had almost three times higher parasite burden than better-nourished children (1,434 AE/mL vs. 341.5 AE/mL) and (1,305.9 AE/mL vs 344.2 AE/mL), respectively for both measurements. Parasite burden was more than three times higher among the deceased patients (1,069.8.2 AE/mL vs. 479.9 AE/mL), and also was higher in those with higher risk of death, sepsis syndrome, pneumonia and jaundice. All differences were statistically significant at the *p*-value less than 0.05. Parasite burden had the same age distribution profile of mortality.

4 Conclusions

The data indicate that parasite burden in patients with kala.-azar is controlled by age- and gender-associated factors as well as by HIV infection and acute malnutrition. Additionally, it influences the clinical picture, disease severity and mortality. The unifying explanation seems to be imunosuppression leading to uncontrolled parasite replication followed by a clinical picture correspondent to progressive systemic inflammation.

C0860 HYPERNOCICEPTION IN EXPERIMENTAL INFECTION BY LEISHMANIA (LEISHMANIA) AMAZONENSIS IN BALB-C MICE

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1 Background

American tegumentary leishmaniasis is one of the six most important infectious diseases worldwide. Normally, the skin lesions are painless, but many patients report local pain. Nociception was studied with *L. major*, but there are no publications related to nociception with *Leishmania (Leishmania) amazonensis*infection. This study aimed to investigate the inflammatory and hypernociceptive response induced by the inoculation of *L. (L.) amazonensis* in BALB/c mice.

2 Methods

The Ethics Committee on Animal use of the Universidade Estadual de Londrina (process number 7689.2015.72) and Universidade Estadual de Maringá (process number 003/2015) approved this project. Male BALB/c mice were subcutaneously infected with $1x10^2$, 10^3, 10^4, 10^5, 10^6, 10^7 and 10^8 *L. (L.) amazonensis*/mL, following analysis were made for nine weeks. Hypernociceptive response was evaluated in the footpad of infected and non-infected BALB/c mice using Von Frey analgesic meter. Footpad volumes were measured using plethysmometer. At the ninth week of infection, footpad tissues were processed for histological analysis and stained with heatoxilyn and eosin. Slides were observed in a common optical microscope. The descriptive statistics and variance analysis (ANOVA) followed by Tukey test were performed using the softwars BioEstat 5.3, GraphPadPrism 5.0 and Microsoft Excel 2010. Values of p <0.05 were statistically significant.

3 Results

Pain intensity was proportional to parasite concentration and peaks were achieved at the seventh week of infection for all groups, followed by a slightly decrease or stabilization. At the third week of infection was observed a significant difference of infected and control footpad edema (p<0.05) with a progressive increase during next weeks. Histological analysis also noticed these findings observing inflammation with inflammatory cells and exudate that increased with parasite concentration.

4 Conclusions

Hypernociceptive response and edema in leishmaniasis are concentration and time dependents. Besides, immunological factors can be involved.

C0865 BONE MARROW EMERGENCY RESPONSE FAVORS THE PROGRESSION OF VISCERAL LEISHMANIASIS

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1 Background

All cells of the host immune system are derived from hematopoietic stem cells (HSCs) that are normally found in a dormant state in the bone marrow. HSCs become activated in response to stress, such as acute infections, which adapt the bone marrow output to the needs of the immune response. Bone marrow homeostasis is usually restored after the infection is cleared, and HSCs return back to their dormant state with little to no functional loss. However, the role and extent of infection-adapted HSC activation and differentiation on the persistence of chronic infections is poorly understood.

2 Methods

We have examined here the bone marrow outcome of experimental infection with *Leishmania donovani* in mice on C57Bl/6 background using flow cytometry, cell culture and microscopy.

3 Results

Our results demonstrate that *L. donovani* induces HSC proliferation and expansion in the bone marrow and spleen during the chronic phase of the infection. The parasite induces a variety of inflammatory cytokines and chemokines that drive HSC differentiation towards non-classical myeloid progenitors with a regulatory phenotype. More importantly, emergency hematopoiesis directly contributes to the pathogenesis of visceral leishmaniasis: Fzd6-/- mice display reduced HSC expansion concomitant with decreased parasite burden. This was not due to altered T cell function of macrophage-mediated parasite killing but stemmed rathed from the generation of fewer Sca1+ emergency myeloid progenitors and consequently fewer Ly6Cint regulatory monocytes. Furthermore, the extracellular milieu from infected Fzd6-/- mice was less favorable to HSC expansion and myeloid differentiation in culture.

4 Conclusions

Our results demonstrate that *L. donovani* is able to subvert host bone marrow emergency responses to drive the production of immature myeloid cells. Myeloid-biased HSC differentiation would serve to facilitate parasite persistence by providing "safe targets" for the parasite. Based on these findings, we propose hematopoiesis as an attractive therapeutic target in chronic parasitic infections.

C0872 INTERLEUKIN-35 ALONG WITH TGF-? IS CRUCIAL FOR REGULATORY T CELL MEDIATED SUPPRESSION OF PROTECTIVE TH1 RESPONSE DURING MURINE VISCERAL LEISHMANIASIS

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1 Background

Visceral leishmaniasis (VL) or kala-azar, a deadly and disseminated infection of the lymphoreticular system, is caused by the protozoan parasites of *Leishmania donovani* complex.Active VL is characterized by suppression of cell-mediated immunity. Unfortunately, the mechanism of downregulation of host immune response during the disease is not well understood. Moreover, the role played by regulatory T cells and their cytokines in VL progression is still not clear.

2 Methods

In the present study, we investigated the cells involved in the enlargement of spleen and liver with the progression of *Leishmania donovani* infection in BALB/c mice through flow cytometry. By capture ELISA, we studied the cytokines implicated during the disease. Furthermore, we co-cultured the purified $CD4^+CD25^-$ and $CD4^+CD25^+$ regulatory T cells obtained from infected mice spleen and the proliferative index of $CD4^+CD25^-$ cells were measured by Carboxyfluorescein succinimidyl ester labelling. By administrating neutralising antibodies against regulatory T cells and its cytokines, we confirmed the role of the cytokines in disease progression.

3 Results

We found that among various splenic cell types, increase in the T cell population with time of infection was most pronounced. By profiling various immune cells and their cytokines during murine VL we also found that $CD4^+CD25^+FOXP3^+$ and $CD8^+CD25^+FOXP3^+$ T cells were the most significant sources of immuno-suppressive cytokines, IL-10, TGF-β and IL-35. Among these IL-35 and TGF-β may act synergistically for creating an immuno-suppressive environment, resulting into exacerbation of the disease. Further, a combined neutralization of IL-35 and TGF-β led to induction of protective response resulting in the arrest of the infection.

4 Conclusions

$CD4^+CD25^+Foxp3^+$ regulatory T cells are the most dynamic cells during progression of murine visceral leishmaniasis and are the major sources of disease progressive cytokines, IL-10, IL-35 and TGF-β. Moreover, IL-35 and TGF-β are criticalfor regulatory T cell mediated suppression of protective Th1 response during the disease.

C0887 IMMUNOPATHOLOGICAL STUDY OF THE SKIN LESION CAUSED BY NON-ULCERATED ATYPICAL CUTANEOUS LEISHMANIOSIS IN CENTRAL AMERICA.

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1 Background

Leishmaniosis presents a large spectrum of clinical manifestations depending on the parasite specie and the host immune genetic background. In Honduras, visceral leishmaniosis (VL) and non-ulcerated or atypical cutaneous leishmaniosis (NUCL) have as etiological agent *L. (L) infamtum chagasi.* NUCL is the most common clinical form in southern of the country, affecting mainly young people. NUCL lesions is defined as papule, asymptomatic nodule, non-ulcerative, erythematous or not. Considering the lack of knowledge about the pathogenesis caused by *L. (L) infamtum chagasi*, in individuals affected by NUCL, our aim was to characterize the histopathological changes and the cellular immune response *in situ*.

2 Methods

Biopsies (n=21) from human cutaneous non-ulcerative lesions with positive parasitological diagnosis were collected and processed by usual histological techniques. Paraffin sections stained by HE were used to characterize the histopathological changes. *In situ* cellular immune response was evaluated by immunohistochemistry using anti-macrophage (CD68), lymphocytes (CD4, CD8, CD20), and NOS2 antibodies. For development of the reaction, NOVOLINK kit was used. The number of positive cells was assessed by quantitative morphometric analysis using an image analysis system (Zeiss).

3 Results

Macroscopically the lesions varied between 3 to 5 mm. Microscopically, the skin lesions were characterized by mononuclear inflammatory infiltrate in the dermis composed of lymphocytes, vacuolated macrophages or not and few plasma cells. The intensity of the infiltrate varied from discrete to intense; in both, parasitism was discrete. Granulomas were present in 47% (10/21) of cases and it was associated to intense inflammation ($p<0.002$). Immunohistochemistry showed inflammatory response composed by $CD8^+$ (750 cells/mm^2), followed by $CD4^+$ (297), $CD68^+$ (220) and $CD20^+$ (165). The granulomatous reaction was correlated with higher number of $CD8^+$ cells and increase on $NOS2^+$ macrophages (220); while non-granulomatous reaction showed discrete and focal mixed mononuclear response with similar number of T, B lymphocytes and macrophages.The time of evolution of the lesions varied from 1 month to 20 years and did not show correlation with tissue inflammatory response.

4 Conclusions

The data showed an efficient cellular immune response characterized by high number of $CD8^+$ cells related to the activation of macrophage, which is probably responsible for low tissue parasitism preventing the evolution of the lesion size.

C0889 FURTHER EVIDENCES ON THE ABILITY OF LEISHMANIA (V.) BRAZILIENSIS AND L. (L.) AMAZONENSIS IN ORCHESTRATING THE MODULATOR EFFECT OF TNF-?, IL-10 AND TGF-? IN AMERICAN CUTANEOUS LEISHMANIASIS

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1 Background

Leishmania (L.) amazonensis and *L. (V.) braziliensis* are the most pathogenic agents of American cutaneous leishmaniasis in Brazil, responsible for a clinical-immunological spectrum of the disease: localized cutaneous leishmaniasis ($LCL^{DTH+/++}$), borderline disseminated cutaneous leishmaniasis ($BDCL^{DTH\pm}$), anergic diffuse cutaneous leishmaniasis ($ADCL^{DTH-}$), and mucosal leishmaniasis ($ML^{DTH++++}$), respectively. Recently, we have showed a dichotomy between these parasites and the human T-cell immune response; while *L. (V.) braziliensis* leads the infection fromthe moderate hypersensitivity LCL form toward to ML at the hypersensitivity pole, *L. (L.) amazonensis*, in contrast, drives the infection to ADCL at the hyposensitivity one. This study evaluatedthe TNF-α, IL-10 andTGF-β cytokines expressions within the clinical-immunological spectrum of ACL.

2 Methods

43 patients were examined: 6 cases ADCL, 5 BDCL, and 11 LCL by *L. (L.) amazonensis* $DTH^{(-)}$; 10 cases LCL, 4 BDCL, and 6 ML by *L. (V.) braziliensis* $DTH^{(+)}$. Paraffin-embedded biopsies of cutaneous and mucosal lesions were carried out for immunohistochemical analysis of stained cells($TNF\text{-}\alpha^{+}$, $IL\text{-}10^{+}$ and$TGF\text{-}\beta^{+}$), using goat anti-human TNF-α, and rabbit anti-human IL-10 and TGF-β polyclonal Abs. A Zeiss image analysis system was used to quantify these cytokines in 5-8 fields per histological section (400x). Cytokines expressions were analyzed by Kruskal-Wallis test using Biostat 5.0 ($P< 0,05$).

3 Results

It was found an inverse progression on $TNF\text{-}\alpha^{+}$ expression compared to those of $IL\text{-}10^{+}$ and $TGF\text{-}\beta^{+}$; while $TNF\text{-}\alpha^{+}$ showed an increase cell-density from the hyposensitivity ADCL to the hypersensitivity ML (ADCL/*L.a*= 162 → BDCL/*L.a*= 330 → LCL/*L.a*= 732 → LCL/*L.b*= 1154 → BDCL/*L.b*= 532 → ML/*L.b*= 1376 [$p< 0,05$]), $IL\text{-}10^{+}$ and $TGF\text{-}\beta^{+}$, in contrast, showed an increase cell-density from the hypersensitivity ML to the hyposensitivity ADCL (IL-10: ML/*L.b*= 479 → BDCL/*L.b*= 992 → LCL/*L.b*= 729 → LCL/*L.a*= 939 → BDCL/*L.a*= 1172 → ADCL/*L.a*= 1995 [$p< 0,05$]); $TGF\text{-}\beta^{+}$: ML/*L.b*= 708 → BDCL/*L.b*= 958 → LCL/*L.b*= 820 → LCL/*L.a*= 991 → BDCL/*L.a*= 1098 → ADCL/*L.a*= 1595 [$p< 0,05$]).

4 Conclusions

These findings strongly evidence the role of *L. (V.) braziliensis* and *L. (L.) amazonensis* in orchestrating the modulator effect of the inflammatory (TNF-α) and anti-inflammatory (IL-10 and TGF-β) cytokines in the T-cell immune response of ACL.

C0894 CUTANEOUS AND MUCOSAL LEISHMANIASIS DUE TO LEISHMANIA INFANTUM: BEHIND GRANULOMAOTUS LESIONS

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1 Background

Cutaneous and mucosal leishmaniasis are underdiagnosed in *Leishmania infantum* endemic areas. This vector-borne disease is frequently not suspected due to the polymorphism of its clinical form. On other occasions, the low sensitivity of direct microscopic techniques does not allow the diagnosis of leishmaniasis. These facts highlight the need of the development of novel, sensitive and specific diagnostic methods. Therefore, the aim of this study is to contribute to the improvement in the diagnosis of cutaneous and mucosal leishmaniasis through the establishment of a diagnosis protocol from histological samples.

2 Methods

Forty-six samples were collected from 44 patients throughout a retrospective study from 2014 to 2015. These samples were labelled in 4 categories according to their diagnosis:

- 9 samples from 9 *Leishmania* negative patients
- 17 samples from 15 confirmed leishmaniasis patients
- 4 samples from 4 patients with histology suspected leishmaniasis
- 16 samples from 16 patients with Granulomatous Lesions with Unknown Origin (GLUO)

The samples were analysed with two different PCR techniques: GRANALEISH Multiplex qPCR, which is able to differentiate among *L. infantum, L. tropica* and *L. major,* the main etiological agents of CL in the Old World; and a PCR-ELISA technique, specific for *L. infantum*.

3 Results

The diagnosis of the 9 negative samples and the 17 positive samples was confirmed with both PCR techniques, one of them was identified as *L. major*. The aetiological agent of the 4 samples with suspected leishmaniasiswas confirmed as *L. infantum*. Notably, *L. infantum* infection was confirmed in 75% (12 out of 16) of the samples categorized as GLUO, while the 4 remaining cases were negative. The clinical diagnosis of GLUO group were determined as dermatofibroma, sarcoidosis, dermatitis or Melkersson-Rosenthal syndrome.

4 Conclusions

A high percentage of GLUO are due to *L. infantum* infection. Given that the success of direct microscopy analysis is associated to the experience of the analyst, we suggest the generalized implementation of a PCR technique, such as those indicated: these show high sensitivity and specificity and could help overcome the drawbacks of direct diagnosis. Additionally, we recommend that all granulomatous cutaneous lesions from *L. infantum* endemic areas should be systematically investigated in by these techniques.

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C0906 ASSESSMENT OF LIVER HISTOLOGICAL ALTERATIONS IN DOGS NATURELLY INFECTED WITH LEISHMANIA CHAGASI/INFANTUM

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1 Background

Visceral leishmaniasis (VL) is a disease caused by *Leishmania chagasi/infantum* and is endemic in many regions of the world. Dogs are the main domestic reservoir. Canine VL shares many clinical and pathological aspects with human disease. Therefore, canine VL seems to be an useful model for the study of different aspects of the disease. The aim of this study was to evaluate liver disorders in dogs naturally infected with *L. chagasi/infantum*.

2 Methods

We examined 152 animals from an endemic area. The animals were clinically examined and ELISA tests and culture of splenic aspirates were performed to determine the infection. The number of clinical signs of LV defined the clinical status: no signs, asymptomatic; with any clinical signs, symptomatic. The animals were also ranked according to structural organization of the splenic white pulp: (1) well organized, (2) slightly disorganized, and (3) intensely disorganized. The histological evaluation was performed in a blinded way.

3 Results

The frequency of portal inflammation was higher in the group of animals with symptomatic active infection when compared to the group with asymptomatic inactive infection (p=0.0187). Kupffer cell hyperplasia (p=0.0303), Kupffer cell hypertrophy (p=0.0398), and intrasinusoidal lymphocytosis (p=0.0074) were more frequent in the group of animals with symptomatic active infection when compared to the group with asymptomatic active infection. The presence of intrasinusoidal inflammatory cell aggregates was more frequent in the group with symptomatic active infection when compared to the group with asymptomatic inactive infection (p=0.0063), and when compared with the group with asymptomatic active infection (p=0.0009). The frequency of portal inflammation was higher in the group with active infection and type 3 spleen when compared to the group with inactive infection and type 1 spleen (p=0.0252). Sinusoidal congestion (p=0.0340) and hepatocyte necrosis (p=0.0279) were more frequent in the group with active infection and type 3 spleen when compared to the group with active infection and type 1 spleen.

4 Conclusions

Similarly to that observed in the spleen, some inflammatory changes in the liver are significantly more frequent in dogs with positive culture for *Leishmania* and with more symptoms. Also, some liver changes were correlated with the disruption of the splenic white pulp. These liver changes may be related to the severity of leishmaniasis and provide important information on the evolution the disease.

C0909 EXPRESSION OF MICRORNAS IN LEISHMANIA (VIANNIA) BRAZILIENSIS-INFECTED HUMAN MACROPHAGE CELL LINE

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1 Background

Different clinical manifestations in human American tegumentary leishmaniasis (ATL) are strongly influenced by the immune status of the host. The infection development is also influenced by the parasite, which seems to be able to interfere in mechanisms of regulation of the inflammatory response in its favor, modifying microRNAs (miRNAs) expression in infected macrophages. Thus, the aim of this work was to analyze the expression of these small RNAs in *Leishmania (Viannia) braziliensis*–infected THP-1 human macrophages and to identify possible interactions with target genes related to the pathogenesis of ATL.

2 Methods

Human THP-1 macrophage cell line infected or non-infected with stationary phase *L. (V.) braziliensis* were incubated for 4h (32 °C / 5% CO_2). After washing, infections were followed for 6h or 24 h. Total RNA was extracted from harvested cells and qPCR were performed to evaluate the expression of 84 miRNAs specific for inflammatory response (miScript miRNA PCR Array – Qiagen), using the cDNA template (miScript II RT Kit – Qiagen).Results were obtained by the comparative Ct method and the significant ones (t-student test; $p < 0.05$) were selected for target prediction performed with the DIANA-miRpath 3.0 server (TargetScan algorithm).

3 Results

After 6 h of incubation, some miRNAs were significantly up- or down-regulated in relation to the uninfected control (miR-106b-5p, miR-211-5p, miR-29b-3p, miR-195-5p, miR-302a-5p, miR-34c-5p) and others after 24 h (let-7i-5p, miR-130a-3p, miR-520d-3p, miR-545-3p). Comparing the modulated miRNAs in 6 h and 24 h, some miRNAs expression was maintained (up-regulated: let-7i-5p, miR-30e-3p, miR-302b-3p, miR-34c-5p, miR-372-3p; down-regulated: miR-106b-5p, miR-29b-3p and miR-29c-3p), but some miRNAs had an increased expression (miR130a-3p, miR-211-5p, miR-520d-3p, miR-545-3p) or reduced (miR-195-5p and miR-30a-5p). Target prediction included cytokine coding genes, MHC II proteins and signaling pathways important in several cellular processes. However, some of the significant transcripts could not have their targets mapped by the chosen algorithm.

4 Conclusions

miRNAs expression profile exhibited by human THP-1 macrophages was altered by *L. (V.) braziliensis*, which may interfere with the immune and inflammatory responses of the host. This can represent an evasion mechanism of the parasite to the host's defenses, given the suggested interactions with identified target genes.

C0930 INTEGRATED ANALYSIS OF SPLENIC LYMPHOID TISSUE STRUCTURAL CHANGES AND IMMUNE GENE-EXPRESSION SIGNATURE IN HUMAN VISCERAL LEISHMANIASIS

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1 Background

In visceral leishmaniosis (VL), the spleen develops hyperplasia followed by atrophy and white pulp disorganization which may be associated with the severity of the disease. In this work, we studied the spleens of patients severe VL non-responsive to conventional treatment.

2 Methods

Patients with VL from Teresina, Brazil, with or without HIV infection, non-responsive to conventional treatment with amphotericin B and secondoray prophylaxis were subjected to splenectomy as complementary therapeutic. The spleens were processed for histology, immunohistochemistry (CD3, CD4, CD8, B20, CD79a, CD68, CD138, FOX-P3, IFN-g, TNF, IL17, IL6, IL4, TGF-band caspase 3) and gene expression analysis (immune response, *L. braziliensis* and HIV). As control, spleens surgically removed due to mechanical traumatic lesions and samples from the Genotype-Tissue Expression Consortium were used.

3 Results

The spleens of patients with VL showed a decrease in white pulp (WP) size (4/5 patients), lymphoid follicle (LF) and marginal zone (MZ) atrophy (4/5 and 2/5 patients respectively), and WP disorganization (2/5 patients). LF hypertrophy was observed in 1/5 patient and plasmacytosis in 4/5 patients. Heavily *Leishmania*-infected macrophages were observed in all spleen compartments. WP disorganization was associated with a decrease of IL6- and IL17-producing cells, and an increase in TNF-producing cells. Gene expression profile was accessed in 3/5 patients. All 3 patients presented high counts of *Leishmania*-related genes, but undetected counts for HIV-related genes. Corrected Multiple t-test revealed 171 genes significantly modulated (151 up-regulated and 20 down-regulated). Clusterization methods (PCA and hierarchical clustering) correctly classified normal and VL spleen samples. We performed an integrated analysis considering histopathological findings with high variance and gene expression data, building a correlation network supported by a $P<0.05$. Based on Louvain method, ten clusters were identified, including gene clusters associated to the MZ (IL-10 and PRDM1), WP (CCL19, IL-6, LTA and TNF), infected macrophage (IFN-g and SOCS1) and leishmania genes (CCL2, IL-4, IRF4 and TGFB1).

4 Conclusions

The observed gene expression patterns allowed the grouping of patients into two clusters: VL and normal samples, revealing an underlying transcriptomic modulation during VL. Furthermore, the spleen WP disorganization and corresponding gene expression patterns may relate to severe irreversible forms of the disease.

C0940 LEISHMANIA INFANTUM-SPECIFIC IFN-? PRODUCTION IN STIMULATED BLOOD FROM DOGS WITH CLINICAL LEISHMANIOSIS AT DIAGNOSIS AND DURING TREATMENT

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1 Background

The aim of the present study was to investigate the kinetics of *L. infantum* specific IFN-γ production in stimulated whole blood of dogs with leishmaniosis at the time of diagnosis and during treatment. IFN-γ concentrations were correlated with specific *L. infantum* antibodies, blood parasitemia and clinicopathological findings.

2 Methods

Thirty-three dogs were diagnosed based on physical examination, routine laboratory tests and *L. infantum*-specific antibody levels by ELISA. *Leishmania* real-time PCR in blood was also performed. Whole blood was stimulated with *L. infantum* soluble antigen (LSA) for 5 days. IFN-γ concentration was measured in supernatants of stimulated blood using an ELISA. Dogs were treated with a month of meglumine antimoniate and 12 months of allopurinol and were followed-up at 30, 180 and 365 days

3 Results

16 dogs were classified as IFN-γ non-producers after LSA stimulation (mean (X) ± standard deviation (SD): 0 ± 0 pg/ml) and 17 dogs as IFN-γ producers (X±SD: 3046 ± 4518 pg/ml) at the time of diagnosis. Production of IFN-γ after LSA stimulation was significantly increased (P=0.018) during treatment at day 365 in the IFN-γ non-producers (X±SD: 1744 ± 4201 pg/ml) in agreement with clinical improvement comparing with day 0. In contrast, IFN-γ producers maintained their IFN-γ production during treatment follow up. At diagnosis, IFN-γ non-producers showed a statistically higher blood parasitemia (P=0.003) *versus* IFN-γ producers. Interestingly, IFN-γ non-producers drastically reduced blood parasitemia (P=0.08) to minimum values at day 365 when compared with day 0 while no statistical differences were observed in IFN-γ producers. At diagnosis, *L. infantum* specific antibodies were higher (P=0.011) in IFN-γ non-producers (X±SD: 18575 ± 26250EU) than IFN-γ producers (X±SD: 3059 ± 4853EU). As expected and correlated with clinical improvement, a marked reduction of antibody levels was found at day 365 when compared with day 0 in IFN-γ non producers (P=0.008) and also in IFN-γ producers (P=0.002) with a X±SD of 702 ± 1125EU and 251 ± 361, respectively.

4 Conclusions

The results of this study demonstrate that IFN-γ concentration increases with long-term anti-*Leishmania* treatment and is associated with clinical improvement in IFN-γ lacking production dogs at the time of diagnosis.

C0966 ASSOCIATION OF TICK-BORNE CO-INFECTIONS WITH SEVERITY OF CANINE VISCERAL LEISHMANIOSIS IN DOGS FROM NORTHEASTERN ARGENTINA

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1 Background

Canine Visceral Leishmaniosis (CVL) is an endemic parasitic disease in Northeastern Argentina. This region is also known to be endemic for tick-borne parasitic diseases, particularly those transmitted by the tick vector *Rhipicephalus sanguineus*, such as *Ehrlichia canis*, *Anaplasma platys*, and *Babesia vogeli*. The objective of this retrospective study was to assess the relationship between seropositive results to tick-borne pathogens and the severity of CVL in a canine patient population for which the full medical history was available.

2 Methods

Serum samples were collected from 112 dogs between 2009 and 2016. Seventy-eight (78) samples were obtained from dogs diagnosed with CVL based on cytology and/or PCR, categorized as symptomatic (n=45) or asymptomatic (n=33), and staged according to LeishVet guidelines. An additional 34 samples were collected from asymptomatic dogs with no evidence of *Leishmania* infection to serve as a control sampling of the common population. All samples were tested by genus-specific Enzyme-Linked Immunosorbent Assays (ELISA) for *Leishmania*, *Ehrlichia* spp, *Anaplasma* spp, and *Babesia* spp. The ELISA seropositive rates for each clinical group were calculated and Chi-square tests were performed to identify statistically significant differences between groups.

3 Results

Leishmania seropositive rates were 76% in the CVL group and 14% in the control group. In addition, seropositive rates for *Ehrlichia* spp., *Anaplasma* spp., and *Babesia* spp. were 13%, 23%, and 14% for the CVL group and 6%, 20%, and 6% for the control group. The frequencies of dogs testing positive to *Leishmania* and at least one tick-borne pathogen were 0%, 32%, and 43% for the control group, asymptomatic CVL group, and symptomatic CVL group, respectively. A statistically significant difference was observed in seropositive rates for *Ehrlichia* spp. between symptomatic and asymptomatic CVL groups (20% vs 3%, P=0.0274).

4 Conclusions

These results suggest that co-infection with tick-borne pathogens such as *Ehrlichia canis* may play a role in the clinical progression of CVL. Prospective studies are needed to further understand this apparent relationship and the associated immunopathology involved.

C0969 EFFECT OF SPLENECTOMY IN THE COURSE OF EXPERIMENTAL LEISHMANIA INFANTUM INFECTION IN HAMSTER

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1 Background

Spleen is involved in all the cases of visceral leishmaniasis (VL) and may have an important role in the control of host response to the parasite. Furthermore, chronic VL is associated with hypersplenism, hematological disturbances and high parasite load that may contribute to the progression of the disease. Hence, splenectomy has been used as a complementary therapy in patients with severe refractory cases of VL. In this work, we studied the course of *Leishmania infantum* infection in hamsters previously subjected to splenectomy.

2 Methods

7 hamsters were subjected to splenectomy and injected with $2x10^7$ metacyclic promastigotes of *L. infantum* into the dermis of the ear. The animals were followed 120 days after infection and euthanized. Retroauricular lymph nodes, ear skin, lung, liver, kidney and intestine were examined by conventional histology.

3 Results

Liver granulomas were observed in 6 out of 7 splenectomized-infected hamsters and in 4 out of 8 non-splenectomized-infected hamsters (P=0.28). The score for liver granuloma density was 1.3±0.9 in splenectomized-infected and 0.9±1.1 in non-splenectomized-infected animals (P=0.36). Portal tract inflammatory infiltrate was present in all 7 splenectomized-infected hamsters and in 5 out of 8 non-splenectomized-infected hamsters (P=0.03). The portal inflammatory infiltrate was more intense in splenectomized-infected hamsters than in non-splenectomized-infected animals. Granulomas were also more frequent in the ear-draining lymph nodes of splenectomized-infected (6/7) than of non-splenectomized-infected hamsters (0/8, P=0.02).

4 Conclusions

The data presented herein suggest that splenectomized hamsters develop a more severe form of VL than non-splenectomized animals.

C0970 RECURRENT VISCERAL LEISHMANIASIS IN KIDNEY TRANSPLANT RECIPIENT: A CASE REPORT

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1 Background

The incidence of adult visceral leishmaniasis (LV) has increased in recent years because of the growing number of organ transplantations and the improvement of associated immunosuppressive treatments. Moreover, transplant recipients seem to have an increased risk of disease recurrence or reinfection.
In this study, we report the case of a recurrent visceral leishmaniasis in kidney transplant recipient.

2 Methods

A 35-year-old Tunisian man, with end-stage renal disease which etiology was not determined, received a kidney allograft in 2010. He originally came from the North of Tunisia (Tunis), where he lived, before and after transplantation. Immunosuppression was induced with mycophenolate mofetil (MMF). He was hospitalized in June 2014, four years after transplantation, with complaints of high fever, diarrhea and abdominal pain. Physical examination revealed splenomegaly and laboratory tests revealed pancytopeny. The serum protein electrophoresis revealed a polyclonal γ-globulin pattern. Diagnosis of VL was done by direct finding of the parasite in smears of bone marrow, an increase of anti-*Leishmania* antibody and PCR positive result.
This episode was successfully treated with 0,5 mg/kg of Amphotericin B for 32 days. At that time, immunosuppressive treatment was decreased. After the course, the PCR was negative.
However, three months later, the patient had to be hospitalized for a new episode of VL that was confirmed by positive peripheralblood PCR for *Leishmania.* Hewas treated with Glucantime®, a pentavalent antimonial, at 20 mg/kg/day. However, he presented at the 7th day a pancreatitis. The antimonial was stopped and Amphotericin B was initiated at the dose of 35 mg per day and was continued for a total of 58 days. PCR became negative, with complete resolution of clinical and laboratory findings. Since then, there has been no evidence of relapse.

3 Conclusions

VL may complicate the clinical course of organ transplantation and can be fatal, particularly when untreated. Relapses may occur after the completion of the apparently effective treatment. But most case series have relatively short follow-up periods and do not differentiate relapse of disease from reinfection.
Close follow-up and meticulous surveillance are mandatory in leishmaniasis, because relapse is reported in 28.5% of cases, occurring between one month and five years after completion of treatment.

C0979 WHAT ABOUT VISCERAL LEISHMANIASIS IN TUNISIAN ADULTS?

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1 Background

Visceral leishmaniasis (VL) is a parasitic disease, caused by protozoa of the genus *Leishmania*, transmitted by the phlebotomine sandflies. It is endemic in numerous countries, on four continents. In the Mediterranean basin, it mostly affects young children. But, since 1985, its prevalence in adults has increased, significantly in many areas, mainly in Southern Europe, due to HIV infection. Moreover, many cases of VL are reported with other immunosuppressive states, such as neoplasic disease, use of corticosteroids, organ transplantation.
The aim of our study was to reveal the epidemiological, clinical, biological and therapeutic profiles of VL diagnosed in Tunisian adults.

2 Methods

It is a retrospective study conducted between January 2010 and November 2016. It included 132 cases of suspected VL in adult patients. The diagnosis was established by parasite detection in bone marrow smear examination after MGG coloration, or/and serology (Indirect immunofluorescence), or/and PCR.

3 Results

Visceral leishmaniasis was diagnosed in seven cases (5.3%). All patients were men. Their mean age was 39 [20-60 years]. They came from the north of Tunisia. Three patients have received kidney transplantation. The others have no history of immunosuppression. Splenomegaly and fever are the major clinical symptoms in all cases. Episodes of diarrhea were found in two cases (two renal transplantation recipients). Six patients had pancytopenia.
The diagnosis was made by:

- Serology (positive for all cases)
- Finding Leishmania in all bone marrow smears (four cases)
- PCR when it was performed (Three cases)

Five patients were treated with a pentavalent antinmonial (Glucantime®). However, one patient presented a pancreatitis and the antimonial was substituted by liposomal Amphotericin B (Ambisome®). Three patients were treated by conventional Amphotericin B. The evolution was fatal for one case.

4 Conclusions

Visceral leishmaniasis is increasing among adults in Tunisia. It is mainly an opportunist disease. But in endemic areas, cases in immuncompetent patients are possible. An early diagnosis is essential for establishing an adequate treatment.

C0994 IMMUNE AND PATHOLOGICAL PARAMETERS IN BALB/C MICE INFECTED WITH DIFFERENT DOSES OF LEISHMANIA (LEISHMANIA) AMAZONENSIS

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1 Background

Currently, several experimental vaccines against leishmaniasis have been developed; these often are ineffective due to the high number of parasites employed in the challenge, resulting in a high susceptibility of the mice. Since the dose can influence the development of the disease, the aim of this study was to investigate the impact of the inoculation of different parasite dose (low, medium and high) on immunopathological response in BALB/c mice after infection with *L. (L.) amazonensis* (MHOM/VE/84/MEL).

2 Methods

Five mice BALB/c per group were intradermally injected into the right footpad with different inoculum of *L. (L.) amazonensis* promastigotes per mouse (0; 10^2; 10^4; 10^6). During 13 weeks their swelling footpad were evaluated. After 13 weeks of infection, levels of anti-*Leishmania* total IgG, IgG1 and IgG2a antibody were determined in the sera from each animal by enzyme-linked immunosorbent assay. At the end of protocol (week 13) animals were killed, splenic index was evaluated and the percentage of CD11b+ cells at infection site and spleen was detected by flow cytometry.

3 Results

These show that using 10^4 and 10^6 parasites per mice, an increase in footpad swelling was observed accompanied by high levels of total IgG. Higher levels of IgG1 than IgG2a isotype with a significant increase of splenic index was shown. Only animals infected with the highest dose of *L. (L) amazonensis* promastigotes showed a significant increase of percentage of CD11b+ cells at spleen, and animals infected with moderated (10^4) and high (10^6) doses showed this effect at infection site. Inoculation of the lowest dose of *L. (L.) amazonensis* (10^2) did not lead to the pathology development. These animals showed no footpad swelling and their anti-*Leishmania* antibodies levels, spleen index and the amount of inflammatory cells at spleen and infection site were similar to uninfected group.

4 Conclusions

According to results, it can be concluded that the infection of susceptible BALB/c mice with a high (10^6) and medium (10^4) inoculum results in progressive disease with a predominant Th2-like immune response, being 10^4 parasites per mice a good choice to be used in our experimental vaccines studies.

C1009 CONTRASTING EFFECTS OF IL-33 IN RESPONSE TO LEISHMANIA DONOVANI INFECTION IN THE SPLEEN ACCORDING TO GENETIC BACKGROUND

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1 Background

Little is known about the determinants leading either to progressive visceral leishmaniasis (VL) or to asymptomatic carriage. We previously showed that IL-33 had a deleterious effect on the hepatic immune response to *L. donovani*. As the spleen is also a major target organ during VL and is often associated with clinical relapse, we aimed to describe the role of IL-33 and its receptor ST2 in this organ, using various mouse models.

2 Methods

In several experiments, BALB/c wild type (BWT) or BALB/c ST2-deficient ($ST2^{-/}$) mice, C57BL/6 wild type (B6WT) or C57BL/6 IL-33-deficient ($IL\text{-}33^{-/-}$) mice, BWT and B6WT treated with recombinant il-33 (BWT+rIL-33 and B6WT+rIL-33), or not treated (NT), were infected intraperitoneally with 10^8 *L. donovani* promastigotes and sacrificed at day 15 (D15), D30 or D60 post-infection to collect spleen for histological and transcriptomic analysis.

3 Results

Both IL-33 and ST2 mRNA were highly induced in BWT mice and peaked on D30 (x6.6 and x7.6, respectively, confirmed by immunohistochemistry), whereas they were only moderately induced in B6WT (x2.2 and x1.7, respectively). The kinetics of infection over time also differed, with increasing and decreasing parasite loads in BWT and B6WT, respectively. In both genetic backgrounds, parasite loads were lower in case of deficient IL-33/ST2 pathway ($ST2^{-/-}$ or $IL\text{-}33^{-/-}$), compared to their WT counterparts, and this was associated with a trend towards higher expression of Th1 cytokines (IFN-g, IL-12) in deficient mice. Treatment with rIL-33 had contrasting effects, with an early control of infection in B6WT+rIL-33, but not in BWT+rIL-33 ($p<0.01$ compared to NT BWT), and the opposite effect on D60, with higher parasite loads in B6WT+rIL-33 ($p<0.05$) despite increased Th1 response (IFN-g, IL-12, CXCL10, IL1-b), and decreasing parasite loads in BWT+rIL-33 despite abolition of Th1 response.

4 Conclusions

Taken together, these results underline the complex interplay of the IL-33 ST2 axis in the immune response to *L. donovani* according to genetic background, but confirm that the lack of IL-33/ST2 pathway warrants a better immune response during VL whatever the genetic background.

C1065 UNUSUAL BALANITIS PRESENTATION IN A NATURALLY INFECTED DOG BY LEISHMANIA

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1 Background

Canine visceral leishmaniasis (CVL) is an infectious disease caused by *Leishmania infantum chagasi*. The host immune response and the parasite genetic background may influence directly on the onset of CVL, as well as on its prognosis. It is considered a chronic and severe illness, and the clinical manifestations are usually non-specific and may be extremely divergent from one animal to another. Furthermore, sometimes it may appear unusual signs or sites of lesions that can cause a misleading diagnosis. The aim of this report was to describe an atypical lesion on the penis caused by *Leishmania* infection.

2 Methods

A 1-year-old, intact male mongrel dog was attended at the Universidade Estadual do Maranhão Veterinary Hospital, for evaluation of a mass located at the glans penis, similar to a typical canine transmissible venereal tumor (TVT) lesion. Fine-needle aspiration was performed, and the smears were prepared and stained by the Romanowsky method. Unexpectedly, the cytological analysis showed the presence of intrahistiocytic *Leishmania* amastigotes shapes . An excisional biopsy of the lesion was performed and this fragment was collected and fixed in 10% neutral buffered formalin, and further processed for histological and immunohistochemistry (IHC) analyses.

3 Results

Cytology showed an mononuclear inflammatory infiltrate associated to the presence of *Leishmania* amastigotes, free in the preparation or within the histiocytes; and the absence of malignant cells, which excluded the possibility of being a TVT case. Besides cytological identification, histological and immunohistochemistry examinations confirmed the presence of the parasite, diffusely distributed throughout the tissue.

4 Conclusions

This report demonstrates a case of an asymptomatic infection caused by *Leishmania* sp., with an atypical presentation similar to TVT, a very common type of dog transmissible neoplasia. Because of this similarity, the accurate diagnosis of canine leishmaniasis can be masked, which should serve as a warning for clinical and pathologists veterinarians.

C1066 TISSUE LESIONS AND DEATH IN HUMAN VISCERAL LEISHMANIOSIS. AN AUTOPSY-BASED STUDY COMPARING PATIENTS WITH AND WITHOUT THE DISEASE

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1 Background

A variety of tissue lesions is associated with human visceral leishmaniosis (VL). However, any lasting disease is associated unspecific tissue changes that may also contribute to death. In this study, we compare the tissue changes associated with death in patients with and without VL.

2 Methods

Autopsy records and histological slides from 18 patients with VL and 31 age matched controls that died of other diseases were obtained from the Edgar Santos Hospital, Federal University of Bahia, Salvador, Brazil. Details of clinical history and laboratory test results were collected from the autopsy records. The histological slides were reviewed by a pathologist without knowledge of the clinical data from the patient.

3 Results

The male:female ratio was similar between patients with (11/18) or without (18/31) VL. Disease duration was similar between both groups. Patients with VL had higher frequency of diarrhea (P=0.04), more intense anemia (P<0.03), serum dysproteinemia (P<0.01) and lower WBC counts (P<0.01) than patients without VL. Bleeding and co-infections were the main cause of death in both patient groups. However, bleeding was more frequent as cause of death of patients with (5/18) than in patients without (4/31) VL. The weight of spleen (P<0.01), liver (P=0.01), pancreas (P=0.04) and Kidney (P=0.02) were higher in patients with than in patients without VL. Histological review showed a high frequency of splenic lymphoid atrophy in patients with (8/11) than in patients without (6/14) VL.

4 Conclusions

Besides the usual changes described in patients that dye of VL, bleeding and splenic lymphoid atrophy emerge as the most distinctive changes associated with death in these patients.

C1070 MIR155 GENE DEFICIENT MICE FAIL TO MOUNT AN EFFICIENT TH1 RESPONSES BUT CONTROL THE EXPERIMENTAL VISCERAL LEISHMANIASIS CAUSED BY L. DONOVANI.

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1 Background

Micro RNAs are becoming increasingly investigated in the study of immune regulation during disease because of their impact on gene expression. Of particular interest is micro RNA 155 (miR155) which is vital for T cell mediated immune responses, and Th1 associated IFN- γ production. Since IFN-γis required for a protective host response against infection with *Leishmania*, we investigated the role of miR155 during the course of *L. donovani* infection.

2 Methods

Mir155 KO and WT C57BL/6 mice were infected with *L. donovani* amastigotes, and disease progression as well as immune responses were monitored at 15, 40, 60, 90 and 120 days post infection.

3 Results

L. donovani infected miR155KO mice displayed significantly higher parasitic loads in their livers and spleens compared to WT controls but they eventually resolved the infection. As expected, *Leishmania* antigen (LmAg) stimulated spleen cells from miR155KO mice produced significantly less IFN-γ compared to WT controls. Interestingly, infected livers from miR155KO mice expressed significantly higher gene transcript levels of IFN-γ and NOS2 suggesting that distinct tissue-specific host immune mechanisms are involved in the infected spleens and livers of miR155 mice. Furthermore, miR155KO mice contained more PD-L1 expressing inflammatory monocytes in their livers and spleens compared to WT controls and blockade of inflammatory monocyte recruitment in these mice using a CCR2 antagonist significantly reduced parasitic loads.

4 Conclusions

In conclusion, our results reveal that although miR155 is involved in controlling parasite loads in the liver and spleen during *L. donovani* infection, it is not required for eventual disease resolution. Furthermore, our data show that miR155 may play distinct roles in regulating IFN-γ production in spleen versus liver during *L. donovani* infection.

C1071 CORRELATION BETWEEN CD4+ AND CD8+ T CELLS FROM PERIPHERAL BLOOD AND SPLENOMEGALY IN DOGS NATURALLY INFECTED BY LEISHMANIA (LEISHMANIA) INFANTUM

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1 Background

Canine visceral leishmaniasis is characterized by the inability of lymphocytes response to *Leishmania* antigen. Thus, the quantification of $CD4^+$ and $CD8^+$ T cells in peripheral blood in dogs with visceral leishmaniasis (VL) is relevant to investigate the immunologial aspects of the illness. Spleen is one major organ affected by *Leishmania* infection and suffers a marked morphological and cellular imune profile alterations which are associated with resolution or progression of VL. Since studies have demonstrated in human and canine VL the importance of ultrasonographic findings in the diagnosis VL and its correlation with severity of the disease, we propose to evaluate $CD4^+$ and $CD8^+$ T cells in peripheral blood of dogs naturally infected by *Leishmania (Leishmania) infantum* in association with splenic size.

2 Methods

Twenty dogs with VL were clinically evaluated and diagnosticated by parasitological test. All them were examined by abdominal ultrasonography. Blood samples were collected for haematological and biochemical analysis. $CD4^+$ and $CD8^+$ T cells from peripheral blood were quantified by flow cytometry.

3 Results

The main clinical features of the dogs were lymphadenopathy 100% (20/20), skin lesion 75% (15/20), pale mucous membrane 45% (9/20), onychogryphosis 40% (8/20), ocular lesion 40% (8/20) and weight loss 35% (7/20). The main haematological findings were anaemia in 75% (15/20) and thrombocytopenia in 50% (10/20). Leukocytosis was observed in 30% (6/20) and leukopenia in 5% (1/20) of dogs. Biochemical changes most frequent were increased alkaline phosphatase in 60% (12/20), hypoalbuminaemia in 50% (10/20), hyperproteinaemia in 75% (15/20) and hyperglobulinaemia in 85% (17/20) of cases. The main ultrasonographic changes were hepatosplenomegaly in 55% (11/20) of dogs VL. Parenchymal lesion was not seen in both liver and spleen of all animals. There was positive correlation between $CD4^+$ and $CD8^+$ T cells quantity from peripheral blood of animals ($r= 0.4263$, $P= 0.0344$, Spearman´s test). Interestly, there was a positive correlation between $CD4^+$ and $CD8^+$ T cells quantity from peripheral blood and size of spleen ($r= 0.4334$, $P= 0.0319$ and $r= 0.5102$, $P= 0.0128$, Spearman´s test, respectively).

4 Conclusions

Determination of $CD4^+$ and $CD8^+$ T cells profile and spleen size may help in the establishment of additional tools for follow up the immmunological status in the canine VL.

C1081 INCREASE OF CD14+CD11B+ CELLS IN BONE MARROW AND ITS ASSOCIATION WITH ANAEMIA IN CANINE VISCERAL LEISHMANIASIS

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1 Background

Hematological disturbances are hallmark of canine visceral leishmaniasis (VL) caused by *Leishmania (Leishmania) infantum* being anaemia the most common alteration. Lately studies have been shown that the genesis of hematological alterations in canine VL are associated with bone marrow dysplasia. Macrophage expansion is present in bone marrow of *Leishmania*-infected animals that may have a role in the alterations in haemopoiesis. Since our group have observed dyshaematopoiesis and an increase of macrophage in bone marrow in canine and experimental VL and since canine bone marrow-macrophages are characterized by expression of CD11b and CD14 markers,we aimed to evaluate the monocyte/macrophage $CD14^+CD11b^+$ cells in bone marrow of dogs naturally infected by *L. (L.) infantum* to analyze their correlation with development of anaemia.

2 Methods

We examined 20 *L. (L.) infantum* naturally -infected and four control dogs. All dogs were clinically evaluated and the diagnosis confirmed by parasitological test. Blood samples were collected for haematological analysis and bone marrow aspirates to quantify $CD14^+CD11b^+$ cells by flow cytometry.

3 Results

The main clinical features in 20 dogs were lymphadenopathy present in 20 (100%), skin lesion in 15(75%), pale mucous membrane in 9 (45%), onychogryphosis in 8 (40%), ocular lesion in 8 (40%) and weight loss in 7 (35%) animals. The main haematological findings were anaemia present in 18 (90%) and thrombocytopenia in 10 (50%) dogs. Leukocytosis was observed in 6 (30%) and leukopenia in one (5%) dogs. Flow cytometric analysis showed that $CD14^+CD11b^+$ were increased in bone marrow of infected dogs compared with control dogs ($P= 0.0207$, Mann Whitney test). Interestingly, there was a negative correlation between $CD14^+CD11b^+$ cells and peripheral red blood cell counts ($r= -0.3800$, $P= 0.0492$, Spearman´s test).

4 Conclusions

The increase of monocyte/macrophage in bone marrow may affect the dynamic of hematopoiesis that indicate the need to further study the role of $CD14^+CD11b^+$ cells in the development of anaemia in canine VL.

C1108 CELLULAR IMMUNE RESPONSE IN THE LESION SITE OF PATIENTS WITH AMERICAN CUTANEOUS LEISHMANIASIS DUE TO LEISHMANIA (VIANNIA) SPP. IN AMAZONIAN BRAZIL

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1 Background

American cutaneous leishmaniasis (ACL) constitutes a serious public health problem in Brazil, especially in the Amazon region that is responsible for 37% of notified cases. The interaction between *Leishmania* species and host response results in a wide spectrum of clinical, histopathological and immunopathological presentations in human infection. The clinical manifestations of ACL caused by *L.(Viannia)* spp. are variable, ranging from self-healing skin ulcers to disfiguring mucosal lesions. The aim of this study was to investigate the histopathological changes and immune response in the lesion sites of ACL caused by species of the *Viannia* subgenus from the Amazonian region of Maranhão State, Brazil.

2 Methods

The cellular density was determined through immunohistochemistry of CD68$^+$, lysozyme$^+$, CD1a$^+$, factor XIIIa$^+$, CD4$^+$, CD8$^+$, CD56$^+$, interferon (IFN)-γ$^+$, and inducible NO synthase (iNOS)$^+$ cells in lesions of 22 patients with ACL caused by *Leishmania* (*Viannia*)spp.

3 Results

Histopathological analysis showed a dense mononuclear inflammatory infiltrate in the dermis composed of lymphocytes, macrophages, plasma cells, and granulomas in the majority of the samples. The activated macrophages density (4824 cells/mm^2) was higher than inactivated macrophages (3459 cells/mm^2) in the lesions. In the epidermis, the densities of Langerhans cells (CD1a$^+$) were 310 cells/mm^2, while in the dermis the densities of dermal dendritic cells (factor XIIIa$^+$) were 634 cells/mm^2. The CD8$^+$ T lymphocytes density (2374 cell/mm^2) was higher than CD4$^+$ T lymphocytes (1268 cell/mm^2). By comparing the immunostained cell population according to the *Leishmania* species that caused ACL, was observed a higher density of IFN-γ-expressing cells (P=0.034) in patients infected by *L. (Viannia)* sp. when compared to *L. (V.) braziliensis*. The evaluation of these markers according to the time of infection did not show any differences in the cell densities.

4 Conclusions

Our data showthat *L.* (*Viannia*) *sp* infection leads to favorable immune response to the host which there are predominantly activated macrophages (lysozyme$^+$), dermal dendrocytes (Factor XIIIa$^+$), CD8$^+$ T and IFN-γ$^+$ cells in the lesion site, resulting in a low parasitism. However, the regulatory T cells and the immunoregulatory cytokines TGF-β and IL-10 can be responsible to maintain the inflammatory process and the parasite persistence.

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C1111 SERUM LEVELS OF PROSTAGLANDIN E2 AND LEUKOTRIENE B4 IN DOGS WITH VISCERAL LEISHMANIASIS

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1 Background

Some eicosanoids as Prostaglandin E2 (PGE2) and Leukotriene B4 (LTB4) display an important role during cutaneous and visceral leishmaniasis (VL) infection. There are results showing that PGE2 benefits parasite survival and LTB4 is related with parasite killing. We hypothesize that not only the unbalanced immune response but also the chronic production of inflammatory mediators are the involved in the development of multiple organ lesions and disease progression resulting in emaciation. The aim of this study was to evaluate serum levels of PGE2 and LTB4 in dogs with VL and varied corporal score.

2 Methods

Naturally infected dogs with a mean age of 1-5 years, both sexes and diagnosed by ELISA were included in this study. The determination of LTB4 and PGE2 concentrations in serum was performed by ELISA kit (*R&D Systems*®). 27 dogs were enrolled into the group of infected dogs (GA) and 6 uninfected dogs into the control group (GB). These animals were grouped according to their body score (PURINA®). Dogs that showed emaciation (GC1,n=12,score 1-2) and dogs with good body condition (GC2, n=15, score 3-4), and control group (GB, n=6).

3 Results

There was no difference between the amount of LTB4 in the serum when compared to dogs grouped according to the body score. There was a significant difference ($p<0.0323$) when both, GC1 and C2 were compared to GB. When GA was compared with GB, a significant difference was also observed ($p<0.0071$). The concentration of PGE2 did not show significant difference between dogs with different body scores, and the dogs of GB. There was no difference between serum PGE2 of animals with VL.

4 Conclusions

Our results shows that there is a high concentration of LTB4 in serum of dogs with VL. The overproduction of LTB4, would stimulates the production of ROS and NO, and the polarization of immune responses for Th1 and Th17 pattern. It would be expected that dogs with good body condition, presented high levels of serum LBT4. The pro-inflammatory functions of LTB4 would be contributing for enhancer of leukocyte functions, including cytokine secretion with inflammatory effect as described for IFN-γ and TNF-α, in the spleen, bone marrow and lymph node. Interestingly PGE2 possess potential anti-inflammatory effects in late phases of VL. The lack of modulator effect of PGE2 on inflammation induced by LBT4, would result in inappropriate systemic inflammatory response that contribute to the worsening of the clinical presentation of the disease.

C1112 LESION MORPHOMETRICS AND HISTOPATHOLOGICAL PATTERNS IN PATIENTS WITH LOCALIZED CUTÁNEOS LEISHMANIASIS

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1 Background

Histopathological patterns may classify different stages in the pathogenesis of American cutaneous leishmaniasis, and morphometrics is a precise tool to measure cell and tissue structures in a disease. Our aim was to perform a morphometric analysis of lesions and relate the results with the histopathological patterns of Ridley and Magalhaes in patients with localized cutaneous leishmaniasis.

2 Methods

Eighteen patients with localized cutaneous leishmaniasis were studied. Lesion samples were stained with hematoxylin/eosin, and were classified according to the histological patterns of Ridley (1976) and Magalhaes (1983). For morphometry, images of the histological sections were obtained to determine the following variables: epidermal thickness , granulomatous infiltrate area (GIA), necrosis area and cell count of the inflammatory infiltrate from three GIAs distinct from the dermis.

3 Results

The predominant histological pattern according to Ridley was IV (61%) and according to Magalhaes were the patterns III and IV, with 39% each. Measurement of epidermal thickness and cell density of GIA fail to show statistically significant differences regarding the histopathological patterns. Necrosis was present in 8 lesions = 44.44% of patients. According to Ridley, Necrosis predominates in patterns III and V of Ridley, and in pattern V of Magalhaes ($p < 0.0001$). The greater area of ??the infiltrate was evidenced in patterns IV and V of Ridley and in pattern V of Magalhaes ($p < 0.02$). The multivariate analysis of principal components showed a positive correlation between the variables regarding histopathological patterns: 1) For Ridley: variables were associated in group 1: lesion size and thickness of epidermis, group 2: leishmanine and GIA, group 3: necrosis and cell density of GIA. In addition, a negative correlation was observed between the time of evolution and GIA (Spearman $r = -0.52$; $p = 0.02$); 2) For Magalhaes: Group 1: lesion size and leishmanine, group 2: GIA, GIA cell density and disease progression time, a negative correlation was observed between leishmanine and GIA (Spearman $r = -0.50$, $p = 0.03$).

4 Conclusions

The predominant histopathological patterns were characteristic of this stage of leishmaniasis. However, this study emphasizes the importance of histological and morphometric indicators for patients belonging to other patterns associated with increased immunopathology, such as those cases that may evolve to Mucocutaneous Lesihmaniasis.

C1113 BONE MARROW AND PERIPHERAL BLOOD IN SEVERE VISCERAL LEISHMANIASIS

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1 Background

Visceral leishmaniasis (VL) is a protozooan disease caused by *Leishmania infantum* in Americas and *Leishmania donovani* in Indian subcontinent and in East Africa, regions where the disease is endemic. VL can be classified according to gravity. Patients with severe disease present classical manifestations as fever, anemia and hepatosplenomegaly besides an intense inflammatory condition, presence of bleeding and infections. In Brazil, 5 to 10% of patients with VL presented fatal outcome. To understand the severe disease pathogenesis is important to the development and application of new therapies. Thus, this research aimed to verify the bone marrow (BM) and peripheral blood (PB) role in VL pathogenesis, in particular, of severe disease.

2 Methods

The present study was composed by 29 patients with symptomatic VL (VL group), 12 patients with clinical suspicion of VL (no VL group/non-healthy control). Patients were evaluated for clinical and laboratorial parameters, membrane surface and intracellular of leucocytes immunophenotyping, besides plasmatic cytokines and BM supernatant dosage.

3 Results

VL patients presented an increase in B lymphocytes and neutropenia in PB, an increase in T CD4 Lymphocytes in PB and BM when compared to non-healthy controls. With respect to circulating cytokines, VL patients presented higher IL-6 and IL-10 levels in PB when compared to the control group, while in BM were recorded higher IL-6, IL-10, INFγ, TNFα e IL-1β levels. Concerning to cytokines expression by leucocytes, the PB monocytes of VL patients produced significantly more IL-10 when compared to the control group. The IL-6 expression increase by B lymphocytes, monocytes and T CD8 Lymphocytes was observed only in VL patients PB when compared to the control group. T CD4 cells presented higher IL-6 expression in PB and BM. T $CD8^+IL6^+$ and T $CD4^+IL6^+$ lymphocytes presented positive correlation with IL-6 plasmatic levels.

4 Conclusions

The results obtained in this study allow us to suggest that bone marrow and peripheral blood of VL patients presented significantly higher inflammatory and immunosuppressive cytokine levels. However, T lymphocytes in these compartments appear to exhibit different behavior.T lymphocytes, in severe patients, when present in BM they exert regulatory activity because only in PB it was recorded a significant IL-6 expression by T cells, this cytokine has inflammatory activity and it is associated with disease severity.

C1115 IS CANINE EHRLICHIOSIS CAUSING PROGRESSION OF CLINICAL LEISHMANIOSIS IN DOGS?

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1 Background

Canine ehrlichiosis (CE) and canine leishmaniosis (CanL) are systemic diseases caused by intracellular pathogens that have been shown to co-infect dogs, potentiating the pathogenesis of each disease. CE is caused by gram-negative bacteria including *Ehrlichia canis*, *Ehrlichia ewingii* and *Ehrlichia chaffeensis*. CanL is caused by the kinetoplastid obligate intracellular protozoan parasite, *Leishmania infantum*. These microorganisms have been hypothesized to alter the host immune response in ways that increase progression to clinical disease. The specific relationship of *Ehrlichia* infection as a risk factor in causing progression of clinical leishmaniosis has not been rigorously evaluated controlling for confounding variables. In this longitudinal case-control study we evaluated how co-infection with *Ehrlichia spp*. impacted progression of CanL.

2 Methods

200 dogs were studied longitudinally over 9 months to determine the causal relationship between *Ehrlichia* infection and development of clinical leishmaniosis. The cohort included dogs from seven states and ten kennels across the United States. Dogs were tested via serology and PCR prior to, during the middle of, and after tick season. IDEXX SNAP® 4Dx®Plus Test, confirmatory ELISA and PCR were used to identify infection with specific species of *Anaplasma*, *Ehrlichia*, *Borrellia, Rickettsia spp.*,*Bartonella spp.*,.and *Hepatozoon spp.* All dogs were evaluated for clinical signs via physical examination by licensed veterinarians. Each dog's age, sex, core vaccination status, deworming status, topical insecticides, geographic location, and *Leishmania* status (determined via PCR, serology, and clinical signs) was obtained. A backward stepwise multivariate logistic regression will be used to assess the data and determine significant risk factors.

3 Results

Based on previous findings from case reports we hypothesize thatdogs infected with *Ehrlichia spp*. have a significantly higher risk of developing clinical CanL and vice versa.

4 Conclusions

This study is important in the United States, where CanL transmission is mostly vertical, mom to puppies, as well as in areas endemic for vector transmitted CanL. Prevention of tick-borne diseases, and particularly ehrlichiosis could lead to a significantly increased lifespan for dogs. These findings will additionally contribute to better understanding the factors that lead to progression of human leishmaniasis by preventing immune-response altering comorbid infections.

C1117 BONE MARROW FAT AS BODY CONDITION AND NEGLIGENCE PARAMETER FOR DOGS WITH VISCERAL LEISHMANIASIS

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1 Background

Visceral leishmaniasis (VL) can lead to weight loss and emaciation of dogs, and the presence of other infectious diseases associated with malnutrition can contribute to worsening of clinical signs. The law in Brazil determines that in the case of a positive diagnosis of VL the dog must be euthanized. Treatment is not recommended because it does not lead to clinical cure and there is no evidence of complete elimination of the parasite. Nonetheless, despite the risk of violating the law, owners decline euthanasia procedures performed by the Zoonosis Control Center (CCZ) of Araçatuba - SP and keep the sick dogs, sometimes under inhumane conditions Seropositive dogs from the CCZ were grouped as asymptomatic/oligosymptomatic and multisymptomatic. Our hypothesis was that dogs with low BM fat content would be found at boundaries of the city, where the diagnosis of VL is more prevalent and the socioeconomic conditions are poor.

2 Methods

Twenty-three adult dogs, both sexes, naturally infected with Leishmania spp, were separated according to PURINA body score 1-2 (n = 14) and 3-4 (n = 9) After euthanasia and necropsy, the femur and humerus were sawn, from which the bone marrow was removed and homogenized. Approximately 1g of lipid material was extracted by ethyl ether extraction, following a Soxhlet model, in addition to three grams of bone marrow that were homogenized in 8 mL of distilled water and centrifuged in a 15mL (4000 rpm, 5 min) for measuring the amount of fat (in mm).

3 Results

The mean fat content of the samples of animals of score 1-2 was 1.86 mm (0-3.5). This value was significantly lower ($p < 0.05$) than the observed in dogs of score 3-4, whose average amount was 4.94 mm (2-17). Regarding the Soxhlet quantification, animals with a lower body score had 24.51% (10.68-45.06%) of fat in the bone marrow, differing ($p < 0.05$) from the animals with the best score, whose mean Was 57.61% (20.66-81.56%)

4 Conclusions

In a study of animals with good body score, is reported a mean percentage of fat (59.4%) similar to that observed in the dogs with the best score of this experiment. Bone marrow fat serves as an effective parameter for determining body conditions, serving as a basis for neglect studies in cases involving forensic veterinary medicine. The observation of dogs in these conditions serves as an alert for the adoption of educational policies, aiming to reduce animal abuse.

C1125 INNATE LYMPHOID CELLS IN PERIPHERAL BLOOD OF PATIENTS WITH AMERICAN CUTANEOUS LEISHMANIASIS

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1 Background

Innate lymphoid cells (ILCs) are a described heterogeneous cell population classified into three groups based on the expression of specific transcription factors: ILC1(Tbet+) includes conventional NK cells and other non-NK ICLs, responding to IL-12 and IL-18 and producing IFN-g; ICL2 (GATA3+), also called natural helper cells, responding to IL-33 and IL-25, producing IL-5, IL-9, IL-1; ILC3 (ROR?t+) includes tissue inducers and those expressing the natural cytotoxicity receptor (NCR), responding to IL-1beta and IL-23, producing IL-22 and IL-17.
ILCs contribute to inflammation and modulate adaptive immunity; however their characterization in the clinical spectrum of American Cutaneous Leishmaniasis (ACL) has not been performed.

2 Methods

Circulating ILCs were characterized using flow cytometry in 20 patients (LCL = 7, MCL = 10, ICL= 3, DCL = 3), and controls from endemic area (CE = 9) and non - endemic area (CS = 8), immunophenotyping as follows: ILC 1 ($CD3^-$T-bet^+), ILC2 ($CD3^-$GATA-3^+) and ILC3 ($CD3^-$ROR-gt^+).

3 Results

Preliminary results showed a higher proportion of ILC in LCL (5,080 ± 1,331) than CS (1,140 ± 0,5858), CE (1,370 ± 0,1,448) and MCL ± 0.376) ($p<0.05$). ILC3 was more abundant in patients with MCL = 2,480 ± 0.5939 and DCL = 4.300 ± 0.3000 with respect to CS (0.7000 ± 0.2934) and CE (1.900 ± 0.2698) ($p <0.05$).

4 Conclusions

These results suggest that ILC1 in LCL may be associated with disease resolution whereas ILC3 is associated with immunopathology in MCL and DCL patients.

C1143 MULTIVARIATE ANALYSIS OF CYTOKINES AND MOLECULES SUPPRESSING CYTOKINE SIGNALING (SOCS) IN THE MURINE MODEL OF INFECTION WITH LEISHMANIA INFANTUM/CHAGASI

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1 Background

In the BALB /c mouse model of experimental visceral leishmaniasis, the immune response to *Leishmania infantum / chagasi* is organ specific: in the liver the infection is resolved by creating immunity to reinfection, whereas in the spleen the parasite persists, however, molecular patterns associated with chronicity of infection in spleen are not elucidated. OBJECTIVE: Perform a multivariate analysis of the activity of splenic cytokines Th1 (IFN-γ and IL-12), Th2 (IL-4), Th17 (IL-17), Treg (IL-10, TGF-beta), TNF-α, and SOCS-3 and SOCS-5 in BALB /c mice infected with *Leishmania infantum / chagasi*.

2 Methods

Those molecules were evaluated by immunocytochemistry in frozen sections of spleen isolated on days 7, 14, 21, 28, 35 and 42 post-infection (PI) and the parasite load was determined on smears of spleen sections stained with Giemsa. Data was analyzed using multivariate principal components and correspondence analysis dependent on the course of infection.

3 Results

A positive correlation between the variables evaluated was observed in Group-1: Parasitary load, splenomegaly and IL-17; Group-2: SOCS-3 and SOCS-5; Group-3: IL-4, IL-10 and TGF-β; Group-4: TNF-α, IFN-γ, IL-12. TGF-β ($r = -0.5708$ $p = 0.004$) and IL-4 ($r = -0.48$ $p = 0.01$) were negatively correlated with parasite load, whereas IL-12 with splenomegaly ($r = -0.55$ $p = 0.007$). The correspondence analysis represented by 45% indicated that the course of infection (days post-infection) correlates as follows with the cytokines: 1) **Day 1, Day 7**: IL-12; **Day 14**: TNF- α, IL-17, SOCS-3, IL-12. 2) **Day 21**: TNF-α, Parasite load and splenomegaly, IL-17, TGF-β, SOCS-3 and SOCS-5; **Día 28:** IL -12, TGF-β and SOCS-5; **Day 35**: IFN-γ. 3) Chronic infection: TGF-β, SOCS-3 and SOCS-5, IL-10, IL-4.

4 Conclusions

These results suggest that infection by *Leishmania infantum / chagasi* begins with an IL-12 production that is overtaken by TNF-α and the Th17 phenotype that allows parasite multiplication and splenomegaly. A response of regulatory cytokines and SOCS-5 (mainly inhibiting Th2 signaling) is elicited to establish a Th1 response (IFN- ?) that is consistent with the free reduction of parasite burden. Chronic infection is finally associated with SOCS-3 and SOCS-5 favoring the expression of regulatory cytokines.

C1154 IDENTIFICATION OF LEISHMANIA PERUVIANA-RELATED SPECIES CAUSING CUTANEOUS LEISHMANIASIS IN FEDERAL DISTRICT, BRAZIL

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1 Background

Background: Leishmaniasis in Federal District, Brazil, is an emerging disease and it is important to identify the Leishmania species causing cutaneous leishmaniasis in this area.

2 Methods

Methods for Leishmania species identification: ITS1 and *HSP70* gene amplification by PCR and RFLP. The PCR products were cloned into pGEM-T Easy vector (Promega) and submitted to automatic sequencing.

3 Results

Here we present four case reports of ACL patients: 1) SCS, F, 24 years, ulcer with 2cm of diameter on her right ankle. Laboratorial exams (LE): culture and Leishmanin Skin text (LST) positive (10x5mm); indirect immunofluorescence (II): 1:40. Biopsy showed necrotic exudative granulomatous dermatitis. Treatment: N-methyl glucamine (NMG) 20mgsbV/kg/day for 20 days, with healing. After four months of the initial treatment superficial ulcerations developed on the scar. Patient was retreated with NMG20mgsbV/kg/day and pentoxifylline 1200mg/day for 20 days with clinical cure. 2) DLA, F, 6 years resident of Brasilia- DF, ulcer on the right leg with 2X2cm. LE: culture and smear positive; LST positive (7x8mm); II: 1:80. Biopsy showed granulomatous infiltrate where amastigotes were observed. Treatment: NMG20mgsbV/kg/day for 20 days, with complete healing of the ulcer. 3) CCS, F, 62 years, vesicle that developed into an ulcer (3x3 cm) with raised erythematous border on her left leg. Laboratorial exams: positive culture and positive smear for Leishmania spp. Leishmanian skin text (LST): positive (21x20mm). Biopsy showed lymphohistiocytic infiltrate with granulomatous dermatitis. Treatment: N-methyl glucamine (NMG) 20mgsbV/kg/day for 4 days, and 4mgsbV/kg/day for 20 days after interruption for 2 weeks due to low treatment adherence, with complete healing.4) MPC, F, 46 years and resident of Planaltina-GO, ulcer (5X3cm) on her right arm with raised erythematous border associated with local lymphadenitis. LE: LST positive (11x11mm); II: 1:40. Biopsy showed lymphohistiocytic infiltrate. Treatment: NMG20mgsbV/kg/day for 20 days, with complete healing.We identified the four Leishmania cultures isolated from these patients and all four were related to *Leishmania peruviana* species based on the ITS1 and HSP70 gene sequences. Three patients reported that they have travelled to north or northeast of Brazil just before the appearance of the skin lesions and the other lived in the north.

4 Conclusions

We showed that four patients presented *Leishmania peruviana*-related species causing ACL in Brazil.

C1156 FURTHER EVIDENCES ASSOCIATING IGG1 BUT NOT IGG2 WITH THE SUSCEPTIBILITY TO CANINE VISCERAL LEISHMANIASIS DUE TO LEISHMANIA (L.) INFANTUM CHAGASI-INFECTION

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1 Background

American canine visceral leishmaniasis (CVL) is a parasitic zoonosis widely spread throughout Latin America and caused by *Leishmania (L.) infantum chagasi* (=*Leishmania chagasi*). With regards to immunology of canine *L. (L.) i. chagasi*-infection, it is well known that no consistent association has till now been described concerning the IgG1 and IgG2 immune responses and disease resistance in dogs. This study evaluated the meaning of identifying resistance and susceptibility markers to CVL based on these IgG subclasses against *L. (L.) i. chagasi*-infection, taking in account their relationship with the clinical status and specific delayed-type hypersensitivity (DTH) response to *L. (L.) i. chagasi*-antigen of the infected dogs.

2 Methods

This was a cross-sectional study which analyzed a cohort of 50 mongrel dogs with seropositive diagnosis (IFAT-IgG with amastigote *L. (L.) i. chagasi*-antigen from hamster infection and cut-off 80) for CVL, twenty-eight (56%) with a subclinical status (=apparently healthy) and twenty-two (44%) sick, living in endemic area of American visceral leishmaniasis (AVL) in the Brazilian Amazon. The IgG1 and IgG2 responses were measured by ELISA while DTH reaction was elicited by a leishmanin skin test (LST) with crude promastigote *L. (L.) i. chagasi*-antigen. Data analysis used Chi-square and coefficient *r* of Pearson with 95% confidence interval.

3 Results

With regards to DTH reaction and the clinical status of dogs, it was noted that 100% (8) of dogs with positive DTH were from the subclinical group (apparently health), while 100% of dogs with negative DTH were from the sick group. By the other side, it was observed higher IgG2 response compared to IgG1 one in both clinical groups of dogs. However, when this comparison was between both clinical groups, it was recorded that IgG1 response in the dogs from the sick group was higher than that in the dogs from the subclinical one, while IgG2 response in the dogs from the sick group was similar to that in the dogs from the subclinical one. In addition, it was found lower IgG1 response in the dogs from the subclinical group with positive DTH than that in the dogs from the subclinical and sick groups with negative DTH, whereas no difference was found in the IgG2 response in both groups of the infected dogs, subclinical or sick.

4 Conclusions

These findings strongly suggest that IgG1 but not IgG2 response is associated with the susceptibility to CVL due to *L. (L.) i. chagasi*-infection in Brazilian Amazon.

C1176 IN VITRO INFECTIVITY OF LEISHMANIA INFANTUM STRAINS ISOLATED FROM NATURALLY INFECTED DOGS DISPLAYED DISTINCT VIRULENT AND PATHOGENIC PROFILE.

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1 Background

Visceral leishmaniasis (VL) is the most severe manifestation among diseases transmitted by *Leishmania* protozoa. The dog is the main domestic reservoir of the *L. infantum* parasite favoring its transmission in the urban area. Considering the diversity of strains, it is important to understand their behavior *in vitro* and *in vivo*, until even the use of these parasites in several studies. We analyzed *in vitro* and *in vivo* characterization of *L. infantum* isolates from naturally infected dogs in an Brazil endemic region based on infectivity and pathogenicity descriptions.

2 Methods

For this assays, DH82 canine macrophages were infected *in vitro* with different isolates for comparing infectivity. The isolated more and less infective were used for in vivo infection in hamsters. We performed analysis regarding mortality rate, biochemical and hematological parameters, histopathology and parasite load by real-time PCR.

3 Results

It was confirmed that all isolates were *L. infantum*. The cultivation of isolates in DH82 canine macrophages showed distinct profiles of infectivity. We identified isolates with polarized *in vitro* profile, takin in account infection index and parasite load increased or reduced (isolates 616 and 614, respectively). These isolates were selected to *in vivo* evaluation using hamsters, as well as the standard PP75 strain. All the hamsters survived to infection using 616 isolate whereas 614 induced 50% of death. The isolated 614 induced: more evident clinicopathological changes, biochemical abnormalities in the liver and kidney, high levels of inflammatory cells in the liver, high splenic and hepatic parasite loads. We confirmed high variability of pathogenicity and infectivity in *L. infantum* isolates from naturally infected dogs considering *in vitro* and *in vivo* analysis. Surprisingly, it was observed that the isolated less infective *in vitro* was the most infective and pathogenic *in vivo*.

4 Conclusions

This result behind an important contribution to studies using these parasites, such as drug testing, experimental infection studies, among others.

C1181 IMMUNOPATHOLOGICAL CHANGES IN SPLEEN FROM NATURALLY INFECTED DOGS BY LEISHMANIA (LEISHMANIA) CHAGASI

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1 Background

The spleen is the largest lymphoid organ and it is the organ most affected during the course of canine visceral leishmaniasis (CVL). Splenic parasitism exerts a marked influence on the histopathological and functional processes in the spleen during CVL.

2 Methods

Due to the importance of the spleen as a target organ of parasitism and tissue changes due to *Leishmania chagasi* infection, this study evaluated the splenic histopathology in different clinical forms of CVL: asymptomatic dogs (AD, n = 12), oligosymptomatic dogs (OD, n=12), symptomatic (SD, n = 16) and uninfected dogs (UD, n = 11). Parasitism was investigated through anti-*Leishmania* immunohistochemical reaction and Giemsa-stained imprint for evaluation by the Leishman Donovan Units index (LDU). In addition, we evaluated the correlation between the histological and parasitological findings versus immunophenotypic profile and cytokines levels in splenocytes.

3 Results

The histological study by optical microscopy (HE stain) revealed a mononuclear cell infiltrate, predominantly characterized by plasma cells located primarily in the red pulp. The SD group presented higher frequency of capsule inflammation and parasitism by anti-*Leishmania* immunohistochemical assay and LDU index when compared to AD group. In addition, OD and SD groups presented higher intensity of capsule inflammation in relation to AD and NID groups. These results point to a direct relation between splenic impairment and clinical evolution of LVC. Positive correlation was observed between LDU and IL-10 levels, as well as the positive correlation between the percentage of CD4 + cells and the IL-10 levels. In addition to the CD4+ cell percentage, IL-10 production also correlated positively with IFN-gamma levels.

4 Conclusions

Our major results pointed to a central role of spleen from dogs presenting visceral leishmaniasis to induce immunopathological changes that were related to the intensity of clinical manifestations in canine disease.

C1184 T CD8+IL-6+ LYMPHOCYTES ARE ELEVATED IN SEVERE VL PATIENTS

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1 Background

Visceral leishmaniasis (VL) is a protozooan disease caused by *Leishmania infantum* and *L. donovani*, capable of causing severe disease, mainly, in infants less than 1 year of age, adults between 50 and 60 years and HIV/Leishmania infected ones. Severe patients presented higher IL-6 plasmatic levels, severe neutropenia, bleeding and bacterial infections, and IL-6 has been correlated with the presence of bleeding. Considering these severity parameters, it would be of great relevance to identify the severe VL pathogeneses directly related cells. Thus, the present research had as objective to identify severity markers in VL patients.

2 Methods

The present study involved 27 VL patients, evaluated for clinical and laboratorial parameters, membrane surface and intracellular of leucocytes immunophenotyping, besides plasmatic cytokines and bone marrow (BM) supernatant dosage. The VL patients clinical and/or laboratorial results were added to the *Kala-Cal*® program, that allows to calculate death probability, *P* (death).

3 Results

Of these 27 patients, only 4 presented *P* (death) above 15%, the cut-off point used to define the severity value were the higher IL-6 plasmatic levels. The most severe patients presented the higher IL-6 and CXCL-8 levels, and lower IL-12 levels in peripheral blood (PB) and in BM. Less severe patients presented in BM a significantly higher number of $CD14^+IL\text{-}6^+$ cells, $INF\gamma^+$ neutrophils, and $IL\text{-}10^+$ neutrophils. While in PB, the most severe patients presented a significant increase in $CD8^+IL\text{-}6^+$ cells. Only the plasmatic and bone marrow supernatant IL-6 levels presented positive correlation with *P* (death). Only the T $CD8^+IL6^+$ and T $CD4^+IL6^+$ lymphocytes in PB presented positive correlation with plasmatic IL-6 levels and *P* (death).

4 Conclusions

The results obtained in this research suggest that T CD4 and, mainly, T CD8 lymphocytes in PB produce more IL-6 in severe patients, contributing to the increase of plasmatic levels of this cytokine. The stimulus for the increase in IL-6 synthesis by these cells appears to occur in the bloodstream and/or peripheral organs such as the spleen. Under these conditions T $CD8^+IL6^+$ appear to participate significantly in the severe disease pathogenesis and can be considered as VL severity markers.

C1227 IN MICE, ARE THE ADIPOSE TISSUES SANCTUARIES OF LEISHMANIA INFANTUM?

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1 Background

Leishmania (L.) infantum, agent of visceral leishmaniasis, is known to persist in humans when asymptomatic carriage or after treatment of visceral leishmaniasis. We hypothesized that adipose tissue could be a sanctuary of *Leishmania* parasite after treatment.

2 Methods

BALB/c mice were infected with *L. infantum* by intravenous administration. Different typesof adipose tissues were tested for the presence of *L. infantum* by quantitative PCR.

3 Results

L. infantum was detected in abdominal and inguinal (white tissues) and dorsal adipose (brown tissue) tissues 3 months post-infection. Moreover, from these adipose tissues we obtained one positive culture.

4 Conclusions

Adipose tissue may be a reservoir for *L. infantum* to persist for prolonged periods in asymptomatic patients or after apparent clinical cure. Mouse model of *L. infantum* infection may be used to understand the relapse of visceral leishmaniasis and provide new perspectives to the follow-up of patients.

C1231 CD3+CD4NEGCD8NEG (DOUBLE NEGATIVE) T LYMPHOCYTES AND NKT CELLS AS THE MAIN CYTOTOXIC CELLS IN LESIONS OF HUMAN CUTANEOUS LEISHMANIASIS CAUSED BY LEISHMANIA (VIANNIA) BRAZILIENSIS

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1 Background

Cutaneous leishmaniasis (CL) is caused by *Leishmania (Viannia) braziliensis,* which cause an intense tissue inflammation and a skin ulcer that heal spontaneously or after antimonial therapy. The resolution of lesions depends on immune response and cytotoxic cells seem to have an important role for that. The aim of this study is to better understand the role of cytotoxicity-mediated mechanisms that occur during the immune response in lesion milieu, considering distinct cytotoxic cells, such as $CD8^+$, $CD4^+$, $CD4^{neg}$ $CD8^{neg}$ (DN) and $CD4^+CD8^+$ (DP) T lymphocytes, as well as NK and NKT cells.

2 Methods

After lesion tissue maceration, supernatant was collected and cells were assessed to surface markers, CD107a expression and to quantify cytokines and granzyme B concentrations by flow cytometry.

3 Results

Flow cytometry analyses from CL lesions revealed that NKT cells are the major CD107a-expressing cell population committed to cytotoxicity, although we observed high frequencies of cytotoxic $CD4^+$ and DN T cells Analyzing the pool of $CD107a^+$-cell populations, we observed the higher distribution of DN T cells (44%), followed by approximately 25% of NKT cells. Interestingly, NK and $CD8^+$ T cells represented only 3 and 4% of the $CD107a^+$-cell pool, respectively.

4 Conclusions

The cytotoxicity-mediated mechanisms that occur in the lesion milieu of CL patients seem to be ruled mainly by DN T and NKT cells. These findings incite us to rethink the role of classical-cytotoxic NK and $CD8^+$ T cells in the pathogenesis of CL and lead us to have a critical look upon the cytotoxic events that might occur at lesion environment, considering the variety of cell populations, which present cytotoxic profiles in CL lesions.

C1267 DEVELOPMENT OF REAL-TIME PCR ASSAYS FOR EVALUATION OF IMMUNE RESPONSE AND PARASITE LOAD IN GOLDEN HAMSTER (MESOCRICETUS AURATUS) INFECTED BY LEISHMANIA (VIANNIA) BRAZILIENSIS

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1 Background

Cutaneous leishmaniasis is widely spread in Brazil and represents a public health issue, in which *Leishmania (Viannia) braziliensis* stands out as the most prevalent specie. Experimental studies with *L. braziliensis* are scarce, given the resistance of murine infection and the underutilization of the hamster, that despite being the most suitable model, is impaired by the lack of available immunological and molecular supplies. Theaim of this study was to develop and evaluate a SYBR Green RT-qPCR assay to estimate the cytokine gene expression profile in distinct tissues of hamsters infected with *L. braziliensis*. Herein, we standardized a RT-qPCR gene expression assay to evaluate a panel of immunological markers and a qPCR assay to quantify with high sensitivity and reproducibility the parasite load in skin lesions.

2 Methods

Hamsters were infected with 10^5 promastigotes of *L. (V.) braziliensis* and 110 days after RNA and DNA were extracted from skin lesions and lymph node. Gene expression of IFN-?, IL-10, TGF-β TNF, IL-4, IL-6, iNOS and arginase were measured using non-infected animal tissue as calibrator. Parasite load was quantified from DNA extracted from lesion by qPCR targeting *Leishmania* kDNA and normalized by hamster GAPDH, using a SYBR Green-based absolute quantification methodology.

3 Results

PCR efficiencies ranged from 92.3% to 116.4%.Uninfected animals showed higher basal mRNA levels in lymph node for IFN-?, TGF-β, TNF and IL-4 compared to skin. Infected animals exhibited increased expression of all immunological markers evaluated, ranging from 2.7±0.2 for TGF-β to 1018.5±809.0 for iNOS in skin lesions, and 2.4±1.6 for TGF-β to 600.2±666.4 for iNOS in popliteal lymph nodes. Of note, significant higher levels of IFN-?, TNF and IL-10 mRNA were observed in skin in comparison to lymph node, while a lower significant level of arginase mRNA was observed in skin. Parasite loads ranged from 27.0 to 6647.0, with a median of 553.4 [416.7-1504.0] parasites/mg skin equivalents, with lesion size variation from 0.3 to 3.1 mm. Despite the tendency of bigger lesions to present higher parasite load, the correlation observed was not statistically significant.

4 Conclusions

Herein we describe for the first time a sensitive, reproducible and cheaper molecular assay to quantify gene expression of immunological markers and parasite load in skin lesions observing a mixed profile of immune response in the hamster model infected by *L. (V.) braziliensis*.

C1273 BONE MARROW DYSFUNCTION IN LEISHMANIA DONOVANI-INFECTED C57BL/6 MICE REVEALS ASSOCIATION BETWEEN F4/80+ CELLS AND ANAEMIA

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1 Background

Hematological disturbances are a hallmark of human and canine visceral leishmaniasis (VL) caused by *Leishmania donovani* or *L. infantum* infection for which the pathogenesis is poorly known. Mouse models of VL have been extensively used to explore pathogenic mechanisms, but little information is available on hematological dysfunction in this animal model. We therefore investigated the VL-associated hematological alterations in *L. donovani*-infected C57BL/6 mice.

2 Methods

Blood samples were collected for haematological analysis and F4/80, CD169and CD11bexpressing cells in the bone marrow were quantified by confocal microscopy and flow cytometry.

3 Results

At 28 days post-infection, hematological changes characterized by mild anaemia, leucopenia and thrombocytopenia were observed. In bone marrow aspirates, cellular morphological alterations, an increase in myeloid:erythroid ratio, myeloid maturation index alteration, and an erythroid hypoplasia were seen. Moreover, we observed a significant increase in the percentage of lymphocytes and macrophages in infected animals. Histopathological examination of bone marrow biopsy revealed significant increases in cellularity accompanied by infiltration and/or proliferation of mononuclear cells similar to macrophages. By confocal microscopy, these cells were characterized as F4/80$^+$ cells, a typical marker of resident and/or stromal macrophages. By flow cytometry, there was a significant increase in the percentage of F4/80$^+$ cells. Interestingly, there was a negative correlation between F4/80$^+$ cells and hemoglobin levels ($r= -0.7917$, $P= 0.0022$, Pearson´s test). In addition, using additional markers of macrophage subsets such as CD169 and CD11b, we saw a decrease in the F4/80$^+$CD169$^+$CD11b$^+$ population in infected mice.

4 Conclusions

These data suggest *L. donovani*-infected C57BL/6 mouse as a good model for the study of dyshematopoiesis in VL. Further they suggest the role of stromal macrophage in the pathogenesis of hematopoietic alterations during VL to be explored.

C1301 CANINE VISCERAL LEISHMANIASIS: HEMATOLOGICAL AND BIOCHEMICAL EVALUATION OF NATURALLY INFECTED DOGS WITH LEISHMANIA INFANTUM.

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1 Background

Visceral leishmaniasis (VL) in recent years have been increasingly urbanized, with a large number of human and canine cases. The dog is an important reservoir of infection in the domestic environment and main link in the urban transmission cycle of the disease. Therefore, this project has the objective of characterize the clinical aspects, hematological and biochemical profiles of dogs naturally infected with *Leishmania infantum*.

2 Methods

Blood samples from 52 non-breed dogs with Leishmaniosis reagents in the rapid immunochromatography Dual Path Plataform (DPP®) and Enzyme Linked ImmunonoSorbent Assay (ELISA) were collected for hematological and biochemical tests following the protocols of Bioclin kits. After blood collection, the dogs were euthanized and spleen fragments were used in the Polymerase Chain Reaction to characterize the parasite species responsible for the infection.

3 Results

Leishmania infantum was responsible for the infection in all animals evaluated. Dogs were classified according to clinical signs, 29 dogs were symptomatic, and 23 were asymptomatic. A third group composed of nine healthy dogs was used as negative control. Among the infected dogs, macrocytic and normochromic anemia were found in 73.58% of these animals. The mean values of creatinine, total bilirubin, alkaline phosphatase, glutamic oxaloacetic transaminase and glutamic pyruvic transaminase remained in accordance with the reference standards.The infected dogs presented high values in the averages for total proteins and urea and a reduction in the serum levels of albumin.However, after statistical tests, only serum levels of albumin showed a statistically significant difference ($p < 0.05$). The levels were lower in infected animals (symptomatic and asymptomatic group) in relation to uninfected dogs.

4 Conclusions

The clinical evolution of Canine Visceral Leishmaniasis (CVL) in naturally infected dogs promotes clear serological changes and hematological and biochemical parameters, which are directly related to the clinical status of dogs. Thus, the investigation of these laboratory parameters associated with clinical aspects of the disease is extremely important to be considered in clinical practice and in the application of therapies against CVL.

Keywords: *Leishmania infantum*, Hemogram, Biochemical tests.
Support: Bioclin/Quibasa, Belo Horizonte-MG, Brazil; FAPEMIG and CNPq.

C1312 CONTROL OF HUMAN LEISHMANIA BRAZILIENSIS INFECTION: THE ROLE OF NITRIC OXIDE AND REACTIVE OXYGEN SPECIES IN SUB CLINICAL INFECTION AND IN CUTANEOUS LEISHMANIASIS

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1 Background

Human Cutaneous leishmaniasis (CL) caused by *Leishmania braziliensis*, presents an exaggerated Th1 immune response that is associated with ulcer development. Approximately 17% of the individuals living in an area ?? of *L. braziliensis* transmission, and exposure to the parasite, do not show evidence of clinical disease and are considered to have a subclinical (SC) *L.braziliensis* infection. Macrophages are the primary cells infected by *Leishmania* parasites and two distinct groups of oxidants are important in the control of *Leishmania* by these cells: reactive oxygen species (ROS) and nitric oxide (NO). However, it is not known how SC individuals control leishmania infection. The aim of this study is to evaluate the role of ROS and NO in the control of *L. braziliensis* infection by monocytes from CL patients and SC individuals infected with *L. braziliensis*.

2 Methods

Monocytes from CL patients and SC individuals were infected with *L. braziliensis* at 5:1 ratio. The determination of oxidative radicals production was performed by dihidrohodamine 123 (DHR 123) oxidation and evaluated by flow cytometry. The intracellular production of NO and ROS was determined by specific intracellular probes (DAF-FM diacetate and 2DCFDA-HCM). Promastigotes viability was assessed by counting of extracellular motile promastigotes after the inhibition of NO and ROS pathways.

3 Results

After infection with *L. braziliensis*, the expression of the oxidative burst by monocytes from CL patients was higher when compared to monocytes from SC individuals. The production of ROS was higher than the production of NO by monocytes from CL patients and SC group. However, the NO and ROS production was higher in monocytes from CL patients when compared with cells of SC individuals. There is a positive correlation between the production of NO and lesion size in patients with CL and after therapy and cure, a decreased in production of NO and ROS by cells from CL was observed. While in CL group the inhibition ROS pathway allowed the parasite growth, in SC group the inhibition of both pathways had no effect in the parasite viability.

4 Conclusions

These results indicate that in subclinical individuals there is no evidence that NO and ROS molecules are involved in the control of infection. While in CL patients the production of NO seems to be more related to lesion development, ROS participate of parasite control.

C1317 EVALUATION OF EXPERIMENTAL INFECTION OF LEISHMANIA BRAZILIENSIS STRAINS ISOLATED FROM PATIENTS WITH CUTANEOUS LEISHMANIASIS IN MINAS GERAIS, BRAZIL

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1 Background

This study reports intraspecific variations of five native isolates of *Leishmania braziliensis* from patients with typical and atypical manifestations of cutaneous leishmaniasis in a transmission area in the State of Minas Gerais, Brazil.

2 Methods

L. braziliensis preparations containing 10^6 parasites, including the reference strain (MHOM/BR/75/M2903) were inoculated in the right hind paw of male golden hamsters. The lesion development was followed for 30 and 60 days by size measurements. In these same times post infection (PI), samples of skin, spleen, liver and whole blood were investigated to check histopathological changes and parasite burden by parasitological and molecular methods.

3 Results

Four native isolates induced skin lesions in golden hamsters, initially showing rapid development between 18 and 24 days PI, however, the isolated RR 444 was characterized by initial low infectivity in the experimental animals. Histopathological analysis of lesions showed an exudative inflammatory pattern with acanthosis and hyperkeratosis and predominant lymphocytes in the dermis during early infection, while well-defined granulomas and inflammatory infiltrate with predominance of macrophages and plasmocytes were detected at a later time. Amastigotes of all isolates were detected and quantified by direct parasitological method in the skin lesions in both periods PI. In two samples, including the reference strain, the parasite burden decreased sixty days PI, and in four strains these number increased. These results were also observed in quantitative PCR (qPCR) with statistical correlation between the methods (Pearson correlation coefficient). Low parasite burden (qPCR) were observed in the whole blood, spleen and liver, with no alterations in these tissues in the macroscopic examination and in the histopathological analysis.

4 Conclusions

The different patterns of infectivity observed during early infection by *Leishmania braziliensis* strains in hamsters suggests that the modulation in the immune response that occurs in an early phase of the infection could dictate the disease progression and the patogenicity of the chronic phase.

C1318 HOST-MEDIATED MECHANISMS OF PARASITE DISSEMINATION IN VISCERAL LEISHMANIASIS

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1 Background

People are infected with Leishmania donovani when the parasite is deposited in the dermis during the bloodmeal of the sand fly vector. A latent infection without clinical disease is most common, but some individuals develop progressive visceral leishmaniasis.The mechanism of parasite dissemination from the skin and the reason that only a minority of infected individuals develop full-blown disease are not understood, but malnutrition has been identified as a major risk factor for the development of active VL.

2 Methods

We established a model of moderate acute malnutrition by placing BALB/c mice on a diet low in protein, iron and zinc for 28 days. Mice were then infected intradermally with 10e6 metacyclic L. donovani promastigotes. Trafficking of inflammatory cells and parasites from the skin to the draining lymph node (LN) and spleen was determined over the first 72 hrs of infection.

3 Results

Within 3 days of intradermal infection the malnourished (MN) mice showed increased parasite dissemination to the spleen, but a reduced parasite load in the draining LN, suggesting a loss of LN barrier function in the MN mice. There was enhanced capture of parasites by neutrophils and inflammatory monocytes early after delivery to the skin of MN mice. The parasitized neutrophils and monocytes showed increased migratory capacity, increased expression of CCR7, and increased trafficking through the LN to the spleen in the MN host. There was increased expression of CCR7 ligands in the spleen. Parasitized inflammatory monocytes were the primary means of early dissemination from the skin to the spleen.PGE2 production at the site of skin infection was increased in the MN host, and the early dissemination from the skin to viscera was reproduced by delivery of PGE_2 to the site of infection in normal mice. The malnutrition-related increase in parasite dissemination in inflammatory monocytes was abrogated by blocking PGE_2 synthesis.

4 Conclusions

These data suggest a three-component model of parasite dissemination in the malnourished host that involves (1) increased influx of inflammatory cells and parasite capture in the skin; (2) hypermigration of infected monocytes and neutrophils from the skin via expression of PGE2 and chemokines, and (3) increased escape of migrating infected myeloid cells from the draining lymph node. Control programs should consider the potential impact of malnutrition on the large population of subclinically infected people who are at risk of developing VL.

C1326 INTERMEDIATE MONOCYTES ARE ASSOCIATED WITH IMMUNOPATHOLOGY IN CUTANEOUS LEISHMANIASIS PATIENTS

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1 Background

The activation of inflammasomes components leading to high production of IL-1β is associated with the pathogenesis of a variety of inflammatory diseases. Recent work has shown the importance of the inflammasome activation for IL-1β production in murine leishmaniasis and a role for NLRP3 in IL-1β production was reported. Cutaneous leishmanisis (CL) due to *Leishmania braziliensis* infection is an inflammatory disease where skin ulcer development is associated with mononuclear cells infiltrate and high levels of inflammatory cytokines. Therefore, our hypothesis is that CL patients have increased expression of NLRP3 and produce high levels of IL-1β. Intermediate monocytes (CD14+CD16+) are inflammatory cells associated with immunopathogenesis of some inflammatory diseases.

2 Methods

Bone marrow-derived macrophages were obtained from C57BL/6 wild type and knockout mice for CASPASE-1, NLRP3, PYCARD, AIM2 and IL-1R. Peripheral blood mononuclear cells (PBMC) and lesion biopsies were obtained from CL patients. PBMCs and biopsies from CL patients were maintained in culture in presence of SLA for 72 hours and IL-1β and TNF levels determined by ELISA. We also identified the source of these molecules by flow cytometry by stimulating PBMC with SLA for 8 hours andstaining for CD14 and CD16.

3 Results

In our murine experiments we found that IL-1β production in response to *L. braziliensis* was dependent on NLRP3 and CASPASE-1. Our patients' results showed that intermediate monocytes (CD14+CD16+) were the main source of IL-1β and TNF. Treatment of PBMCs from CL patients with inhibitors of NLRP3 decreased IL-1β production.

4 Conclusions

Intermediate monocytes produce high levels of TNF and IL-1β in CL patients. Targeting of NLRP3 inhibited IL-1β production in these individuals. Inhibition of inflammasome activation may be an alternative treatment for CL patients.

C1334 THYMUS IS A TARGET OF L. INFANTUM INFECTION DURING PROTEIN MALNUTRITION

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1 Background

The thymus is one of the most affected organs in several infectious diseases, displaying thymic atrophy and thymocyte depletion. Interestingly, malnutrition share these characteristics. In experimental models, the detrimental effects of malnutrition and infection on immunity have long been recognized. The thymus has been barely studied during malnutrition-infection association, and information is limited to *Trypanosoma cruzi* and viral infections. The aim of this study was to analyze the effects of protein malnutrition and infection with *L. infantum* on the thymus of a BALB/c mice model.

2 Methods

BALB/c mice were fed with control protein (14%) or low protein (4%) isocaloric diets, followed by infection with *L. infantum*. Thymocytes and interstitial fluid of the thymus were isolated. The effects of protein malnutrition and infection on T cell migration were evaluated by measurement of chemotactic factors by qPCR, ELISA and by stimuli-driven chemotactic assays. We compared protein abundance in the interstitial fluid samples by a quantitative proteomics approach using iTRAQ as follows: animals fed 14% protein (CP: iTRAQ 114), animals fed 4% protein (LP: iTRAQ 115), animals fed 14% protein and infected (CPi: iTRAQ 116), and animals fed 4% protein and infected (LPi: iTRAQ 117). Samples were analyzed in a LTQ-Orbitrap-XL.

3 Results

L. infantum infected animals displayed parasites in the thymus. Protein malnutrition modified thymic chemotactic factors diminishing the CCL5, CXCL12, IGF1, CXCL9 and CXCL10 levels in infected animals. However, T cells preserve their migratory capability, as they were able to migrate *ex vivo* in response to chemotactic stimuli, indicating that malnutrition compromises the thymic microenvironment rather than thymocyte migration *per se*. Proteomic analyses showed a total of 280 secreted proteins, 90% of which were secreted by exosomes. LPi animals displayed an increase in the relative abundance of proteins related with lipid metabolism, oxidative phosphorylation and TCA cycle, suggestive of a non-proliferative microenvironment.

4 Conclusions

L. infantum was found in the thymus of infected animals and the malnutrition along with infection affected mainly molecules involved in T cell migration in this organ. In addition, the LPi proteomic profile was related to dysfunctions on cellular migration, proliferation and essential thymic aspects, as thymopoiese, which can contribute for the thymic atrophy observed in malnourished and *L. infantum* infected animals.

C1343 CO-INFECTION WITH TICK-BORNE DISEASES CAUSES CLINICAL PROGRESSION OF LEISHMANIOSIS IN DOGS

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1 Background

Zoonotic vector-borne diseases have dramatically increased in prevalence in both animals and people over the past decade. Little is understood about the magnitude or significance of tick-borne infection on disease progression in animals already infected with *Leishmania infantum*. Canine leishmaniosis (CanL) is enzootic within United States hunting dogs, a population with frequent, repeated, exposure to ticks. As tick-borne diseases have been shown to occur concomitantly with leishmaniosis in dogs, it is important to understand the causal nature of the relationship between tick-borne and *Leishmania* infections in leading to clinical CanL.

2 Methods

200 dogs from Western, Midwestern, Eastern, and Southern regions of the United States were studied longitudinally over 10 months to determine how tick borne diseases impact development of clinical CanL. Dogs were tested via serology and PCR both pre- and post-primary tick season. IDEXX SNAP® 4Dx®Plus Test, ELISA and PCR were used to identify infecting species of *Anaplasma*, *Ehrlichia*, *Borrelia, Rickettsia, and Bartonella spp.* All dogs were evaluated via physical exam for clinical signs of ehrlichiosis, anaplasmosis, and Lyme disease at time 0, 7, and 10 months. Each dog's age, core vaccination status, deworming schedule, topical insecticide treatments, sex, geographic location, and *Leishmania* status (determined via PCR, serology, and clinical signs) was obtained. A backward stepwise multivariate logistic regression was used to determine significant risk factors in the outcome of developing clinical CanL.

3 Results

Dogs infected with at least two tick borne diseases were 10 times more likely to develop clinical leishmaniosis than dogs with no tick borne co-infections (OR: 9.87 95% CI: 2.08-46.76). Dogs were 3 times more likely to develop clinical leishmaniosis in comparison to dogs with only one tick borne co-infection (OR for one tick borne co-infection 3.70 95% CI: 0.35-39.62). The relative contribution of each tick-borne infection and their effects additively will be addressed.

4 Conclusions

As dogs infected with multiple tick-borne co-infections are more likely to develop clinical leishmaniosis than those with a single tick-borne co-infection, reduction of tick burden is necessary to reduce risk of clinical CanL. Both owner education and through tick-prevention are necessary. Reduction of tick-borne diseases may reduce the progression of *Leishmania* infection and increase the potential for eliminating *Leishmania* transmission.

C1370 STUDIES ON LEISHMANIA (VIANNIA) BRAZILIENSIS AND L. (V.) NAIFFI MIXED INFECTIONS

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1 Background

In some endemic regions more than one species of *Leishmania* affect humans. The mixed infection in an individual may represent a rare event, however, this information might be underestimated due to lack of sensitive diagnostic methods available. Golden hamster (*Mesocricetus auratus*) is the choice model for *Leishmania (Viannia)* species infection, since mice are resistantto some strains.

2 Methods

We developed studies aiming to elucidate clinical and immunopathological aspects of mixed infection with two sympatric species from *L. (Viannia)* subgenus. We detected and analyzed changes in susceptibility, virulence and disease progression. Golden hamsters were infected with *Leishmania braziliensis* and *L. naiffi* strains individually or as mixed infection. The infection course was follow up to ten weeks and there-isolated parasites present in fragments of skin lesions were characterized by performing isoenzyme assay (MLEE). Also DNA from lesion' fragments were used for PCR-RFLP hsp70. The parasite load was assessed by qPCR, and histopathological analysis were performed by H&E staining.

3 Results

We observed changes in the course of experimental infections with animals mixed infected showing larger and more severe lesions in relation to single infections. It was also possible to characterize by MLEE the presence of *L.braziliensis* and *L.naiffi* in cultures derived from mixed infected animals. But, after several passages we could not observe the electromorph relative to *L. naiffi*. PCR-RFLP hsp70 gene analysis was able to detect only *L.braziliensis* in lesion of mixed infected animals.

4 Conclusions

In conclusion, the mixed infection of *L.braziliensis* and *L.naiffi* seems to induce more severe lesions than the single infections. Aspects of the immune response observed by the mRNA expression for cytokines, may clarify the mechanism involved in this phenomenon.

C1377 VISCERAL LEISHMANIASIS IN ADULTS

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1 Background

Visceral leishmaniasis (VL) is endemic in areas bordering the Mediterranean Sea, including Tunisia. It is caused by *Leishmania infantum* and it is transmitted by the bite of hematophagous sand fly belonging to phlebotomusspp; dog constitutes the main reservoir of the infection. The incidence of adult visceral leishmaniasis in Tunisia has increased in recent years.

2 Methods

We conduct a retrospective study which describes clinico-hematological, epidemiological and therapeutic features of VL cases in adults, admitted in university hospital, during 1999–2016 in Monastir Tunisia.

3 Results

A total of 10 VL cases were recorded in which 5 (50%) were male. The patients Mean age was 42.5 years (19-78years). Fever (n=10,100%), asthenia (n=8, 80%) and hepato and spleen enlargement (n=6,60%) were the most common clinical patients features. Pancytopenia was noted in 40%, anemia in 100% and thrombocytopenia in 50% of cases. We notice an increase of the level of CRP (C-Reactive Proteins), AST (aspartate aminotransferase), and alkaline phosphatase, LDH in 100%, 30% and 10 % of cases, respectively. Parasitological examinations confirmed diagnosis in all cases, it was positive in blood (n=8) and in bone marrow (n=7). Serological tests were performed in 7 out 10 patients, with 5 positives results. The PCR was positive in 7 cases. Especially, it was negative for two patients who had systemic lupus with positive anticorps. In these two cases we confirm VL diagnosis by PCR.

All patients were treated with meglumine antimoniate (3g/day) for 17 days (14- 28 days).

For these patients we noted 4 cases of intolerance causing the stop of treatment in two cases and switch by amphotericine B. But the evolution was favorable in all cases.

4 Conclusions

In comparison with pediatric visceral leishmaniasis, adult disease is characterized by inconsistence occurrence of some major symptoms such as fever and spleen enlargement. For this reason the clinical disease syndrome may be incomplete and diagnosis may be difficult.

C1381 INVOLVEMENT OF THE NON-INFLAMMASOME FORMING NUCLEOTIDE-BINDING DOMAIN LEUCINE-RICH REPEAT PROTEIN 12 (NLRP12) IN MURINE VISCERAL LEISHMANIASIS (VL).

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1 Background

Leishmania infantum (Li) causes VL, with suppression of type 1 immune responses. The NLR proteins include >20 cytosolic proteins that regulate inflammation and immunity. Activation of three NLRs or AIM2 can cause assembly of an inflammasome leading to IL-1β and IL-18 release. Functions of non-inflammasome forming NLRs are not as well understood. **We hypothesized that non-inflammasome forming NLR proteins influence the course of VL by modifying the localized inflammatory response to *Li*.**

2 Methods

We screened for NLR effects by infecting NLR pathway gene knockoutor wild type (WT) mice with *Lic* co-expressing luciferase and mCherry. Progressive parasite expansion was monitored by in vivo imaging (IVIS), qPCR and, Luciferase assay. The screens suggested involvement of the non-inflammasome forming Nlrp12 in progression of VL.

3 Results

Li parasite loads expanded early (day 28) but were controlled in WT mice, whereas *Li* continued to expand and were 2-fold higher than WT on day 56 of *Nlrp12*$^{-/-}$ infection. Consistently, liver-derived infiltrating cells from *Nlrp12*$^{-/-}$ mice released less antigen-induced IFNgamma than WT cells on infection day 56 (24 vs. 41 pg/mL). Flow Cytometry showed inflammatory monocytes expanded on day 28 in WT but not *Nlrp12-/-* mice, preceding parasite clearance from WT. Instead, resident macrophages expanded in *Nlrp12-/-* mice in parallel with the late expanding parasite load (day 56). The kinetics of monocyte derived dendritic cell (MNDC) recruitment paralleled parasite load, with recruitment at 28 days in WT but recruitment at 56 days in *Nlrp12-/-* mice.

4 Conclusions

. These data suggest that Nlrp12 playsa protective role in VL, associated with recruitment of both inflammatory monocytes and MNDCs at the time of peak parasite growth, followed by parasite clearance. Infiltration of inflammatory monocytes is impaired in the absence of Nlrp12, leading to delayed MNDC influx, impaired IFN-gamma with lower CD4+ expansion and but increased expansion of resident macrophages, which permit parasite growth.

C1384 HISTOPATHOLOGICAL INTESTINAL ALTERATION IN HAMSTERS EXPERIMENTALLY INFECTED WITH LEISHMANIA (L.) INFANTUM.

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1 Background

Visceral leishmaniasis (VL) is a systemic parasitic disease, affecting mainly liver and bone marrow, however others organs are involved during the systemic infection, such as intestinal systems. Leishmania infantum has been detected in intestinal mucosal from human and naturally infected dog and in experimental models. Presence of parasite is related to inflammatory alterations in this organs and rupture of the intestinal barrier can allows microbial products and viable bacteria to translocate to other organs through hematogenous dissemination, inducing greater severity during active visceral leishmaniasis. In this work we evaluated the histopathological alterations in colon and small intestine from hamsters experimentally infected with Leishmania infantum.

2 Methods

Hamsters (*Mesocricetus auratus*) were infected intraperitoneally with $2x10^7$ *Leishmania (L.) infantum* amastigotes. After 48, 72 hours and 15, 45 and 90 days post-infection were euthanized under anesthesia and the follows organs were collect: spleen, small and large intestine and peripheral blood. Histopathology analyses were performed in intestines to detect parasite and inflammatory process. Spleen were used to count parasite burden according to Stauber' method.

3 Results

We observed an increase of parasite burden in spleen following times of infection. After 48 hours post-infection the mean of parasite burden was $3.92x10^4$ and after 90 days post-infection we observed $10.79x10^4$ parasites. Histopathological analyzes show alterations in both small and large intestine. Mild to severe inflammatory infiltrate, enteritis, histiocytosis, and presence of amastigotes in the intestinal lumen were observed in infected-hamsters. These changes occur from 48 hours of infection, differentiating the population of the inflammatory infiltrate between Neutrophil, Lymphocyte, and Lymphohistiocytic. We observed presence of eosinophils in the inflammatory infiltrates only at 90 days pos-infetion. Inflammatory infiltrates of the small intestine are mostly located near the peyer's patches, in some cases, causing lymphocytolysis of this region, occurring after 15 days post-infection. We also observe that only from 45 days of infection occurs white-ear hyperplasia, followed by depletion of the same in spleen.

4 Conclusions

Histological alterations in intestine could be related to increase of parasite burden and presence of amastigotes in intestine must inducing histopathological alteration.

C1429 POLYMORPHISM OF TOLL INTERACTING PROTEIN (TOLLIP) GENE IS NOT ASSOCIATED AS A RISK FACTOR FOR VISCERAL LEISHMANIASIS

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1 Background

Etiology of establishment of *Leishmania donovani* infection is not well understood and host immune system plays a key role in pathogen entry, survival and propagation. The innate immune responses are vital for recognition and subsequent elucidation of inflammatory responses for pathogen killing and clearance in the host. This is evident from the fact that not all persons infected with visceral leishmaniasis show same pathogenesis and disease progression. Toll like receptors and its signaling pathways are the hallmark of the innate immune system and Toll interacting protein (TOLLIP) is a key regulatory protein of this pathway. Genetic polymorphisms of TOLLIP gene is known to be associated as a risk factor for wide range of bacterial diseases like sepsis, leprosy, tuberculosis and also in protozoan infections like cutaneous leishmaniasis. However, its role in visceral leishmaniasis as a risk factor is not known. Therefore, we carried out a case control study to evaluate the role of TOLLIP gene polymorphism and its association with visceral leishmaniasis

2 Methods

A total of 212 participants including 76 cases and 136 controls with no history of VL were enrolled in this study. The TOLLIP gene polymorphism at rs5743899 was screened by PCR-RFLP method. DNA was extracted from all the blood samples and PCR was performed for amplification of the gene. Genotyping was done by RFLP method followed by agarose gel electrophoresis separation

3 Results

The G and T alleles of the rs5743899 SNP were present in both cases and controls. The findings suggest no evidence of deviation from Hardy-Weinberg equilibrium for the polymorphism in either groups of patients with VL or healthy controls. In the rs5743899, distribution of genotypes in VL cases were statistically similar compared to controls (p=0.09, OR=1.59, 95 % CI) and thus statistically insignificant.

4 Conclusions

Findings indicate that the presence of rs5743899 polymorphism may not be attributed as a risk factor for visceral leishmaniasis in the endemic population of Assam. However, further studies are needed to support this view. Studies in other VL endemic ethnic populations may yield association of rs5743899 polymorphism in the population as a risk factor for developing visceral leishmaniasis.

C1442 INFLUENCE OF LEISHMANIA RNA VIRUS-1 ON VIRULENCE FACTOR AND PRO-INFLAMMATORY BIOMARKER EXPRESSION IN A HUMAN MACROPHAGE MODEL OF AMERICAN TEGUMENTARY LEISHMANIASIS

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1 Background

Leishmania RNA virus-1 (LRV-1) is a double stranded RNA virus identified in 20-25% of the *Viannia* complex endemic to Latin America, and is believed to accelerate 10% of cutaneous leishmaniasis (CL) to mucosal leishmaniasis (ML) over time. LRV-1 is correlated to increased levels of proinflammatory cytokines and chemokines in both human and murine models. Virulence factors (VFs) are endogenous molecules known to exacerbate the pathogenicity of *Leishmania* spp. Our objective was to quantify proinflammatory biomarkers from human macrophages infected with LRV-1-positive and negative strains of *Leishmania,* and understand known VF expression according to LRV-1 status.

2 Methods

Eight cultured isolates of *Leishmania*, including 5 clinical and 3 ATCC strains were used, 4 of which were LRV-1-positive (*L. V. braziliensis* [n=1], *L. V. guyanensis* [n=1], *L. V. panamensis* [n=2]), and 4 were LRV-1-negative (*L. V. panamensis* [n=3], *L. V. braziliensis* [n=1]). Human monocytes (U937 cells) were transformed to macrophages over 72 hours. Promastigotes of LRV-1-positive and negative strains of *Leishmania* were inoculated into macrophage cultures, and supernatants were collected at 24- and 48-hrs. Proinflammatory biomarkers measured by ELISA at 24-, and 48-hrs included: IL-1β, IL-6, TNF-α, CXCL10, CCL5, and SOD. VF expression analysis of HSP20, HSP70, HSP83, MPI, CPB, and GP63 was quantified by qPCR.

3 Results

Overall expression of LRV-1 positive isolates did not differ significantly when compared to the LRV-1 negative isolates (IL-1 β [p=0.88], TNF-α [p=0.27], CCL5 [p=0.71], CXCL10 [p=0.47], IL-6 [p=0.52] or SOD [p=0.63]). Similarly, LRV-1 positive *L. V. panamensis* strains did not show differential expression. A clinical strain of *L. V. braziliensis* that is LRV-1 positive demonstrated significantly higher expression of TNF-α (p=0.01), SOD (p=0.02); and significantly lower expression of CXCL10 (p=0.004) and IL-6 (p<0.001) compared to the LRV-1 negative strain. Pooled VF analysis of LRV-1 negative *L. V. panamensis* strains (n=3) showed markedly higher expression of CPB (2.3-fold), MPI (987-fold), HSP20 (136-fold) and HSP100 (868-fold) compared to the LRV-1 positive strains.

4 Conclusions

Our findings suggest that LRV-1-positive *L. V. braziliensis* produces a hyper inflammatory response promoting parasitic persistence, while LRV-1-negative *L. V. panamensis* exhibits higher overall expression of VFs, supporting different mechanistic approaches to disease severity by infecting strain.

C1489 EVALUATION OF BALB/XID MICE INFECTION BY LEISHMANIA AMAZONENSIS

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1 Background

Leishmaniasis is a neglected disease and *Leishmania* amazonensis is the etiological agent of diffuse cutaneous leishmaniosis in Brazil. This work aims to study the role of B lymphocyte using BALB/Xid in infection by *Leishmania amazonensis*. BALB/Xid mice has a mutation on Bruton's tyrosine kinase's (Btk) coding gene, this kinase is related to B1 lymphocyte's development and B2 lymphocyte's maturation. Thereby, those animals have lower frequency of B1 and B2 lymphocyte's than wild type mice, mostly B1 lymphocyte.

2 Methods

The infection was performed inoculating stationary promastigotes form of *L. amazonensis* in the footpad's of mice. The lesion's growth was weekly checked using caliper rule for register the increased thickness of the infected foot (in mm). Animals were euthanized at day 60th post infection and the infected footpad and the draining lymph node were macerated, we used the limiting dilution analysis to determine the parasite load. Furthermore, cells from lymph nodes were used for analysis by flow cytometry.

3 Results

Our results demonstrated that BALB/Xid mice displayed smaller lesions in comparison to wild type. However, the parasite load obtained on the infected footpad and on the lymph nodes were very similar. Besides, we observed an increase in the frequency of CD4+ T cells, CD8+ T cells, regulatory CD4+ T cells on the BALB/Xid's lymph nodes. We did not observe differences in effector cells producing INF- γ on the lymph nodes. Cytokines' analysis in the homogenate of infected footpad, draining lymph node and spleen showed a decrease of IL-10 production by BALB/Xid mice when compared with control group, what is probably related to the low level of B lymphocytes. BALB/Xid mice had high amounts of INF-γ and IL-4 on the lesion paw and lower levels of IgM and IgG1 in the serum when compared to wild type. We performed *in vitro* interaction using peritoneal B1 lymphocytes in the presence or absence of LPS and/or *L. amazonensis*. IL-10 levels' analysis were made and as result we showed that, in the presence of both stimulus, B1 lymphocytes were capable to produce higher levels of IL-10 when compared to the control, suggesting that the low level of this cytokine *in vivo* could be related to the low numbers of B1 lymphocytes on BALB/Xid mice.

4 Conclusions

In conclusion, B cells are associated to pathogenesis in murine cutaneous leishmaniasis caused by *Leishmania amazonensis* through production of IgG1 and IL-10.

C1494 RISK FACTORS FOR MORTALITY OF CHILDREN WITH ZOONOTIC VISCERAL LEISHMANIASIS IN CENTRAL TUNISIA

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1 Background

Zoonotic visceral leishmaniasis (ZVL) caused by *Leishmania infantum* is endemic with an epidemiological profile of a paediatric disease in Tunisia. In the context of a high fatality rate, identifying risk factors for in-hospital mortality in children treated for ZVL is of major epidemiological importance

2 Methods

A retrospective (case-control) study included 230 immuno-competent children diagnosed and confirmed with primary ZVL in the paediatric department of the University Hospital of Kairouan between 2004 and 2014.

3 Results

Forty-seven per cent (47%) were children under 18 months of age, and with a male / female ratio of 1.01. The overall case-fatality was 6 % (n = 14). The risk factors for in-hospital death identified by a multivariate analysis were: bleeding at admission (OR=25.5, 95% CI :2.26-287.4 ; p=0.009), White cells count less than 4000/mm3 (OR=5.66, 95% CI :1.16-27.6 ; p=0.032), cytolysis (OR=28.13, 95% CI :4.55-173.6 ; p < 0.001), and delay between onset of symptoms and admission ≥ 15 days (OR=11, 95% CI :1.68-72 ; p=0.012).

4 Conclusions

The results strongly suggest that paediatric patients admitted 15 days after onset of symptoms, with bleeding, white cells counts bellow 4,000/mm^3, and cytolysis at admission should be considered severe cases and subsequently, they are at high risk of mortality. A better understanding of factors associated with children death from ZVL may contribute to decrease mortality.

C1548 PARASITISM AND INFLAMMATION IN EAR SKIN AND IN GENITAL TISSUES OF SYMPTOMATIC AND ASYMPTOMATIC MALE DOGS WITH VISCERAL LEISHMANIASIS

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1 Background

Visceral leishmaniasis (VL) constitutes an important public health problem in several regions of the world (WHO, 2014). The principal mode of transmission of *Leishmania infantum*, the main causative agent of VL in Brazil, is via the bites of infected female sandflies (Spiegel et al., 2016). Furthermore, domestic dogs are considered the main reservoirs of the parasite in the urban environment (Silva et al., 2006). The present study aimed to evaluate the parasitic loads and inflammatory processes in genital tissues from seropositive (symptomatic and asymptomatic) male dogs. To the best of our knowledge, *our study is the first to compare* parasitic loads and inflammatory profiles of samples from the genital region and ear tip skin obtained from *Leishmania*-infected male dogs.

2 Methods

Fourty-five seropositive dogs were separated into groups containing symptomatic (n = 23) and asymptomatic (n = 22) animals. The control group (n = 2) healthy animals with seronegative and negative results in direct parasitological test. Samples of ear tip skin, prepuce, glans penis, testis, epididymis and prostate were collected for evaluation of parasitic load and inflammatory *infiltrate*.

3 Results

Although ear tip skin was the most intensely parasitized, prepuce and epididymis revealed no difference in parasitism when compared with ear tip skin (P>0.05). Parasitic loads in testis and prostate were lower than other tissues (P<0.05). Parasitism in glans penis was high, similar to prepuce and epididymis, but lower than ear tip skin. High parasitism was more frequent in symptomatic dogs than asymptomatic animals. Severe inflammatory processes were more frequent within the symptomatic animals compared with asymptomatic and more predominant in prepuce and epididymis. Ear tip skin and genital tissues presented signs of chronic inflammation. There were weak and moderate positive correlations between parasitic loads and inflammatory processes.

4 Conclusions

Our results demonstrate that likewise with the ear tip skin, the genital of seropositive dogs can carry a large number of *Leishmania infantum*. amastigotes and this process is more intense in symptomatic animals. These data have important implications for the understanding the possibility of venereal transmission of CVL.

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C1558 INFESTATION BY RHIPICEPHALUS SANGUINEUS INTERFERE WITH THE COURSE OF THE DISEASE AND IMMUNOLOGICAL PARAMETERS IN RESISTANT MICE INFECTED WITH LEISHMANIA MAJOR.

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1 Background

Dogs are considered the main domestic reservoirs of visceral leishmaniasis in Brazil and many regions of the world. In nature, a wide range of coinfections may take place in dogs, especially those caused by brown ticks. Tick's saliva has many properties aiming the vector to circumvent the clothing, inflammatory and perception mechanisms of the host. However, the role of the immunomodulation driven by ticks in the outcome of leishmaniasis is poorly known and is still a missing step of the host-parasite interface.

2 Methods

Mice (C57BL/6 and BALB/c) were previously infested once by two adult females of *Rhipicephalus sanguineus* on the dorso-thoracic skin, for 7 to 10 days and infected with 10^5 metacyclic promastigotes of *Leishmania major* in the left hind paw as well as a non-infested group. As a control group for some experiments, mice infested by *R. sanguineus* were inoculated in the footpad with sterile PBS. Footpad thickness was monitored weekly. Parasitism and immunologic parameters were measured at five and eight weeks post infection. The titers of anti-*Leishmania* antibodies and IL-10 were measured in sera by ELISA and the parasite load in the skin and spleen was measured by qPCR.

3 Results

Infestation with *R. sanguineus* promoted a minor increase in the thickness of the infected footpad when compared to non-infested mice. However, the parasite load in the paw and spleen was slightly higher in infested groups. Moreover, no difference in footpad swelling was observed in BALB/c mice, but the parasite load in the footpad was slightly higher in the infested group. Interestingly, titers of anti-Leishmania IgG1 and IgG2 antibodies were lower in infested mice eight weeks after the end of infestation. Serum levels of IL-10 were higher in both groups infested by *R. sanguineus* five weeks after infection, suggesting a long term effect of tick's driven immunomodulation.

4 Conclusions

Those data strongly suggests that ticks may affect the immunity and the course of the disease in co-infected mice harboring *Leishmania major*.

C1596 "EFFECT OF INFECTIOUS DOSE ON LEISHMANIA INFECTION: COMPARISON OF LEISHMANIA TROPICA WITH LEISHMANIA MAJOR"

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1 Background

Infectious dose is an important variable in leishmanial infections. In the current paper, we have studied the effect of infectious dose on pathologic and immunologic aspects of *Leishmania (L). tropica* infection in BALB/c mice and compared these phenotypes with those of *L. major*.

2 Methods

High and low doses of *L. tropica* and *L. major* were used for infection of mice and lesion development, parasite load, and cytokine response were assessed. Pre-infected mice were challenged by *L. tropica* and level of protection was evaluated

3 Results

Results showed that: 1) *L. tropica* has more potential for growth in lymph nodes at early phases of infection, 2) lower levels of IL-10 are produced in response to *L. tropica*, 3) For *L. tropica*, lower dose infection results in lower levels of IFN-g and IL-10 at all intervals after infection. 4) For *L. major*, lower dose infection results in lower levels of these cytokine at one week and one month after infection. However, similar levels of these cytokines were produced against low and high dose infection at four months after infection. 5) *L. tropica* at high dose disseminates to spleen while *L. tropica* at low dose and *L. major* at high or low dose do not disseminate at intervals of up to four months after infection.

4 Conclusions

Infectious dose is an important variable in *L. tropica* infection. Higher infectious dose of *L. tropica* results in visceralization of parasite to spleen, induction of higher levels of IFN-g and IL-4, and protection against challenging infection. Substantial differences between *L. tropica* and *L. major* may help to dissect the immunity against *L. tropica* infection

C1639 IMPLICATION OF LEISHMANIASIS VECTOR BITES IN THE LOSS OF TOLERANCE IN PEMPHIGUS

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1 Background

The role of the bite of blood-sucking vectors has been suspected in the Brazilian endemic pemphigus that presents both clinical and epidemiological similarities with pemphigus in Tunisia. We recently investigated a possible etiological link between pemphigus and zoonotic cutaneous leishmaniasis (ZCL). We showed that pemphigus patients have significantly higher titers of antibodies against *P. papatasi* salivary proteins particularly against its immunodominant target, PpSP32. We hypothesized that the direct interaction between PpSP32 and desmogleins (Dsg1 and Dsg3), the specific target autoantigens in pemphigus, could induce a loss of tolerance to the latter proteins and initiate the autoimmune response in pemphigus

2 Methods

By Far Western blot, we showed that the recombinant form of PpSP32 (rPpSP32) binds to an epidermal protein with a molecular weight of about 160kD, possibly corresponding to the Dsg3. Co-immunoprecipitation of the epidermal extract/rPpSP32 mixture using Dynabeads® coated with anti-histidine antibodies revealed the interaction of rPpSP32 with either Dsg1 or Dsg3. A specific binding between PpSP32 and Dsg1/3 was further demonstrated by ELISA assays in which plates were coated with recombinant forms of Dsg1, Dsg3 or an irrelevant protein then incubated with a decreasing amount of rPpSP32. Finally, Swiss mice immunized with rPpSP32 twice a week during 120 days exhibited significantly increased levels of anti-Dsg1 antibodies from day 75 to day 120.

3 Conclusions

Herein, we demonstrated, for the first time, an interaction between PpSP32 and Dsg 1/3. This could explain the production of anti-Dsg antibodies in pemphigus patients leading to the development of the disease. Our findings further support that an environmental component may initiate the autoimmune response in pemphigus. A persistent exposure to *P. papatasi* may be a possible etiologic link to pemphigus in Tunisia.

C1743 RELATIONSHIP BETWEEN THE OXIDATIVE CAPACITY OF NEUTROPHILS AND ANTIBODIES LEVELS IN THE CANINE LEISHMANIOSIS. PRELIMINARY RESULTS

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1 Background

In canine leishmaniosis, high levels of antibodies production are associated with the presence of several clinical signs, because of the deposition of soluble immune complexes in tissues and organs. The nitroblue tetrazolium test (NBT) has remained as an efficient and simple screening method to evaluate the oxidative capacity of neutrophils.The objective of this study was to correlate the oxidative capacity of neutrophils with the antibodies levels in canine leishmaniosis.

2 Methods

The physical and laboratorial examination, complete blood count (CBC), urinalysis and biochemical profile, were performed to 3 healthy non-infected dogs, 14 dogs with moderate canine leishmaniosis (stage II) and 9 dogs with severe canine leishmaniosis (stage III), classified according to the LeishVet Consensus. All dogs from stage II and III, showed amastigotes forms in bone marrow and lymph node smears stained by Giemsa and examined under optical microscopy, and positive antibodies levels (^{3}1:80) by immunofluorescence antibody test (IFAT). The NBT was used to evaluate the oxidative capacity of neutrophils.

3 Results

Healthy dogs showed a mean NBT reduction of 94±2%, as well as negative antibodies levels by IFAT, normal CBC and biochemical and urinalysis profile. Dogs with moderate disease showed a mean NBT reduction 88±15%, as well as clinical signs, non regenerative anemia, hyperglobulinemia and hypoalbuminemia; and normal renal profile with creatinine (0.7-1.3 mg/dL), urea (19-66 mg/dL), and non proteinuric with UPC ≤0,95. The antibodies levels were elevated, median (1:640). Dogs classified in the stage III showed a mean NBT reduction 83±13%, as well as the clinical signs listed in stage I and II. The values ??of creatinine (0.6-7.3 mg / dL) and urea (21-333 mg / dL) in blood were higher, as well as urinary excretion of proteins with UPC >1; The median antibodies titers were 1:320. The differences observed in the oxidative capacity of neutrophil between healthy and diseased dogs were not statistically significant ($p = 0.266$), nor was the difference in antibodies titers ($p = 0.144$) between the two stages. However, the correlation between antibody titers and NBT % reduction in all dogs infected dogs was significant ($r^2=0.23$, $p = 0.02$).

4 Conclusions

The general tendency observed was a decrease in the oxidative capacity of neutrophils and antibodies levels, from stage II to III in canine leishmaniosis, a low positive correlation was observed in both variables.

C1746 CLINICAL AND OCULAR ALTERATIONS IN DOGS NATURALLY INFECTED BY LEISHMANIA INFANTUM

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1 Background

Clinical findings of canine visceral leishmaniasis (CVL), as well as of human visceral leishmaniasis (HVL) are variable and nonspecific. Few studies describing the clinical and histopathological alterations of these infections are found in the literature. Thus, highlighting the need to obtain data that could contribute to these studies, the objective of this work was to study the clinical and ocular abnormalities.

2 Methods

In the present study were used twenty five dogs with CVL from the locality Cidade Operária, Sao Luís - Maranhão/Brazil by conducting ophthalmic and clinical examination, ophthalmological tests (fluorescein staining and Schirmer tear test), hematological and biochemical measurements, isolation and characterization of the parasite, histopathology and PCR analysis in different samples, such as blood, bone marrow, spleen, aqueous humor, eyeball and annex tissues.

3 Results

The results showed that lymphadenopathy was the most common symptom, followed by cachexia, alopecia, skin lesion, onychogryphosis, and hepatosplenomegaly. Ocular clinical manifestations observed most frequently were the presence of some type of secretion and conjunctivitis, followed by blepharitis, uveitis and corneal opacity. Eight animals were positive in the Schirmer tear test and seven in fluorescein staining indicating the presence of keratoconjunctivitis sicca and cornea ulcer, respectively. The most common hematologic abnormalities were anemia and thrombocytopenia, while biochemical were the increased serum total protein and globulin and decreased albumin. Histopathological analysis showed lesions on the eyelids, sclera, glands and muscles retrorbital. The presence of plasma cell type inflammatory infiltrate was the most frequent observed alteration.

4 Conclusions

These results show that the ocular manifestations are present in animals with CVL and can occur concurrently with other systemic signs of illness. So, We suggest that in endemic areas, CVL should be included as differential diagnosis of dogs with eye injuries or of unknown etiology.

C1750 DO EXTRACELLULAR VESICLES PARTICIPATE IN THE IMMUNE RESPONSE OF HUMAN CUTANEOUS LEISHMANIASIS CAUSED BY LEISHMANIA (VIANNIA) BRAZILIENSIS?

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1 Background

The resolution of cutaneous leishmaniasis (CL) lesions caused by *Leishmania (Viannia) braziliensis* depends on cellular immune responses that acts in peripheral blood and lesion site of infected patients. The contribution of extracellular vesicles (EVs) in the immunopathogenesis of CL is much unexplored. The aim of our study was to isolate and characterized the EVs released from T lymphocytes, macrophages and NK cells to determine its contribution in the immune response of CL patients, which might be involved in triggering immunological mechanisms, leading to cure or disease progression.

2 Methods

Human plasma and supernatants from Leishmania-stimulated cell cultures have EVs isolated and characterized by differential-centrifugation steps and flow cytometry (Cytoflex, Beckman Coulter), respectively. A flow cytometric staining panel including lactadherin, calcein, anexin V, anti-CD14, anti-CD3, anti-CD56, anti-HLA-DR, and anti-TCRα/β was used to characterize the origin of EVs. To identify the particles smaller than 900nm we adjusted the settings of CytoFlex flow cytometer using a mix of well-known-size beads (between 100 and 900nm – BioCytex) and use the Side Scatter of Violet Laser as a parameter to identify these small particles.

3 Results

Preliminary experiments were performed tostandardize the flow cytometry protocols and adjust flow cytometer to define EVs, using the mix of beads with determined diameters (100, 160, 200, 240, 300, 500 and 900 nm). This approach allow us to define areas in acquisition dot plots corresponding to EVs sizes. Triggering cells in side scatter of violet laser allow noise reduction, which is obligatory to EVs/debris discrepancy.

4 Conclusions

As a first approach, we were able to identify EVs and determined their sizes. CytoFlex flow cytometer was very sensitive and with an optimum resolution for such research. Foremost experiments are underway in our lab and data will be discussed further.

C1762 HEMATOLOGICAL DISORDERS AND CHANGES IN THE BONE MARROW OF SEMI-DOMICILED DOGS NATURALLY INFECTED WITH ACTIVE INFECTION BY LEISHMANIA INFANTUM

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1 Background

INTRODUCCION: As human patients, dogs with visceral leishmaniasis often has several hematological disorders during the disease. **AIM**: This study aimed to evaluate the bone marrow of dogs naturally infected with *Leishmania infantum* and the alterations in the hemogram during the active infection determined by positive spleen culture.

2 Methods

We have studied the distribution of cell lineages and the profile of cytokine expression in the bone marrow of semi-domiciled dogs. After obtaining the results of laboratory tests, samples were collected and analyzed by conventional microscopy. Molecular biology techniques were used for detection of *L. infantum* and verification of cytokines expression in the bone marrow. Cases of severe canine visceral leishmaniasis were determined by splenic disorganization and were also studied.

3 Results

On the analysis, the active infection was detected by splenic culture in 18/57 (31.6%) of the dogs. The majority of dogs with positive spleen culture had detection of *L. infantum* in the bone marrow ($p = 0.0004$) followed by histiocytosis ($p=0.0046$). Dogs with active infection had a lower red blood cell count (4.4 ± 1.1) than animals with negative spleen culture (5.2 ± 1.1), p=0,0079. They also had a higher amount of circulating leukocytes (19477.8 ± 11863.3) accompanied by an increase in neutrophils (14124.4 ± 10308.9) than animals with negative splenic culture (13554.4 ± 5405.0), (8583.5 ± 4212.8), p=0,0306 and p=0,0173, respectively. Dogs with active infection and severe visceral leishmaniasis had a more intense decrease of red blood cells ($p=0.0066$) accompanied by a decrease amount of erythroid lineage cells in bone marrow than the dogs with negative culture and organized spleen ($p=0.0127$). These changes were accompanied by elevated gene expression of IFN-γ ($p=0.0015$) and TNF ($p=0.0091$) in the bone marrow microenviroment.

4 Conclusions

After analyzing the results we concluded that dogs with active infection present anemia and leukocytosis with neutrophilia. When they have active infection and severe visceral leishmaniasis, presented more intense hematological disorders that are accompanied by erythroid hypoplasia and are associated with changes in the expression of cytokines in the bone marrow microenvironment, such as the increase of IFN-γ and TNF.

C1775 INFECTIVITY OF DIFFERENT SPECIES AND STRAINS OF LEISHMANIA (VIANNIA) SUBGENUS IN J774. A1 MURINE MACROPHAGES

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1 Background

It is well known that in cutaneous leishmaniasis (CL) some patients do not respond to treatment, leading to therapeutic fail and persistent infection. The causes for these different outcomes is not clear although these could be related to intrinsic characterises of leishmania parasites. The CL caused by *Leishmania (Viannia) braziliensis* is more frequent and severe than those caused by *L. (V.) lainsoni* and *L. (V.) naiffi*. Different outcomes in CL can also be observed among distinct strains of *L. (V.) braziliensis.* This study aimed to investigate the infectivity of different species and strains of *Leishmania (Viannia)* subgenus in J774. A1 murine macrophages. Previous results showed that these species are different in terms of size, form and enzymatic profile. This is a preliminary study to characterize the virulence of species and strains that will be used in an *in vivo* experiment to investigate prognostic factors associated with CL.

2 Methods

In this study, we used three species of *Leishmania (Viannia)* subgenus and two strains of *L. (V.) braziliensis*: *L. (V.) lainsoni* MHOM/BR/8l/M6424 (LVl), *L. (V.) naiffi* MDAS/BR/79/M5533 (LVn), *L. (V.) baziliensis* MHOM/2003/2314 (LVb2314) and *L. (V.) baziliensis* MHOM/BR/1987/M11272 (LVb11272). To estimate the infectivity, each J774. A1 murine macrophage was infected with seven *Leishmania* promastigotes in 24-well plates with one coverslip in each well. After a 24h incubation in 5% CO_2 atmosphere at 37ºC, coverslips were stained with hematoxylin and eosin. In microscopy, 200 cells were counted and the infection index was calculated multiplying percentage of infected macrophages and number of parasites per macrophage. For statistical analyses, we used one-way ANOVA and Tukey's Test, being $p<0.05$ significant.

3 Results

The infection indexes were statistically different among studied strains and species ($p<0.001$), being the infection index in descending order: LVb2314 (225.41), LVn (185.24) statistically equal to LVb11272 (170.18) and LVl (115.50).

4 Conclusions

The differences found in infection indexes might be related to the different progression and incidence of CL caused by studied species and strains.

C1788 CLINICO-HEMATOLOGICAL FINDINGS OF PEDIATRIC VISCERAL LEISHMANIASIS IN NORTHEAST OF IRAN

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1 Background

Visceral leishmaniasis (VL) also known as kala-azar as a main public health problem in the Mediterranean countries,is a disseminated parasitic disease caused by leishmania infantum. The disease is described as a vary spectrum of clinical and hematological manifestations,especially in children less than 12 years old.

2 Methods

The Clinico-hematological features, laboratory results and demographic information of 40 pediatric VL patients between 8 months to 6 years with a final diagnosis by microscopic demonstration of Leishmania spp. amastigotes in active bone marrow aspiration (BMA) were reviewed from 2005 to 2015 at hospitals in northeast of Iran.

3 Results

From 33 VL cases, 69.7% were female and 30.3% were male.Children *between* the *ages* of 8 month to 6 *years* participated in the study. The majority of them (78.8%) were between 1-4 years of age. About 80% were from rural areas or nomads. The most common presenting features were Anemia (78.9%), fever (69.7%), hepatosplenomegaly (45.4%).Other clinical manifestations such as cutaneous rash, chilling, gastroenteritis and lymphadenopathy were seen in less than 5% of cases.Various hematological parameters showed that most patients were suffering from moderate to severe microcytic hypochromic anemia (74.2% had RBC count less than 4 million cells/ul , 47.4% Hb less than 8 fl). 70.9% were leukopenic)WBC: less than $5\times10^3/\mu L$)and 86.7% had decreased platelet counts. Pancytopenia was observed in 18.2% of cases. MCV, MCH and MCHC *levels* were *below* the *normal values* in 88%, 90% and 85.1% of the patients. Moreover, Hepatic Enzymes,aspartate transaminase (AST) and alanine transaminase (ALT) levels had increased in53.33% and 6.66% of the patients respectively. 96.9% of cases were CRP positive. Bone marrow was found hyper-cellular in all of them and myeloid to erythroid ratio (M/E) more than 4was found in 39.1% of cases. Plasma cells slightly increased in 60% and megakaryocytes were decreased in thrombocytopenic patients.

4 Conclusions

However there are no specific clinico-hematological features for pediatric VL, characterization of the overall symptoms can help *determine a quick way* for diagnosis and treatments.

C1819 INVESTIGATING THE CORRELATION OF TH1, TH2 AND TH17 CYTOKINES DURING VISCERAL LEISHMANIASIS PATHOGENESIS IN HAMSTER MODEL

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1 Background

Generation ofpotential host pro-inflammatory response is indispensable for the control ofvisceral leishmaniasis. Among the several pro-inflammatory cytokines secreted by Th17 cells, IL-17 has emerged as an important one as it reported to play a protective role in treated *Leishmania*-infected patients. However, its role during active *Leishmania donovani* infection remains poorly defined.Herein, an attempt to correlate the relationship between Th1, Th2 and Th17 cytokines has been initially made in serum and splenic tissue samples of infected hamsters, a suitable chronic model of this disease.

1 Methods

Purifiedsplenic-derived*L. donovani* amastigotes ($1x10^6$ per animal) were injected in naïve hamsters *via* theintracardiac route. Infected hamsters were necropsied on days 15, 30, 45 and 60 post infection (p.i.) and blood and splenic tissue samples were collected for the estimation of various Th1, Th2 and Th17 cytokines both by ELISA and real-time RT-PCR respectively.

3 Results

Amongst the Th1 cytokines, mRNA expression of IL-12 and TNF-α was found to be up-regulated on day 15 p.i., which later declined by day 60. However, mRNA transcript of Th2 cytokines like IL-6 and IL-10 increased with the disease progression up to day 60 p.i. while those of IL-4 and TGF-β were, though, down-regulated initially but later increased by day 60. The mRNA transcripts of Th17 cytokines *viz.* IL-17A and IL-22 in splenic tissue were earlier found to be up-regulated. However with disease progression, the expression of IL-17A slightly declined while that of IL-22 remains highly up-regulated. Similar observations were obtained with serum samples performed by cytokine ELISA.

4 Conclusions

From the above study, it can be inferred that there exist a direct correlation between cytokine levels in serum as well as mRNA transcript in splenic tissue samples. Further study is needed for ascertaining the role of these cytokines in other organs of reticuloendothelial system of the disease model.

C1834 EARLY DISSEMINATION OF LEISHMANIA MAJOR IN MICE LACKING EOSINOPHILS.

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1 Background

Eosinophils are granulocytes mainly involved in helminth infections and allergic responses increasing rapidly in numbers in the bloodstream in those conditions. Both in mice and humans, eosinophils compose the cellular infiltrate in tissue infected by *Leishmania* spp., in association with the inflammatory milieu at the site of infection during early and late disease. Δdbl-GATA1 mice has a double deletion on the high-affinity GATA binding site in the GATA1 promoter, which leads to a defect in the maturation of eosinophils. This lineage has been used for allergy research and functional studies on eosinophils, but has never been studied as a model for *Leishmania* spp. infection. Since no data is available on the infection of this lineage with Leishmania parasites, the aim of this work is to characterize the infection in this murine model.

2 Methods

Δdbl-GATA1 were infected in the footpad with stationary phase promastigotes of *Leishmania major* (friedlin). Lesions were measured weekly and their development were expressed by the difference among infected and non-infected footpad. Parasite load were accessed by a modified limiting dilution assay (LDA). Metastatic lesions were counted as absolute number of new ulcers or nodular lesions in any part of the body after infection.

3 Results

We found no differences on the lesion development in the footpad among Δdbl-GATA1 mice and its background control 8 weeks after infection. In addition, no differences in parasite load were found among mice lineages at the same time of infection. However, nine weeks after infection, a massive grow of metastatic lesions appeared in several parts of the skin in eosinophil deficient mice. Thirteen weeks after infection, metastatic lesions were found in members, nose, ear and tail of respectively 100%, 90%, 70% and 10% of the eosinophil deficient mice, against 60%, 30%, 40% and none control mice.

4 Conclusions

Although this work suggests the potential of eosinophils in controlling the dissemination of *Leishmania major* in BALB/c mice, more data is needed to clarify the role of this cell during leishmaniasis.

C1867 CARRIER MEDIATED DELIVERY OF AMPHOTERICIN B THROUGH MANN0SYLATED LIPOSOMES FOR EFFECTIVE MANAGEMENT OF LEISHMANIASIS

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1 Background

Leishmaniasis is a vector-borne zoonotic infection caused by an obligate intra macrophage protozoan parasite '*Leishmania*'. Despite of a number of remedies available, leishmaniasis is still a speedy migrating and deadly infection due to the resistance of the parasite to the drugs as well as their toxicity.Conventional models of administration have limited utility because anti-leishmaniasis therapeutics in such system lack efficient selectivity towards macrophages cells in RES. To avoid the possible shortcomings in the treatment of Leishmaniasis, Mannosylated liposomes have been selected as drug carrier. This system is hypothesized to provide enhanced permeation and active targeting to RES.

2 Methods

The cationic and mannosylated liposomes were prepared using the cast film method and compared for their antileishmaniasis activity. The surface of the Amphotericin B (Amp B)-bearing cationic multilamellar liposomes was covalently coupled with p-aminophenyl-α-D-mannoside using glutaraldehyde as a coupling agent, which was confirmed by agglutination of the vesicles with concanavalin A. The prepared liposomes were characterized for shape, size, percent drug entrapment, vesicle count, zeta potential, *in-vitro* drug release, *in-vivo* antileishmanial activity was performed on *Leishmania donovani*–infected golden hamster.

3 Results

The In-vivo results revealed that AmpB solution was reduced in the parasite load, whereas the placebo cationic liposomes and drug-containing cationic liposomes showed a reduced parasite load. The mannose-coupled liposomes showed a maximum reduction in parasite load. The biodistribution study clearly showed the higher uptake of mannosylated liposomes in the liver and spleen and hence the active targeting to the reticular endothelial system, which, in turn, would provide a direct attack of the drug to the site where the pathogen resides, rendering the other organs free and safe from the toxic manifestations of the drug.

4 Conclusions

Thus, Mannose coupled liposomes can deliver drug to the macrophages in an effective way and hold great potential for RES targeting. Through Mannosylated liposomes, therapeutic level of drug can be achieved at desired site. This system is proposed to carry dual functions, firstly through retention of drug and secondly through selective delivery to macrophages in RES.

C1873 SURFACE TAILORED NANOSTRUCTURED LIPID CARRIER FOR EFFECTIVE MANAGEMENT OF LEISHMANIASIS

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1 Background

Leishmaniasis is a set of diseases including a range of clinical manifestations, initiating from self-healing cutaneous ulcers to lethal visceral diseases. It is a zoonotic infection in the vertebrate hosts, caused due to a protozoan parasite of the genus Leishmania & spreaded worldwide by the carrier sand fly. Nanostructured lipid carriers are a new type of delivery system offering improved performance in terms of drug loading and long-term stability with the ability to form highly concentrated dispersions. The Fucose-Mannose Ligand (FML) of *Leishmania donovani* is a complex glycoproteic fraction. Macrophages selectively express a receptor that binds glycoproteins with terminal L-fucose and D-mannose residues.

2 Methods

Paromomycin is suitable candidate for the topical treatment of cutaneous leishmaniasis. There is an urgency to develop safe and effective drug delivery strategy for existing molecule which can reduce related toxicity and at the same time can target the drug to phagolysosomes of the mononuclear phagocytic system where leishmania parasite resides. The proposed drug delivery system may serve as an optimum carrier to target macrophages as well as effective against leishmania parasite.

3 Results

NLC formed were circular and stable with good entrapment. Fluorescence microscopy suggested that bilosomes were taken up by gut associated lymphoid tissues. *In-vivo* data demonstrates that combination produced both systemic as well as mucosal antibody responses but fail to produce any synergistic effect. *In-vitro* release was in controlled manner. The high fluorescence intensity present in the photographs reveals that NLC were taken in sufficient amount.

4 Conclusions

Fucose coupled NLCs can deliver drug to the macrophage in an effective way and hold great potential for RES targeting. The fucose-coupled NLCs proved to be an efficient carrier for delivering paromomycin sulphate to macrophage and can play a crucial role in the treatment of visceral leishmaniasis. The carbohydrate-coated NLCs appear to be promising carriers that can expand the specificity of their delivery to the target i.e. Leishmania affected macrophages.

C1909 INTESTINAL PARASITE CO-INFECTIONS OF PATIENTS WITH VISCERAL LEISHMANIASIS DOES NOT IMPACT ON DISEASE SEVERITY

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1 Background

Visceral leishmaniasis (VL) is a neglected tropical disease that affects the poorest communities and cause long-term chronic disabilities and substantial morbidity and mortality. Active visceral leishmaniasis is characterised by the presence of Leishmania parasite in the spleen, hepatosplenomegaly, pancytopenia, systemic inflammation and low body mass index (BMI). The factors impacting on the severity of VL are poorly characterised.

2 Methods

a cross-sectional study to assess whether co-infection of VL patients with intestinal parasites influences disease severity.

3 Results

clinical and haematological data, inflammation, cytokine profiles and BMI of VL patients are similar in VL patients and in VL patients co-infected with intestinal parasites.

4 Conclusions

co-infection of VL patients with intestinal parasites does not impact on disease severity.

Clinical and experimental prophylactic and therapeutic vaccination. Clinical and experimental immunology

C0084 ANTIPLASMODIAL, ANTIPYRETIC, HAEMATOLOGICAL AND HISTOLOGICAL EFFECTS OF THE LEAF EXTRACTS OF MORINGA OLEIFERA IN PLASMODIUM BERGHEI BERGHEI INFECTED MICE

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1 Background

Theaqueous and ethanolic extracts of *Moringa oleifera* leaf were evaluated for safety, antimalarial and antipyretic activities because it is commonly used for various types of ailments especially malaria and fever.

2 Methods

The various concentration of these extracts (0-800mg/kg) were tested against *Plasmodium berghei berghei* infected mice albino mice of either sex, in a Peters'four day antiplasmodial test while antipyretic activities were evaluated in malaria- induced mice. Their possible effects on haematological parameters of PCV, WBC, neutrophil, lymphocytes count and liver enzymes were also determined as well as on the liver and kidney architecture

3 Results

All the tested doses of the aqueous extract were comparable in antiplasmodial activity ($p>0.05$) to each of the tested doses of the ethanolic extract which were significantly different ($p<0.05$) from the negative control. Only the highest doses of both extracts gave significantly higher ($p<0.05$) antiplasmodial activity than the standard drug. For the ethanolic extract, a significant antipyretic activity was not observed until at 800mg/kg in time T_3 while the aqueous extract exerted no significant antipyretic activity at all doses and at all times. The ethanolic extract gave significantly higher PCV values than that of the aqueous. The administration of the aqueous extract gave significantly lower WBC than the ethanolic extract while comparable increase in lymphocyte count was noticeable at each of the doses of both extracts when compared to the negative control. Only 100mg/kg and 800mg/kg of the aqueous extract maintained a relatively high neutrophils count in this study. Also, the aqueous extract elicited higher concentration of ALT (greater than fourfold of normal) but the ethanolic extract produced the highest level of AST at the highest dose of 800mg/kg when compared to the ethanolic extract. Histological examination of the kidney showed progressive degeneration while that of the liver showed recovery when compared to negative control as a result of progressive increase in dosage of the extracts in malarial treatment.

4 Conclusions

In conclusion, though both aqueous and ethanolic extracts of *M. oleifera*leaves exert chemosuppressive antiplasmodial activities in *Plasmodium berghei berghei*infected mice and exhibited antipyretic activity with some improved haematological parameters, caution should be taken in its indiscriminate use because of probable toxic effects on the kidney and the liver.

C0122 CELLULAR IMMUNE RESPONSE ANALYSIS IN DOGS WITH VISCERAL LEISHMANIASIS IN THE PRESENCE OF NEW RECOMBINANT ANTIGENS

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1 Background

The canine immune response against Visceral Leishmaniasis (VL) plays an important role in the control and treatment of the disease, as well as in resistance to re-infection. The Th1 response induces a resistance profile through macrophage activation, which leads to parasitic destruction. A Th2 response favors parasite multiplication, through the inhibition of nitric oxide production by the macrophages. Proteins that stimulate a Th1 profile are being explored as vaccine candidates. The aim of this study is to evaluate the cellular immune response of dogs (*in vitro*) to new recombinant proteins, in order to support vaccine development studies.

2 Methods

First, blood from VL positive dogs, resident in an endemic area of the Pernambuco state-Brazil, were collected and used for separation of Peripheral Blood Mononuclear Cells (PBMC). Cells from three dogs were pooled and then subjected to stimulation with the Lci10, Lci13 and chimera 1 (Q1) antigens through a kinetic of concentration (2.5, 5, 10 µg/mL) vs. time (24, 48 and 72 hours). For comparison, crude *Leishmania infantum* soluble antigen (LSA) was used at 10 and 25 µg/mL for the same time periods. After culturing, RNA extraction was performed, and the gene expression for cytokines of Th1 (IFN-γ, TNF-α and IL-2) and Th2 (IL-4, IL-10 and TGF-β) profiles was evaluated. This was performed through the comparative CT method, after real time RT-PCR.

3 Results

Lci10 was able to induce a balanced Th1/Th2 response after 72h of culturing, with the best results generated using 5 µg/mL of the antigen. For Lci13, was observed a response for all three cytokines of the Th1 profile in 24h and 48h of cultivation at 5 µg/mL, with good regulation of the Th2 response. For Q1, in 24 hours of cultivation was observed expression for Th1 profile only at 2.5 µg/mL. When compared with LSA, the crude antigen was not able to induce a protective response in the concentrations and times tested.

4 Conclusions

Thus, during the kinetic experiment, the three new recombinant antigens demonstrated to be capable of inducing a protective response in dogs with VL. However, the Lci13 was able to induce a Th1/Th2 balance in less cultivation time (24 hours) with continued stimulation of Th1 response (IL2). Therefore, the new antigen Lci13 seems to be a promising candidate for use in immunomodulatory and vaccinology experiments and it was chosen to be evaluated in polisymptomatic, oligosymptomatic and negative dogs for VL.

C0171 DEVELOPING MULTI-SUBUNIT B AND T CELL EPITOPE BASED THERMOSTABLE VACCINE USING IMMUNOINFORMATICS APPROACH TO CONTROL VISCERAL LEISHMANIASIS DYNAMICS

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1 Background

Visceral Leishmaniasis (VL) is a fatal form of leishmaniasis which affects 70 countries, worldwide. Increasing drug resistance, HIV co-infection and poor health system requires effective vaccination strategy to control the VL transmission dynamics. Therefore, a holistic approach is needed to generate T and B memory cells to mediate long term immunity against VL infection. Therefore, immunoinformatics approach was applied to design the multi-subunit B and T cell epitope based thermostable vaccine to control the VL infection in affected countries.

2 Methods

Initially, 58 secretory protein sequences were selected to generate T cell epitopes by using SVMHC and PROPRED server for MHC class I and II representation, respectively. While, B cell epitopes were generated using DISCOTOPE and BCEPREDS software. Based on physio-chemical parameter, best secretory proteins were selected for vaccine design. SHANNON entropy was used to measure the variability and conserved region of proteins. Further, two protein involved in secretory pathways were modelled using SWISS model to yield 3Dstructure of final chimeric vaccine. Finally, molecular dynamics (MD) was performed to develop thermostable and flexible regions of the protein for disulphide engineering, using GROMACS.

3 Results

T cell and B cell epitopes were obtained to elicit both cellular and humoral immunity. Further, all the epitopes of two proteins were combined together to build the final vaccine protein model. By the help of modelled protein structure, we found suitable linkers. RMSDs and RMSFs were also evaluated to check the stability of modelled proteins and residues also. Now, we identify the most flexible region in the refined vaccine model through MD simulation and thus, on the basis of RMSFs we predicted the disulphide bonds. Finally, a multi-epitope subunit vaccine was designed which could generate effective Th1 immune response against *Leishmania donovani* infection.

4 Conclusions

Vaccination is a well-established preventive strategy to control the vector borne diseases. To improve the existing vaccines, we applied a holistic approach which helps to generate a multi subunit T and B cell epitopes based thermostable vaccine. We believe that this immunoinformatics approach based vaccine will be able to improve the transmission dynamics of VL infection by generating effective immunological memory.

C0180 EVALUATION THE DNA VACCINE CONTAINING LEIF AND TSA GENES AGAINST MURINE CUTANEOUS LEISHMANIASIS CAUSED BY LEISHMANIA MAJOR

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1 Background

Leishmania eukaryotic initiation factor (LeIF) antigen (Ag) is a prototype of the DEAD-box protein family that is conserved in both amastigote and promastigote forms. Thiol-specific antioxidant (TSA) protein of *L.major* with a molecular weight about 22.1kDa is a homologue to eukaryotic thiol-specific-antioxidant. This protein presents homogeneously on the surface of both amastigote and promastigote forms. Several copies of the TSA exist in all species of *Leishmania*

2 Methods

Transfection of pEGFP-LeIF-TSA in Human Embryonic Kidney 293 (Hek-293). After 48 hours, GFP expression into HEK-293 cells was evaluated by an inverted fluorescent microscope.
The mice were immunized intramuscularly (i.m.) three times at 3 week intervals with 100 µg of each plasmid DNA containing the gene of interest or with DNA alone (empty vector) prepared in PBS. The mice were divided into five groups (15 mice per group) for DNA vaccination; group I, group II, group III, group IV and group V were injected with pEGFP-LeIF, pEGFP-TSA, LeIF-TSA fusion, pEGFP-N1 (empty vector) and with PBS only, respectively. Three weeks after the last immunization, five mice from each group were sacrificed to assay the cytokines. Remained animals of all groups were subcutaneously challenged into their tail base with 2×10^6 stationary-phase *L. major* (MRHO/IR/75/ER) promastigotes. The challenged mice were divided into two sub-groups; one sub-group for measuring lesion diameter and another sub-group for cytokine assay.

3 Results

All immunized mice with plasmid DNA increased IFN-γ and IgG2a levels compared to control groups ($p<0.001$).
Furthermore, the immunized mice showed significant reduction in mean lesion sizes than empty vector and phosphate buffer saline (PBS) groups ($p<0.05$).IFN/IL-4 and IgG2a/IgG1 ratios indicated that group receiving LeIF–TSA fusion had the highest IFN-γ and IgG2a levels. In the present study,DNA immunization promoted Th1 immune response characterized by higher IFN-γ and IgG2a levels and also reduction of lesion size.

4 Conclusions

These results showed that a bivalent vaccine containing two distinct antigens may induce more potent immune responses against leishmaniasis.The obtained results in the present study revealed that fusion construct induced more potent responses in comparison to TSA and LeIF groups individually. Altogether, our findings in BALB/c mice suggest that LeIF when fused with TSA, LeIF indicatedmore potent immunity against leishmaniasis.

C0194 TRANSCUTANEOUS IMMUNIZATION USING SLA OR LACK SKEWS THE IMMUNE RESPONSE TOWARDS A TH1 PROFILE BUT FAILS TO PROTECT BALB/C MICE AGAINST A L. MAJOR CHALLENGE

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1 Background

Transcutaneous immunization (TCI) is a novel vaccination method which exploits the abundance of antigen presenting cells in the skin to induce both mucosal and systemic immunity. Leishmaniasis continues to be a health threat worldwide complicated by the absence of an effective vaccine. In our effort to define a *Leishmania* vaccine against cutaneous and mucosal forms of the disease, we investigated TCI as an alternative route to deliver *Leishmania* antigens directly to the skin, a barrier that represents a natural defense mechanism.

2 Methods

L. major soluble antigens (SLA) or recombinant LACK (*Leishmania* homolog of receptors for activated C-kinase) antigen were delivered transcutaneously onto the back of BALB/c mice with cholera toxin (CT), a potent adjuvant. BALB/c mice were immunized 3 times at weeks 0, 3, and 6 with the vaccine formulations (PBS, CT, SLA/CT or LACK/CT). Two weeks after the final boost, we assessed humoral (Antigen-specific IgG titers) and cellular immune responses (IFN-γ ELISpot and cytokine levels) in half of the mice and challenged the other half with an ear intradermal injection of 10^5 *L. major* metacyclic parasites. Mice were monitored weekly and sacrificed after 7 weeks to determine parasite burden and to study ear immunohistopathology.

3 Results

We showed that TCI with SLA or LACK resulted in high titers of anti-SLA IgG and anti-CT IgG. A high frequency of IFN-γ secreting cells was also seen in groups of mice immunized with SLA/CT or LACK/CT. A Th1-type immune response was demonstrated with high levels of IFN-γ production and lower levels of IL-10 resulting in a significantly higher IFN-γ/IL-10 ratio compared to the control groups ($p=0.00002$). After challenge, SLA/CT and LACK/CT immunization were associated with larger ear lesions and increased parasite load in the ear and draining cervical lymph nodes. Immunohistochemistry of ear biopsies using iNOS (nitric oxide synthase) revealed that staining intensity was diminished in the SLA/CT as compared to the control group. This finding suggests that less parasite killing occurred at the site of the infection.

4 Conclusions

Based on the demonstrated strong Th1 profile and the subsequent *Leishmania* challenge outcome, our results indicate that factors other than cytokine profiling are important for a protective immune response. The resulting suppression of iNOS production was unanticipated and further investigation of the mechanism of this exacerbation of infection is planned.

C0210 PHOTO-INACTIVATION OF LEISHMANIA AMAZONENSIS AS AN IMMUNOPROPHYLAXIS TOOL FOR CUTANEOUS LEISHMANIASIS

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1 Background

Photodynamic therapy (PDT) uses photosensitizers to generate cytolytic singlet oxygen and other reactive oxygen species (ROS) upon exposure to light. Transgenic *Leishmania* can be photoinactivated through endogenous accumulation of cytoplasmic uroporphyrin or by taking up exogenous photosensitizers. Herein we employed the exogenous and the endogenous strategies of photoinactivation to *Leishmania amazonensis* to generate a whole-cell vaccine against experimental cutaneous leishmaniasis.

2 Methods

For the exogenous strategy *L. amazonensis* promastigotes were exposed to allumin~~i~~um (AlPh) or silicon-phthalocyanines (Pc2) and white light whereas for the endogenous strategy transgenic *L. amazonensis* expressing PGBD and ALAD were exposed to ALA and longwave UV light. Photoinactivated parasites were used to infect bone marrow derived macrophages (BMDM) and inoculated into mice to evaluate infectivity and immunogenicity.

3 Results

Photoinactivation with Pc2 or ALA-exposure inhibited parasite growth as evaluated by MTT assay and growth in culture medium. BMDM infected with Pc2-photoinactivated or ALA-exposed parasites showed a 4-fold decrease in infection rate compared to untreated controls. BALB/c mice inoculated with Pc2-photoinactivated parasites developed a positive DTH response to *Leishmania* antigen. Mice inoculated with photoinactivated parasites and later challenged with *L. amazonensis* promastigotes showed a smaller disease burden compared to controls. On the other hand, mice inoculated with ALA-induced photoinactivated parasites failed to confer protection against a challenge infection. Double photoinactivation using ALA-induced uroporphyrin and AlPh inhibited lesion development upon parasite inoculation into BALB/c mice. Following challenge, mice failed to develop lesions and showed a significantly smaller parasite load.

4 Conclusions

The results obtained thus demonstrate that complete photoinactivation of *Leishmania* leads to an effective and safe immunization strategy against experimental cutaneous leishmaniasis caused by *L. amazonensis*.

Source of funding: CNPq and FIOCRUZ.

C0247 ROLE OF IL-17 IN THE PROTECTIVE IMMUNITY BY LIVE ATTENUATED L. DONOVANI CENTRIN DELETED PARASITES AGAINST EXPERIMENTAL VISCERAL LEISHMANIASIS

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1 Background

Visceral leishmaniasis (VL) is the fatal form of the leishmaniasis-disease complex.. Existing drugs are toxic, and the emergence of drug-resistant parasites makes *Leishmania* treatment challenging; till date there is no licensed vaccine available. We have previously reported protective role of live attenuated centrin gene-deleted *L. donovani* (*LdCen1-/-)* parasites through induction of Th1 type immune response in mice, hamsters and dogs. Previous and recent studies showed that along with Th1 cytokines, IL-17 plays a complementary role in human protection against *L. donovani* infection, and acts synergistically with IFN-γ to promote protection against *L. infantum* Infection in C57BL/6 mice, respectively. In the present study we explored role of IL-17 in protection induced by *LdCen1-/-* in C57BL/6 mice.

2 Methods

Female C57BL/6 was immunized i.v. with 3×10^6 Ldcen-/- parasites and were challenged with1×10^5 LdWT metacyclics 5wks after immunization. Sorting of Leishmania (WT-RFP, LdCen-/--mCherry) infected splenic DC was performed using FACS Aria-IITM. cDNA prepared from total RNA extract from splenocytes were analyzed for Gene expression levels using the premade TaqMan gene expression assays. Secreted cytokine levels were determined from leishmania antigen stimulated (LAg) splenocyte cell culture supernatants using multiplex ELISA. Splenocytes stimulated with LAg were analyzed for production of intracellular level of cytokines through Flowcytometry.

3 Results

Our results show that LdCen-/- parasites induce expression of Th17 promoting cytokines in infected DC sorted from spleen cells of immunized mice. It also enhances expression of Th17 inducing cytokines and inhibits Th17 suppressing cytokines both at mRNA and protein levels in immunized spleen before and after challenge. Moreover, both CD4+ and CD8+ T cells are involved in the production of IL-17A in LdCen-/- immunized group with concomitant suppression of IL-10 producing Treg and Tr1 cells. Our ex vivo study shows that Recombinant IL-23 induce Th17 and Tc17 effector memory cell in spleen after immunization.

4 Conclusions

CD4+ and CD8+ cells producing IL-17 is induced by LdCen-/- parasites in spleen which might have role in protection through production of effector memory cells upon induction with IL-23 after challenge infection. To our knowledge this is the first attempt showing role of IL-17 in vaccine induced protection by live attenuated parasites in VL.

C0319 CHALLENGES AND SOLUTIONS TO TAILOR AN ANTI-LEISHMANIA VACCINE TO THE NEEDS OF SPECIFIC REGIONS: A NANOMEDICAL APPROACH

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1 Background

Scientists have developed several vaccine candidates against visceral leishmaniosis (VL) which often do not pass clinical trials due to various legal barriers and other related issues. In this context, international research community must make an effort to help speed strategies for infectious disease control in developing countries. DNA vaccine technology offers a number of potential advantages over licensed conventional vaccines, but the safety issues based on the activation of oncogenes as a result of the unlikely integration of plasmid DNA into the host genome, represent a barrier to the production of DNA vaccines for veterinary or human use. Recently, **nanotechnology** has emerged as an attractive alternative to have greater clinical applicability in humans against different diseases. The zoonotic VL due to *Leishmania infantum* is transmitted by the bites of the sand fly *Phlebotomus perniciosus* in Spain. Several studies suggest that some immunogenic salivary proteins from this VL vector, could potentially be used to improve vaccine effectiveness.

2 Methods

Murine bone marrow-derived DC (dendritic cells) and canine macrophage DH82 cell line represent appropriate *ex vivo* models to study the immune response to *L. infantum* infection. We put forward the concept that both immunogenicity and safety can be improved by combination of a vector salivary molecule (**SP03B**) from the insect vector and several *Leishmania* antigens (polyprotein **HisAK70** and nucleoside hydrolase **NH36**) encapsulated in poly(lactide-co-glycolide) nanoparticles (**PLGA NPs**)

3 Results

Our data showed that *ex vivo* stimulation of macrophages and dendritic cells with PLGA-based nanoparticles encapsulating several of the most efficacious protective *Leishmania* antigens, increased the production of immune biomarkers which plays a key role in the resistance against *L. infantum* infection. Thus, **HisAK70–PLGA NPs** and **NH36-PLGA NPs** can reduce the ability of parasites to replicate within host cells.

4 Conclusions

The *ex vivo* proof of concept concerning this nanomedical approach have been encouraging, but further efforts will still be necessary to optimize the efficacy of this method using the *in vivo* model system to improve its clinical applicability to dogs and humans. Such a set of experiments are currently being carried out in our laboratory to evaluate whether these vaccine formulations can be administered in conjunction in order to elicit a synergistic effect on antileishmanial immunity.

C0322 VACCINATION WITH GENETICALLY ATTENUATED LEISHMANIA INFANTUM (KO-LIHSP70-II) ELICITS ROBUST PROTECTION IN C57BL/6 MICE AGAINST VIRULENT LEISHMANIA MAJOR INFECTION

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1 Background

Apart from leishmanization, no effective vaccine has been developed against human leishmaniasis. Approaches using parasite subunits usually fail to confer long-lasting immunity. In the last years, genetically live attenuated parasites have been tested as promising vaccine candidates in various experimental models. They elicit robust and durable immunity in absence of the potential risks of leishmanization. An attenuated *Leishmania infantum* strain lacking the *hsp70-II* gene *(KO-LiHSP70-II)* was developed in our group showing immune-prophylactic properties against *L. major* when assayed in BALB/c mice.

2 Methods

C57BL/6 mice were immunized subcutaneously with $1x10^7$ *KO-LiHSP70-II* promastigotes in the footpad. Four and twelve weeks after immunization, parasite burden was measured by limiting dilution, and humoral and cellular responses against soluble *Leishmania* antigen (SLA) were analyzed by ELISA. Vaccinated mice were intradermally challenged in the ears with 1000 metacyclic *L. major* promastigotes four or twelve weeks after immunization. Inflammatory lesions were monitored weekly using a digital caliper. Parasitological and immunological analyses were performed at different times after infection. T cell populations present in the ear and the draining lymph nodes were characterized by flow cytometry.

3 Results

KO-LiHSP70-II parasites inoculated in the footpad were limited to the popliteal draining lymph node, persisting in very low number for at least 12 weeks. Vaccinated mice challenged four or twelve weeks after immunization did not show clinical manifestations of disease; mice developed an early and moderate Th1 immune response sufficient to control the infection, avoiding tissular damage. Three days after *L. major* challenge, $CD4^+$ and $CD8^+$ IFN-y-producing T cells were detected in vaccinated mice but not in control groups.

4 Conclusions

Immunization with *KO-LiHSP70-II* parasites elicits concomitant immunity able to confer complete protection against infection with *L. major* in C57BL/6 mice. The mechanism of protection is associated with an early and moderate Th1 response elicited in the site of infection and in the draining lymph nodes, mimicking naturally acquired protection following cure. These results and others obtained in the BALB/c model support the usefulness of genetically attenuated parasites for the development of anti-*Leishmania* vaccines and the study of hallmarks of protection.

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C0347 INOCULATION OF A GENETIC VACCINE BASED ON THE ANTIGENIC S-ADENOSYLMETHIONINE SYNTHETASE PROTEIN OF LEISHMANIA INFANTUM INDUCES PARTIAL PROTECTION IN DIFFERENT MURINE MODELS OF CUTANEOUS LEISHMANIASIS.

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1 Background

Nowadays a vaccine against human leishmaniasis is not available and the characterization of new *Leishmania* protective antigens is an open field of investigation. In this study, we have analyzed the protective potential of a genetic vaccine based on *Leishmania* S-adenosylmethionine synthetase (MAT2). This enzyme, involved in the catabolism of the methionine, was found to be recognized by DTH+ Brazilian patients recovered from visceral leishmaniasis (VL).

2 Methods

DNA region encoding MAT2 was cloned in a prokaryotic expression vector to obtain the recombinant protein. For genetic vaccination, the same region was cloned in a eukaryotic vector and its expression was demonstrated in mammalian cells. DNA vaccine was assayed in three different mouse models of cutaneous leishmaniasis: *Leishmania major*-BALB/c mice (susceptible);*L. major*-C57BL76 mice (resistant), and *Leishmania braziliensis*-BALB/c mice. Evolution of the disease was followed by measuring clinical lesions and determining parasite burdens. Humoral and cellular responses was analysed after vaccination and after infection. The humoral response was studied by ELISA using the recombinant MAT2 and total parasite extracts. The cytokine response against both protein preparations was analyzed by sandwich ELISA.

3 Results

Inoculation of three doses of the MAT2 DNA vaccine results in the expression of MAT2 gene in the mammalian host and in the induction of a Th1-like response: MAT2 IgG2a specific antibodies and IFN-γ secretion by spleen cells. Vaccinated mice showed a decrease in clinical evolution of the CL and a moderate decrease in parasite burdens (*L. major* resistant model and *L. braziliensis* model). On the contrary, BALB/c mice infected with *L. major* showed the progressive form of the disease (delayed in time with respect unvaccinated controls) and *high parasite burdens in the target organs. Partial protection was concomitant with loss of the Th1 response against the MAT2 protein.*

4 Conclusions

*This is the first report demonstrating that Leishmania*MAT2 protein can be considered of interest in the design of anti-*Leishmania* vaccines. However, more immunogenic vaccination methods should be employed to increase the degree of protection.

Supporting. Grants from the Brazilian grant from CNPq (Ciencia sem Fronteiras-PVE 300174/2014-4) and Spanish Ministerio de Economía y Competitividad-FEDER FUNDING (FIS PI14/00366).

C0348 A NEW PEPTIDE-BASED VACCINE AGAINST HUMAN LEISHMANIASIS – OPTIMIZED PRECLINICAL DEVELOPMENT TO PREVENT LATE-STAGE FAILURE

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1 Background

Vaccine development against leishmaniasis is supported by evidence of natural immunity against infection, mediated by a dominant Th1 response and production of IFN-γ, IL-2 and TNF-α by polyfunctional CD4+T cells. A main challenge is how to ascertain which immunodominant epitopes are responsible for natural protection. Excreted-secreted proteins are important virulence factors present throughout *Leishmania* life stages and induce durable protection in dogs, a good model for human infection. We describe the preclinical development of a 2nd generation vaccine from the *Leishmania* secretome, with the potential for cost-effective large scale dissemination.

2 Methods

The secretome of 6 main pathogenic species was identified by Mass Spectrometry and conserved candidate antigens were searched. *In silico* class-I and –II epitope binding prediction analysis was performed (IEDB, NetMHCpan3.0, SYFPEITHI), with world coverage regarding HLA restriction. Strong binders were selected through an automated R script developed in-house, according to strict criteria (100% identity among species, predicted by >2 algorithms, low homology to host, promiscuity and solubility). Synthetic epitopes will be tested by *in vitro* immunoscreenings, firstly with human T lymphocytes from naïve individuals, measuring IFN-γ production and frequency of specific T cell repertoire.

3 Results

A high number of common proteins was found in the secretome (33-50%), consistent with high antigenic conservation among species. We selected a total of 52 protein candidates. Interestingly, we found 28 previously described vaccine candidates, and an additional 24 new candidates through a reverse vaccinology approach. The automated script allowed us to greatly restrict the epitope list, further selected to 50 synthetic peptides according to optimal IC_{50}, homology to host and solubility properties. Preliminary results for class I epitopes indicate strong immunogenicity.

4 Conclusions

Combining proteomic analysis and *in silico* tools, we reached a rapid selection of the best epitopes, with great immunogenic and antigenic potential. Validation of candidate peptides will be performed exclusively in human samples from naïve and healed individuals in medium-throughput immunoscreenings, avoiding high-cost animal trials with limited extrapolation for human immunity. Together, the preclinical validation assays will provide us a very strong base for a vaccine formulation and allow to fast-track translation to the field.

C0352 IMMUNODOMINANT POTENTIAL OF MULTIEPITOPE PEPTIDE VACCINE CANDIDATES TO RECALL A SPECIFIC MEMORY T CELL RESPONSE IN INDIVIDUALS CURED FROM CUTANEOUS LEISHMANIASIS

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1 Background

The host immune control of Leishmaniasis is associated to Th1-dominated response that promotes cytokine, activators of cell-mediated immunity such as IFN-γ. Healing is correlated with resistance to reinfection due to the development of memory T cells, suggesting that vaccination is feasible. However no vaccine is available. Believing that synthetic peptide antigens are easy to produce, distribute and administer at low cost, their use is the most suitable approach for the design of vaccine against leishmaniasis. On this basis, our objectives are the identification and the immunogenicity evaluation of multiepitope peptide vaccine candidates against human leishmaniasis.

2 Methods

Three *Leishmania* protein candidates, PSA (Promastigote Surface Antigen), LmlRAB (*L.major* large RAB GTPase) and H2B (Histone), described as immunogenic in previous studies performed in humans, mice and dogs, were screened *in silico* for T cell epitopes able to bind to the most frequent HLA class-I and class-II alleles.

Twelve multiepitope peptides were designed, chemically synthesized, combined in peptide pools and used to screen peripheral blood mononuclear cells isolated from subjects cured of cutaneous leishmaniasis due to *Leishmania major*. IFN-γ and IL-10 production was evaluated by ELISA.

3 Results

Some peptide pools were strong and specific inducers of FN-γ production in cured individuals compared to healthy controls. A pool composed of peptides containing HLA class-I and class II-binder epitopes sets apart with its highest IFN-γ levels. No IL-10 production was detected. A theoretical world population coverage calculation of this peptide pool showed that more than 90% and 75% of individuals are predicted to respond to the set of HLA class I-binder epitopes and to the set of HLA class II-binder epitopes, respectively.

4 Conclusions

In conclusion, some peptide combinations were able to elicit IFN-γ, a crucial cytokine for parasite elimination, arguing for their vaccine potential.

In order to better characterize the immune response induced by these peptide combinations, other cytokines correlated with protection such as IL-2, granzyme B and TNF-a will be evaluated by cytometric beads array, and cellular sources of cytokines will be identified by flow cytometry.

In addition to the results of induced immune response by the proposed peptide candidates, HLA typing of recruited individuals will be performed to validate the theoretical vaccine world coverage.

C0370 THE ROLE OF BILAYER COMPOSITION ON INTERACTION OF DENDRITIC CELLS WITH SOLUBLE LEISHMANIA ANTIGEN-CONTAINING LIPOSOMES

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1 Background

Dendritic cells (DCs) are antigen presenting cells which can process antigens, undergo maturation and introduce antigens to lymphocytes. Activation of DCs and their maturation can lead to a T-helper response which is favorable for immunity against antigens. Moreover, nanoparticles can play an effective role in antigen delivery. Antigen presenting cells like DCs have a negative cell surface charge, though cationic nanoparticles can be used to improve antigen delivery, immune responses against antigens and DCs maturation. In this study, we prepared, characterized and used different soluble leishmania antigen (SLA)-containing cationic liposomes to evaluate human and/or murine DC maturation in vitro. Both BALB/c and C57BL/6 mice was used as a source of DCs to study their interction with liposomes.

2 Methods

Murine bone marrow cells were isolated from femur and tibia, exposed to GM-CSF and IL-4 until converted to immature DCs. Monocyte drived DCs (MoDC) were also generated from magnetic cell-sorted CD14+ monocytes purified from human peripheral blood mononuclear cells (PBMC). Then, the cells were encountered to free SLA, liposomal SLA, free LPS as a positive control or buffer as a negative control. Liposomes' uptake and induction of surface markers such as CD40, CD80 and MHC-II, which are indicative of DC maturation, was investigated by flow cytometry in immature and mature DCs and Geometric mean (G_m) as an indicative of marker's expression level was determined.

3 Results

Liposomes' uptake by DCs was dependent on their lipid composition but there was no significant difference in liposomes' uptake between immature and mature DCs. The expression of CD40, CD80 and MHC-II in liposomal SLA was higher than buffer and again it was dependent on their formulations. Interestingly, most of the formulations induced a higher amount of maturation factors compared to LPS as a positive control.

4 Conclusions

In conclusion, cationic liposomal SLA consisting with high Tm lipid distearoyl-phosphatidylcholin (*DSPC*) can induce more DC uptake and maturation than free SLA or LPS. Moreover, presence of a cationic lipid called DOTAP in formulation, due to its adjuvant effect, is also necessary in liposomal SLA formulation.

C0380 EVALUATION THE EXPRESSION OF TLRS IN INFECTED MACROPHAGES WITH LEISHMANIA MAJOR TREATED WITH MORPHINE BY QUANTITATIVE REAL TIME-PCR

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1 Background

Toll-like receptors (TLRs) are one of the primary innate immune receptors and thirteen genes have been identified in mammals. Pattern recognition receptors (PRRs) are expressed on phagocytes that are involved in the detection of *Leishmania* by pathogen associated molecules (PAMPs). A well characterized class of PRRs are the Toll-like receptors.

2 Methods

Medium containing 10^6 promastigotes of *Leishmania magor* (MRHO/IR/75/ER) in stationary phase were added to wells including J774 macrophages (10^5) (*Leishmania*: macrophage; 1:10) and then plates were incubated in CO_2 at 37 ^oC. To detect and confirm macrophage infection with *Leishmania* parasites, plates were checked with inverted microscope and then treated with morphine in sulfate (0.1, 10 and 100 µg/ml), glucantime (50 µg/ml) or left untreated as control. After 24 hours the cells were harvested and centrifuged, the supernatant was discarded, RNA extracted from the pellet and cDNA was constructed from extracted mRNA,. Expression of TLRs (TLR1, TLR2, TLR3, TLR4, TLR7 and TLR9) was evaluated by real time PCR and specific primers.Relative gene expression normalized to beta-2 microglobulin then calculation was performed using$2^{-??CT}$ method.

3 Results

TLRs1,2, and 3 expressions were significantly increased and TLR7 expression was decreased at all concentrations of morphine in comparison with control group. TLR9 expression was increased in the morphine 0.1, and 10 µg/ml group. TLR4 expression decreased in the morphine 0.1, and 10µg/ml groups and TLR9 expression was decreased in the 100µg/ml group. Expression of all TLRs was increased in the glucantime group.

4 Conclusions

Vaccine adjuvants and immunotherapy for leishmaniasis have already yielded promising results. The adjuvants seem to improve the efficacy of vaccine therapy and immunotherapy by stimulation of cellular immunity through TLRs .This study showed that significant increase of expression of TLRs 1,2, and 3 in all f morphine treated groups. TLR3 is important in *Leishmania* recognition and plays a role in IFN-g production.
TLR7 is decreased at all concentrations of morphine. Accordingly one could use imiquimod; a TLR7 agonist to increase the expression of TLR7. TLR9 is increased at 2 concentrations of morphine, and this will activate IL-12, a very important cytokine in leishmaniasis. Overall morphine could increase some of TLRs in infected macrophages and have positive role in limiting the pathogenesis of leishmaniasis.

C0386 COMPARISON OF INTRAMUSCULAR AND SUBCUTANEOUS VACCINATION APPROACH WITH LIVE RECOMBINANT LEISHMANIA TARENTOLAE AGAINST CUTANEOUS LEISHMANIASIS

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1 Background

Leishmaniasis is a major neglected tropical disease and according to ranking after malaria it is a second most prevalent parasitic disease**.** For many years making an effective vaccine remained as a dreamy desire especially for tropical and subtropical country .The use of the *Leishmania tarentolae* as a live vaccine to deliver specific *Leishmania* antigens is a recent approach and we used this species as a live vaccine vector in protecting BALB/c mice against cutaneous Leishmaniasis.

2 Methods

Protective Efficacy of recombinant *L.tarentolae*-KMP-NTGp96-GFP Fusion that was designed in our previous studies was tested with intramuscular (leg muscle) and subcutaneous (footpad) vaccination approach in susceptible BALB/c mice. 3 weeks after the last immunizations, the mice were challenged with stationary phase promastigote of *L. major* by subcutaneous injection in the tail base. Clinical evaluations were performed by measurement of lesion diameter, and survival rate of the mice. Both humoral and cellular immune responses were assessed before and at 4 and 10 weeks after *Leishmania* challenge.

3 Results

The strong protective effects were observed with *L. tarentolae*-KMP- NT-Gp96-GFP *by both vaccination strategies* that provides significant protection against cutaneous leishmaniasis and was able to induce an increased expression of IFN-γ and IgG2a.Lesion size was less for immunized animals than control groups in two approaches. Spleen parasite burden decreased in all immunized groups in comparison with control groups ($p<0.05$).

4 Conclusions

The present study is the first to use a combination of leishmania antigen with an immunologic antigen in live recombinant *L. tarentolae* and this vaccination strategy could provide a potent rout for future vaccine development. Subcutaneous injection of live parasites into the foot-pad is the common approach for vaccination, but in this study, we found that we could use the intramuscular approach for this purpose. More additional studies need for determining the immunological basis of this approach.

C0498 COMPARISON OF IMMUNE RESPONSES ELICITED BY THIOL-SPECIFIC ANTIOXIDANT-BASED PROTEIN VACCINES AND DNA VACCINES WITH ADJUVANTS IN BALB /C MICE INFECTED WITH LEISHMANIA MAJOR

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1 Background

Leishmaniasis is caused by parasitic protozoa of the genus *Leishmania*. No effective vaccine has been developed yet against the disease. The purpose of this study was to study efficacy of DNA and protein vaccines with adjuvants in BALB /c mice infected with *Leishmania major.*

2 Methods

Protein vaccines were included with the recombinant TSA protein (TSA , thiol-specific antioxidant, a main antigen of *Leishmania major*) and montanide ISA 70 oil adjuvant and chitosan nanoparticle . DNA vaccines were designed with TSA recombinant plasmid and dendrimer and PMMA nanoparticles (as adjuvants). After expression and purification of the recombinant TSA protein and preparation adjuvants , BALB/c mice were divided into six groups [TSA/ high chitosan , TSA/ low chitosan , TSA/ montanide and high chitosan, low chitosan, PBS groups] and subcutaneously immunized with 20 µg of recombinant protein TSA vaccine and 40 µg of adjuvants at three times intervals on days 0, 14 and 28. In DNA vaccine,After plasmid construction and preparation adjuvants ,The mice were divided into 7 groups [pcDNA3/TSA, pcDNA3/TSA +dendrimer and pcDNA3/TSA +PMMA and pcDNA3, dendrimer, PMMA and PBS] . Immunization schedule was performed with 3 inoculations (equal doses of 100 µg), with intramuscular injections at 3 week interval . The booster peptide (20 µg) and incomplete Freund's adjuvant were subcutaneously injected two weeks after the last nano-vaccines injection. The mice were challenged intradermally with parasites ($1*10^6$ promastigotes of *L.major*) , 21 days after the final immunization . The animals were then sacrificed the spleens and serum samples were harvested for immunological analysis 3 weeks after the last immunization and 3 weeks after the challenge infection.
The lymphocyte proliferation was assayed with Brdu method. Cytokines(IFN-γ,IL-4) and antibodies (Total IgG,IgG1,IgG2a) responses were assayed with ELISA method.

3 Results

This study showed that the new formulated vaccines were strong and effective in inducing the specific antibody and cellular responses and reducting the parasite burden in spleen compared to control groups .

4 Conclusions

Based on this results, we may suggest that the formulated vaccines are suitable candidates to undergo further studies in the context of leishmaniasis control.

C0553 COMBINATION OF IN SILICO METHODS IN THE SEARCH FOR POTENTIAL CD4+ AND CD8+ T CELL EPITOPES IN THE PROTEOME OF LEISHMANIA BRAZILIENSIS

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1 Background

The leishmaniasis are neglected tropical diseases widespread throughout the globe which are caused by protozoa from the genus *Leishmania* and are transmitted by infected phlebotomine flies. The development of a safe and effective vaccine against these diseases has been seen as the best alternative to control and reduce the number of cases. To support vaccine development, this work has applied an *in silico* approach to search for high potential peptide epitopes able to bind to different Major Histocompatibility Complex Class I and Class II (MHC I and MHC II) molecules from different human populations.

2 Methods

First, the predicted proteome of *Leishmania braziliensis* was compared and analysed by modern linear programs to find epitopes with the capacity to trigger an immune response. This approach resulted in thousands of epitopes derived from 8,000 proteins conserved among different *Leishmania* species. Epitopes from proteins similar to those found in host species were excluded and epitopes from proteins conserved between different *Leishmania* species and belonging to surface proteins were referentially selected. The resulting epitopes were then clustered, to avoid redundancies, resulting in a total of 230 individual epitopes for MHC I and 2,319 for MHC II. These were used for molecular modeling and docking with MHC structures retrieved from the Protein Data Bank (PDB). Molecular docking then rankedepitopes based on their predicted binding affinity to both MHC I and II.

3 Results

Peptides corresponding to the top 10-ranked epitopes were synthesized and evaluated *in vitro* for their capacity to stimulate PBMC from post-treated cutaneous leishmaniasis patients, with PBMC from healthy donors used as control. From the ten peptides tested, 50% showed to be immunogenic and capable to stimulate the proliferation of lymphocytes from recovered individuals.

4 Conclusions

The methodology developed in this work was capable to identify peptides in the whole proteome of *L. braziliensis* with potential to be used as vaccine candidates.

C0554 EVALUATION OF IMMUNOLOGICAL PARAMETERS AFTER STIMULATION OF LEISHMANIASIS PATIENTS CELLS WITH CD4+ AND CD8+ T CELL EPITOPES FROM THE PROTEOME OF LEISHMANIA BRAZILIENSIS

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1 Background

Leishmaniasis are a group of neglected diseases which affects populations in 98 countries and territories. Drugs and vector control are currently available methods to limit these diseases. Up to today, there is no safe and effective vaccine against *Leishmania* to be applied in humans. This work tested the immunogenicity of ten different $CD4^+$-$CD8^+$ 15-mer epitopes from the proteome of *Leishmania braziliensis* with PBMC from cutaneous leishmaniasis (Active disease-AD; Post-treatment-PT) and resistant (RT) individuals.

2 Methods

Initially, cells were labelled with CFDA-SE and stimulated with 20µg/mL of each peptide, individually, for 96h at 37°C with 5% CO_2. Cell supernatant was stored for Th1, Th2, and Th17 cytokines measurements, and the cells were processed for flow cytometry. T-bet expression was assessed in $CD4^+$ and $CD8^+$ T cells.

3 Results

A significant high level of cell proliferation for the RT group (ranging from 28.6% up to 38.9%) was found for all peptides tested. T-bet was significantly up-expressed in $CD4^+$ (2.58% to 5.07%) and $CD8^+$ (3.32% to 5.31%) T cells from PT group after stimulation with six peptides. Significant levels of IFN-γ, TNF, and IL-6 were observed for the cells from the PT group stimulated with four tested peptides. High levels of the same cytokines were also observed for the AD group. IL-10 was also present in PT and AD group, and very low levels of IL-4.

4 Conclusions

The peptide epitopes investigated have the potential to stimulate human cells from AD, PT patients and RT individuals, suggesting its immunogenicity.

C0555 DEVELOPMENT OF VACCINE POTENTIAL POLYEPITOPE ANTIGENS BASED ON THE COMBINATION OF IMMUNOGENIC CD4+ AND CD8+ T CELL EPITOPES FROM THE LEISHMANIA BRAZILIENSIS PROTEOME

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1 Background

Leishmaniasis is a neglected tropical disease which is widespread throughout the globe and highly endemic in Brazil. The best alternative to control this disease or improve the current therapy would be using a safe and effective vaccine. In order to support vaccine development, this study was carried out using ten peptide epitopes to construct two chimeric polyepitope proteins to be evaluated as potential vaccine candidates.

2 Methods

Modern linear and structural methods were used to search for combined $CD4^+$ and $CD8^+$ T cell epitopes in the predicted proteome of *Leishmania braziliensis*. The 10 best ranked sequences were selected to construct the chimeric polyepitopes. For this, the softwares A Plasmid Editor (APE v. 2.0.49) and Gene Designer 2.0 (v. 2.0.191) were used to design two genes containing the sequences of the polyepitopes. The softwares OPTIMIZER and CAI Calculator 2 were applied to optimize the genes for expression in *Escherichia coli* K12 and to verify the codon adaptation index. In one gene the combination of spacer sequences Pan Dr Epitope (PADRE) and GPGPG were added in frame between each pair of peptides to enhance the immune response. The other gene was composed with the epitopes and the PADRE spacers between each pair of peptides. Both genes were submitted to protocols of transformation in chemically competent *E.coli* cells; plasmid purification by a manual miniprep protocol; sub cloning in the expression vector prSETa and induction with Isopropyl β-D-1-thiogalactopyranoside (IPTG).

3 Results

After synthesis, the genes were resuspended in ultrapure water and transformed in *E.coli* TOP10. Then, the confirmation of transformation success was visualized through the growth of colonies on the culture dish. Plasmid purifications were performed with a manual miniprep, and the DNA was further digested releasing the inserts. The digestion confirmation was done visualizing the predicted bands in a SYBR safe agarose gel. Afterwards, the genes were sub cloned in the expression vector prSETa, transformed in BL21 Star competent cells and submitted to

induction with IPTG. The induction confirmation was observed in a polyacrylamide gel, where only the gene containing both spacers succeeded.

4 Conclusions

One gene was expressed successfully and it is necessary to purify the proteins and perform other biological assays to evaluate their capacity of eliciting a satisfactory immunological response.

C0570 EVALUATION OF A NOVEL LENTIVIRAL VACCINE EXPRESSING KMP11-HASPB FUSION PROTEIN AGAINST LEISHMANIA INFANTUM IN BALB/C MICE

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1 Background

According to the latest reports, more than 98 countries of the world are involved in leishmaniasis, and about 2 million new cases are diagnosed annually. Vaccine development against leishmaniasis, apart from chemical therapeutics, has become an inevitable issue. Hydrophilic acylated surface protein B is an immunogenic Leishmania protein and kinetoplastid membrane protein 11 is another protein antigen of Leishmania which is reported as a promising candidate for vaccination of visceral leishmaniasis. We have evaluated the immunogenicity of the recombinant lentiviral vaccine expressing L. infantum's KMP11-HASPB fusion protein in BALB/c mouse model of L.infantum infection.

2 Methods

he coding sequences of KMP11 and HASPB of L.infantum JPCM5 were synthesized by Genscript, and sub-cloned in pCDH513B using XbaI and BamHI restriction enzymes. HEK293T cells were transfected using transfer lentiviral vector with packaging plasmids by calcium phosphate transfection method. The gene expression was confirmed using RT-PCR and western blot methods. Then injected subcutaneously with $1x10^6$ TU of recombinant lenti-KH (10^6 TU/ml), and $1x10^6$ TU of recombinant Lenti-NON ($1x10^7$TU/ml) or 0.1 ml PBS. Three weeks post-injection, splenocytes and the sera of mice were analyzed for the expression of cytokines and antibody level. The mice were injected intraperitoneally 3 weeks after the last vaccination. Four weeks post-infection, the parasite burden of the liver and spleen of the vaccinated mice were quantified by limiting dilution.

3 Results

A significant increase of IFN-γ and IL-4 as well as IgG1 and IgG2a was observed compared to the control group ($Pv<0.05$). Furthermore, the number of parasites in the liver and spleen of vaccinated mice decreased significantly compared with the control group ($Pv<0.05$).

4 Conclusions

the results of our study indicate that the lentiviral vaccine expressing KMP11-HASPB fusion protein is capable of inducing the immune system to produce cytokines and specific antibodies against *L.infantum* and resulted in decreased parasite burden. Considering the fact that mouse immune response is different from that of human, it is important to evaluate the vaccine in other animal models. Furthermore, since this is the first report of the protective effects of KMP11-HASPB fusion protein against *L.infantum*, more studies and experiments are needed to verify the capability of this fusion protein as a candidate vaccine.

C0607 ALTERNATIVE STRATEGIES AGAINST VISCERAL LEISHMANIOSIS: HISAK70-SALMONELLA CHOLERAESUIS-PULSED DCS

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1 Background

Attenuated *Salmonella* can transfer plasmids that encode foreign antigens under the control of eukaryotic promoters to the host cells, being considered as suitable candidates for the delivery of DNA vaccines (Salmofection). We describe a new approach by using an antibiotic resistance free expression plasmid (pVAXasd-HisAK70) that encodes the polyprotein HisAK70 carried by an attenuated Δ*asd* Δ*rpo*S Δ*omp*A triple mutant of *Salmonella enterica* serovar Choleraesuis (*S.* Choleraesuis ARO). Subsequently, murine bone marrow derived DC (DCs) were pulsed with this vaccine vector. The prepared HisAK70 - *S.* Choleraesuis-pulsed DCs represent an alternative approach for DNA vaccine delivery due to their ability to confer long term resistance against visceral leishmaniosis in mice.

2 Methods

S. Choleraesuis ARO were transformed by electroporation with pVAXasd-HisAK70. *In vitro* plasmid stability in *S.* Choleraesuis, and HisAK70 expression in DCs were assessed. For pulsing DCs with *S.* Choleraesuis, bacteria were grown to stationary phase and adjusted to a multiplicity of infection of 100:1 (bacterial/eukaryotic cell ratio). After 24h, DCs were washed, collected and counted. Then, cells were used for immunization assays. BALB/c mice were immunized intravenously with $5x10^5$ *S.* Choleraesuis pulsed-DC twice every two weeks. Challenge with $1x10^6$ stationary-phase promastigotes of *L. infantum* (M/CAN/ES/96/BCN150, zymodeme MON-1) was done two weeks (n=5) or 10 weeks (n=5) post immunization. Groups of infected mice were euthanized previously to the challenge (n=2), and at day 30 post-challenge (n=5). Draining lymph nodes, spleens and livers were removed in order to determine parasite burdens and other immunological parameters.

3 Results

In this study, we developed an elegant strategy to drive DNA vaccines into immune target cells (DC) by attenuated intracellular bacteria. In our *ex vivo* model of DC infection, the prepared vaccine formulation could reduce parasite growth by up to 80% by reducing arginase activity. Also, mice when immunised with this formulation showed significant reduction in parasite burden in both spleen (20% of reduction) and liver (75% of reduction) through decreased IL-10 levels.

4 Conclusions

Our results suggest that HisAK70-*S.* Choleraesuis-pulsed DCs could be considered as an alternative vaccine approach against visceral leishmaniosis.

C0652 GENE SIGNATURES ASSOCIATED WITH PROFILE OF CD8+ T-CELLS DURING VISCERAL LEISHMANIASIS

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1 Background

Visceral leishmaniasis (VL) is one of the major neglected tropical diseases where the role of CD8 has been poorly understood. Following infection, $CD8^+$ T cell activation generates several effector mechanisms for killing the target cell. In addition to the naïve $CD8^+$ T cells, effector/memory $CD8^+$ T cells and CD8+ Treg cells employ different mechanisms for regulating immune responses. Given these various fates for CD8+ T cells in response to infection, there has been a lot of interest in the factors that regulate these processes. We hypothesize that $CD8^+$ T cell function is compromised in VL patients and recovers following successful drug treatment.

2 Methods

This has been tested by nanostring technology for studying the transcriptional profile of $CD8^+$ T cells in VL patients, and compared after 30 days of drug treatment, as well as with endemic controls to measure 130 mRNA species involved in T-cell development, activation, function and regulation. The results were analyzed for the difference in the expression level in terms of adjusted p-values and subsequently IPA (Ingenuity Pathway Analysis) was done to uncover the alterations in the signaling pathways.

3 Results

We have found significant changes in the effector functions that were compromised in diseased state as compared to the healthy subjects which was further confirmed from the transcriptional factors that regulate the differentiation, activation and proliferation of CD8+ T-cells.These results were further validated at protein levels by looking at the significant targets obtained from transcriptional profiling by flow cytometry; this was extended for the functional aspects by selectively targeting the key pathways of immune responses by CD8+ T-cells.

4 Conclusions

This study will further open avenues for new insights into the immunomodulatory roles of $CD8^+$ T cells during chronic infectious diseases and identify new immune checkpoint molecules for manipulating this T cell subset for therapeutic advantage in VL.

C0670 SURVEY OF CLINICAL AND MOLECULAR FORMS OF ATYPICAL CUTANEOUS LEISHMANIASIS IN BAM IRAN

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1 Background

Due to the presence of atypical forms of cutaneous leishmaniasis especially in endemic areas with lack of access to advanced diagnostic methods, This study aimed to investigate the clinical and molecular forms of atypical cutaneous leishmaniasis in endemic region of Bam, Iran.

2 Methods

Materials and Methods: This cross-sectional study from patients admitted to the medical center in Bam city as Leishmanial endemic foci of Iran in the period 1393-1395 performed.Clinical evaluation and providing wound smear sampling of 40 typical and 40 atypical lesions was performed by a dermatologist.The slides were examined under a microscope and the severity of the infection was evaluated by counting the number of parasites. DNA extraction for molecular analysis were performed using by slides.

3 Results

from 40 prepared slides of Typical ulcer, The microscopic examination And PCR were positive.But the each of 40 slides prepared from atypical wounds That All of them in microscopic view were negative ,30 of them in PCR was Positive And all of them were L-tropica.

4 Conclusions

results of this study show That cutaneous leishmaniasis In endemic areas is importantand in Of atypical forms Be worth pursuing For the correct treatment of patient.

C0696 IMMUNE RESPONSE EVALUATION AGAINST LIVE LEISHMANIA VS. CRUDE LEISHMANIA ANTIGENS IN VOLUNTEERS WITH HISTORY OF CUTANEOUS LEISHMANIASIS

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1 Background

Leishmaniasis is a major health problem in some of the endemic areas, cutaneous leishmaniasis (CL) is a major health problem in many endemic foci, treatment of leishmaniasis is difficult especially CL and is challenging issue in endemic areas. The sole recommended chemotherapy is using antimonite derivatives which serves side effects and is not always effective. Development of vaccine against different forms of leishmaniasis has a long history, several first generation *Leishmania* vaccines have been tested in clinical trials, but so far no vaccine is available against any form of human leishmaniasis. The only effective prophylactic method to control CL so far is leishmanization(LZ).
In this study, volunteers with history of CL caused by either *L. major* or *L.tropica* were recruited, management of the lesions of the volunteers were done by the same group. The immune responses of the volunteers along with control groups was checked using Live *L.major, L.tropica,* crude *Leishmania* antigens.

2 Methods

Patients :Blood samples were collected from volunteers with history of CL caused by either by *L. major* or *L. tropica* (recently cured from CL), peripheral blood mononuclear cells (PBMC) was isolated and cultured in the presence of PHA, either Live *L.major* , *L.tropica*, or SLA. The culture supernatants were collected at 72 hours after stimulation and IFN-γ and IL-5 were titrated using ELISA method.

3 Results

The mean of IFN-γ produced by PBMC stimulated with PHA, SLA, Live *L.major*, *L.tropica* and no stimulation was 3578pg/ml ,1345 pg/ml,2850 pg/ml,2570 pg/ml, 257 pg/ml respectively.IL-5 was low and there was no significantly difference between the different groups.

4 Conclusions

The results showed that Th1 type of immune response generated in volunteers with history of CL is significantly stronger against live *Leishmania* than crude *Leishmania* antigens, that might be a reason that LZ is effective to control CL but not any one of killed *Leishmania* vaccines so far checked

C0708 LEISHMANIA ACTIVATION OF MURINE NEUTROPHILS IS SPECIES SPECIFIC

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1 Background

Innate immunity plays a crucial role in the initial phase of *Leishmania* infection. After parasite inoculation in the host dermis, leukocytes accumulate at the infection site. Neutrophils, the first cells to arrive exhibit different mechanisms able to provoke microbicide killing at the intracellular and extracellular space. Molecular oxygen can be converted in superoxide anion (O2-) that can promote intracellular parasite lysis. Neutrophil extracellular traps (NET) and enzyme exocytosis are additional effector mechanisms aiming to trap and compromise the parasite viability in the outside space. Thus, the present study investigates the direct effect of visceral and cutaneous *Leishmania* species on activation of BALB/c mouse neutrophils, exploring the activity of oxidative and non-oxidative pathways and NET release.

2 Methods

Neutrophil isolated from BALB/c mice were exposed to *L. (L) infantum, L. (L) amazonensis, L. (V) shawi* and *L. (V) guyanensis* cultured promastigotes. To access neutrophil activity the production of O_2^- was measured by colorimetric nitroblue tetrazolium assay, the extracellular activity of elastase (NE) and catepsine G (CatG) were evaluated using specific colorimetric substrates and NET emission was observed by scanning electron microscopy.

3 Results

Mouse neutrophils internalized promastigotes of all species and tolerated amastigote differentiation. All the infected cells presented high levels of O2-, however *L. infantum* and *L. guyanensis* was the species that induced the lower production. *L. amazonensis* stimulates NE and CatG enzymatic activity. *L. shawi* induced CatG and, *L. infantum* and *L. guyanensis* elicited NE, indicating species specific exocytosis of these enzymes. Although neutrophils have emitted NET against visceral and cutaneous species of *Leishmania*, *L. amazonensis* avoided being trapped by NET fibers.

4 Conclusions

Despite mouse neutrophils respond differently to the species evaluated in the present study, parasites of *Leishmania* subgenus appear to be better tolerated by neutrophils. It is possible that neutrophil tolerance favors *L. infantum* spread to internal organs and the development of skin lesions rich in *L. amazonensis* amastigotes. Although the establishment of visceral and cutaneous diseases depends on a complex parasite-host interplay a proper immune response displayed right away parasite inoculation can determine the infection outcome.

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C0725 MULTI-EPITOPE BASED IMMUNIZATION WITH OUTER MEMBRANE PROTEINS OF LEISHMANIA DONOVANI EMPLOYING IMMUNOINFORMATICS TO PRODUCE THERMODYNAMICALLY STABLE VACCINE

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1 Background

Visceral Leishmaniasis (VL) is a vector borne disease which affects 70 countries worldwide. Incompetence nature of vector control methods, increasing drug failure cases and HIV co-infection requires effective vaccination strategy to control the VL in the affected countries. Novel vaccine designed on the basis of B cell and T cell epitopes can become the next generation vaccine for the prevention and elimination of visceral leishmaniasis.

2 Methods

Here, we have implemented protein structure based strategies to design multi-epitope based vaccines against VL infection. For this study, we took 50 outer membrane protein sequences from the amastigote stage of *Leishmania donovani* parasites. Later on, it was used to generate B and T cell epitopes by using T cell showing MHC class I representation and (PROPRED) for MHC class II representation, B cells (DISCOTOPE, BCEPREDS) specific software. Further, epitopes were selected from the five protein involved in secretory pathways and were modelled using SWISS model and PHYRE2 to yield 3Dstructure of final chimeric vaccine. Allergic nature, antigenicity and stable nature were also confirmed using different software. Finally, we propose a stable multi epitope based vaccine, on which we also performed molecular dynamics to develop thermostable and most flexible regions of the protein for disulphide engineering by using GROMACS.

3 Results

Isoelectric point, molecular weight and localization of 50 outer membrane proteins data provide the involvement of five proteins in the secretory pathways. Further, all the epitopes of five proteins were combined together with the conserved amino acid residues and the highly conserved peptide were chosen to build the final vaccine protein model. To obtain a high quality improved 3D model of vaccine protein without any structural irregularities; energy minimization of the predicted 3-dimensional model was carried out. Energy minimization were refined the model and the vaccine protein gained the most stable model. RMSDs and RMSFs were also evaluated to check the stability of modelled proteins and residues also. Final chimeric vaccine has shown good antigenicity, negligible allergic nature and thermodynamically stable existence.

4 Conclusions

Immunoinformatics based approached were employed to generate a holistic and thermodynamically stable vaccine candidate, which can provide a next generation epitope based efficient vaccine against visceral leishmaniasis.

C0735 NOVEL CLASS II T CELL EPITOPES PREDICTED FROM THE SECRETOME OF LEISHMANIA BRAZILIENSIS EVOKE INTERFERON-GAMMA-PRODUCING T CELLS IN ACTIVE CUTANEOUS LEISHMANIASIS PATIENTS

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1 Background

Cutaneous leishmaniasis (CL) is a parasitic disease of public health importance in Latin America. *Leishmania braziliensis* is one of the most important CL-causing species because of its prevalence, association with treatment failure, and risk of mucosal compromise. No effective vaccine against CL is yet available. We aimed to predict potentially immunogenic class-II T cell epitopes from the *L. braziliensis* secretome and assess their potential to induce cellular immune responses in active CL patients.

2 Methods

The *L. braziliensis* partial secretome (39 proteins) was screened using immunoinformatics tools to identify putative highly immunogenic HLA class II-restricted T cell epitopes. Epitopes conserved across *Leishmania* species and showing low cross-reactivity with the human genome and microbiome were selected. HLA-peptide binding affinity was verified *in vitro*. Peripheral blood mononuclear cells (PBMC) derived from active CL patients and healthy individuals were pre-stimulated for 14 days with individual peptides or a peptide pool, followed by identification of IFN-g-producing T cells by enzyme-linked immunospot assay (ELISpot). Phytohemagglutinin and total crude leishmanial antigen were included. After the 14 days, cells were harvested and cultured with each stimulus on pre-coated IFN-g ELISpot plates during 24 hours. Spot-forming units (SFU) were counted using an ELISpot reader by subtracting background values and expressed as SFU per million PBMC.

3 Results

Thirty-one MHC class II epitopes were predicted from the *L. braziliensis* secretome, of which 8 peptides showed less cross-conservation with the human genome. The HLA binding assay confirmed 7 of the 8 peptides as strong binders to at least one HLA allele. Our first experiments with PBMC from 4 CL patients using single peptide stimulation did not induce IFN-g-producing T cells. We next used a peptide pool consisting of 5 peptides displaying the highest affinities on the HLA binding assay. IFN-g-secreting T cells were produced most frequently in response to the peptide pool in CL patients (mean ± standard deviation, 393 ± 353 SFU/10^6 PBMC; n=3) as compared to healthy donors (19 ± 12 SFU/10^6 PBMC; n= 2).

4 Conclusions

We identified *L. braziliensis* epitopes with high capacity to stimulate IFN-g-producing T cells derived from PBMC of active CL patients. Further work is required to compare T cell responses among patients with active CL, healed CL, and healthy subjects from *L. braziliensis*-endemic areas.

C0743 PRELIMINARY EVALUATION OF CROSS-REACTIVITY BETWEEN COMMERCIALLY AVAILABLE ANTI-HUMAN MONOCLONAL ANTIBODIES AND MONONUCLEAR CELLS /CYTOKINES FROM DOGS FOR FLOW CYTOMETRY ASSAYS

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1 Background

Studies of immunological responses developed by dogs are extremely important to elucidate about immune profiles that are involved in resistance or susceptibility to diseases. The cytokines are associated with several important physiological processes, such as the pro- and anti-inflammatory equilibrium, related with the control of diseases like canine visceral leishmaniasis. Furthermore, they may help to identify prognostic markers of several diseases and to evaluate new vaccine candidates. For this immune characterization, the Flow Cytometry (FC) is a powerful tool with high analytical capacity, able to precisely indicate several cellular parameters. Nevertheless, the availability of conjugated antibodies for dogs is commercially restricted, for both cell surface and intracellular cytokine staining. The aim of this study was to perform a preliminary evaluation of cross-reactivity between anti-human monoclonal antibodies (mAb) and mononuclear cells /cytokines from dogs for FC assays.

2 Methods

Heparinized periferal blood from one human and one dog (both healthy) was aliquoted and cultured in RPMI medium during 24h with phytohemagglutinin (PHA) at 5µg/ml and without stimulation (WSt). Immunophenotyping of human and canine leukocytes was performed with the following conjugated mAbs: anti- CD3, CD4, CD8, CD14, CD25, FOXP3, CD28 and CTLA-4. Intracytoplasmic staining of cytokines was performed with: anti- IL-4, IL-10, TNF-α, IFN-γ and granzyme B. The experiments were performed in FACSCalibur™ flow cytometer and analyzed in CellQuest Pro software. The ratio between the percentages of positive human and canine cells for each staining was used to indicate cross reactivity.

3 Results

The following ratios were obtained (PHA [%HUM/DOG] and WSt [%HUM/DOG]): CD3 ([7.39] and [23.17]), CD4 ([24.95] and [55.94]), CD8 ([54.85] and [38.34]), CD14 ([1.25] and [0.79]), CD25 ([9.37] and [10.19]), CD28 ([24.24] and [41.05]), FOXP3 ([1.24] and [2.84]), CTLA-4 ([2.05] and [1.11]), IL-4 ([0.38] and [5.0]), IL-10 ([0.85] and [0.44]), TNF-α ([0.4] and [1.1]), IFN-γ ([7.2] and 0.69]) and granzyme B ([1.67] and [1.13]).

4 Conclusions

Satisfactory cross-reactivity was suggested with important pro- and anti-inflammatory cytokines: CD14, FOXP3, CTLA-4, IL-4, IL-10, TNF-α and granzyme B. Further analyses are necessary, but these results already indicate the possibility of fewer restrictions to develop searches in immunology/vaccinology with dogs using FC.

C0776 PRE-CLINICAL TRIAL USING VACCINE NANOFORMULATIONS COMPOSED BY LEISHMANIA SP. ANTIGENS AGAINST VISCERAL LEISHMANIASIS

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1 Background

Leishmaniasis is a vector-born disease widely disseminated in the Brazilian territory and the *Leishmania* (*Leishmania*) *chagasi* specie is the main etiological agent of canine visceral leishmaniasis (CVL). The development of an efficient vaccine would be the best approach to control the CVL.

2 Methods

In this way, our study has employed hamsters (*Mesocricetus auratus*) as experimental model in anti-*Leishmania* pre-clinical vaccine assays to analyze the safety, antigenicity and levels of protection induced by two innovative nanoformulations prepared by the combination of polymer poly-*D,L*-lactic acid (PLA) with *Leishmania* sp. antigen (Ag). These formulations were identified as Submicrometric Particle I associated with *Leishmania* sp. antigen ($PSm^{I}Ag$) and Submicrometric Particle II associated with *Leishmania* sp. antigen ($PSm^{II}Ag$). The LBSap vaccine, already established by our research group, was used as a reference. The animals were previously immunized with the immunobiologicals and, then, challenged with 10^7 stacionary *L. chagasi* promastigotes forms.

3 Results

The clinical and laboratorial analyses performed after the vaccination protocol ($T3^{dose}$) have showed that the nanoformulations employed were safe and harmless, besides antigenic (total IgG anti-*Leishmania* induction) when employed in hamsters. The data obtained 200 days after the experimental challenged (T^{PD}) had shown less pronounced hepatosplenomegaly in the LBSap, $PSm^{I}Ag$ and $PSm^{II}Ag$ groups when compared to the control groups. In the spleen, the LBSap and $PSm^{II}Ag$ groups showed a decrease in the intensity of inflammatory infiltrate and granuloma formation. In the humoral immune evaluation, in the LBSap and $PSm^{I}Ag$ groups, was observed an increase in the anti-*Leishmania* antibody titles. Furthermore, was observed significant reduction in the spleen's parasite burden in LBSap, $PSm^{I}Ag$ and $PSm^{II}Ag$ groups when compared to the control groups.

4 Conclusions

In this way, the $PSm^{II}Ag$ formulation showed better results in vaccinated and challenged animals to reduce further the parasitic load in the spleen and attenuating lesions in liver and spleen tissues, making it a safe and harmless nanoformulation vaccine with great immunoprotective potential.

C0784 FATE OF LEPTIN IN MALNOURISHED COUPLED EXPERIMENTAL VISCERAL LEISHMANIASIS

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1 Background

Visceral leishmaniasis (VL) is responsible for several deaths in malnourished children accompanied by diminished circulating leptin and impaired cell-mediated immunity. Leptin as a cytokine regulates innate and adaptive immunity, hematopoiesis, and angiogenesis. Typically, leptin deficiency is associated with Th2 polarization that markedly coincides with the pathogenesis of VL. Hence, the present study aims to unravel the immunotherapeutic role of leptin in malnutrition-coupled VL BALB/c mice.

2 Methods

The promatigotes stage of L. donovani parasites were cultured in the complete M199 medium at 25oC. Before infection mice were fed with diet-A consists of 21% of protein, sufficient zinc, and iron, and diet-D consists of 1.25% of protein, deficient in zinc and iron for three weeks. Later mice were infected with stationary phase promastigotes (1×107) through tail vein injection. Simultaneously, leptin (5µg/day for 3-weeks) was administrated to the diet-D infected group by the subcutaneous route. Completion of total 6-weeks, mice were euthanized, and blood, spleen, and liver were collected for serum leptin, TGs, IgG1, IgG2a analysis and parasite burden were determined in spleen and liver by serial dilution method. Cytokines were analyzed in spleen and arginase enzyme activity in BMDM.

3 Results

Interestingly, our results show that L. donovani infection itself reduces the serum leptin levels as in malnutrition. Leptin administration in malnourished infected mice controls severe body weight loss and parasite load in spleen and liver. Furthermore, leptin increases functional CD8+ T-cell count, Granzyme-A expression and downregulates anergic T-cell markers such as PD-1 and CTLA-4. Simultaneously, Leptin induces the secretion of IFN-γ, IL-2, and TNF-α into the culture supernatant upon SLA stimulation of the splenocytes. Moreover, leptin might interfere with the proliferation of parasite favored monocytes as observed by downregulation of splenic GM-CSF mRNA expression. Evidently, leptin induces the granulomatous response to clear the infection in the liver and also reduced the arginase enzyme activity in the bone marrow indicates active effector T-cell response. Furthermore, leptin significantly upregulates serum IgG2a level, a crucial humoral antibodies for host immune response.

4 Conclusions

Altogether, our findings suggest that the exogenous recombinant leptin treatment augments the humoral and cell-mediated immunity in malnurished coupled VL.

C0800 INVOLVEMENT OF B-CELL ACTIVATING FACTOR IN ANTIBODY PRODUCTION DURING EXPERIMENTAL LEISHMANIASIS

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1 Background

Strong humoral responses are a hallmark of human visceral leishmaniasis as well as experimental cutaneous leishmaniasis, and are often speculated as an outcome of Th2-dominant immunity. However, host molecules involved in the humoral responses are not fully understood. We recently reported higher levels of B-cell activating factor (BAFF) in sera from VL patients compared with healthy controls.

2 Methods

To examine the role of BAFF in induction of humoral responses during experimental leishmaniasis, BALB/cA mice lacking a baff gene (BAFF-KO) were generated, infected intravenously with *Leishmania donovani* into the tail vein or intradermally with *L. major* into the auricle, and examined for their serum immunoglobulins/antibody levels.

3 Results

L. donovani-infected wildtype (WT) mice had significantly higher levels of serum BAFF compared with the uninfected controls. These infected mice also showed significantly higher serum levels of total IgG as well IgG antibodies to leishmanial antigens. In contrast, production of anti-SLA IgG antibodies in response to *L. donovani* infection was suppressed in BAFF-KO mice. During *L. major* infection, although the difference between BAFF-KO and WT mice was not as significant as that seen in *L. donovani* infection, anti-SLA IgG antibody level was also lower in BAFF-KO mice. However, production of IFN-γ and IL-4 upon antigen recall of splenocytes with SLA was comparable between the *L. major*-infected WT and BAFF-KO mice. Interestingly, BAFF dependency in antibody production differed among defined antigens.

4 Conclusions

These results suggest that BAFF is involved in induction of humoral responses during *L. donovani* and *L. major* infection independent of Th1/Th2 balance. Dependency on BAFF in antibody production is more significant in *L. donovani* infection, and is also variable on the antigens, suggesting that places and processes for antigen presentation affect BAFF dependency on antibody production during *Leishmania* infection.

C0801 LEISHMANIA IINFANTUM FOCUSE-MANNOSE LIGAND-SAPONIN INDUCED PREDOMINANT TH1 IMMUNE RESPONSES IN VACCINATED DOGS IN AN AREA OF VL ENDEMIC IN IRAN

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1 Background

Canine visceral leishmaniasis (CVL), is endemic in Northwest and south of Iran. *Leishmania infantum* is cause of visceral leishmaniasis in human and dogs in Mediterranean basin. Protective immunity of Fucose Mannose ligand (FML) antigen of *Leishmania donovani* against CVL was reported in a few studies. This study was conducted to evaluate the immune responses of vaccinated dogs against FML antigen of *Leishmania infantum*.

2 Methods

A total of 40 dogs which were negative for *Leishmania,* both by PCR and DAT were selected for the study in an endemic area of VL in Iran. FML antigen isolated from native *L. infantum*. The selected dogs were divided into three groups. The first 20 dogs, as a test group, received FML+ saponin; the second group, 10 dogs as control group, received saline injection and the third group, 10 dogs as second control group, received saponin injection alone. The animals in all three groups received three subcutaneous injections, every three weeks, for 42 days. Ten days after the last injection, peripheral blood sample was taken from each dog for culturing of peripheral blood mononuclear cells (PBMCs). Serum samples were collected from dogs for detection of anti-FML antibodies by FML-ELISA. The PBMCs were separated from the blood samples of the 40 dogs. Cells of each dog were cultivated in RPMI media in a four well plate and FML, concanavalin A (Con A) and FML+ Con A were added to three of the wells while one well remained untreated. The cells were harvested after 12 hours and washed with phosphate buffered saline before RNA extraction. Total RNA of cultivated PBMCs was extracted by TRIzol reagent. Quantitative real-time PCRwas used forevaluation of the expression of IL-10, IL-12A, IL-13, IFNγ in vaccinated along with controls dogs.

3 Results

A significant increase in expression of IFNγ, IL-10 and IL-13, but not IL12A, gene transcripts in PBMCs of FML-saponin injected dogs in comparison with controls. Also a significant difference in ratio of IFNγ/IL-10 mRNA expression was obtained in FML-saponin injected dogs in comparison with control groups. Moreover, a significant level of anti-FML antibodies was detected in serum of vaccinated dogs.

4 Conclusions

These findings showed that FML-saponin stimulates both Th1 and Th2 immune responses, with predominant Th1 responses, as well as strong humoral immune responses to produce protective immunity against CVL.

C0848 STUDY OF EFFICIENCY OF BCG AS AN ADJUVANT PLUS LIVE LEISHMANIA MAJOR IN VACCINATION OF ANIMAL MODEL

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1 Background

Cutaneous leishmaniasis (CL) is one of chronic diseases in the tropic and sub tropic regions. The disease is caused by protozoal parasites of the genus *Leishmania.* For some reasons such as vector and reservoir variety little can be done to control the disease and prophylactic immunization with an effective vaccine is very important. According to immunization history with live cultures of *Leishmania major* difficulties can arise in certain individuals due to use such a vaccine. To avoid from such side effects with live parasite injection one way is reducing the dose of inoculum plus an appropriate adjuvant. In regard to proved effects of BCG in activating cell immune system in this study BCG was used in an immunization protocol with different doses of live *L.major* in BALB/c mice.

2 Methods

L.major (MRHO/IR/75/ER) was cultured in N.N.N medium and RPMI 1640. Different Groups of BALB/c mice were inoculated with different doses of harvested promastigotes in stationary phase. Two groups of ten mice were selected for each dose. In one group parasites were given with BCG and the other was received parasites without BCG. After four months all mice were challenged with 2×10^6 active virulence parasites. In all groups clinical examination and measurement of the lesions continued twelve weeks. Leishmanin Skin Test (L.S.T) was done in all test and control groups 60 days before and 60 days after infectious challenge.

3 Results

Results of the skin test, clinical examinations and ulcer measurement showed that immunization with low dose of parasite (120 and 1200 promastigotes per mouse) with or without BCG could stimulate cell immunity to produce protection against the disease. Moderate doses of parasite (12000 promastigotes per mouse) produce only partially protection and here the role of BCG in helping the process of immunization was appeared. There was no protection with high dose of parasite (200000 promastigotes) with or without BCG.

4 Conclusions

It is suggested that low doses of live parasites plus BCG could be used in leishmanization for prophylactic purposes in human.

C0903 POTENTIAL SYNTHETIC ALPHA-GAL-CONTAINING NEOGLYCOPROTEIN-BASED VACCINE AGAINST CUTANEOUS LEISHMANIASIS

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1 Background

Protozoan parasites from the genus *Leishmania* cause extensive clinical manifestasions affecting humans. Cutaneous leishmaniasis (CL) is a neglected tropical disease affecting about 1.2 million people worldwide. Despite current advances, there are no preventive or therapeutic human vaccines available. Glycoinositolphospholipids (GIPLs) are major molecules on the plasma membrane of all *Leishmania* spp. In *L. major*, GIPL-2 *and* GIPL-3 contain nonreducing, terminal α-galactopyranosyl (α-Gal) residues, which are highly immunogenic to humans, due to their absence in human tissues. Consequently, we hypothesize that targeting α-Gal glycotopes abundantly expressed on *L. major* surface could lead to an effective vaccine for CL. Here, we evaluate a synthetic α-Gal-containing neoglycoprotein (αGal-NGP) termed KM5 as a potential vaccine against *L. major* infection.

2 Methods

Groups of six female *α-1,3-galactosyltransferase*-knockout (α1,3GalT-KO) mice were immunized with 10 µg KM5/animal/dose (subcutaneously) at 7-day intervals, in a total of 4 immunizations. Serum was collected from all groups for anti-α-Gal antibody analysis by chemiluminescent-ELISA. Th1/Th2 cytokines and mouse Ig isotyping were assessed by Multiplex Luminex immunoassays. Mice were challenged in the footpad with 10^5 *L. major* metacyclic promastigotes.

3 Results

Mice immunized with KM5 showed higher levels of anti-α-Gal antibodies, accompanied by 84% protection from disease (cutaneous lesion) after challenge with parasites. Additionally, we observed that KM5 induced a predominantly Th1 immune response in immunized mice, with an increase of IL-12 and IFN-gamma, and significant higher IgG2a/IgG1 ratio in comparison to the control group.

4 Conclusions

Immunizations with KM5 resulted in significan protection against infection, likely due to the increase in proinflammatory cytokines and a high IgG2a/IgG1 ratio, which are associated with protective immune response against *L. major*. For future vaccination experiments, the protective effect of KM5 will be further explored in combination with adjuvants such as CpG ODNs and αGal-ceramide.

C0947 VACCINATION WITH ATTENUATED LEISHMANIA INFANTUM HSP70-II NULL MUTANT INDUCES PROTECTION IN LEISHMANIA BRASILIENSIS MURINE MODEL

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1 Background

Despite being a global parasitic disease, there are no effective vaccines against human leishmaniasis and live attenuated parasites are emerging as a promising immunization strategy. We showed previously that a deletion mutant of *Leishmania infantum* lacking both HSP70-II alleles (Δ-HSP70-II) does not induce pathology in BALB/c mice and provide protection against *L. major* infection. In this study we showed that immunization with Δ-HSP70-II confer cross-protection against *L. braziliensis* in the BALB/c infection model.

2 Methods

Mice were immunized once with Δ-HSP70-II via intravenous route for short (4 weeks) or long periods (15 weeks) before challenge with *L. braziliensis* promastigotes. Disease evolution was followed by measuring clinical lesions and determining parasite burdens. Humoral and cellular responses were evaluated after vaccination and after infection.

3 Results

Immunization with Δ-HSP70-II induced a Th1-like response translated by *Leishmania*-specific IgG2a antibodies and increased IFN-γ secretion by spleen cells, while IL-4 and IL-10 levels remained similar to those in control mice. Upon challenge with virulent *L. braziliensis* promastigotes, mice immunized either for short or long periods showed significant protection. In mice immunized for 15 weeks and challenged in the ear, lesions were completely absent and parasites could not be detected at the inoculation site. Immunized/challenged mice showed sustained secretion of IFN-γ by *L. braziliensis* Ag-stimulated lymph node cells compared with non-immunized/challenged mice with established lesions.

4 Conclusions

Immunization of BALB/c mice with Δ-HSP70-II induces sustained IFN-γ production and protection against *L. braziliensis* experimental infection. Experiments are under way to further evaluate the immune mechanisms involved in protection in this model.

C0978 A NOVEL CATIONIC LIPOSOMAL VACCINE FORMULATION FOR SUSTAINED PROTECTIVE IMMUNITY AGAINST VISCERAL LEISHMANIASIS

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1 Background

Despite reports of a number of potent antigens, a vaccine for human leishmaniasis is still lacking. Although various studies suggest live vaccines as the most effective measure for combating leishmaniasis, this strategy is restricted from clinical use due to safety concerns. The major challenges with subunit vaccines, the safer alternatives to live vaccines, are to generate and maintain protective immunity. Stable cationic liposome can be a proficient tool for antigen delivery for optimum uptake and presentation by antigen presenting cells (APCs) and also to ensure antigen persistence for generation of memory responses.

2 Methods

In this study we have identified a novel antigen, Leishmanial EF1-α as an immunodominant component of soluble leishmanial antigen (SLA) that reacts with visceral leishmaniasis (VL) sera and induces cellular proliferative and cytokine response in PBMCs of active and cured VL subjects. The antigen both in its native and recombinant forms were formulated in stable cationic liposome and evaluated for in vivo vaccine potential in BALB/c mice against virulent *Leishmania donovani* challenge infection. Protective efficacy was determined by estimating the parasite load in liver and spleen by microscopic observation (LDU) and limiting dilution assay (LDA). The immune correlates of protection were analyzed by assessment of DTH, antibody and cytokine responses after immunization as well as infection. Stimulated splenocytes were assayed for expansion of polyfunctional CD4 and CD8 T cells and associated memory response.

3 Results

EF1-α entrapped in cationic liposomes exhibit strong resistance to parasitic burden in both liver and spleen of BALB/c mice through induction of a robust DTH, IgG2a dominant antibody and Th1 biased cytokine responses. Multiparametric analysis of splenocytes for generation of antigen specific IFN-γ, IL-2 and TNF-α producing lymphocytes indicate that cationic liposome facilitate expansion of both CD4 as well as CD8 polyfunctional T cells to ensure protective as well as memory response against VL.

4 Conclusions

Liposomal EF1-α is a novel and potent vaccine formulation against VL that imparts long term protective responses. Moreover, the flexibility of this formulation opens up the scope to combine additional adjuvants and epitope selected antigens that can be used for other disease forms including leishmaniasis and cancer.

C0989 THE POTENTIAL IMPACT OF VISCERAL LEISHMANIASIS VACCINES: EXPLORATIONS WITH DIFFERENT DETERMINISTIC AGE-STRUCTURED TRANSMISSION MODELS

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1 Background

Current interventions to control visceral leishmaniasis (VL) focus on diagnosis and treatment of cases and on vector control. At this point there is no human vaccine available. However, vaccine development has been ongoing for decades and the results of various studies strongly support the possibility for immunoprophylaxis of VL in the future, suggesting vaccines to be of great additional benefit for individuals at risk.

2 Methods

This study explores the potential health impact of different hypothetical vaccines at population level, using two age-structured mathematical models that capture the VL transmission dynamics between humans and sandflies. The models differ regarding the main reservoir of infection; in symptomatic and asymptomatic individuals. We incorporate different types of vaccines that 1) lower the risk of infection, 2) lower the risk of developing clinical VL, 3) lower the risk of developing PKDL and 4) a vaccine after which susceptible individuals become immune. Other assumptions include different durations of the vaccines' effect and various population coverage levels. The models output is presented as a reduction in VL incidence over time for different endemic settings.

3 Results

In the model with the main reservoir of infection in asymptomatic individuals, the largest decrease in VL incidence is reached with the vaccine protecting individuals from developing infection. In an endemic setting with annual pre-control incidence of 5 new VL cases per 10,000 population, the target of less than 1 VL case per 10,000 population per year can be achieved within 5 years. The vaccine that decreases the development of PKDL has the least impact, only causing a minor decrease in incidence after at least 10 years of an active vaccination programme. The same is true for the model with the main reservoir of infection in symptomatic individuals, however showing a slower decline in VL incidence. The vaccine target product profiles (TPP) are also identified for specific VL targets including the minimum required vaccine efficacy, duration of effect, coverage levels and which age groups best to include in a vaccine program would one become available.

4 Conclusions

These new insights aid in guiding vaccine development and support policy makers with the development of potential future vaccination strategies for the elimination and control of VL.

C0999 FIRST GENERATION VACCINE FORMULATED WITH A LIGAND OF TOLL-LIKE RECEPTOR 3 PROTECTS BALB/C MICE AGAINST INFECTION WITH LEISHMANIA (LEISHMANIA) AMAZONENSIS.

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1 Background

Increasingly cases of cutaneous leishmaniasis in different countries have been detected. In Argentina, there are three principal species: *L.* (*Viannia*) *braziliensis*, *L.* (*L.*) *amazonensis*, *L.* (*L.*) *guyanensis*. Therefore it is very important to develop an effective vaccine this disease. The objective of the present study was to evaluate the efficacy of a first-generation vaccine against leishmaniasis, formulated with the poliinosinic-policitidilic acid [Poly (I:C)] as adjuvant, which is a double-stranded RNA and it acts as TLR3 ligand.

2 Methods

100 µg of Total *L. (L.) amazonensis* Antigen (TLA) plus 50 µg of Poly (I:C) was tested in mice BALB/c using a homologous prime/boost immunization. The humoral and cellular immune response generated by each formulation was evaluated. Subsequently, mice were infected with 1 x 10^5 of *L. (L.) amazonensis* (MHOM/VE/84/MEL) promastigotes. The progress of the infection was assessed during 11 weeks, the parasite load was quantified and histopathologic at the primary site of infection studies were made.

3 Results

The TLA-Poly (I:C) formulation generated an increase of IgG anti-TLA ($P<0.05$), with increases of IgG1 and IgG2a isotypes. This generated an increase in IFN-γ with low levels of IL-4, a reduction in the swelling, histologic index and parasitic count.

4 Conclusions

The immunization of BALB/c mice with a vaccine formulation based on TLA and Poly (I:C) generated a protective Th1-type immune response against infection with *L. (L.) amazonensis*, characterized by intense production of IgG2a and IFN-γ. Accordingly, Poly (I:C) could be an appropriated adjuvant to develop a first generation vaccine against leishmaniasis.

C1015 CHARACTERIZATION OF INFLAMMATORY CYTOKINE PRODUCTION WITH DIFFERENT TLR AGONISTS BEFORE OR AFTER LEISHMANIA INFANTUM INFECTION IN A CANINE MACROPHAGE IN VITRO MODEL

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1 Background

Leishmaniosis is a group of infectious vector-borne diseases caused by obligatory intracellular protozoa parasites of monocyte/macrophage cell lineages. The immune response against *L. infantum* is also highly dependent on macrophages, and although they are the host cells targeted by infection, they are capable of antigen-presentation and killing intracellular parasites. Toll-like receptors (TLRs) are crucial regulators of the innate immune repose against various pathogens including *Leishmania* parasites. Nevertheless, the effect of stimulation of macrophages receptors such as TLRs during *L. infantum* infection has been vaguely investigated.The aim of this study was to evaluate the pro-inflammatory cytokine profile of *L. infantum* infected canine DH82 cells before and after stimulation with TLR2, TLR3, TLR4 and TLR7 agonists.

2 Methods

Cells of the DH82 canine macrophage line were cultured in 24-well tissue cultures platesand treated with a pre-infection dose of 0,3µg/ml TLR2 agonist (Pam3CSK4), 1 mg/ml TLR4 agonist (MPLA-SM), 10 mg/ml of TLR3 agonist (Poly I:C), or 5 ug/ml of TLR7 agonist (Imiquimod). Twenty four hours after agonist TLR stimulation, cells were infected with *L. infantum* promastigotes at a 10:1 parasite:host cell ratio. The cultures were incubated for 3 hours and then re-stimulated with the post-infection dose of TLR agonists described above. Supernatants were collected at 24 and 48 hours andIL-6 and TNF-α were measured by ELISA.

3 Results

TLR2 and TLR4 agonists significantly induced TNF-α and IL-6 production at 24 and 48 hrs. Higher amounts of both cytokines were measured in infected cells treated with TLR2 and TLR4 agonists, especially in post-infected cells when compared with untreated cells. DH82 cells treated with TLR3 secreted significantly larger concentrations of TNF-α and IL-6 in post-infection treated cells but no differences were observed in pre-infection treated cells when compared with control macrophages. Finally, TLR7 agonist induced lower levels of IL-6 and TNF-αin all treatments. However, at 24 hrs, statistically higher levels of IL-6 were detected in infected and non-infected cells treated with TLR7. TNF-α was poorly produced after TLR7 agonist treatment.

4 Conclusions

TLR4, TLR2 and TLR3 agonists were able to stimulate a strong pro-inflammatory response in DH82 macrophages after *L. infantum in vitro* infection while TLR7 agonist did not.

C1031 A VACCINE THERAPY FOR CANINE VISCERAL LEISHMANIASIS PROMOTED SIGNIFICANT IMPROVEMENT OF CLINICAL AND IMMUNE STATUS AND STRONG REDUCTION IN PARASITE BURDEN SUGGESTING THE BLOCKADE INFECTION TO SAND FLIES

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1 Background

Visceral leishmaniasis (VL), caused by *L. infantum*, is the most fatal form of leishmaniasis. Dogs are extremely susceptible to infection presenting clinical, pathological and immunological alterations very similar to human disease and are considered the most important model for evaluation of new treatment strategies (immunotherapy and immunochemotherapy). Herein, we evaluated the treatment strategy employing a therapeutic heterologous vaccine composed by antigens of *Leishmania braziliensis* associated with MPL adjuvant (LBMPL vaccine) for visceral leishmaniasis (VL) in symptomatic dogs naturally infected by *L. infantum*.

2 Methods

Sixteen dogs received immunotherapy with MPL adjuvant (n=6) or with a vaccine composed by antigens of *L. braziliensis* associated with MPL (LBMPL vaccine therapy, n=10). Dogs were submitted to an immunotherapeutic scheme consisting of 3 series composed of 10 subcutaneous doses with 10-day interval between each series. The animals were evaluated before (T0) and 90 days after treatment (T90) for their biochemical/hematological, immunological, clinical and parasitological variables.

3 Results

Our major results showed that the vaccine therapy with LBMPL was able to restore and normalize main biochemical/hematological parameters. In addition, in an *ex vivo* analysis using flow cytometry, dogs treated with LBMPL vaccine showed increased $CD3^+$ T-lymphocytes and their subpopulations ($TCD4^+$ and $TCD8^+$), reduction of $CD21^+$ B-lymphocytes, increased NK-cells ($CD5^-CD16^+$) and $CD14^+$ monocytes. Under *in vitro* antigen stimulation, the animals developed a strong antigen-specific lymphoproliferation mainly by $TCD4^+$ and $TCD8^+$ cells; increasing in both $TCD4^+IFN\text{-}g^+$ and $TCD8^+IFN\text{-}g^+$ as well as reduction of $TCD4^+IL\text{-}4^+$ and $TCD8^+IL\text{-}4^+$ lymphocytes with an increased production of TNF-a and reduced levels of IL-10. Concerning the clinical signs of CVL, the animals showed an important reduction in the number and intensity of it; increase body weight as well as reduction of splenomegaly. In addition, the LBMPL immunotherapy also promoted a reduction in parasite burden assessed by Real Time PCR in the bone marrow and skin as well a reduction of parasite load in sand flies after xenodiagnosis.

4 Conclusions

The results obtained in this study highlighted the strong potential for the use of this heterologous vaccine therapy as an important strategy for VL treatment.

C1055 PRE-CLINICAL STUDIES IN MICE OF AN INNOVATIVE MULTIVALENT VACCINE FOR HUMAN VISCERAL LEISHMANIASIS

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1 Background

Leishmaniasis is caused by different *Leishmania spp.*, intracellular parasites with a complex life cycle requiring a susceptible host and a permissive vector. The most severe form of the disease is caused by *L. donovani* and *L. infantum*, species able to reach internal organs. The chemotherapeutic and vector control strategies used to fight Leishmaniasis present several limitations. Disease prevention by vaccination is a strong option, since in endemic areas previous infection leads to anti-*Leishmania* immunity. Nevertheless, till date there is no vaccine available for human Visceral Leishmaniasis (VL).

2 Methods

Our objective was to assess the safety and immunogenicity of an innovative vaccine candidate against human VL, consisting on Virus-Like Particles (VLP) loaded with 3 antigens, adjuvanted with a Th1 inducer TLR4 agonist. The VLP formulation has the potential to generate specific CD8+ T cell responses due to cross-presentation. Antigens are both parasite (KMP-11/LeishF3+) and vector saliva (LJL-143) derived (PD and VD, respectively), which will contribute for the induction of both a Th1 anti-Leishmania and a Th1-DTH anti-sandfly saliva responses. Importantly, each antigen were demonstrated individually effective in VL models, and both the adjuvant and the virosomes are approved for human use.
Initially, studies of optimal vaccine composition were performed to assess adjuvant contribution and to define the ideal antigens/adjuvant dosages. Subsequently, the pre-clinical trial was designed to assess the contribution of the virosome to immunogenicity, the possible immunodominance of the VD antigen, the effect of priming with the VD protein and the safety and immunogenicity of the formulations.

3 Results

Overall, all the vaccine components were prooved safe. Furthermore, the antigen-specific cellular and humoral responses determined confirmed the immunogenicity of the formulation. Interestingly, the responses detected against the VD antigen were always more robust than the obtained for the PD ones, but this immunodominance seems not to be a problem in our model. Curiously, to prime with the VD protein contributed for a response increment to the PD antigens. Also, virosome improved the specific humoral immune response.

4 Conclusions

Overall our results point to the candidate tested as a promising anti-*Leishmania* vaccine. Protection studies are ongoing, using a natural infection animal model, to properly evaluate the full vaccine candidate protective potential.

C1116 SCREENING OF LEISHMANIA INFANTUM PEPTIDES SELECTED BY BIOINFORMATICS TOOLS IN NATURALLY INFECTED DOGS

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1 Background

Canine visceral leishmaniasis (CVL) is a zoonosis in Latin America and dogs play a central role in the urban cycle of *Leishmania infantum*. In Brazil, the Current strategies to control CVL are inefficient and there are no vaccines that could be employed in public health control program. In this context, rational vaccinology appears as a strong approach that will allow the discovery of potential antigens. In previous studies, our group, has identified 38 peptides with immunogenic potential from five *L. infantum* proteins, using bioinformatics tools (BT).

2 Methods

The aim of this work was to screen *L. infantum* peptides selected by bioinformatics tools in *Leishmania* naturally infected dogs. Thus, we performed *in vitro* and *in vivo* evaluations in naturally infected dogs selected through leishmanin skin test (LST), clinical signs and leukogram to identify the best immunogenic peptides. For the *in vitro* assay, peripheral blood mononuclear cells (PBMC) from dogs were stimulated by three peptides per mix using combinatorial analysis (CA). At that point, we evaluated *in vitro* capacity of $CD4^+$ and $CD8^+$ T cells to proliferate and produce IFN-γ. Then, we developed a score matrix to evaluate the individual response of five dogs, based on index of stimulated culture/control culture. In concerning to the in vivo assay, we performed the reverse antigen screening based on Delay Type-Hypersensitivity (DTH). For that, ten peptides selected by *in vitro* assay, a negative control and positive control (LST) were inoculated singly in the dog´s skin and we evaluate the formation of erythema and induration after 48 hours.

3 Results

Regarding the PBMC stimulation with mixes, 13 mixes met the criteria and were selected. Next, these mixes were combined resulting in 13 peptides selected. Afterwards, they were individually evaluated with the same parameters of the mixes to confirm the CA, thus 10 out of 13 peptides were capable of inducing T cell proliferation and IFN-γ production in dogs PBMC. In the in vivo assay, six of ten peptides demonstrated an interestingly DTH response in the dog´s skin after 48 hours with formation of erythema and induration.

4 Conclusions

Peptides selected using BT were capable to induce *in vitro* proliferation and produce IFN-γin T cells and were able to induce a strong cellular response *in vivo*. In the next step, the best immunogenic peptides selected will be combined in multi-peptide or chimeric vaccines to the preliminary immunogenicity tests.

C1135 EVALUATION OF THE PROTECTION PROVIDED BY THE HEPARIN BINDING PROTEIN OF LEISHMANIA INFANTUM CHAGASI IN BALB/C MICE AS A VACCINE CANDIDATE AGAINST VISCERAL LEISHMANIASIS.

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1 Background

Visceral leishmaniasis is caused by the intracellular parasite *Leishmania infantum chagasi*, specie in the Americas that has a strong tendency to generate severe visceral lesions, which makes this more severe clinical form of leishmaniasis. Lectins that bind to specific carbohydrates have been described in some species of *Leishmania,* among them the heparin binding proteins (HBP). These lectins participate in the regulation of host cell biological activities, and act directly on the adhesion and internalization of the parasites in the tissues and cells of both vertebrate and invertebrate hosts. Recent experiments in our group have shown the presence of HBP in *L. infantum chagasi* promastigotes (HBP*Lc*). We also showed that the native protein is immunogenic, inducing mixed response Th1/Th2 in immunization of BALB/c mice assays (unpublished data). The known participation of lectins in parasite adhesion to the host cell and the confirmation of the presence of HBP*Lc* led us to investigate the protective efficacy of this protein in experiments of immunization against infection by *L. infatum chagasi* in mammalian host. In these experiments we quantified the parasitic load in the spleen and liver of BALB/c mice immunized with HBP*Lc* four weeks after challenge with *L. infantum chagasi* promastigotes, in addition to the detection of important cytokines present in the parasite infection course.

2 Methods

Four weeks after challenge of BALB/c mice immunized with PBS, Saponin adjuvant, HBP*Lc* and HBP*Lc* associated with Saponin adjuvant, spleen and liver were extracted and the organs were used for the quantification of parasites by the limiting dilution technique. Cytokines IFN-γ, IL-17, IL-10 and IL-4 were assessed by flow cytometry (BD Cytometric Bead Array Kit) from leukocyte culture supernatants from spleen after 24 h or 72 h of incubation at 36°C. The result of parasites quantification was expressed as the average of the logarithm of the parasites number per organ and the total average of the logarithm of the parasites number per tissue milligram.

3 Results

The results showed decreased parasite load in the spleen and liver and high production of IFN-γ, IL-17 and IL-10 in mice immunized with HBP*Lc* + Saponin adjuvant compared to control groups - mice immunized with PBS or Saponin adjuvant. No IL-4 production was detected in the evaluated groups.

4 Conclusions

These results suggest that HBP*Lc* administered with the Saponin adjuvant is a strong vaccine candidate against visceral leishmaniasis.

C1155 PRE-CLINICAL TRIAL USING HAMSTERS FOR IMMUNOGENICITY ANALYSIS OF NANOFORMULATION VACCINES AGAINST VISCERAL LEISHMANIASIS

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1 Background

Visceral leishmaniasis is caused by *Leishmania infantum* and the euthanasia of the infected dog is the recommended method for preventing the spread of the disease in Brazil. Due to the impacts that euthanasia has in society, the development of an anti-canine visceral leishmaniasis (CVL) vaccine becomes a necessary means of controlling the parasite transmission. As such, the aim of this study was to evaluate the antigen-carrying potential of submicrometric particles composed of poly-D, L lactic acid (PLA) and chitosan with a low molecular weight.

2 Methods

We performed a preclinical study of potential vaccines applied in different experimental groups utilizing hamsters (*Mesocricetus auratus*) as the experimental model: (i) Control group (saline solution at 0,85%); (ii) saponin (SAP); (iii) small, empty submicrometric particles (PSmP); (iv) large, empty submicrometric particles (PSmG); (v) crude *L. amazonensis* antigen associated with saponin adjuvant (LASAP); (vi) small submicrometric particles containing crude *L. amazonensis* antigen (LAPSmP) and (vii) large submicrometric particles containing the crude *L. amazonensis* antigen (LAPSmG). Hamsters were inoculated with three subcutaneous doses of the vaccines at intervals of 21 days. The experimental infections were performed with 10^7 *L. infantum* promastigotes in stationary phase, 30 days following the first dose of the vaccine. In the time points T0 (prior to the first dose of the vaccine), T3 (21days following the experimental infection) and T180 (180days following the experimental infection), blood samples were collected from the animals. The renal and hepatic function, hemograms and the levels of anti-*Leishmania* IgG were analyzed. Spleen and liver samples were collected for analyzing the parasitic load through the limiting dilution technique.

3 Results

The data showed that LAPSmP and LAPSmG were innocuous, safe, showing immunogenicity, and low parasite load. Our major results were supported by: (i) local and clinical alterations compatible with the maintenance of the animals; (ii) unaltered hepatic and renal function; (iii) absence of anemia and hemolysis; (iv) lymphocyte induction after vaccination; (v) antigenicity related to the total anti-*Leishmania* IgG; (vi) reduction in spleen size; and (vii) a reduction of parasitic load in the liver and spleen.

4 Conclusions

These findings indicate that the encapsulated *Leishmania* crude antigens were immunogenic and induced a high protection levels against visceral leishmaniasis.

C1164 IMMUNOGENICITY ANALYSIS TO TRIAL ANTIGENIC PROTEINS TO NEW VACCINE FORMULATIONS AGAINST LEISHMANIA INFECTION

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1 Background

Leishmaniasis is caused by different species of *Leishmania* genus and is transmitted to vertebrate host through the bite of infected sandfly. The dog is considered the major domestic reservoir of the parasite in natural infections. However, in experimental *Leishmania* infections, the hamster (*Mesocricetus auratus*) is considered an important animal model for vaccine testing against *Leishmania* parasites.

2 Methods

We have established a platform for rapid *in vivo* testing to analyze vaccine candidates using hamsters. Herein, we performed a pre-clinical trial, including the following groups: (*i*) control (sterile 0.85% saline), (*ii*) adjuvant (saponin), (*iii*) crude *Leishmania amazonensis* antigen (LA), (*iv*) soluble *L. amazonensis* antigen (LA^{Sol}), (*v*) particulate *L. amazonensis* antigen (La^{Part}), (*vi) L. amazonensis* crude antigen and saponin (LASap), (*vii*) soluble antigen and saponin ($LASap^{Sol}$), and (*viii*) *L. amazonensis* particulate antigen and saponin ($LASap^{Part}$).

3 Results

The results showed that the vaccine formulations were innocuous (local and systemic analysis). We observed lymphocytosis restricted to the groups that received vaccine antigens associated or not with saponin adjuvant. Similarly, antigenicity analysis revealed increased levels of total IgG anti-*Leishmania*. The immunoblotting analysis showed in LASap, $LASap^{Sol}$, $LASap^{Part}$ groups the presence of three bands with distinct molecular weight (40kDa, 30kDa and bands between 20 and 30 kDa).

4 Conclusions

The immunogenic proteins found in this work present as possible antigens candidate to new vaccine formulations against *Leishmania* parasites.

C1170 SUBMICROMETRIC PARTICLE DEVELOPMENT AND ANALYSIS AS POTENTIAL FORMULATION FOR VACCINE AGAINST VISCERAL LEISHMANIASIS

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1 Background

In the last years, studies suggested a possible increase in anti-*Leishmania* vaccine immunogenicity when performing the antigen encapsulation. This study aimed to produce and analyze the inflammatory profile and toxicity of sub-micrometric particles (SMP) and to apply the nanoformulation using *Leishmania* antigens encapsulated into SMP.

2 Methods

Particle production was performed reacting different polylactic acid (PLA) and chitosan concentrations, obtaining two formulations (SMP^1 and SMP^2). After a subcutaneous single dose of SMP^1 or SMP^2, in mice, were analyzed inflammatory reaction, cell migration, and inflammatory mediators at the inoculation site.

3 Results

SMPs promoted cell recruitment, without tissue damage. In addition, they induced selective migration of neutrophils and macrophages, and inflammatory response was reduced at 72 h, in all analyzed groups. The SMPs led to N-acetyl-beta-D-glucosaminidase (NAG) and myeloperoxidase (MPO) production before 24 and 48 h, and IL-6 and TNF-α production. Additionally, mice were vaccinated with three doses of encapsulated *L. braziliensis* antigen. After the 14 days of third immunization, the immunophenotypic profile demonstrate an increase of monocytes and B-lymphocytes in SMP^1 group, and increase in T-lymphocytes and B-lymphocytes, in SMP^2.

4 Conclusions

Our data demonstrate that the both formulation stimulate the early events of innate immune response, suggesting their ability to increase the co-administered antigens immunogenicity.

C1331 FIELD TRIAL TO ASSESS LEISHMANIASIS VACCINE EFFECTIVENESS AS A POTENTIAL IMMUNOTHERAPY IN ASYMPTOMATIC DOGS

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1 Background

Leishmania infantum, the causative agent of zoonotic visceral leishmaniasis (ZVL), is enzootic within United States hunting dogs, maintained in this population through vertical transmission. Within endemic countries, dogs serve as a reservoir host for *L. infantum* infection, infected-dog ownership increases the risk of human infection several fold. Because of this relationship, there is a need for safe and efficacious vaccines to prevent disease transmission in dogs. A vaccine that can function as an immunotherapeutic to prevent disease progression and transmission from already infected animals is crucial for the success of any elimination effort. Efficacy of this type of vaccination/immunotherapy is difficult to assess as vaccine trials in Brazil are unable to maintain asymptomatic dogs within their trial cohort due to mandatory culling of serologically positive dogs. Here we report the efficacy of a double-blind, placebo, randomized field trial to assess the efficacy of a commercially available canine vaccine, LeishTec®, to prevent disease progression within all dogs, as well as the ability of this vaccine as an immunotherapy in infected, non-clinical dogs to reduce progression to clinical leishmaniasis.

2 Methods

A total of 606 *Leishmania*- negative or asymptomatic dogs were enrolled in the vaccine trial in seven states within the United States of America. *Leishmania* infection status was determined via a combination of qPCR, serology, and clinical signs (assessed by a licensed veterinarian). Dogs were declared non-clinical if they had less than two clinical signs but were positive via qPCR, serology, or both. Additional information collected included each dog's age, sex, tick and heartworm serological status, deworming and core vaccine history and geographic location. Dogs were vaccinated and boosted 3 times at 14 day intervals then followed every 3 months for 12 months. A safety assessment was completed after the 3 dose- vaccination series.

3 Results

This study, the first of its kind to include asymptomatic dogs, underscores the important need for vaccines against leishmaniasis that can be targeted at both uninfected and asymptomatic dogs. Evaluation of vaccines as immunotherapies to prevent the progression of leishmaniasis are critical in preventing the spread of leishmaniasis vertically, from mother to offspring, and horizontally, via sand fly vectors, in both dogs and humans.

C1415 VISCERAL LEISHMANIASIS AND SYSTEMIC LUPUS ERYTHEMATOUS: RARE, SERIOUS AND ATYPICAL ASSOCIATION

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1 Background

To determine the clinical features, treatment and results of systemic lupus erythematous (SLE) associated to visceral leishmaniasis (VL) occurring in an inaugural way.

2 Methods

We report the observation of a patient with a particularly, severe and atypical association of SLE and LV.

3 Results

A 32 year-old woman, without a significant pathological history, presented in the department of infectious diseases of Fattouma Bourguiba university hospital of Monastir for inflammatory arthralgia, weight loss, and bicytopenia, without associated fever, evolving since 3 months. The highlights on physical examination were intense pallor, edema of the extremities without hepatosplenomegaly. Blood tests showed pancytopenia, hyperproteinemia with hypoalbuminemia and hypergammaglobulinemia. Microbiology investigation was negative for the most common pathogens, including tuberculosis. There were no signs of hematologic malignancy in the bone marrow smear. PCR for Leishmania was positive in bone marrow. However, the serology using ELISA and western blot was negative. The immunological tests showed the presence of antinuclear antibodies (ANA), anti native DNA, anti Sm and anti nucleosomes with hypocomplementemia. The direct coomb test was positive. initially the patient had received Glucantime (3g / day) interrupted three days after, due to stibiointolerance and stibiointoxication and replaced by Amphotericin B. At a cumulative dose of 260 mg of amphotericin B, the patient presented a pericardial tamponade requiring emergent drain. The analysis of the pericardial liquid showed an exudative appearance positive for ANA. The diagnosis of SLE was confirmed, the patient was treated with 3 bolus of methylprednisolone (1 g / d) relayed by prednisone 1mg / Kg / d in combination with amphotericin B. The evolution was marked by the pericardial drying and the negativity of proteinuria .

4 Conclusions

The clinical and serological presentation of VL during SLE is often atypical. The concomitant declaration of both pathologies poses an etiological problem of possible complications which may be multifactorial

C1460 ANTIBODIES ELICITED BY PRIMARY VACCINATION AND ANNUAL BOOSTER WITH CANILEISH® IN DOGS FROM SPAIN: A CLINICAL FIELD STUDY

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1 Background

To confer protection against canine leishmaniosis (CanL) a combined approach including the use of repellents and vaccine is recommended. This study examines the antibody response induced in dogs vaccinated with CaniLeish® (Virbac, France) using two serology techniques targeting antibodies against *L. infantum*.

2 Methods

Twenty-three veterinary practices in different endemic areas of Spain were included in the study. A physical examination form was designed to collect information about clinical status and vaccination. In addition, an owner informed consent form was collected for each participating animal. One baseline (day of primary vaccination or 1-year booster) and 4 follow-up visits were scheduled at: Days 0 (V1), 30 (V2), 120 (V3), 240 (V4) and 365 (V5). Check-up visits consisted in a physical exam and serological tests using IFAT and Speed-Leish K™ (Virbac, France). Nested-PCR was performed in dogs suspected of suffering from CanL during the follow-up year.

3 Results

In total, 186 dogs were enrolled: 94 receiving the vaccine for the first time (P) and 92 given a booster (B). Some of the revaccinated animals (B) were followed beyond 1 year: 2 years (n=36), 3 years (n=20) and 4 years (n=16).

On Day 0 (V1), 100% of the dogs in group P tested negative with both the IFAT and Speed-Leish K™tests while 9.8% in group B only returned a positive IFAT result. In following visits, IFAT seropositivity rates were: V2 = 31.9% (P) and 40.3% (B); V3 = 15.9% (P) and 8.9% (B); V4 = 4.3% (P) and 6% (B); and V5 = 3.2% (P) and 3.2% (B). IFAT seropositivity rates in the dogs followed for 2, 3 and 4 years were 11.1%, 45%, and 56%, respectively. Antibody titres in all these seropositive dogs were low, at 1/100. No significant differences in seropositivityagainst *L. infantum* infection were found with respect to sex, age, size, primary *versus* re-vaccination, and geographical area.

Over the 1-year period, only two dogs (1 in P and 1 in B) tested positive with the Speed-Leish K™test at both the time points V4 and V5. Blood and bone marrow samples were taken for these 2 dogs in order to determine the presence of *L. infantum* DNA by nested-PCR. These samples were all PCR negative for the protozoan.

4 Conclusions

One month after CaniLeish®vaccination, anti-*L. infantum* antibodies were detected by IFAT in 31.9-40.3% of the dogs included in this study. One year later, 3.2% of the dogs were still able to show an antibody response to the vaccine.

C1462 TOLERANCE OF THE CANILEISH® VACCINE IN 314 HEALTHY DOGS IN SPAIN: A CLINICAL FIELD STUDY

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1 Background

A vaccine inducing a cell-mediated immunity against the protozoan responsible for canine leishmaniosis has been available in Europe since 2012 (CaniLeish®, Virbac, France). Although full protection against the disease is not conferred, this vaccine reduces the risk of an active infection progressing to clinical leishmaniosis. In addition, as suggested by a recent xenodiagnosis study, it may reduce disease severity in dogs that become infected despite vaccination. However, the tolerance of CaniLeish®remains a worry for some practitioners. This study examines this parameter in a large group of client-owned vaccinated healthy dogs.

2 Methods

Twenty-nine veterinary clinics from various regions of Spain were included in the study. A clinical form was designed to record information about each dog (sex, size, age) and vaccination (date, adverse events and treatment if applied). In addition, an owner informed consent form was collected for each participating animal. All data were entered in a computerized database.

3 Results

In total, 314 dogs wereenrolled: 116 after primary vaccination and 198 after a booster injection. The population was homogenous in terms of sex, age and size. The following adverse events potentially associated with the vaccination were recorded in 20 of the 314 dogs (6.4%): apathy (n=8), digestive disorders (vomiting and/or diarrhoea) (n=6), anaphylactic reactions (n=2), facial erythema and facial angioedema (n=2), urine or faecal incontinence (n=2) and inflammation, oedema and/or pain at the injection site (n=4). Most of these adverse reactions are mentioned in the SPC of the vaccine. Although 12 of the affected dogs required a symptomatic treatment, all animals showed a quick clinical recovery. No significant correlation was observed between adverse events, sex, age, size and primary vaccination or booster. Of note, in 10 dogs practitioners used NSAIDs before vaccination to lower the risk of possible adverse events.

4 Conclusions

CaniLeish® demonstrated a general good safety profile with some dogs showing adverse events potentially linked to the vaccination, resolving quickly either spontaneously or after treatment.

C1469 IMMUNE RESPONSE IN PATIENTS INFECTED WITH LEISHMANIA (VIANNIA) GUYANENSIS IN NORTH REGION OF BRAZIL

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1 Background

American Cutaneous Leishmaniasis is an infectious-parasitic disease caused by protozoa of the genus *Leishmania*. The main defense mechanism of the human host against this infection is the immune system and the cell-mediated response that plays a fundamental role because the microorganism is an obligate intracellular agent. The immune system acts in a network of cooperation involving the participation of many structural, molecular and cellular components. IFN-γ is an essential cytokine in the TH1 response profile due to its ability to increase the microbicidal effect of phagocytes leading to parasite death. IL-10 is a cytokine that has as one of its main functions inhibit TH1 profile cells, whereas IL-4 induces differentiation of the cells into a TH2 profile, thus leading to a humoral immune response. In *Leishmania* infection cytokines play an important role in the resolution and control of the disease. Therefore, it is extremely important to determine the serum level of these citokines in the clinical course before and after treatment with pentavalent antimonial, the first choice medication determined by the Ministry of Health in Brazil.

2 Methods

IFN-γ, IL-4 and IL-10 were quantified in sera of 15 individuals infected with *L. guyanensis* before and after treatment with pentavalent antimonial (Glucantime®). The same number of samples from individuals (1: 1) that weren't infected was tested using flow cytometric analysis ("Cytometric Beads Array"/CBA Kit (BD).

3 Results

The results demonstrate higher cytokine production in infected individuals ($p < 0.0001$) both before and after treatment, revealing the presence of TH1 and TH2 response in *L. guyanensis* infection.

4 Conclusions

When comparing serum levels of cytokines in *L. guyanensis* infection with other *Leishmania* species, a significant variation is observed, suggesting a possible relationship between the etiologic agent species and the mediators of the immune response.

C1495 EFFICACY OF INTRANASAL LAAG VACCINE AGAINST LEISHMANIA AMAZONENSIS INFECTION IN PARTIALLY RESISTANT C57BL/6 MICE

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1 Background

We have previously demonstrated that intranasal vaccination of highly susceptible BALB/c mice with whole *Leishmania amazonensis* antigens (LaAg) leads to protection against murine cutaneous leishmaniasis. Here, we evaluate the response of partially resistant C57BL/6 mice to vaccination as a more representative experimental model of human cutaneous leishmaniasis.

2 Methods

C57BL/6 mice from different animal facilities were infected with *L. amazonensis* (Josefa strain) to establish the profile of infection. Intranasal vaccination was performed before the infection challenge with two doses of 10 µg of LaAg alone or associated with the adjuvant ADDAVAX® by instillation in the nostrils. The lesion progression was measured with a dial caliper and the parasite load by limited dilution assay in the acute and chronic phases of infection. Cytokines were quantified by ELISA in the homogenates of infected footpads.

3 Results

C57BL/6 mice from different animal facilities presented the same *L. amazonensis* infection profile, displaying a progressive acute phase followed by a controlled chronic phase. Parasites cultured in M199 and Schneider's media were equally infective. Intranasal vaccination with LaAg led to milder acute and chronic phases of the disease. The mechanism of protection was associated with increased production of IFN-gamma in the infected tissue as measured in the acute phase. Association with the ADDAVAX® adjuvant did not improve the efficacy of intranasal LaAg vaccination. Rather, ADDAVAX® reduced vaccination efficacy.

4 Conclusions

This study demonstrates that the efficacy of adjuvant-free intranasal vaccination with LaAg is extendable to the more resistant C57Bl/6 mouse model of infection with *L. amazonensis*, and is thus not exclusive to the susceptible BALB/c model. These results imply that mucosal immunomodulation by LaAg leads to peripheral protection irrespective of the genetic background of the host.

C1510 PROSPECTS OF A TH1 STIMULATORY PROTEIN(S) BASED VACCINE AGAINST VISCERAL LEISHMANIASIS

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1 Background

Control strategies against visceral leishmaniasis (VL) rely on generation of protective Th1-type immune response as the parasite hides themself within the host macrophages and therefore evades the defensive immune mechanism of the host. The fact that healing and recovery protects individuals from re-infection indicates towards the feasibility of a VL vaccine. Therefore, efforts are required to identify such biomolecules which can skew immune system towards Th1 type. During the last decade, our lab was successful in identifying an antigenic protein fraction from soluble *Leishmania donovani* antigen, stimulating Th1 type of immune response both *in vitro* as well as in hamster model. Further proteomic analysis of this sub-fraction led to the identification of several proteins, which were later developed as recombinant ones and subjected to their immunological characterization. Herein, we present the immunological and prophylactic/therapeutic studies of these recombinant proteins. Further identification of potential T-cell epitopes of the most immunogenic proteins for developing synthetic polyvalent vaccine against VL, is also deliberated.

2 Methods

The cellular responses (Lymphoproliferative and cytokine) of the recombinant proteins were assessed in *L. donovani* infected/treated hamsters as well as in endemic contacts and infected/treated VL patients. Their protective (prophylactic as well as therapeutic) responses were studied in hamsters against *L. donovani* challenge. Identification of peptides from the potential proteins was done using immunoinformatics tools and these were further evaluated for their prophylactic efficacy in hamsters.

3 Results

Six recombinant proteins - elongation factor-2, p45, aldolase, enolase, triose phosphate isomerase and protein disulfide isomerase were found to be highly immunogenic and have shown more than 75% prophylactic efficacy in combination with BCG in hamsters against *L. donovani* challenge. These proteins have also exhibited moderate therapeutic efficacies when administered alone. Among the several antigenic peptides belonging to above stated potential proteins, 3 peptides were found to be highly immunogenic and offered considerably good prophylactic efficacies.

4 Conclusions

The results will enable us to design polyvalent synthetic vaccine for treating VL which will expands host protective immune responses, thus allowing them to produce beneficial T-cells that reduce parasite burden and alleviate disease symptoms more quickly.

C1512 THE YELLOW PROTEINS AND APYRASE OF THE PHLEBOTOMUS PAPATASI SALIVA ARE CANDIDATES OF VACCINE AGAINST HUMAN CUTANEOUS LEISHMANIASIS.

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1 Background

Several experimental data demonstrate that the pre-exposure to saliva of phlebotomes confers protective immunity against leishmaniasis. Our previous work in humans indicates that salivary proteins of *Phlebotomus papatasi*, vector of *Leishmania major*, elicit essentially IL-10-producing CD8+ T lymphocytes. Interestingly, blocking IL-10 enhanced the activation of IFN-g-producing CD4+ T lymphocytes of the phenotype Th1

2 Methods

Herein, we used several immunological approaches targeting the salivary components activating a Th1 immune response that could constitute potential vaccine candidates against human leishmaniasis. We first analyzed the cellular immune response elicited by fractionated salivary extracts of *Phlebotomus papatasi* in magnetically separated CD4+ and CD8+ T lymphocytes of immune donors. A proliferative response and an IFN-g induction were demonstrated in CD4+ T lymphocytes stimulated with >30 KDa salivary fraction. We then usedNucleofection™technique to transfect peripheral blood mononuclear cells (PBMC) from immune donors with plasmids derived from the cDNA library from *P. papatasi* (Tunisian strain) salivary glands

3 Results

Our data showed that the "yellow proteins" (PpSP42 and PpSP44) and "apyrase" (PpSP36) elicit a cellular immune response with IFN-g-production. Strikingly, PpSP44 triggers the highest level of PBMC proliferation and IFN-g secretion. Multiplex cytokine analysis confirmed the Th1-polarized immune response induced by such proteins and showed as well that PpSP15, a candidate of vaccine in rodent and non primates, trigger a Th2-polarized immunity. By using, the recombinant form of PpSP44, we confirmed the results obtained above and further support that such protein constitutes a potential vaccine candidate against human leishmaniasis.

4 Conclusions

altogether, our results suggest that yellow proteins PpSP42 and PpSP44 as well as apyrase PpSP36 are candidates of vaccine against human cutaneous leishmaniasis.

C1539 PHAGE-FUSED EPITOPES FROM LEISHMANIA INFANTUM USED AS IMMUNOGENIC VACCINES CONFER PARTIAL PROTECTION AGAINST LEISHMANIA AMAZONENSIS INFECTION

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1 Background

Two mimotopes of *Leishmania infantum* identified by phage display were evaluated as vaccine candidates in BALB/c mice against *L. amazonensis.*

2 Methods

The epitope-based immunogens, presented as phage-fused peptides; were used without association of a Th1 adjuvant, and were administered isolated or in combination into animals.

3 Results

Both clones showed a specific production of IFN-g, IL-12, and GM-CSF after *in vitro* spleen cells stimulation, and were able to induce a partial protection against infection. Significant reductions of parasite load in the infected footpads, liver, spleen, bone marrow, and paws' draining lymph nodes were observed in the immunized mice, in comparison to the control groups (saline, saponin, wild-type and non-relevant clones). Protection was associated with an IL-12-dependent production of IFN-g, mediated mainly by $CD8^+$ T cells. Protected mice also presented low levels of IL-4 and IL-10, as well as increased levels of parasite-specific IgG2a antibodies.

4 Conclusions

The association of both clones resulted in an improved protection in relation to their individual use. More importantly, the absence of adjuvant did not diminish the cross-protective efficacy against infection. This study describes for the first time two epitope-based immunogens selected by phage display against *L. infantum*-infected dogs sera, which induced a partial protection in mice against *L. amazonensis*.

C1569 EVALUATION OF A CANINE LEISHMANIASIS VACCINE UNDER FIELD CONDITIONS IN SPAIN: AN ONGOING FIELD TRIAL

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1 Background

In recent years, there has been an increase in canine leishmaniasis cases, both in endemic and non-endemic areas in Spain, France, Italy and Portugal. Several methods have been suggested for disease control, including dog immunization. There are currently three licenced vaccines for CanL, one in Brazil and two in Europe. After 11 years of use, the first CanL vaccine commercialized in Brazil was recently withdrawn by the Brazilian Ministry of Agriculture due to lack of effectiveness evidence. This reinforces the need for properly designed and independent field studies to support vaccine's safety and efficacy observed in laboratory conditions. The project presented here consists of a random field trial of CaniLeish® vaccine, licenced in Europe in 2011. The main objectives of this study are to assess the vaccine safety and effectiveness in field conditions.

2 Methods

A total of 170 dogs of different breeds, sex and age have been enrolled in the vaccine field study. These dogs live in 21 dog kennels in Girona province (Catalonia, north-east Spain), an endemic area for CanL. Inclusion criteria were the same as indicated in CaniLeish® leaflet for vaccine use: healthy animals, aged more than 6 months and serologically negative for *Leishmania infantum*. Selected dogs were randomly allocated to two groups: vaccinated (N=85) and control (N=85). Each animal in the vaccinated group received a three vaccine course, following the manufacturer's instructions. Dogs in the control group did not receive any vaccine or placebo. Blood samples were collected at pre-determined time-points and whole blood, plasma and PBMC's from each animal were preserved for subsequent processing. Dogs were exposed to natural *L. infantum* infection during a transmission season and followed-up for detection of infected subjects. Finally, results obtained between vaccinated and control groups will be compared and analysed.

3 Results

No severe adverse reactions were observed in vaccinated dogs. Apart from one case of transient anorexia and apathy following the first vaccine dose administration, no other adverse reactions were reported. Field and laboratory assessment of vaccine-induced immune response and vaccine effectiveness are currently underway.

4 Conclusions

There were no observed severe adverse reactions associated with the use of CaniLeish® vaccine in the dog population studied in this field trial.

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C1597 KMP11-HASPB FUSION PROTEIN-EXPRESSING LENTIVIRAL VACCINE PROTECTS BALB/C MICE AGAINST LEISHMANIA MAJOR INFECTION

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1 Background

Leishmaniasis is an important zoonotic disease caused by facultative intracellular protozoa belonging to Leishmania genus, a Kinetoplastida that infects the macrophages of vertebrates and has more than 20 of which are pathogenic for the human. According to the WHO, nearly 350 million individuals are susceptible to the infection, and nearly 12 million are infected annually, among which 1.5-2 million suffer from cutaneous (CL). Hydrophilic acylated surface protein B is an immunogenic *Leishmania*-specific protein that antibodies are produced against it in the sera of Leishmania-infected individuals. Kinetoplastid membrane protein 11 is another *Leishmania* antigen and considered as suitable candidate for vaccine development leishmaniasis. It is a highly conserved surface protein expressed in both promastigotes and amastigotes. In this study, KMP11 and HASPB coding sequences were cloned into a pCDH-cGFP lentiviral vector as a fusion protein to be used as a DNA vaccine against *L.major*.

2 Methods

The coding sequences of HASPB and KMP11 were retrieved from GeneBank. After codon-optimization, the sequences were synthesized by Genscript and isubcloned into pCDH plasmid. According to Trono protocol, HEK293T cells were transfected using transfer lentiviral vector (pCDH-KH or pCDH-empty) in conjunction with three plasmids (pLP1, pLP2, and VSV-G). KMP11-HASPB fusion protein was successfully expressed as evidenced by RT-PCR and western blot assays. The effect of the vaccine was determined by evaluating the level of IFN-γ, IL-10, IgG1, and IgG2a performed using ELISA as well as determining the parasite load after challenge with L.major in vaccinated mice

3 Results

The results revealed that IFN-γ, IL-10, IgG1, and IgG2a significantly increased after vaccination using KMP11-HASPB-expressing lentiviruses in BALB/c mice. It is noteworthy that the level of IFN-γ and IgG2a was higher than that of IL-10 and IgG1, respectively. Moreover, the parasite load in the spleen and liver of vaccinated mice after challenge was significantly lower than that of controls.

4 Conclusions

Our results are in line with other researchers', and this indicates a promising fusion protein has been probably discovered for vaccination against different species of Leishmania. According to the results of our study, was indicated that the lentiviral vaccine expressing KMP11-HASPB is capable of inducing the Th1 response and resulted in decreased parasite burden.

C1613 APPLICATION OF HIGH THROUGHPUT QPCR ANALYSIS IN VACCINE CANDIDATE'S STUDIES AGAINST LEISHMANIA INFANTUM: A PRACTICAL CASE FOR BALB/C MICE IMMUNISED WITH RLIPABP3

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1 Background

Visceral leishmaniasis (VL) is the most severe form of leishmaniasis and cause between 200.000 and 400.000 new cases per year. In the last years, *L. infantum* has been responsible of an important outbreak in Spain. The parasite enters the host through the bite of a sand-fly and migrates to liver, spleen and bone marrow for its replication. The symptoms of VL are fever, weight loss, fatigue, anaemia, splenomegaly and hepatomegaly, and without the appropriate treatment, the death. There are drugs for treat VL but due to their toxicity, treatment regimes and parasite resistance, the development of effective vaccines is necessary. For this purpose, cutting edge techniques like RT-qPCR are required to understand gene expression changes and shed light on the immunological mechanisms underpinning protective immune responses.

2 Methods

The gene Poly(A)-Binding Protein 3 of *L. infantum* (LiPABP3) was cloned in the vector pQE30 to produce the recombinant protein PABP3 in *E. coli* cell cultures. rLiPAPB3 expression was induced using IPTG and its purification done by ion exchange chromatography. The immunisation of BALB/c mice required the subcutaneously administration of 1 ug rLiPAPB3 per mice four times, one every 14 days. The serum was extracted 2 weeks after each immunisation to analyse the humoral response by ELISA. For mice infection, 10^6 *L. infantum* promastigotes were injected via tail vein. After euthanasia, spleen and liver were extracted and the parasite load was analysed by limiting dilution. RNA was extracted from spleen to perform retro-transcription (RT) and qPCR to analyse expression of 106 immune system-related genes.

3 Results

rLiPABP3 promoted the production of antibodies in mice since the second immunisation. Also, the immunised and infected mice had a significant decrease of parasite load after 2 months-post-infection (mpi) both liver and spleen. The gene expression analysis performed in spleen revealed that rLiPABP3 induced the infra-expression of inflammatory immune response genes. At 2 mpi, TNF-alpha was infraexpressed in spleen, meanwhile, Ccr7 did not have any expression changes.

4 Conclusions

During VL, the infraexpression of inflammatory immune response genes generated by rLiPABP3 immunisation seems to enable the correct migration of dendritic cells from marginal zone towards PALS. This allows the appropriate antigens presentation and activation of some protective immune responses.

C1640 F1 DOMAIN OF THE LEISHMANIA DONOVANI NUCLEOSIDE HYDROLASE PROMOTES A T1 RESPONSE IN LEISHMANIA INFANTUM CURED PATIENTS AND IN ASYMPTOMATIC INDIVIDUALS LIVING IN AN ENDEMIC AREA OF LEISHMANIASIS.

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1 Background

The nucleoside hydrolase of NH36 is the Leishmune® main antigen and one of the promising candidates for vaccination against visceral leishmaniasis (VL).

2 Methods

The antigenicity of the N-terminal domain (F1), the central domain (F2) or the C-terminal recombinant domain (F3) of NH36 was evaluated in PBMC from individuals from a *Leishmania infantum* endemic area of Spain.

3 Results

Both NH36 and F1 domain (and no F2 and F3) significantly increased the PBMC proliferation stimulation index of cured patients and asymptomatic individuals compared to healthy controls. In patients cured from visceral leishmaniasis, proliferation to NH36 and F1 was accompanied by a statistical increase of IFN-g secretion. High levels of IFN-g were also found in patients cured from cutaneous leishmaniasis (CL) due to *L. infantum* and in asymptomatic individuals. The same profile was observed for the granzyme-specific secretion. The levels of IFN-γ and TNF-α were higher in response to F1 than to NH36 in patients cured of VL. IL-17 secretion, on the other hand, was stronger in response to both NH36 and F1 in asymptomatic subjects and patients cured of CL.

4 Conclusions

These data demonstrate that F1 peptide induces a recall cellular response similar to the one induced by NH36, a recombinant antigen that has protected mice from infection by several *Leishmania* species. The F1 domain of *Leishmania donovani*-NH36 may be consequently involved in generation of a protective response against *Leishmania infantum* human infection and represents a potential vaccine candidate for the control of human leishmaniasis by itself, or in combination with other HLA epitopes/antigens.

C1701 MICRONEEDLES INTRADERMAL VACCINATION WITH DNA COKTAIL ENCODING NUCLEOSOMAL HISTONES OF LEISHMANIA

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1 Background

Nucleosomal histones are intracellular proteins that are highly conserved among Leishmania species. Their ability to induce an effective immune response and protect from infection have been previously demonstrated in *L. major* and *L. braziliensis* mice model using different immunization strategies. In this study, we coated a microneedle device with DNA encoding nucleosomal histones and compare the elicited protective immunity with sc or id administration of the plasmid injected in mice using conventional needle and syringe.

2 Methods

BALB/c mice were immunized three times at three-weeks interval with 60 ug of a plasmid DNA cocktail encoding four *Leishmania* nucleosomal histones LiH2a, Lih2b, LiH3, LiH4 delivered subcutaneously (SC) or intradermally (ID) using either a needle and syringe or 30 solid microneedles previously coated with the plasmid DNA. Four weeks after the last immunization spleens and lymph nodes of some immunized mice were collected for cytokines determination with Multiplex Kit (spleens) or quantitive real-time PCR (lymph nodes). Other mice were challenged 2 x 105 infective metacyclic promastigotes of *L. major* in the base of the tail. Lesions were measured for 15 weeks with a digital caliper and parasite load determined at the end of the experiment by quantitative limiting dilution.

3 Results

Mice immunized with the microneedles showed increased expression ratio of IFN-g/IL-10 and IFN-g/Il-4 in the spleens and lymph nodes compared with mice that received the plasmid DNA by id or sc route using a needle and syringe ($p<0.05$). However, none imunization strategy was able to protect mice from *Leishmania major* infection, as indicated similar lesion development and parasite load determination in immunized and non-immunized mice after parasite challenge.

4 Conclusions

The results support the use of microneedle devices for eliciting stronger immune responses that the same dose of SC/ID delivery. The non efficacy of the vaccination with plasmid DNA encoding *Leishmania* nucleosomal histones observed in the current study underlines the importance of parasites number and administration route used for *Leishmania* challenge in mice.

C1703 EVALUATION OF A NEW LEISHMANIA-SPECIFIC HYPOTHETICAL PROTEIN FOR THE SERODIAGNOSIS OF CANINE VISCERAL LEISHMANIASIS AND ITS PROPHYLACTIC PROPERTIES AS A VACCINE AGAINST LEISHMANIA INFANTUM INFECTION

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1 Background

Leishmaniasis is a disease complex with a large incidence in the world, presenting high morbidity and mortality. Brazil accounts for approximately 95% of the visceral leishmaniasis (VL) cases in Americas, being the dog the main domestic reservoir of the parasites. Due to the severity of this disease and the various problems related to the control measures, the development of new diagnostic and prevention strategies for leishmaniasis has become a priority.

2 Methods

In the present study, a *Leishmania*-specific hypothetical protein, LiHyD, in a recombinant form (rLiHyD), was evaluated in ELISA experiments for the CVL diagnosis. Three B cell epitopes of LiHyD were synthesized (Peptide-1, Peptide-2 and Peptide-3) and also evaluated as diagnostic markers. In the search to also select a candidate antigen for composing a vaccine against visceral leishmaniasis, a combination of rLiHyD and saponin was tested in BALB/c mice against the infection by *Leishmania infantum*.

3 Results

The recombinant protein and the Peptide-3 showed the best results, being recognized by antibodies in sera of dogs with asymptomatic and symptomatic VL, and did not show cross-reactivity with antibodies in sera of dogs with Chagas disease, ehrlichiosis, babesiosis or animals without leishmaniasis and/or vaccinated with the Leish-Tec® vaccine. The immunogenicity of rLiHyD/saponin vaccine was evaluated, and the results showed that immunized mice produced higher levels of IFN-γ, IL-12 and GM-CSF after splenocytes *in vitro* stimulation with rLiHyD or *L. infantum* protein extract. After challenge, vaccinated animals showed significant reductions in the parasite burden in all evaluated organs, when compared to those that were inoculated with saline or immunized with saponin alone. The protection obtained with the rLiHyD/saponin vaccine was associated with a *Leishmania*-specific production of nitric oxide as well as with a parasite-specific IL-12-dependent IFN-γ production, which was produced mainly by CD4+T cells. In these animals, a decrease in the parasite-mediated IL-4 and IL-10 responses, associated with the presence of high levels of LiHyD and parasite-specific IgG2a isotype antibodies, were also found.

4 Conclusions

The present study showed that the hypothetical protein LiHyD, which was firstly identified in *L. infantum* and is also conserved in other *Leishmania* species, can be used for the CVL serodiagnosis and, combined with a Th1 adjuvant, can compose a vaccine and confer protection against visceral leishmaniasis.

C1727 TREATMENT OF VISCERAL LEISHMANIASIS PATIENTS OUTSIDE CLINICAL TRIALS; THE CASE OF THE LEISHMANIASIS EAST AFRICA PLATFORM

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1 Background

Treatment of patients outside clinical trials in the clinical research setting can be challenging especially for neglected tropical disease research, where trials are often conducted in constrained, poor, rural and remote settings and in vulnerable patients. The Leishmaniasis East Africa Platform (LEAP) conducts clinical research in visceral leishmaniasis (VL) in Ethiopia, Kenya, Sudan and Uganda. With the support of the Drugs for Neglected Diseases *initiative* (DND*i*) and other partners, LEAP has delivered one new VL treatment for the region, sodium stibogluconate and paromomycin combination, with more studies for new treatments either ongoing or in the pipeline. The main objectives of the studies are geared towards development of an oral drug for VL in the region.

2 Methods

We conducted a retrospective review of data for leishmaniasis patients at the LEAP clinical trials sites between 2010 and 2016. Five clinical trials were conducted during the review period in Ethiopia, Kenya, Sudan and Uganda. We assessed the number of patients treated for leishmaniasis overall and categorised the patients according to whether they had participated in clinical trials or not, and further assessed other leishmania cases by number treated for PKDL, cutaneous leishmaniasis and mucocutaneous leishmaniasis. Ethically, it was obligatory to treat patients excluded from the clinical trials.

3 Results

During the reporting period, 23,740 suspected cases were screened and 10,823 (45.59%) of these were positive for leishmania. Out of the 10,823, 320 (3%) participated in phase II and III clinical trials, and 1,349 (12.5%) in a phase IV study. There was a total of 9,154 (84.6%) leishmania cases treated outside the trials, corresponding to: VL 8,409 out of 9,154 (91.9%), PKDL 157 (1.7%), cutaneous leishmaniasis 491 (5.4%) and mucosal leishmaniasis 97(1%).These were excluded from the clinical trials due to stringent inclusion exclusion criteria.

4 Conclusions

While the vast majority of patients may not participate in clinical trials, they benefit from a collaborative approach in which VL clinical research has been integrated with the treatment of leishmaniasis in patients excluded from participating in the trials. LEAP has implemented a new VL treatment, bringing benefits to the local communities, and raised standards of treatment through training, resourcing and providing expertise to both participants and non-trial participants.

C1733 CD8+ T CELLS CONTRIBUTE TO PROTECTION AGAINST CUTANEOUS LEISHMANIASIS IN BALB/C MODEL: IMPLICATION FOR VACCINE DESIGN

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1 Background

Leishmaniasis is a neglected tropical disease endemic in more than 98 countries and is spreading over due to transmigration and drug resistance. However the vaccine is still lagging behind. The contribution of CD8$^+$-T-cells to resistance against intracellular infections and evidence indicating their role in induction of Th1 responses and protection against primary and/or secondary *Leishmania major* infection, has impacted effective vaccine design against leishmaniasis. DNA vaccines have revealed promising in protection induction by CD8$^+$-T-cells stimulation and self-adjuvanting potential.

2 Methods

Herein, the protective efficacy of a polytope DNA in Prime-Boost regimen (DNA-DNA immunization) against *L. major* challenge was assessed. Four H-2Kd restricted epitopes from *Leishmania* major proteins were included in the polytope construct (besides 13 HLA-A2 restricted peptides from *Leishmania* well known vaccine candidates). The protective potential was evaluated by footpad swelling, parasite burden and immunological assays (IFN-γ/IL-5 ELISA, IFN-γ ICCS and CFSE) after *L. major*^{EGFP} infectious challenge in BALB/c mice.

3 Results

In this study the CD8+ T cells contribution in protection against cutaneous leishmaniasis due to *L. major* was clearly demonstrated. Lesion size in polytope vaccinated group was significantly lower than other groups during infection. LN parasite burden and footpad imaging confirmed significant lower parasite at 7 weeks post challenge. DNA-DNA immunization resulted in evident Th1 polarization while CD8 depletion **c**ompromised this effect with dominant Th2 response. ICCS successfully detected CD8+/IFN-γ+ T cells in vaccinated group both early after infection and later. IgG2a/IgG1 ratio was at least 2 folds higher in DNA-DNA immunized group compared to controls. Higher IgG1 level in CD8+T-cell depleted group compared to G1 was noteworthy.

4 Conclusions

Proteins or peptides that effectively induce CD8+ T cells should be reconsidered in respect to subunit vaccine design. Therefore Polytope DNA vaccines encompassing multiple immunogenic high avidity peptides to induce CD8+ and CD4+ T cells represent a promising approach for vaccine design against leishmaniasis.

C1740 EVALUATION OF LACK ANTIGEN AS VACCINE CANDIDATE AGAINST VISCERAL LEISHMANIASIS: T-CELL ANTIGENICITY IN HUMANS AND EFFICACY TRIAL IN HAMSTER.

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1 Background

Leishmania homologue of receptors for activated C kinase (LACK) antigen is one of most studied *Leishmania* protein as vaccine candidate, due to its immunogenic properties. A heterologous prime-boost vaccination with DNA and recombinant vaccinia virus (MVA and M65) vectors expressing LACK antigen has been shown to enhance specific cellular immune responses against *Leishmania* and to elicit protection in mice against cutaneous leishmaniasis. However, only one study has been performed to evaluate the efficacy of DNA-LACK/MVA-LACK against visceral leishmaniasis (VL), which demonstrated vaccine-induced protection in experimental canine model. With the aim to find an effective vaccine against human VL, we have assayed the cellular antigenicity of the LACK protein in humans and, for the first time, we have evaluated the efficacy of DNA-LACK/MVA-LACK and DNA-LACK/M65-LACK in a hamster model of VL.

2 Methods

We have measured *in vitro* the cellular antigenicity to LACK antigen in PBMCs isolated from asymptomatic subjects (AS), cured patients from cutaneous (CCL) and visceral leishmaniasis (CVL) and healthy endemic subjects as negative control (EC) from a post outbreak area of *L. infantum* (Fuenlabrada, Spain). Also, we have carried out a vaccine efficacy trial in *L. infantum* infected hamsters, previously immunized with DNA-LACK/MVA-LACK or with DNA-LACK/M65-LACK.

3 Results

LACK antigen was recognized specifically by PBMCs from cured (38% of CVL and 20% of CCL) and asymptomatic individuals (50%). Regarding to cytokine production, LACK produced a significant secretion of IFN-γ in PBMCs from CVL, CCL and AS regarding to EC, and TNF-α in PBMCs from CCL and AS. The efficacy trial carried in hamsters showed that DNA-LACK/MVA-LACK group presented a lower level of anti-SLA IgG antibodies than the vaccine components control group (empty-DNA/empty-virus). Moreover, DNA-LACK/MVA-LACK and DNA-LACK/M65-LACK vaccinated hamsters had higher specific PBMCs lymphoproliferation to SLA when compared with saline control group. In addition, both LACK-immunized groups underwent a significant reduction of parasite burden in liver ($p \leq 0.01$ and $p \leq 0.05$, respectively) and bone marrow ($p \leq 0.01$ and $p \leq 0.01$, respectively).

4 Conclusions

In conclusion, LACK induced a Th1 response in human PBMCs of those individuals who have been in contact with the parasite, and the two assayed DNA-LACK based vaccines generated protection in hamsters, suggesting it is an exceptional vaccine candidate to move to clinical trials.

C1760 COMPARISON OF THE IMMUNOGENICITY AND PROTECTIVE EFFICACY OF LEISHMANIA INFANTUM RECOMBINANT PROTEINS USED IN DIFFERENT VACCINE STRATEGIES AGAINST VISCERAL LEISHMANIASIS

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1 Background

Visceral leishmaniasis (VL) is a major health problem in the world. Due to difficulties in implementing effective prophylactic methods and in the treatment of the disease, the development of alternative measures, such as vaccines, is attractive. Attempting to select different candidate antigens to compose a vaccine against VL, immunoproteomic and bioinformatics tools have been widely used. Several proteins have been identified and tested; however, the search for improving a vaccine keeps going. The aim of this study was to compare the immunogenicity and the protective efficacy of three recombinant proteins isolated (LiHyp1 -LinJ.35.1290, LiHyp6 - LinJ.36.0580, and HRF - LinJ.24.1560), using in a mix, as well as a chimeric protein composed by T cell epitopes derived from these proteins, all of them added with saponin, against VL.

2 Methods

The DNA sequences were cloned, and the recombinant proteins were expressed and purified. The chimera was synthesized with immunodominant regions composed by $CD4^+$ and $CD8^+$ T cell epitopes specific for mice and human haplotypes, from the three proteins. The single or mixed proteins, as well as the chimera, were added with saponin and used to immunize BALB/c mice. Animals were infected with *L. infantum* and the immunogenicity and protective efficacy were evaluated.

3 Results

All the vaccinated mice showed an increase in specific production of IFN-γ, IL-12, and GM-CSF after *in vitro* stimulation, which were maintained after infection; however, the level of these cytokines was higher in the mix and chimera groups, when compared with the isolated proteins and control groups (saline and saponin). Immunized animals also presented significant reductions of the parasite burden in the evaluated organs, but the mix and chimera groups showed a higher protection in relation to the other groups. The vaccine efficacy was associated with a raised production of IFN-γ and nitric oxide, besides of anti-leishmanial IgG2a isotype antibodies. In addition, a decrease in the secretion of IL-4 and IL-10 was also observed in protected animals.

4 Conclusions

In conclusion, recombinant proteins isolated, when added with saponin, can induce protection in BALB/c mice against VL; however, the mix of proteins, as well as the chimeric protein, showed a better efficacy. Due to the efficience and to the possibility of cost reduction, a recombinant chimeric protein plus saponin could be considered as a prophylactic alternative to prevent VL.

C1761 A NEW LEISHMANIA-SPECIFIC HYPOTHETICAL PROTEIN, FIRSTLY IDENTIFIED IN LEISHMANIA INFANTUM, USED AS A VACCINE ANTIGEN AGAINST TEGUMENTARY AND VISCERAL LEISHMANIASIS

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1 Background

Leishmaniasis represents a spectrum of diseases caused by protozoan parasites of the genus *Leishmania* that affect million people in several countries in the world. Due to difficulties found in implementing effective prophylactic methods and in the treatment of the disease,the development of alternative measures, such as vaccines, is attractive. Experimental vaccine candidates have been tested to prevent leishmaniasis, but no commercial vaccine has proved to be effective against more than one parasite species. The aim of this study was evaluate the immunogenicity and protective efficacy of a new hypothetical protein, specific and conserved in *Leishmania* spp, named LiHyT, added with saponin, against *L. infantum, L. major* and *L. braziliensis* infection.

2 Methods

The DNA sequence of LiHyT was cloned and the recombinant protein was expressed and purified. BALB/c mice were vaccinated with LiHyT plus saponin and infected with *L. infantum, L. major* or *L. braziliensis*. The immune response generated was evaluated before and 10 weeks after infection, as well as the parasite burden in spleen, liver, bone marrow, draining lymph node and lesion.

3 Results

The vaccination induced a Th1 response, which was characterized by the production of IFN-γ, IL-12 and GM-CSF, as well as by high levels of IgG2a antibodies, after in vitro stimulation. After challenge, vaccinated mice that were infected with *L. major* or *L. braziliensis* showed significant reduction in footpads swelling, as well as of the parasite burden in this tissue. All the immunized animals, infected with different species of *Leishmania*, demonstrated a significant decrease of the parasite load in the organs evaluated, when compared to the control groups (saline and saponin). The anti-*Leishmania* Th1 response was maintained after infection, being the IFN-γ production based mainly on $CD4^+$ T cells.

4 Conclusions

In conclusion, we described that only one conserved *Leishmania*-specific hypothetical protein could compose a good vaccine against several species of *Leishmania* spp.

C1763 HETEROLOGOUS PROTECTION OF A LEISHMANIA- SPECIFIC HYPOTHETICAL PROTEIN AGAINST TEGUMENTARY LEISHMANIASIS CAUSED BY LEISHMANIA MAJOR AND LEISHMANIA BRAZILIENSIS SPECIES

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1 Background

Leishmaniasis comprises a group of diseases ranging from cutaneous to visceral forms. Tegumentary leishmaniasis (TL) is endemic in more than 70 countries and 90% of the cases are registered in Afghanistan, Algeria, Brazil, Pakistan, Peru, Saudi Arabia and Syria. The disease control has been difficult due to the natural features of reservoir and vectors, making the elimination of both components hard to obtain. Also, therapeutic measures are ineffective and the development of a vaccine would be the most effective measure to eliminate or, at least, control the disease.

2 Methods

The present study aimed to evaluate a *Leishmania*-specific hypothetical protein, named LiHyD (LinJ.33.3150), which is conserved between different *Leishmania* species, as a potential vaccine candidate to protect against TL caused by *Leishmania major* and *Leishmania braziliensis* species. This antigen was firstly identified in *Leishmania infantum* and showed to be protective against infection of BALB/c mice using this parasite species.

3 Results

After the vaccination with rLiHyD/saponin the immunogenicity was evaluated, and the results showed that immunized mice produced high levels of IFN-γ, IL-12 and GM-CSF after in vitro stimulation with rLiHyD, as well as by using *L. major* or *L. braziliensis* protein extracts. After challenge, vaccinated mice showed anti-*Leishmania* IgG2a antibodies and a predominant IL-12 driven IFN-γ production (mainly produced by CD4+ T cells) against parasite proteins whereas unprotected controls showed anti-*Leishmania* IgG1 antibodies and parasite mediated IL-4 and IL-10 responses. The vaccinated animals also presented significant reductions in the infected footpad swellings, as well as in the parasite burden in the infection site, liver, spleen, and infected paws draining lymph nodes, when compared to those that were inoculated with the vaccine diluent (saline) or immunized with saponin. The immunization of rLiHyD without adjuvant was not protective against both challenges.

4 Conclusions

In conclusion, the present study showed that a hypothetical protein that was firstly identified in L. infantum, when combined to saponin, was able to confer a cross-protection against highly infective stationary-phase promastigotes of two Leishmania species causing tegumentary leishmaniasis.

C1768 CLINICAL MANAGEMENT OF SOROPOSITIVE DOGS FOR VISCERAL LEISHMANIASIS, ASYMPTOMATICS AND WITH NO INFECTING POTENTIAL FOR SAND FLIES.

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1 Background

Canine Visceral Leishmaniasis (CVL) is a chronic and fatal disease, when untreated. It is caused by a digenetic protozoan named *Leishmania infantum*, which also infects humans and other animal species. Its transmission occurs by the bite of infected dipteran sandflies insects of the species *Lutzomyia longipalpis*. The dog is the main domestic reservoir of *L. infantum*, but the diagnosis of infection is often difficult, either due to the low anti-*Leishmania* antibodies titers, the absence of parasitic forms in parasitological and molecular tests, absence of physical signs and normality of the complementary tests such as the complete blood count, renal and hepatic profile and serum protein levels. Animals in these conditions are not classified as infected but rather as suspects and do not have a protocol of therapeutic management or established follow-up

2 Methods

In order to monitor suspected CVL animals, thirty-two dogs, of different breeds, varied ages and both sexes, with positive serological results, IFAT and/or ELISA, were followed for one year, but with low titers, parasitological and/or molecular tests negatives, absence of physical signs and alterations in the complementary tests classified as suspected (Stage I) of CVL.

3 Results

Of these thirty-two animals, in the first evaluation, 26/32 were ELISA reagents and in IFAT, 17/32 had titles 1:40, 13/32 1:80 and 2/32 1: 160. All dogs were submitted to immunotherapy, based on the application of the *L. infantum* A2 antigen associated with 1 mg of saponin as adjuvant (two vials of the Leish-Tec® vaccine), in three administrations with 21-day intervals and one administration every six months. These animals were evaluated one year later and 13/32 were maintained ELISA reagents; in IFAT, 21/32 were negative, 3/32 had titles 1:40, 7/32 1:80 and 1/32 1: 160. The physical pattern remained asymptomatic, parasitological and/or molecular exams continued negative and the complementary tests remained within the normality parameters.

4 Conclusions

The absence of disease evolution in these animals, as well as the reduction in the number of reactive animals in the ELISA and the antibody titers in the IFAT, except for those false positives, may indicate a protective immunomodulatory response against the progression of the infection provided by the immunotherapeutic treatment with the A2 antigen associated with 1 mg of saponin. Similar results were demonstrated in dogs treated with immunotherapy and allopurinol in a previous study (RIBEIRO et al., 2013).

C1777 ACTIVE CUTANEOUS LEISHMANIASIS AND CLINICAL CURE: MULTIVARIATED ANALYSIS OF TH17-RELATED CYTOKINES.

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1 Background

In cutaneous leishmaniasis , Th1-like cytokines production (IFN-γ, TNF-α, IL-2), are important for host control of parasite burden and clinical cure. In addition, IL-17, a cytokine produced by Th17-type T cells has been related with resistance and pathology in cutaneous leishmaniasis caused by the infection with *Leishmania braziliensis*. IL-22, IL-23 are also related with Th17 cells. IL-23 is involved in the differentiation and maintenance of Th17 phenotype. Meanwhile, IL-22, is inflammatory during skin inflammation and also plays a role in tissue repair after wounding. The roles of these Th17 cytokines in the resolution of the Leishmania infection have not been investigated.

2 Methods

In this study, serum IL-17, IL-22 & IL-23 levels (pg/mL) were determined by ELISA in healthy controls (CT) and localized cutaneous leishmaniasis patients (LCL) with active or cured disease, and %CD4+ T cells, %CD8+ T cells, %monocytes, %natural killer cells (NK) , %B cells and %neutrophils were determined by flow cytometry. Two principal component analyses (PCA) were performed: the first analysis considered Montenegro and PPD skin tests, %lymphocytes, %monocytes and %neutrophils; the second included all cellular types and Th17 cytokines. To evaluate differences between groups the similarity test: ANOSIM were applied.

3 Results

The PCA 1 showed a variability of 67.3%, and also positive correlations between Montenegro & PPD skin test, %monocytes and %neutrophils. These parameters were orientated to LCL patients with active infection. Meanwhile vector corresponding to lymphocyte, increased its value to cured LCL patients and healthy subjects. For the second PCA (42.4% variability) it was found two well defined groups (p=0.02): group 1 (Ct and cured LCL patients) and group 2 (active LCL). Vectors representing CD4+T cells, CD8+ T cells, IL.17 and IL-22 are projecting to active LCL; these 4 parameters were positively correlated. Furthermore, NK cells vector was associated with cure LCL patients. NK cells are important in the activation of Th1 response, which mediates healing in cutaneous leishmaniasis.

4 Conclusions

Overall results suggest that Principal component analysis could discriminated active to cure cutaneous leishmaniasis. Th17 cytokines (IL-17 & IL-22) were associated with active disease in contrast, clinical cure was related with NK cells and lymphocytes.

This work was supported by CDCH-UCV.

C1785 ASSESSMENT OF THERAPEUTIC POTENTIAL OF TH1 STIMULATORY PROTEINS AGAINST EXPERIMENTAL VISCERAL LEISHMANIASIS

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1 Background

For the control of visceral leishmaniasis (VL), a deadly neglected tropical disease caused by the intracellular parasite- *Leishmania donovani*, an effective therapeutic vaccination strategy requires which can boost an immune response in *Leishmania*-infected individuals as most of them, in endemic regions, exhibits either subclinical or asymptomatic infection.In the present study, therapeutic role of potential Th1 stimulatory proteins was assessed in hamsters, an experimental host, infectedwithaclinical isolate of *L. donovani*.

2 Methods

Four recombinant proteins namely, aldolase, enolase, p45 and triose phosphate isomerase of *L. donovani* were purified using affinity chromatography. Cytotoxicity of these proteins was assessed in peritoneal macrophages of a naïve hamster with MTT dye. For assessing their therapeutic efficacy, each protein was administered subcutaneously at different concentrations (12.5, 25 and 50 µg) in 21 days old infected hamsters (with 5 X 10^5 purified amastigotes). Two booster shots of these proteins were given at 14 days interval. All animals were euthanized on day 60 post infection and assessment of splenic parasite load as well as delayed type hypersensitivity (DTH), cytokine and antibody responses were made.

3 Results

All the four recombinant Th1 stimulatory proteins were successfully purified and none of the proteins exhibited cytotoxicity in peritoneal macrophages, even at higher concentration (100 µg/ml). Vaccination studies revealed a significant parasite inhibition (%) in *Leishmania*-infected hamsters immunized with a lower dose (12.5 µg) of aldolase (56%), enolase (46%), and p45 (31%) in comparison to higher doses (25 and 50 µg) of the same. However, there was no therapeutic effect of TPI observed in infected hamsters. DTH response was also found to be enhanced in hamsters vaccinated with lower doses of aldolase, enolase, and p45. Furthermore, these animals also exhibited higher serum IgG2 response measured by ELISA, an indicative of Th1 type of immune response, along with significant expression of splenic mRNA transcript of Th1 cytokines through real-time RT-PCR.

4 Conclusions

This study indicates towards the therapeutic potential of three out of four recombinant proteins- aldolase, enolase, and p45 in *Leishmania*-infected hamsters. Further assessment of these proteins in combination with potent immunomodulators will offer a better approach for the control of this disease.

C1822 IMMUNITY RESPONSE IN VACCINATED DOGS AGAINST LEISHMANIASIS IN A BRAZILIAN ENDEMIC AREA

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1 Background

The number of new human cases of visceral leishmaniasis (VL) is increasing in some areas of Brazil in spite of prevent and control measures that have been taken. A vaccine for human and canine populations is a convenient and efficacious method for the control of zoonotic VL. An ideal vaccine against canine VL should have immunological features that include the elicitation of a long-lasting cell-mediated immune responsecharacterized by antigen specific poly-functional T-cells expressing IFN-γ, TNF-α and IL-2. So, we aimed evaluate the efficacy of two vaccines against *Leishmania*.

2 Methods

In this study we have immunized seronegative dogs with Leishmune® fromPfizer-Zoetis –V1 Group (N= 17), and Leish-Tec® from Hertape Calier -V2 Group (N=19), and 12 animals were not immunized (control group). All of them were naturally exposed to sandfly bites in an endemic area of Brazil (Panorama-São Paulo). Blood sample were collected from each animal at six(6M) and 12 months (12M) post vaccination for evaluation the secretion level of IFN-g, TNF-α and IL-2 in serum after whole blood stimulation with soluble leishmanial antigen (WBA-SLA).

3 Results

At month 6 post vaccination, the IFN-γand IL-10 secretion was increased in V2 group in comparison to the negative controls and group V1. The group V1 presented high level of IFN-γ only 12 months after vaccination, however they were lower than those observed in group V2. The TNF-α secretion increased in V1 group at 6M regarding to the negative controls and group V2. However, at 12M, the TNF-α secretion was increased in both vaccinated groups in comparison to the negative controls, although it was more patent in group V2. In vaccinated animals, the high production of IFN-γ showed concordance with the development of a positive DTH response in the skin. In addition, we have also found a statistically significant relationship between the size of the induration produce by the DTH (cm^2) in the skin and the production of IFN-γ.

4 Conclusions

Although further studies are still in progress to associate data from the serology and molecular diagnostic, we can conclude that Leish-Tec® vaccine produced a good immunization six months after inoculation that was maintained until the 12 months.

C1840 ANTIGENICITY OF KMP11, F3+ AND LJL-143 PROTEINS AS COMPONENTS OF A MULTIVALENT VACCINE CANDIDATE AGAINST HUMAN VISCERAL LEISHMANIASIS.

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1 Background

Leishmania infantum KMP-11 protein and the three antigens that composed the chimeric F3+ protein (*L. donovani* nucleoside hydrolase, *L. infantum* sterol 24-c-methyltransferase and*L. infantum* Δ cysteine proteinase B) are protective in animals models against visceral leishmaniasis (VL). *Lutzomya longipalpis* salivary protein LJL-143 generates a Th1 environment in immunized dogs and a parasite detrimental *in vitro* assays. The combination of these three proteins seems to be an accurate approach in order to find a potential vaccine against human VL. With the aim to confirm their suitability to be part of a vaccine candidate, developed in the framework of the FP7 European project MuLeVaClin, in this study we have assayed the antigenicity of KMP-11, F3+ and LJL-143 in human.

2 Methods

Samples from active (symptomatic) patients, cured (healed) patients from visceral and cutaneous leishmaniasis (CVL/CCL respectively), asymptomatic subjects (AS) and healthy endemic subjects as negative control (EC) were collected in a *L. infantum* post outbreak area (Fuenlabrada, Spain). Humoral antigenicity was measured determining in sera the specific IgG levels by ELISA. Cellular antigenicity was evaluated in PBMCs measuring the specific cytokine profile on supernatants after five days culture with different antigens.

3 Results

From the 198 subjects included in this study, we have observed that KMP-11 and F3+ were recognized by sera from a high percentage of symptomatic, asymptomatic or healed individuals. Regarding to cellular antigenicity, KMP-11 produced a significant secretion of IFN-γ in PBMCs from CVL, CCL and AS subjects regarding to EC individuals, whereas F3+ produced IFN-γ and TNF-α in PBMCs from CCL and AS. Finally, LJL-143 was recognized by sera and PBMCs of some individual from all groups, including some endemic healthy control.

4 Conclusions

In conclusion, F3+ and KMP-11 were specifically recognized by sera of an important percentage of individuals who were in contact with the parasite. In addition, F3+ and KMP-11 produced a Th1 cytokine profile in *Leishmania* cured and asymptomatic individuals. As expected, LJL-143 was recognized by the sera and PBMCs of some individuals from all groups, since all of them were exposed to sand-fly bytes. These proteins have been combined and formulated with a Th1 adjuvant and virosomes as a protein delivery system, this formulation also have been evaluated in preclinical immunogenicity, safety and efficacy trials with promising results.

C1843 EFFICACY TRIAL OF A VACCINE CANDIDATE WITH THREE RECOMBINANTS PROTEINS (KMP-11, F3+ AND LJL-143) FORMULATED IN VIROSOMES IN COMBINATION WITH TLR4 AGONIST ADJUVANT IN A HAMSTER MODEL OF VISCERAL LEISHMANIASIS.

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1 Background

With the purpose to generate an efficient multivalent vaccine against human VL, in the framework of the FP7 European project MuLeVaClin, we carried out a preclinical efficacy trial. The vaccine candidate was composed by three proteins: *Leishmania* proteins,KMP-11 and chimeric F3+ (constituted with nucleoside hydrolase, sterol 24-c-methyltransferase and Δ cysteine proteinase B proteins), which are protective in animals models, and vector salivary protein LJL-143, that has immunomodulatory properties because is able to generate a Th1 environment in immunized dogs. These proteins were included in virosomes, that are a potent delivery vehicle targeting dendritic cells, and have the capacity to deliver antigen to the both MHCI and MHCII pathways, resulting in a stimulation of CD4+ and CD8+ T cells. In addition, this formulation was combined with a potent Th1 adjuvant, GLA-SE, a TLR-4 agonist approved for human use.

2 Methods

In the experimental model of VL in hamsters infected with *Leishmania infantum,* we assayed different concentrations of the proteins (five times the amount of KMP-11 and F3+ respect to LJL-143 (5:5:1) or all of them in the same concentration at low doses (1:1:1)) and/or the addition of GLA-SE and/or including virosomes into the formula.

3 Results

We have found that groups immunized with the formulation including virosomes in combination with the GLA-SE, independently of the concentration of antigens (VPA 1:1:1 and VPA5:5:1), presented a lower level of anti-SLA IgG than the other groups. Regarding to protection against *Leishmania* infection, those animals vaccinated with the proteins alone at 5:5:1 (P5:5:1), immunized with VPA 1:1:1 or VPA 5:5:1, showed areduction of parasite burden in spleen, that was significant in VPA 5:5:1 group ($p \leq 0.05$).

4 Conclusions

In conclusion, the vaccine candidate formulation composed by the higher concentration of KMP-11 and F3+ than LJL-143 proteins, targeted in virosomes and administrated with GLA-SE was efficacious agains *L. infantum* infection in hamster. Other data obtained about accurate antigenicity of these proteins in humans, the safety and immunogenicity of different formulation that induced a Th1 profile in vaccinated mice, have been taking into account to carrier out the phase I trial in humans, planned to begin proximately.

C1887 DEVELOPMENT OF CHIMERIC PROTEIN AS A VACCINE TARGET AGAINST TRYPANOSOMATIDS (THE TRYPAN VACCINE)

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1 Background

The Trypanosomatidae Family is formed by unicellular eukaryotic organisms belonging to the class Kinetoplastea, and it is of great medical and economic interest as more than 8 million people are infected worldwide. In this class are included the genera *Leishmania* and *Trypanosoma*, which are responsible for American and African leishmaniasis and trypanosomiasis, respectively. Together these diseases generate approximately 4.27 million disability-adjusted life years (DALYs) due to the symptoms caused by parasitism. The control of these diseases is mainly based on insect vector control and treatment of patients (WHO, 2015). However, these measures are not always satisfactory, since most of the time the complete elimination of the vector insects is difficult and the lack of complete cure of available treatments. In addition, the number of infected individuals continues to expand and a more effective form of control such as the development of a vaccine that are capable of protecting against all these diseases is urgently needed.

2 Methods

In the present work we propose the development and use of a chimeric molecule formed by the fusion of DNA sequences that encode with imunogenic and conserved regions in the different parasites (*Leishmania* and *Trypanosoma*) but absent in host species. The heterologous expression of a chimeric protein comprising 64 different peptides was produced in bacterial expression system, followed by subsequent protein purification for further use in the immunization of Balb/c mice. After 3 doses, administered every 15 days, the animals were challenged *L. infantum*, *L. mexicana* or *T. cruzi* strain Y.

3 Results

So far, we detected the significant reduction by 68% of the parasite load in animals challenged with *T. cruzi*, in relation to the control group (immunized with PBS only).

4 Conclusions

Our result demonstrate the promising use of a potential vaccine against trypanosomatid infection.

C1904 TRYPANOSOMA CRUZI CL-14 AS AN EFFICIENT VACCINE ALTERNATIVE AGAINST LEISHMANIA INFANTUM INFECTION

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1 Background

Visceral leishmaniasis (VL) is a major public health issue, reportedly the second in mortality amongst all tropical diseases. Clinical trials have shown that protection against visceral leishmaniasis is associated with robust T-cells responses, especially those producing IFN-γ. In order to trigger such response, we designed different live vaccine candidates using the Trypanosoma cruzi CL-14 non-virulent strain.

2 Methods

We stably transfected CL-14 with integrative plasmids containing the synthetic gene A2, repeatedly shown to be immunogenic and protective against VL in different animal models, or the coding sequence of LiHyp1 antigen, a novel promising vaccine candidate. BALB/c mice were then immunized intraperitoneally with 10^7 recombinant parasites through homologous prime-boost protocol or subcutaneously with the respective recombinant protein adsorbed in Alum+CpG. Spleen cells were harvested and serum samples were collected and tested by ELISA for specific immunoglobulins and IFN-γ production. 21 days after the last dose, the animals were challenged subcutaneously with 10^7 *Leishmania infantum* promastigotes and the parasite burden of liver and spleen was measured after 30 days.

3 Results

Immunization with the CL-14 transgenic parasites elicited a stronger cellular immune response than control groups, shown by levels of IFN-γ, conferring partial protection in BALB/c mice against L. infantum challenge. Interestingly, the use of the wild type CL-14 alone was enough to boost immunity and confer protection, confirming the previously reported pro-immunogenic potential of the strain.

4 Conclusions

Altogether, these results support the efficiency of T. cruzi CL-14 as a vaccine vector and its ability to induce strong T-cell based immune response against visceral leishmaniasis in animal models. This strategy reveals a promising alternative in the development of new vaccines, including the possibility of association and test of a multi-parasite vaccine.

C1919 EVALUATION OF GALACTOSYLCERAMIDE AND LIPOPOLYSACCHARIDE AS ADJUVANTS FOR IMMUNIZATION AGAINST LEISHMANIA MEXICANA

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1 Background

Leishmania parasites are the causative agents of Leishmaniasis. In mammals, they infect phagocytes such as macrophages, which resolve the infection by their IFN-g activation. BALB/c mice are susceptible to *Leishmania*. Upon infection, they initiate a Th2 response which is characterised by the production of IL-4. Such response inhibits macrophage activation and favours disease progression. Studies have shown that through immunization with specific adjuvants, this response can be polarised towards a protective Th1 response mediated by cytokines as IFN-g and IL-12. α-Galactosylceramide (α-GalCer) and lipopolysaccharide (LPS) are adjuvants which can induce a systemic inflammatory effect. However, while α-GalCer induces a higher production of IFN-g; LPS promotes mainly the production of TNF-α.

2 Methods

BALB/c mice were immunized as follows: non-immunized, antigen of amastigotes of *L. mexicana* (100 µg), antigen of amastigotes of *L. mexicana* (100 µg) + a-GalCer (4 µg), antigen of amastigotes of *L. mexicana* (100 µg) + LPS (25 µg), and antigen of *L. mexicana* (100 µg) + a-GalCer (4 µg) + LPS (25 µg). One week later, the animals were infected with 1x10? parasites in the footpad. The thickness of the footpad was used to determine the progression of the disease and the parasite burden was calculated at week 6 after infection. Additionally, IFN-g and IL-4 secretion by splenocytes was measured.

3 Results

Both a-GalCer and LPS lead to the reduction of the parasite burden. However, only a-GalCer significantly reduced the size of disease associated lesions. There was a ten-fold reduction in parasite burden in skin lesions and additionally there IFN-g production by splenocytes at week 6.

4 Conclusions

a-GalCer is a more effective adjuvant than LPS for immunization against *L. mexicana*, as it helps to control and eliminate the disease via an IFN-g dependant mechanism. This implies that a-GalCer has the potential to be used as an effective adjuvant for immunization against *L. mexicana* towards an effective vaccine against *Leishmania*.

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Diagnostic methodology in practice and under development. Biomarkers in leishmaniasis

C0099 IDENTIFICATION OF LEISHMANIA SPECIES FROM NEGATIVE STAINED SMEARS BY MOLECULAR AND MICROCULTURE METHODS

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1 Background

leishmaniasis is vector-born parasitic disease. The routine diagnosis of CL patients depends on examination of skin lesions using smears and cultures of dermal scrapings. microscopic examination usually has low sensitivity and cannot identify even some confirmed CL cases. The aim of this study was to identify and characterize Leishmania species among the negative direct smears obtained from skin ulcers suspected to CL by PCR and microculture methods.

2 Methods

This study was carried out in during 2011-2013. Among patients with suspicious skin lesions to CL referred to health center laboratories of Gonbad-e-Qabus and Torbat-e-Jam cities, Samples were obtained through skin scrapping and negative Giemsa stained smears were collected. Then improved microculture (IMC) and polymerase chain reaction (PCR) on obtain samples were performed.

3 Results

The positive rates from 131 negative Giemsa stained smears were 29.9%, and 18.36% for PCR and microculture methods, respectively. Thus among the DNA extracted from
smears, L. tropica and L.major were isolated from 11 (8.4%) and 23(17.5%) of the smears.

4 Conclusions

This study revealed Molecular and microculture techniques are along with the advantages of rapidity, adequate sensitivity. Hence we suggest the IMC as a valuable alternative diagnostic method for PCR in diagnosis of CL particularly from endemic regions.

C0105 REPEATED STERNAL BONE MARROW SAMPLINGS IN 115 DOGS LIVING IN CANINE LEISHMANIASIS ENZOOTIC ARE

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Clinique Vétérinaire des Lavandes la Begude de Mazenc France

1 Background

Cytologic and PCR examination of bone marrow aspirates can provide relevant informations to detect early leishmaniasis (Leishmania infantum) infection in enzootic area.

2 Methods

The aim of this prospective study was to determine feasibility, repeatability and relevance of a series of 4 sternal bone marrow aspirations in dogs at intervals of 3 months. One hundred and fifteen privately owned dogs, aged from 6 months to 13 years (4,5 to 67 kg), living in leishmaniasis enzootic area (south of France) were selected. Twenty-two breeds (mainly hunting dogs) were represented. Some dogs (n=32) were previously treated against canine leishmaniasis and selected in this study for the follow up of the disease. Other dogs (n= 83) were included in a vaccine trial against canine leishmaniasis. Materials were surgical spirit, disposable needles (18G, 1.2 x 40 mm), syringes (20 ml), microscope slides and EDTA blood collection tubes.

3 Results

Bone marrow samplings were obtained without sedation or anesthesia in all dogs. Four hundred and sixty samples were obtained for cytologic and PCR examination. In 87% of cases, one attempt was sufficient to obtain bone marrow. In 13% of case, two (n=48) or three (n=12) attempts were necessary. No statistical significance was found between number of attempts and periods of sampling (first to last). We observed no adverse effect (bleeding, hematoma or abscess). In all cases, cytologic and PCR examination were successfully performed : quantity and quality of cells were satisfactory.

4 Conclusions

In conclusion, repeated sternal bone marrow sampling can be obtained with cheap materials from an awake dog in order to follow up their leishmaniasis status.

C0129 DETECTION AND IDENTIFICATION OF LEISHMANIA SPP. IN CLINICAL SAMPLES FROM NEW WORLD COUNTRIES USING TWO HSP70 GENE BASED PCR PROTOCOLS.

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1 Background

Leishmaniasis is highly prevalent in New World countries, where several methods are available for detection and identification of *Leishmania* spp. Two *hsp*70 based PCR protocols (PCR-N and PCR-F) and their corresponding restriction fragment length polymorphism analysis (RFLP) were applied to the study of clinical samples recruited in Colombia, Guatemala and Honduras.

2 Methods

A total of 93 cases were studied. The samples were classified into positive or suspected of leishmaniasis according to parasitological criteria (smear direct examination and culture). Molecular amplification of two different *hsp*70 gene fragments and further RFLP analysis, for identification of *Leishmani*a species, was done.

3 Results

The detection of parasites was higher using PCR-N than PCR-F. The species in most samples were discriminated using RFLP-N. However, RFLP-F was more useful for distinction between *L. panamensis* and *L. guyanensis* (from Colombia). When both protocols were applied to the species identification of the same sample, the results were always concordant. The main species identified in the study were *L. panamensis, L. braziliensis* and *L. infantum* (*chagasi*).Unexpectedly, one sample from Colombia revealed an RFLP pattern corresponding to *L. naiffi.*

4 Conclusions

RFLP-F and RFLP-N are useful for typing *Leishmania* from New World countries. To select the most appropriate restriction algorithm for identification purpose, in different locations, the species circulating in each one must be taken into consideration. More studies are needed to confirm the presence of *L. naiffi* in Colombian territory.

C0146 FIRST DETECTION OF LEISHMANIA KDNA IN CANINE CERUMEN SAMPLES BY QPCR

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1 Background

Nowadays there is still a controversy about which is the best sample for the molecular diagnosis of canine leishmaniosis. The most commonly used are obtained by invasive methods that can cause animal distress and owner reluctance, so it is important to test non-invasive samples as an alternative. Therefore, our objective was to assess the potential use of cerumen taken with cotton swabs for the molecular diagnosis of canine leishmaniosis.

2 Methods

To conduct the study, a population of 38 dogs from a leishmaniosis endemic area (Cáceres, Spain) was selected based on the results for serological techniques SLA ELISA and IFAT and divided in two groups: A (33 seropositive dogs) and B (5 healthy dogs). Cerumen samples were collected from all dogs. Moreover, blood, lymph node and bone marrow samples from most of the animals were obtained. All samples were analyzed by qPCR in order to detect *L.infantum* kinetoplast DNA (kDNA).

3 Results

We were able to detect *L.infantum* kDNA in cerumen samples from dogs of group A, with a 90.9% of sensitivity, higher to that obtained for bone marrow (88.5%). The highest sensitivity (100%) was obtained in lymph node, being the lowest reached with blood samples (57.6%). In addition, the specificity of qPCR assays was 100% in all samples taken from dogs of group B.It is important to highlight that the results obtained with cerumen-qPCR method were strongly correlated with qPCR analyses performed with bone marrow ($r=0.82$, $p<0.01$), lymph node ($r=0.70$, $p<0.01$) and in a lesser extent with the blood results ($r= 0.54$, $p<0.01$).

4 Conclusions

The results showed that *Leishmania* kDNA can be isolated, detected and quantified by qPCR in cerumen samples from dogs with leishmaniosis. As far as we know, this is the first report of the presence of *Leishmania* kDNA in canine cerumen samples. The use of cerumen for molecular analyses presents many advantages: the collection is harmless and easy and there is no need of specific conditions for transport and storage. Moreover, cerumen-qPCR method showed a sensitivity higher to that obtained using invasive bone marrow samples. Thus we propose the cerumen as a new sample to take into account for the molecular diagnosis of zoonotic visceral leishmaniosis.

C0156 SEROLOGICAL SIGNATURES OF CLINICAL CURE FOLLOWING SUCCESSFUL TREATMENT WITH SODIUM STIBOGLUCONATE IN ETHIOPIAN VISCERAL LEISHMANIASIS

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1 Background

In Ethiopia, visceral leishmaniasis (VL) is a growing public health threat. Among the key challenges in VL control in Ethiopia is lack of test of cure. The recommended test of cure is parasite detection. As, sterile cure is not expected with the current widely used drugs, the value of parasite detection as test of cure is questionable. Moreover the samplingis invasive, requires well-equipped facility and highly skilled personnel, which are hardly found in our set-ups. Thus, our aim was to assess the value of soluble CD40 ligand (sCD40L), Matrix metaloprotase -9 (MMP9) and interleukine 10 (IL-10) serum levels as signature biomarkers of clinical cure.

2 Methods

A total of 45 VL cases before and after treatment and 30 endemic health controls were included in the study. Sandwich ELISA was used to measured serum level of sCD40L, MMP9 and IL-10.

3 Results

the mean sCD40L, MMP9 and IL-10 serum levelschanged significantlyat clinical cure.At individual cases level sCD40L and MMP9 showed an increasing treand. Yet, the degree of increase in serum levelof MMP9 seems to be affected by nutritional status of the individual VL case. The mean IL-10 serum level was significantlyreduced at clinical cure. Looking at it on case by case, all demonstrated a decreasing treand but two VL cases had had high IL10 level at clinical cure.

4 Conclusions

our result is suggestive of the possibility of developing signature biomarker to monitor VL treatment in Ethiopia.

C0167 HEMATOLOGIC CHANGES IN VISCERAL LEISHMANIASIS

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University Hospital Center "Mother Teresa", Tirana Albania

1 Background

The aim of the study was to evaluate the changes in blood count of patients diagnosed with Visceral Leishmaniasis at University Hospital Centre "Mother Teresa", Tirana.

2 Methods

This is a retrospective cohort study. In our study were included n = 55 children who were admitted in the Pediatric infectious disease department of the University Hospital Centre "Mother Teresa"Tirana,aged 0-14 years diagnosed with Visceral Leishmaniasis(the diagnosis is confirmed by microscopic identification of Leishmania in bone marrow aspiration) in the period January 2012 to July 2013. We have evaluated the changes of red blood cells, hemoglobin, white blood cells and platelets confronted with normal values ??of pediatric age groups. Our sample was divided in four groups by age (0-1 years old, 1-3 years old, 3-6 years old, 6-14 years old).

3 Results

We have recorded red blood cells decline in 35/55 (63.64%) in which 13/7 (65%) were in group 0-1 y.o., 11/16 (68.75%) group 1-3 y.o., 4/9 (44.44%) group 3-6 y.o., 7/10 (70%) in group 6-14 y.o.. The minimal value found was 1 250 000 and the maximum 5 840 000 red blood cells. The white blood cells was decreased in all four groups : 12/20 (60%) group 0-1 years old, 14/16 (87.5%) group 1-3 y.o., 6/9 (66.66%) group 3-6 y.o., 7/10 (70%) in the last group. In total we have recorded 39/55 (70.91%) of children with decreased white blood cells. The hemoglobin was found decreased in 43/55 (78.18%) of children in which 16/20 (80%) group 0-1 y.o, 10/16 (62.5%) group 1-3 y.o, 9/9 (100%) group 3-6 y.o and 8/10(80%) last group. We have classified anemia in 4 groups (I; Hb 9.6-12.8g%, II; Hb 6.4-9.6g%, III; Hb 3.2-6.4g% and IV; Hb<3.2g%) and the result shows that the most of the cases were in group II (69.09%). Platelets were decreased in 41/55 (74.55%) of children in which 14 (25.45%) were under 100 000 plt/mm^3.

4 Conclusions

Hematological changes in Visceral Leishmaniasis are common. A high degree of suspicion for Visceral Leishmaniasis needs to be maintained by the pediatrician and it should be included in the differential diagnosis of patients presenting with anemia, leukopenia, thrombocytopenia, pancytopenia; particularly in geographical areas where the disease is endemic or accompanied with clinical findings like fever and hepato-splenomegaly.

C0168 DIAGNOSIS AND TREATMENT OF VISCERAL LEISHMANIASIS IN ALBANIA

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University Hospital Center "Mother Teresa", Tirana Albania

1 Background

We have studied the diagnosis approach and treatment for Visceral Leishmaniasis in Albanian children during 2012-2013.

2 Methods

55children aged 0-14 years old were included in this study, all admitted and treated for Visceral Leishmaniasis in Pediatric Infectious Disease Service, University Hospital Center "Mother Theresa" Tirana, Albania. For diagnose confirmation we have used bone marrow aspiration and serological methods (ELYSA, IFA). We have treated this children with meglumine antimonite(Glucantime) and liposomoal amphotericin B (Ambisome).

3 Results

The bone marrow aspiration was positive for leishmaniasis in 46 children (83.6%), suspicious in 2 children and negative in 7 children. In this cases we have used for the diagnosis immunoassays methods (ELYSA in 9 children and IFA in 2 of them). We have treated 50 children (90.91%) with Glucantime for 28 days (i.m. route) and Ambisone in 5 children with 7 doses (day1,2,3,4,5,14,21).

4 Conclusions

Bone marrow aspiration remains the main methods to confirm the diagnosis of Visceral Leishmaniasis. In negative or suspicious result, where the clinical data suggest for leishmaniasis, must be confirmed by serological methods. Glucantime has been used in most of our children and Ambisone has been used less due to the absence of the drug. The control of bone marrow aspiration has been performed to all the children after the treatment, which resulted negative in all of them. Children were followed for one year and there was no relapse of the disease and the cure rate was 100%.

C0178 NEW SEROLOGICAL TOOLS FOR IMPROVED DIAGNOSIS OF HUMAN TEGUMENTARY LEISHMANIASIS

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1 Background

Human tegumentary leishmaniasis (HTL), characterized by skin ulcers that may spread and cause dreadful and massive tissue destruction of the nose and mouth, is considered a neglected tropical disease, and it is a serious threat to global health due to its continuous expansion, favored by the lifecycle of its causative organism that is maintained in domestic animal reservoirs and anthropophilic sand fly species. Serodiagnosis of HTL is a great challenge due to many biological factors, including hampered specificity and/or sensitivity. This investigation addresses the unmet need for new diagnostic markers of HTL, and describes a simple platform to improve the serodiagnosis.

2 Methods

A constrained conformational phage display random peptide library combined with a magnetic microsphere-based subtraction strategy was used to identify ligands with potential diagnostic applications. Six clones were selected against IgG antibodies from HTL patients, characterized by sequencing and confirmed by a phage-ELISA using sera from patients developing visceral leishmaniasis (n=20), Chagas disease (n=10), mucosal (n=30) and cutaneous (n=20) leishmaniasis; as well as from healthy subjects living in endemic (n=20) and non-endemic (n=30) areas of leishmaniasis. A wild-type M13-phage clone and a soluble *Leishmania* antigenic extract were used as negative and positive controls, respectively.

3 Results

Three clones reached 100% sensitivity and specificity, without any cross-reactivity with sera from patients with leishmaniasis-related diseases.

4 Conclusions

Briefly, we describe for the first time a set of serological markers based on three immunodominant mimotopes that showed 100% accuracy, and that could be used in a phage-ELISA assay for the HTL serodiagnosis.

C0183 VALIDATION OF AN ANTIGEN DETECTION RAPID DIAGNOSTIC TEST KIT TO DIAGNOSE SRI LANKAN CUTANEOUS LEISHMANIASIS CAUSED BY LEISHMANIA DONOVANI

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1 Background

Cutaneous leishmaniasis (CL) is a recently emerged and established disease in Sri Lanka. Causative species is Leishmania donovani. Diagnosis is mainly achieved in low resourced local settings by slit skin smear (SSS) and biopsy / histology, with low sensitivity rates. Here, we validated the efficacy of a recently-marketed rapid diagnostic immunochromatographic strip (CL-Direct™ IC-RDT), that captures the peroxidoxin antigen of Leishmania amastigotes, in diagnosing Sri Lankan CL.

2 Methods

Samples were taken from the active edge of 74 clinically suspected CL lesions (papules: 3, nodules: 26, ulcers: 36, plaques: 7, satellite lesions: 2). Slit skin smears were examined for the presence of amastigotes. Samples for IC-RDT were obtained with a dental broach, lysed with 3 drops of lysis buffer. 20µl of the cell lysate was placed on the IC-RDT strip which was then dipped in 3 drops of chase buffer. Results were read after 20-30 minutes at bedside. PCR was done with 2mm diameter punch biopsy samples taken from an adjacent site of same lesions. Clinically non-CL (n=22), discarded skin samples from a CL non-endemic region were taken as negative controls.

3 Results

Out of the 74 clinically suspected lesions 59 (79.7%) were positive by PCR, 43 (58.1%) by Giemsa stained slit skin smear (SSS) and 21 (28.4%) by IC-RDT. All samples those were positive either by SSS or IC-RDT or both were always positive by PCR. The calculated sensitivity rates for IC-RDT and SSS compared to PCR were 35% and 72% respectively. All negative controls were negative by all three methods. Although specificity and positive predictive value (PPV) of both IC-RDT and SSS compared to PCR were 100%, negative predictive value (NPV) of IC-RDT and SSS were 37% and 58% respectively. The median parasite count in SSS positive lesions were 2+ (1-10 parasites/100 High Power Fields).

4 Conclusions

We reported low sensitivity rates of SSS and IC-RDT compared to PCR. This could be due to low parasite counts in the lesions or due to low expression of peroxidoxin antigen in amastigotes of the Sri Lankan strain of L. donovani. An IC-RDT with improved sensitivity would be a valuable future addition for the diagnostic tool kit in this low resource setting.

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C0202 THE PERFORMANCE OF SEROLOGICAL TESTS FOR LEISHMANIA INFANTUM INFECTION SCREENING IN DOGS DEPENDS ON THE FORCE OF TRANSMISSION

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1 Background

Dogs are considered to be the main reservoir for *Leishmania infantum*. This protozoa causes visceral leishmaniasis (VL), an uncontrolled urban zoonosis in Brazil. Serological tests and polymerase chain reaction (PCR) on peripheral blood were performed to identify infected dogs in scenarios of higher and lower incidences (Teresina and Vitoria).

2 Methods

One-hundred infected and 57 non-infected animals from Teresina and 100 non-infected from Vitória were studied. Animal selection was not dependent on previous serology.

3 Results

The sensitivity (Teresina) and specificity (Teresina and Vitória) were as follows: indirect antibody fluorescence (IFAT) cut-off point of 1:40 (IFAT 1:40): 96%, 18%, and 76%; IFAT 1:80: 90%, 33%, and 93%; direct agglutination test (DAT): 96%, 33%, and 98%; fast agglutination screening test (FAST): 93%, 68%, and 100%; immunochromatographic assay with a recombinant rK39 antigen (rK39): 88%, 74%, and 98%; enzyme linked immunosorbent assay (ELISA): 91%, 79%, and 98%; rapid dual-path platform test (TR DPP®): 98%, 60%, and 98%; and blood PCR: 29%, 93%, and 97%, respectively. In the high transmission area, no test adequately discriminated *L. infantum*-infected from non-infected dogs.

4 Conclusions

However, in the high transmission city, the area under the receiver operating characteristic (ROC) curve of FAST, DAT, ICrK39, ELISA and TR DPP® was high.

C0215 THE EVALUATION OF MICRO -CULTURE METHOD FOR ISOLATION OF LEISHMANIA PARASITES FROM CUTANEOUS LESIONS OF PATIENTS IN ETHIOPIA

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1 Background

Cutaneous leishmaniasis (CL) is one of the diseases of public health importance in Ethiopia. The annual incidence is estimated to range between 20, 000 and 30, 000. Slide microscopy and /or culture are being used to confirm clinically suspected CL cases. Both the culture and slide microscopy are less sensitive. Though culture is relatively sensitive as compared to microscopy it needs more than 2 weeks to report as negative. Recently, micro-culture method (MCM) has been shown to be sensitive and rapid method for different *Leishmania* parasite species. Thus our aim was to evaluate MCM for the diagnosis of CL caused by *Leishmania aethopica*

2 Methods

To determine the comparative advantage of MCT in terms of sensitivity and time to culture positivity, 143 lesion aspirates were cultured in duplicate and parallel in conventional culture method (CCM) and MCM. For sensitivity analysis, the consensus standard was considered to be a positive result in any two of the following five tests: Giemsa-stained lesion smear, culture, internal transcribed spacer 1(ITS 1) polymerase chain reaction (ITS1-PCR), or Histopathology.

3 Results

Fifty two lesions fulfilled the consensus criteria for a final diagnosis of CL of which 48 were positive by MCM, 36 by CCM and 35 by stained slide microscopy. The sensitivity of MCM was 92.3%, versus 69.2% for CCM (*P=0.003*). The median times to culture positivity were 3 days for MCM and 6 days for CCM (*P 0.001*).

4 Conclusions

MCM was more sensitive and time-efficient than CCM for isolating *L. aethiopica* from the lesion aspirates in our set up.

C0218 DETECTION OF LEISHMANIA KDNA IN HAIR AND CERUMEN SAMPLES BY QPCR IN DOGS FROM BRAZIL

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1 Background

Despite the control programs stablished in Brazil, visceral leishmaniosis remains a serious public health problem. For an accurate diagnosis of the disease, the combination of serological and molecular techniques is needed. In this way, the association between qPCR and non-invasive samples has shown high sensitivity results. Studies conducted in Spain revealed that both hair and cerumen from dogs with leishmaniosis can harbor parasite kinetoplast DNA (kDNA). Therefore, the objective of this work was to analyze hair and cerumen samples by qPCR of dogs from a Brazilian endemic area.

2 Methods

To carry out the study two groups of dogs, A and B, were analyzed. Group A consisted of 16 dogs with leishmaniosis confirmed by serology and lymph node fine-needle aspiration coming from Araçatuba, a Brazilian endemic area. Group B or control comprised 5 healthy dogs (assessed as negative by parasitological and serological methods) from Londrina, a non-endemic Brazilian region. We analyzed by qPCR dried blood spots, hair and cerumen from all dogs, and also hair of cutaneous lesions from dogs of group A.

3 Results

Regarding dogs with leishmaniosis (Group A), *Leishmania* kDNA was detected in all the samples obtained from nine dogs, being the whole group positive to at least two of them. Cerumen-qPCR showed the highest sensitivity (87.5 %), whereas the lowest was reached by blood-qPCR (68.75%). Comparing hair-qPCR results, it can be observed that sensitivity was higher in hair from lesions (78.57%) than in those from healthy areas (62.5%). On the other hand, the specificity of qPCR assays was the 100% applied to all kind of samples taken from control dogs of the group B.

4 Conclusions

In this work we assessed for the first time hair and cerumen samples for the molecular diagnosis of canine visceral leishmaniosis in dogs from a Brazilian endemic area. The combined use of these non-invasive samples for qPCR analysis provides high reliability, taking into account the high sensitivity and total specificity reached by the method, making it more useful for prevention and control procedures for zoonotic visceral leishmaniosis. Finally, using hair and cerumen can be a breakthrough in the diagnosis of this neglected disease worldwide.

C0222 A COMBINED WET-LAB AND IN SILICO APPROACH TO THE DISCOVERY OF BIOMARKERS OF DISEASE PROGRESSION AND POST-CHEMOTHERAPY RELAPSE IN VISCERAL LEISHMANIASIS

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1 Background

Leishmaniasis is a neglected tropical disease caused by unicellular protozoan parasites of the genus *Leishmania*. There are three main forms of the disease, namely cutaneous, mucocutaneous and visceral leishmaniasis (VL), the later being fatal if untreated. Accurate diagnosis still relies on (invasive) microscopic examination of spleen and bone marrow aspirates. Although less invasive techniques based on antibody detection (DAT, rK39) exist, a biomarker able to predict progression of assymptomatic carriers to active disease and to determine cure after chemotherapy is yet to be discovered. *In silico* techniques can complement wet-lab experiments to improve and optimize the search for such biomarkers.

2 Methods

Mass spectrometry (MS) identified 600+ putative cytosolic promastigote proteins that reacted with IgG1 from VL patients from India (immunoblots). In order to identify the proteins most likely to be in fact antigenic, the following properties were searched *in silico*:

1. Haploid depth (from RNA-seq analysis) for promastigotes and amastigotes greater than the first quartile - Q1 (considering the 2 groups seperately);
2. Confident MS identification (MASCOT score > 100)

All proteins satisfying those criteria were then split in two branches. One with proteins found to be secreted (signal peptide or non-classical pathway) and the other with proteins harboring tandem repeats (TR). All short-listed proteins had their epitopes mapped *in silico.* The epitopes with the highest antigenic scores, AGI (number yet to be defined) are being synthesized and tested for antigenicity against IgG1 using sera from VL patients. Recombinant proteins harboring the epitopes recognized by positive VL sera will be cloned such that the antigenicity of the epitopes versus whole protein can be compared

3 Results

Out of the initial 678 proteins identified by MS, 62 satisfied the first branch i.e. were expressed in both life stages, were found to be secreted and were identified with confidence by MS. The second parallel branch, with proteins harboring tandem repeats expressed in both life cycle stages that were confidently identified by MS, was composed of 3 proteins.

Over 50% of the short-listed proteins from the two branches harbor primary epitopes with predicted AGI higher than that of the rK39, the gold standard rapid diagnostic test for VL confirmation.The primary epitope of one TR protein corresponds exactly to the repetitive region.

The antigenicity validation (of both epitopes and proteins with sera from VL patients will be presented.

4 Conclusions

In silico (bioinformatics) tools can be applied to screen for desired protein properties, dramatically reducing large output sometimes generated by simple wet-lab experiments. Our preliminary *in-silico* epitope mapping suggests potential good performance, which will be confirmed by experimental validation using sera from VL patients.

C0229 FINDING MIMO: DEVELOPING AN ALTERNATIVE RAPID TEST FOR THE SERODIAGNOSIS OF VISCERAL LEISHMANIASIS IN EASTERN AFRICAN POPULATIONS

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1 Background

While the development of rk39 rapid diagnostic tests has revolutionised the serodiagnosis for visceral leishmaniasis (VL) on the Indian subcontinent, sensitivity is sub-optimal in the East African region. The only serological test that has proven highly accurate in all endemic regions of VL is the Direct Agglutination Test (DAT). Unfortunatley this test is not suitable as a point of care test. Therefore we want to develop an alternative rapid serodiagnostic test for the East African region based on the DAT antigen, i.e. entire fixed *Leishmania (L.) donovani* promastigotes; the epitopes responsible for the agglutination reaction are unknown. We however want to mimic these epitopes with short peptides, so called mimotopes.

2 Methods

We used the DAT antigen to isolate specific antibodies from East African VL patient sera. With these antibodies we screened a phage library displaying random dodecamer peptides. The selected phages were confirmed in their target specificity with an enzyme-linked immunosorbent assay (ELISA). Phages that showed reactivity with DAT specific antibodies as compared to DAT negative sera were sequenced. Sequences were analysed for consensus motifs and aligned with the *L. donovani* proteome. Proteomic regions that showed clustered homologies with multiple sequences were tested *in silico* for potential antigenicity. The most promising peptides (phage derived, consensus motifs and proteome derived stretches) were synthesised and tested in ELISA against a panel of East African DAT positive (n=56) and negative (n=22) sera.

3 Results

Within the 30 resulting synthetic peptides one *L. donovani* proteome derived and three phage derived peptides showed high diagnostic potential with an Area Under the Curve (AUC) of 0,74, 0,755, 0,89 and 0,78 respectively. In the phage derived peptide sequences three consensus motifs could be assembled. These are currently under investigation.

4 Conclusions

So far we could identify four potential mimotopes of the DAT. Despite their differential affinity to DAT positive and DAT negative sera in ELISA, the fold-change in reactivity is limited. Further investigations will focus on consensus motifs, increasing peptide reactivity and identifying other antigens with higher or complementary diagnostic potential as compared to the four identified peptides.

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C0252 A NOVEL RAPID, NONINVASIVE AND USER-FRIENDLY DIAGNOSTIC SAMPLING TECHNIQUE FOR CUTANEOUS LEISHMANIASIS

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1 Background

Accurate diagnosis of cutaneous leishmaniasis (CL) is crucial to direct the use of chemotherapy, as well as for epidemiological studies. The most common approach for detection of *Leishmania* parasites involves an invasive method such as collection of a biopsy, to identify *Leishmania* amastigotes in smears microscopically and culture of promastigotes from the infected tissues. Even though these techniques are highly specific, their sensitivity is not adequate and highly skilled health professionals are needed. In this report, we present a novel painless, rapid and user-friendly method of skin tape stripping to collect enough DNA from the lesions of CL patients to perform accurate non-invasive diagnosis of CL.

2 Methods

A total of 119 patients suspected of having CL with different clinical status including acute, chronic and healed lesions were sampled (with duration of sampling shorter than one minute) both from their lesions and uninfected skin areas. In addition, 15 fungal-infected lesions and 54 areas of normal skin were examined as controls. Firstly, all DNA isolated from the skin tape stripping samples, were amplified using kDNA1 minicircle primers. The kDNA1 amplification was positive for all CL patients and yielded 100% sensitivity and specificity. In contrast, none of controls had positive results using the kDNA1 minicircle primers. Standard reference strains of *L. major* and *L. tropica* were used as positive controls. Secondly, all isolated DNA with positive amplification using kDNA1 were subjected to ITS1 PCR-RFLP for species identification.

3 Results

Among the 79 acute CL cases, 45 samples were diagnosed as *L. tropica*, 30 samples as *L. major* and 4 cases were not amplified with ITS1 primers. Furthermore, from 32 chronic CL cases, 18 samples were diagnosed as *L. tropica*, 11 cases as *L. major* and 3 samples were not amplified with ITS1 primers. Among the 8 healed samples, 7 samples were diagnosed as *L. tropica* and one sample as *L. major*. Altogether, using ITS1 primers, showed 94% sensitivity.

4 Conclusions

Sampling of skin lesions by tape stripping is a sensitive, noninvasive and specific alternative to traditional diagnostic assays and it is suitable for field studies as well as health care centers where a large population is in need of diagnosis.

C0273 WORKFLOW FOR MOLECULAR DIAGNOSIS OF VISCERAL LEISHMANIASIS

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1 Background

The definition of visceral leishmaniasis (VL) case is based on clinical, epidemiological and laboratory aspects.The gold standard techniques for laboratory diagnosis of VL are parasitological, smear and culture of bone marrow aspirate, which allow the identification of *Leishmania* in microscopic visualization, and the serological tests, like the immunochromatographic, which presents low sensitivity in immunodepressed individuals.In certain situations, such as *Leishmania*/HIV co-infection, for specific treatment or in epidemiological surveys, it is necessary to identify the etiological agent of visceral leishmaniasis, which is commonly caused by *L.* (*L.*) *infantum* in the Americas. In this way, a workflow for the molecular diagnosis of VL by kDNA PCR (RV1/RV2) and ITS1 PCR (LITSR e L5.8S) was proposed. The performance of the ITS1 PCR RFLP (Restriction Fragment Length Polymorphism (RFLP) analysis to identify the etiological agent was evaluated also.

2 Methods

After standardized (analytical sensivity and specificity evaluation), the molecular techniques (ITS 1 and kDNA) were validated on 82 samples from patients with confirmed VL and 30 from healthy patients.

3 Results

The following sensitivities were presented by the molecular tests: 97.5% (80/82) for the ITS1 PCR and 92.7% (76/82) for kDNA PCR,and 100% specificity for all tests.When comparing the results obtained in molecular tests ITS1 and kDNA with the established gold standard, an almost perfect agreement, with kappa index higher than 0.80 and p value <0.001, was observed.As regard to ITS1 RFLP, from 80 positive samples, 52.5% (42/80) showed a similar profile to that of *L.* (*L.*) *infantum.*Three of the samples that have not demonstrate profile in the ITS1-RFLP obtained more than 90% of identity with strain *L.* (*L.*) c*hagasi*, by analyzing the samples in sequencing.

4 Conclusions

As our conclusion, an workflow for the molecular diagnosis of VL must consider a previous performance of the parasitological and immunochromatographic diagnosis. If they have not defined the VL disease in suspected cases, kDNA PCR (RV1/RV2) and ITS1 PCR, followed by RFLP ITS1, which can define the etiological agent of VL, are recommended.

FINANCIAL SUPPORT

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C0274 EVALUATION OF IMMUNOHISTOCHEMICAL TESTS AS A TOOL FOR THE DIAGNOSIS OF CUTANEOUS LEISHMANIOSIS IN PANAMA.

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1 Background

Cutaneous Leishmaniosis (CL) is one of the most prevalent parasitic zoonosis in Panama. Approximately 3,000 cases are registered annually, but a high underreporting of cases has been described. In the absence of vaccines and effective preventive measures, diagnostic methods play an important role in the control and appropriate treatment of this infection. Immunohistochemistry (IHC) techniques have proven to be a valuable tool to complement the diagnosis of leishmaniosis and to study the cellular immune response developed during infection. At present IHC is not been used in Regional Hospitals as a tool for the diagnosis of leishmaniosis in Panama.

2 Methods

The objective of this study is to evaluate the usefulness of the IHC tests as a complementary tool for the diagnosis of CL in Panamanian patients. For this purpose, 54 biopsy specimens from patients with suspected CL lesions were analyzed by direct diagnostic tests, molecular and histopathological tests. Tissue sections were fixed in formalin, embedded in paraffin, and then evaluated by IHC using anti-Leishmania polyclonal antiserum produced in mice.

3 Results

The presence of *Leishmania* amastigotes was confirmed in 48 (89%) skin biopsies by at least one of the diagnostic tests used in Panama. These results were compared among conventional histopathology (10/54: 19%), IHC (34/54: 63%), direct smear examination (30/54: 56%), parasite isolation from skin scrapings (45/54: 83%) and PCR test (48/54: 89%). Statistical analysis showed that the IHC) was more sensitive (75.0%) than the conventional histopathology (21.7% Sensitivity). Although, a poor concordance of the IHC was obtained when compared to the diagnostic PCR (Kappa index: 0.163); a 49% Overall Agreement and 100% Concordance with Positive Results were obtained compared with histopathology. Comparison results with the other diagnostic tests are presented, and the advantages of implementing IHC for CL diagnosis in Panama are discussed.

4 Conclusions

IHC is a valuable tool for the detection of *Leishmania*, as it facilitates the identification of the parasite and improves the quality of the histopathological diagnosis used in regional hospitals in Panama.

C0283 STANDARDIZATION OF A COST AND TIME EFFECTIVE METHOD FOR MULTILOCUS MICROSATELLITE TYPING (MLMT) STUDIES: APPLICATION OF LEISHMANIA TROPICA ISOLATES AND CLINICAL SAMPLES FROM TURKEY

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1 Background

Molecular techniques are widely used in the field of parasitology to reveal the epidemiological situation of the pathological agent. Population structure and Bayesian based statistical methods needs polymorphic markers to analyze the fragment sizes. The standard method for the studies on fragment analysis should be performed using fluorescently labelled primers in order to obtain primer peaks in ABI systems. In the present study, we aimed to standardize a new method for MLMT analyses using *L. tropica* specimens that is most common causative agent for cutaneous leishmaniasis in Turkey.

2 Methods

Here we standardized a new protocol for Fragment analysis studies, which reduce the spending time at classical PCR step, gel-electrophoresis step and the preparation step for the fragment analysis. This procedure is novel, time saving, cost effective, easy to apply and enables the early detection of heterozygosity in the studied markers.

3 Results

Ten previously studied *Leishmania tropica* strain and one sample obtained from Giemsa stained slide were tested using the new protocol, and results were compared to classical MLMT method that performed previously using ABI system. Overall twelve makers were tested and results were noted as identical to classical protocol.

4 Conclusions

We believe that reducing the time, money and hand manipulations on MLMT studies will help the facilities especially working on epidemiological studies. A non-plate working protocol will also make it possible to work on small sample sizes and genotype the causative agent in one working day from slide to result, especially in leishmaniasis endemic areas.

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C0286 EVALUATION OF URINE AS A DIAGNOSTIC SPECIMEN FOR VISCERAL LEISHMANIASIS IN SUDAN

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1 Background

The diagnosis of visceral leishmaniasis (VL) can either be direct via the demonstration by culture of microscopy of *Leishmania* parasites or indirectly through serology or molecular biology. Direct demonstration is invasive and requires technical expertise and a certain high level of medical precautions, in particular in the case of splenic aspiration. Molecular biology is not yet field applicable and serology requires collection of blood samples which has an inherent risk of accidents, is a taboo in some countries and resented by some groups such as small children. Therefore, non-invasive methods, such as diagnostics on urine would be preferred and studies from the Indian sub-continent describe a good diagnostic performance of rK39 strip tests using urine. Therefore, we initiated a preliminary evaluation of rk39 strip tests and DAT on urine samples collected in Sudan.

2 Methods

Urine samples were collected from individuals in the catchment area of Umkoraa Rural Hospital in West of Gedarif State and Al Azaza Damus Kala-azar Clinic in Sennar State, which is an endemic area for malaria with seasonal transmission, and one of the most important VL areas in the world. VL diagnosis was based on clinical symptoms (including irregular fever, weight loss, splenomegaly and hepatomegaly) and serological testing, unfortunately parasitological testing was not performed. Patient included in the study were 26 VL patients, 18 malaria patients, 17 patients with malaria-VL co-infections, 3 samples from apparently endemic healthy controls from Sudan and 5 urine samples from non-endemic healthy controls, in the Netherlands. In the laboratory, urine samples were tested with the rK 39 Kalazar Detect Rapid Test and the Direct Agglutination test, based on freeze dried antigen.

3 Results

The rk39 test had a sensitivity of 72.1% (95% CI: 57.3 – 83.3) and a specificity of 76.9% (95% CI: 58.9- 88.9) using the initial diagnosis (clinical symptoms combined with positive serology) as reference. The sensitivity of DAT on urine was 62.8% (95% CI: 47.9 – 75.6) and specificity 69.2% (50.0 – 83.5). Tests agreements were fair.

4 Conclusions

The present study has demonstrated some potential of diagnostic testing of urine with DAT and rk39 test for VL, but efforts to further improve test performance should be undertaken. Furthermore, it would be of interest to assess if, and how many (percentage) urine samples stay positive after a complete cure (as a possible test of cure).

C0290 VISCERAL LEISHMANIASIS DIAGNOSIS IN IMMUNOCOMPETENT PATIENTS: COULD THE RK39 IMMUNOCHROMATOGRAPHIC TEST REPLACE THE INVASIVE PARASITOLOGICAL DIAGNOSIS?

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1 Background

The gold standard techniques for laboratorial diagnosis of visceral leishmaniasis (VL) are the parasitological and serological. Parasitological techniques are represented by smear and culture of bone marrow aspirate, which allow the identification of *Leishmania* by microscopic visualization. Serological techniques, like the rK39 immunochromatographic test, diagnose *donovani* complex. Procedures to obtain the bone marrow aspirate for parasitological examination is much more invasive than to obtain the blood samples for rK39, which identify the etiological agent. It is necessary to identify the etiological agent of VL for the specific treatment or in epidemiological surveys.In this way, we compared the sensitivity obtained with the parasitological and rK39 tests results.

2 Methods

80 and 70 samples from patients with visceral leishmaniasis were evaluated by parasitological (smear of bone marrow aspirate) and by immunochromatographic rK39 tests, respectively.

3 Results

The following sensitivities were presented by the parasitological and rK39 tests, respectively, 76,2% (61/80) and 78,5% (55/70). Considering only samples from immunocompromised patients (25) the sensitivity of immunochromatographic test was lower, 60% (15/25).Furthermore, there were differences about the spending time during the procedure of the techniques in the laboratory, since parasitological and rK39 have took approximately 70 and 15 minutes, respectively.

4 Conclusions

rK39 and parasitological tests presented the same sensitivity, but in immunocompromised patients the sensitivity of the rK39 decreased. Nonetheless, the immunochromatographic test applied in blood samples from immunocompetent patients is more advantageous than parasitological, since it is faster and allow the detection of *donovani* complex in the samples, defining the etiological agent for the correct treatment. Furthermore rK39 depends of a lesser invasive procedure to obtain the samples. Therefore rK39 could replace the parasitological test.

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C0313 IDENTIFICATION OF EPIDEMIOLOGICAL BIOMARKERS FOR CANINE EXPOSURE TO PHLEBOTOMUS PERNICIOSUS

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1 Background

Phlebotomus perniciosus is known as the main vector of *Leishmania infantum*, causative agent of canine and human leishmaniasis. When hosts are bitten, the injected saliva of the sand fly elicits an antibody (Ab) response in the host which enables to measure the frequency of sand fly-host contact. Since the use of whole salivary gland homogenate (SGH) is a time-consuming and labor-intensive process, this study searches for the most antigenic biomarkers that can be used to measure dog exposure to *P. perniciosus*.

2 Methods

P. perniciosus 43kDa yellow-related recombinant protein (rSP03B) and 42kDa yellow-related recombinant protein (rSP03) were tested with ELISA on 615 sera samples from naturally exposed dogs (214 from Italy, 341 from Portugal and 60 from Spain). Furthermore, rSP03B was also tested on sera from naturally exposed dogs from Naples sampled at different time points during two consecutive transmission seasons. In order to confirm the presence of similar antigenic epitopes in the native yellow-related protein and rSP03B, an inhibition blot was performed.
Two approaches were used to identify suitable antigenic epitopes. First, B-cell epitopes were defined *in silico* and tested with ELISA on sera from dogs that were experimentally exposed to *P. perniciosus*, negative control sera were used to check for background and non-specific reactions. Second, epitope sequences in *P. perniciosus* salivary proteins were identified by means of randomized peptide phage display.

3 Results

In all the sampling areas a strong correlation was observed between the Ab-response against SGH and rSP03B, suggesting that different populations of *P. perniciosus* share similar antigenic properties of this protein. Furthermore, a clear inhibition of the binding of IgG to the native protein was observed only after pre-incubating the sera with rSP03B, which ensures the specificity of the Ab-response against rSP03B. The dynamics observed for the Ab-response against rSP03B strongly resembled SGH and was clearly related to the seasonal abundance of *P. perniciosus*.
The *in silico* identification of putative B-cell epitopes yielded 8 sequences, for which no difference was observed between sera of experimentally exposed dogs and negative control sera. The phage display studies are in progress.

4 Conclusions

Recombinant salivary protein rSP03B was identified as a valid biomarker in large-scale serological studies to measure dog exposure to *P. perniciosus* throughout its geographical distribution.

C0315 PARASITE LOAD ASSESSMENT BY QPCR IN BLOOD FROM DIFFERENT ORIGINS, INVASIVE AND NON-INVASIVE SAMPLES FROM A L. INFANTUM NATURALLY INFECTED DOG

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1 Background

Visceral leishmaniosis is a systemic disease, being lymphoid organs the most parasitized. Besides, atypical presentation of *Leishmania* has been described in other tissues. This fact motivates a major concern because of the numerous cases of infection in transplant recipients. In this study we analyzed by qPCR several arterial and venous blood samples, tissues and organs from a dog with leishmaniosis to assess the parasite load all in these samples and the possible diagnostic applications of the findings.

2 Methods

A female boxer was attended in the Clinical Veterinary Hospital of Extremadura University (Cáceres, Spain). It showed typical signs of the disease and positive antibody response against *L. infantum* by SLA IgG2 ELISA and IFAT. The dog was euthanized and the following samples were collected: whole blood from different veins (n = 9) and arteries (n = 3), bone marrow, lymph node, liver, skin, pancreas, kidney, lung, heart, and non-invasive samples: hair and conjunctival, oral and cerumen swabs. All samples were analyzed by qPCR in order to detect *L.infantum* kDNA.

3 Results

All samples were positive, with differences in terms of parasite load, being the lowest reached in blood samples. The mean quantity of kDNA obtained in veins was 3 times higher than in arteries. Regarding invasive samples, the highest quantities were observed in bone marrow ($1{,}44 \times 10^7$); lymph node ($3{,}86 \times 10^6$) and spleen ($1{,}64 \times 10^6$), whereas the lowest parasite loads were detected in kidney (2.32×10^3) and heart (547.38). All non-invasive samples were also positive, with variable parasite load, being more than 50.000 in conjunctival swab and less than 300 parasites in hair.

4 Conclusions

The highest kDNA quantities in blood samples were found in cephalic, saphenous and jugular veins, which are precisely the most frequently used for the blood sampling in the clinical practice. Taking into account our results and the similarities between canine and human disease, the importance of screening blood and organs for transplants recipients must be a relevant issue to consider in order to avoid the spread of leishmaniosis in immunocompromised individuals.

C0330 EVALUATION OF AN ENZYME-LINKED IMMUNOSORBENT ASSAY BASED ON THE LEISHMANIA BRAZILIENSIS KINESIN-RELATED RECOMBINANT ANTIGEN FOR SERODIAGNOSIS OF LEISHMANIASES IN BRAZIL

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1 Background

There is an ongoing need for new, improved and reliable diagnostic tests for the leishmaniasis. Various purified and recombinant antigens of *Leishmania* species have been produced and used for serological assays, for example, the rK39 antigen used for the diagnosis of visceral leishmaniasis (VL). The aim of this study was explore the use of *L. tarentolae* as a host for the expression and secretion of *L. braziliensis* kinesin-related recombinant protein (Lbk39) and to evaluate the cutaneous leishmaniasis (CL) diagnostic efficiency of this new antigen by ELISA.

2 Methods

The synthetic Lbk39 gene was designed based on the kinesin-related gene of *L. infantum* and the homology search was performed by BLAST similarity in the TritrypDB database. The Lbk39 gene was inserted into pLEXSY-sat2 recombinant vector, cloned with 6×His-tag and transfected into *L. tarentolae* cells by electroporation. The *L. tarentolae* culturing was carried out and protein purification from the supernatant medium was performed using a nickel affinity column. A total of 108 serum samples were collected from CL and VL endemic and non-endemic regions in Brazil and optimal ELISA conditions were established for the Lbk39 antigen. Crude extract from *L. braziliensis* culture (strain MHOM/BR/84/LTB300) was included as positive control. ANOVA one-way analysis was performed to validate significant differences among groups and student's t test was used to compare the means of each group between antigens. ROC curve, sensitivity and specificity analysis were also performed and for all analyses a significance level of 5% was adopted.

3 Results

The Lbk39 antigen was weakly recognized by CL patients compared to healthy individuals from non-endemic areas, but demonstrated significantly higher antibody levels for VL patients compared to the same control group. Cross-reaction with the Chagas Disease patients was noticed. The ROC curve showed a good accuracy for VL patients (AUC = 0.918±0.0753), but a weak accuracy for CL patients (AUC = 0.631±0.117). Values of sensitivity and specificity for VL patients were 80% and 95.5%, respectively, whilst sensitivity and specificity for CL patients were 88.9% and 40.9%, respectively.

4 Conclusions

Despite of the good outcome of production and expression of the target protein and it being produced from a kinesin-related gene of *L. braziliensis*, it is still strongly recognisable only by antibodies from patients stricken with the visceral form of the disease.

C0332 SEROLOGICAL TECHNIQUES FOR DETECTING ANTIBODIES AGAINST VISCEROTROPIC LEISHMANIA SPECIES IN WILDLIFE

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1 Background

Investigating *Leishmania* in wildlife is important to understand the role of these animals in the transmission cycle. Serology is particularly useful for this purpose, due to its large-scaleapplicability and because it avoids clinically invasive methods to obtain samples. However, it commonly requires species-specific anti-immunoglobulin, which can hinder its application in different species. We evaluated two techniques that do not use species-specific reagents to detect antibodies against viscerotropic *Leishmania* species in wildlife.

2 Methods

We examined serum samples from 82 wild mammals (1 *Mazama gouazoubira*, 1 *Sciurus* (*Guerlinguetus*) *aestuans*, 8 *Callithrix jacchus*, 11 *Didelphis aurita*, 18 *Callithrix penicillata* and 43 *Didelphis albiventris*), captured in an environmentally protected area in Campinas (22°53'20"S 47°04'40"W), São Paulo State, Brazil, from April 2014 to March 2015. A focus of canine visceral leishmaniosis and parasitemia due to *Leishmania* (*L.*) *infantum*, *L.* (*Viannia*) and *Trypanosoma* spp. was previously confirmed in 5/82 *D. albiventris* studied. We performed two tests in duplicate, using positive and negative sera from dogs as controls: the chromatographic immunoassay Kalazar-Detect™ (KD™; INBIOS International®, USA) and the direct agglutination test (DAT) with antigens that we produced with trypsinized and stainedpromastigotes of *L.* (*L.*) *infantum* (MHOM/BR/2002/LPC-RPV) and *L.* (*L.*) *donovani* (MHOM/ET/1967/HU3). Sera were tested at 1:20 to 1:40,960, adopting a cutoff of 1:320, recommended for animal reservoirs.

3 Results

All mammals were negative by DAT, considering the cutoff, but 9/82 (10.9%; 95%CI 4.2–17.7%) had titers of 20 and 40. Only the brocket deer (*M. gouazoubira*) was positive by KD™. None of the five animals with parasitemia by *Leishmania* spp. or *Trypanosoma* spp. had detectable antibodies.

4 Conclusions

It is possible that KD™ is not applicable to *M. gouazoubira* and the positive reaction was unspecific, which could be confirmed by testing a larger number of individuals of this species. In DAT, titers lower than the cutoff may be due to non-specific or cross-reactions. However, negative results in mammals known to be infected with *Trypanosoma* indicate the absence of cross-reactions with this parasite. Negative results in mammals that had parasitemia by *Leishmania* are compatible with recent infection. These results allow us to infer that the tests are useful in epidemiological investigations of *Leishmania* in wildlife.

C0335 USE OF MOLECULAR TECHNIQUES TO SCREEN FOR INFECTION BY LEISHMANIA SPP. IN WILDLIFE

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1 Background

Molecular techniques have been successfully used to detect *Leishmania* spp. DNA, due to their high specificity and sensibility. Different techniques and gene loci have been extensively studied to establish more efficient protocols.

2 Methods

We performed polymerase chain reaction (PCR) on blood samples of 82 free-ranging wild mammals (1 *Mazama gouazoubira*, 1 *Sciurus* (*Guerlinguetus*) *aestuans*, 8 *Callithrix jacchus*, 11 *Didelphis aurita*, 18 *Callithrix penicillata* and 43 *Didelphis albiventris*), captured from April 2014 to March 2015 in Campinas (22°53'20"S 47°04'40"W), São Paulo State, Brazil.
Genomic DNA was extracted using a QIAamp® DNA mini kit in a QIAcube® extractor (Qiagen®, Netherlands). Conventional PCR (cPCR) was performed using different regions of the *Leishmania* spp. genome with previously described cycling profiles and ultrapure water and DNA from cultures of *L.* (*L.*) *infantum*, *L.* (*V.*) *braziliensis* and *L.* (*L.*) *major* standard strains as controls.
We used primers for internal transcribed spacer-1 (ITS-1), LITSR/L5.8S, and to the heat shock protein-encoding gene, HSP70F/R. Specific primers to kinetoplast minicircle (kDNA) of *L.* (*L.*) *infantum*, Lch14/15, were used in cPCR and real-time PCR (qPCR). Sanger genetic sequencing was performed to identify amplicons. High-resolution melting (HRM)-qPCR was performed with HSP70F/R, using DNA of standard strains from different in vitro cultures of *Leishmania* spp.

3 Results

ITS-1 primers amplified 13/82 samples, but only 2/13 had the expected size of 320 base pairs (bp), confirmed as *L.* (*L.*) *infantum* and *L. Viannia* spp. by sequencing. One amplicon with 500 bp was confirmed as *Trypanosoma rangeli*. HSP70 primers amplified expected products of 337 bp in 20/82 samples, including 11/13 positive for ITS-1. However, all amplicons showed low similarity (<90%) with GenBank sequences. All samples were negative with Lch14/Lch15 in cPCR and qPCR. HRM-qPCR differentiated *Leishmania* species from isolated strains, but melting curves and fusion values were not obtained when using clinical samples, even those known to be positive.

4 Conclusions

ITS-1 primers were useful for *Leishmania* screening in blood samples from different wild species and also detected other trypanosomatids. HSP70 primers appeared more sensitive, but failed to identify amplicons by sequencing. Primers for *L. infantum* kDNA could not detect and quantify the parasite. Finally, HRM-qPCR seems applicable only for isolates, not clinical samples.

C0338 CANINE LEISHMANIASIS IS ASSOCIATED WITH VITAMIN D DEFICIENCY

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1 Background

Vitamin D (VitD) deficiency has been associated to increased risk of suffering different disorders, from autoimmunity to infectious diseases. VitD function is mediated by its binding to the vitamin D receptor (VDR) expressed in many immune cells. However, levels of VitD during visceral leishmaniasis have not been characterized. The dog is the main peridomestic reservoir of this parasitic disease, sharing many features with the human disease. The aims of the study were to investigate if VitD levels are associated with canine leishmaniasis and if nucleotide polymorphism in VDR gene is involved in the susceptibility of suffering this disease.

2 Methods

We study a cohort of 68 dogs living in a highly endemic area. VitD levels in serum samples were determined by competitive direct ELISA. Its relationship with parasitological and immunological parameters in non-infected dogs, clinically healthy dogs, and ill dogs was assessed. We genotyped 4 VDR SNPs and the allelic frequencies of the three phenotypic groups were compared. Data were analysed by means of nonparametric tests (SPSS software, SPSS Inc, Chicago, Illinois, USA).

3 Results

Our results showed that dogs suffering clinical leishmaniasis presented significantly lower ($P < 0.001$) VitD levels (19.6 [10.62-25.14] ng/mL) than those detected in non-infected (31.8 [25.95-34.65] ng/mL) and asymptomatic ones (29.6 [24.64-40.03] ng/mL). Furthermore, for every unit (ng/mL) increase in plasma VitD, the odds of having leishmaniasis declined by 14.09 %. VitD levels were also significantly correlated with clinical score, anti-Leishmania specific antibodies, and parasitaemia. However, there was no correlation between VitD levels and Leishmania-specific cellular immune response as measured by leishmanin skin test or by IFN-γ and IL10 cytokine production. Increases in both VitD and leishmanin skin test were independently associated with decreased odds of leishmaniasis, i.e. 21.45 % and 28.73 % per unit, respectively. There were no statistically different allelic frequencies in VDR gene between the three phenotypic classes and between non-infected vs infected dogs.

4 Conclusions

Visceral leishmaniosis in dogs is strongly associated with VitD deficiency. Further studies are needed to determine whether VitD deficiency during canine leishmaniasis is a cause or a consequence of the disease. These results open new possibilities for the design of prospective clinical interventional trials.

C0342 OPTIMIZATION OF THE CONCORDANCE BETWEEN THE IMMUNOCHROMATOGRAPHIC TEST DPP® (BIOMANGUINHOS) AND THE ELISA (BIOMANGUINHOS) IN THE DIAGNOSIS OF CANINE VISCERAL LEISHMANIASIS

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1 Background

In Brazil, Dual Path Platform (DPP® BioManguinhos) immunochromatographic test (ICT) and ELISA (BioManguinhos) are required for the serological diagnosis of Canine Visceral Leishmaniasis (CVL) by public health services. However, the use of the ELISA has faced challenges, such as insufficient production and requirement for laboratory infrastructure for its execution. Considering the practicality of DPP® ICT, the aim of this study was to improve the agreement between DPP® ICT and ELISA, evaluating the possibility of a diagnostic scenario without ELISA.

2 Methods

We did a statistical analysis on the results of DPP® ICT and ELISA performed at the National Reference Laboratory in Diagnosis of Visceral Leishmaniasis (NRL-VL) from January/2015 to July/2016. Furthermore, the influence of different concentrations of positive and negative samples for CVL on the result of DPP® and ELISA was evaluated using samples of dogs from: non-endemic area negative on DPP®, ELISA and parasitological exam (group A, n = 22); endemic area with positive DPP® and negative ELISA (group B, n = 22); positive on DPP®, ELISA and parasitological exam (group C, n = 22). Groups A and B were evaluated at dilutions 1:25, 1:50, 1:75 and 1: 100 (leaflet indication) in ELISA, and groups B and C at dilutions 1: 1 (leaflet indication), 1:50 and 1: 100 in DPP®.

3 Results

Of the 4,657 DPP® ICT reagent samples from the NLR-VL routine, only 66.33% (3,089) were confirmed in ELISA, 28.82% (1.342) were negative and 4.85% (226) were inconclusive. Using lower sample dilution (1:75, 1:50 and 1:25) in ELISA did not improve the agreement with the DPP® ICT. The sensitivity or specificity of the ELISA has not been modified by using 1:75 and 1:50 sample dilution, but the dilution 1:25 drastically reduced the specificity of this assay. However, diluted samples greatly increased DPP® ICT agreement with ELISA, from 50% (22/44) of ELISA confirmation to 95.7% (22/23) when diluted 1:1 and 100% (22/22) when diluted 1:50 and 1:100, respectively. Using 1:100 diluted samples in DPP® increased the agreement between DPP® and ELISA from Kappa 0.4 to 1.0 (perfect agreement) without reducing sensitivity.

4 Conclusions

In this study, by using 1:100 diluted samples in DPP® ICT we obtained the same diagnostic accuracy when using DPP® ICT and ELISA in sequence. So, the use of DPP in 1:100 diluted samples could, in principle, be used by public health laboratories at times of absence of the ELISA.

C0345 IS IFAT STILL USEFUL IN THE DIAGNOSIS OF VISCERAL LEISHMANIASIS IN A SCENARIO WITH RK39 ANTIGEN-BASED IMMUNOCHROMATOGRAPHIC RAPID TEST (IT-LEISH®)?

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1 Background

Visceral Leishmaniasis (VL) is a zoonosis with high lethality and very toxic treatment. Therefore, an accurate diagnosis is of fundamental importance. The immunochromatographic rapid test (ICT) rK39 antigen-based by IT-LEISH® has high diagnostic accuracy and has been used in points of care by the Brazilian public health services since 2014. However, in cases where IT-LEISH® ICT is negative and clinical suspicion continues, Indirect Fluorescent Antibody Test (IFAT) can be performed. Nevertheles, IFAT require a good laboratory infrastructure and qualified professionals, deficient in many endemic areas. Furthermore, many studies have reported a low specificity of IFAT.

2 Methods

Here we evaluated the positive predictive value of IFAT (BioManguinhos) diagnosis in IT-LEISH® ICT negative patients with suspected VL. The laboratory tests were performed at the National Reference Laboratory in Diagnosis of Visceral Leishmaniasis (NRL-VL) with samples collected in the State of Minas Gerais – Brazil, from January/2015 to March/2016 for routine diagnosis. We also considered the results of IT-LEISH® performed at points of care. The data were extract from GAL, a software public laboratory results, and SINAN, the information system for notifiable diseases databases. The positive gold standard was the clinical and epidemiological VL confirmation and the negative gold standard was the confirmed case in others diseases and/or discarded as VL.

3 Results

In this study, we identified 75 cases with IT-LEISH® ICT negative and IFAT positive by crossing the results of the GAL and SINAN databases. IFAT has confirmed as VL only 17,3% (13/75). The remaining cases, 82,7% (62/75), were discarded for VL and, of these, 28% (21/75) cases were confirmed with another pathology, namely: american cutaneous leishmaniasis (11); Chagas Disease (3); Schistosomiasis (1); Spotted Fever (1); Herpes Zoster (1); Acute Toxoplasmosis (1); Chronic Toxoplasmosis (1); Neurotoxoplasmosis (1); and *Klebsiella pneumoniae* infection (1). The positive predictive value of IFAT in the IT-LEISH® ICT negative patients analyzed here was merely 17,33% (95% CI 10,42-27,43).

4 Conclusions

The IFAT positive predictive value is dramatically low in patients with suspected VL but negative IT-LEISH® ICT and, therefore, in these cases, the IFAT should not be indicated, specially in regions endemic to other parasitic diseases such as American Cutaneous Leishmaniasis and Chagas disease.

C0350 THE COMPARISON OF KINETOPLASTID DNA PCR AND PARASITOLOGICAL METHODS IN THE DIAGNOSIS OF CUTANEOUS LEISHMANIASIS USING CLINICAL SAMPLES OF SUSPECTED PATIENTS IN TURKEY

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1 Background

Visceral (VL) and cutaneous (CL) leishmaniasis are seen in Turkey endemically and CL caused by Leishmania tropica is an important public health problem in southeastern as well as other regions of Turkey. The diagnosis has been usually made by clinical view of lesion and/or parasitological using lesion aspiration smears. The aim of the study was to compare a polymerase chain reaction method based on kinetoplast DNA using Uni21/Lmj4 primers with microscopy and culture methods for the diagnosis of CL using clinical samples obtained from the suspected patients in ?anl?urfa province. Where is highly endemic area for CL and L. tropica is main causative agent in the region.

2 Methods

The kinetoplast DNA (kDNA) PCR, parasite culture and microscopical evaluation of stained smears on 62 specimens from suspected CL cases who referred to Cutaneous Leishmaniasis Diagnosis and Treatment Center in ?anl?urfa, Turkey were included in the study.

3 Results

The kDNA PCR showed the highest sensitivity among all diagnostic assays, diagnosing 35/35 (100%) of the samples, followed by the microscopy (25/35 positive, 71.4% sensitivity) and then culture (19/35 positive, 54.3% sensitivity). Culture and microscopy together improved overall sensitivity to 88.6% (31/35).

4 Conclusions

These results suggest that kDNA PCR besides conventional techniques can be used for the diagnosis of CL in highly endemic area, Southeastern Region of Turkey.

C0364 AN ITS1-PCR METHOD FOR COMPARING TISSUE SPOTS ON FILTER PAPERS, UNSTAINED AND STAINED SMEARS IN THE DIAGNOSIS OF CUTANEOUS LEISHMANIASIS

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1 Background

Identifying the species of *Leishmania* causing human cutaneous leishmaniasis (CL) in endemic areas where more than one species exist is essential to its diagnosis, especially in deciding treatment. Dermal tissue scrapings spotted on filter paper, unstained smears and those stained with Giemsa's stain from lesions suspected of being CL were compared and evaluated as samples for, both, the diagnosis of CL and identification of the leishmanial species causing it, employing internal transcribed spacer region 1 (ITS1) –PCR assay. The ITS1-PCRs using the three types of sample were also compared with microscopy of stained smears and *in-vitro* culture to assess the most suitable combination of methods for a multiple test strategy.

2 Methods

From 1994 to 2014, 2232 patients were checked for CL by five different ways. Three were molecular, targeting the ITS1 but using different samples. The other two were microscopy of stained smearsand *in-vitro* culture of tissue aspirates.

3 Results

In the matched comparison based on 100 patients, the three different types of sample used in the ITS1-PCR proved to be equally good with no significant difference (P>0.05). However, tissue spotted on filter paper exposed most positive cases. The kappa statistic for measuring the degree of agreement among the three methods was 83% (83/100) and considered good. Agreement was highest between samples on filter paper and unstained smears, and lowest for stained smears. In the comparison between the molecular and classical methods, the ITS1-PCR using filter papers was the most sensitive method but the difference was insignificant (P=0.32). Moreover, the comparison showed that using smears and filter papers increased the sensitivity significantly to 46%, compared to using the methods individually (P=0.003-0.0008). On comparing the five tests using samples from all 2232 patients, the ITS1-PCR using filter papers, the ITS1-PCR using unstained smears, in-vitro culture, microscopy of stained smears, and the ITS1-PCR using stained smears showed, respectively, sensitivities of 81, 69, 64, 57 and 48%.

4 Conclusions

Of the five tests adjudicated, ITS1-PCR using filter papers proved best in routine laboratory diagnosis of CL. Adding microscopy of stained-smears to it, improved its diagnostic value significantly.

C0401 EVALUATION OF REAL TIME PCR FOR DIAGNOSIS OF POST-KALA-AZAR DERMAL LEISHMANIASIS (PKDL) IN ENDEMIC FOCI OF BANGLADESH

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1 Background

Post-kala-azar dermal leishmaniasis (PKDL) is a sequel of kala-azar or visceral leishmaniasis (VL) that is prevalent in endemic areas including Bangladesh. Because of these vicious cases the success of Kala-azar elimination program (KEP) is under threat. Unlike other endemic regions, mostly macular form of PKDL is found in Bangladesh and diagnosis of these cases is more difficult than other forms of PKDL. As PKDL cases are a key factor in achieving the goal of KEP, therefore, an elimination strategy for these cases through proper diagnosis and treatment should be adopted. Till date, diagnostic method for PKDL cases in endemic regions is limited in clinical examination along with rK39 test or microscopy, where a suitable and accurate alternative method is much appreciated. In this study, we investigated the application of real time PCR as a potential method for diagnosis of PKDL.

2 Methods

Total 105 suspected macular PKDL cases from Mymensingh district, Bangladesh under a clinical trial were diagnosed through clinical examination along with rk39 strip test. Then skin biopsy was collected from each patient and real time PCR was performed for detection and quantification of Leishmania DNA. In addition to cases, 80 controls, those had a past history of kala-azar, were subjected to rK39 dipstick test and real time PCR from blood. All participants were clinically assessed after 12 months.

3 Results

The rK39 rapid test was positive in 103 cases and 48 were positive among controls, indicating the sensitivity of 98.10 %(93.29-99.77:95%CI) and a specificity of 39.24%(28.44-50.87:95%CI). Real time PCR detected 86 cases among all suspected patients with a sensitivity of 81.90%(73.19-88.74:95%CI), where all negative controls were found to be negative with a specificity of 100%(95.49-100:95%CI). The average Ct value for cases in real time PCR was found to be 34.65 with a mean parasite load of 26.72 parasites/µg tissue DNA.

4 Conclusions

From the findings of this study, it can be evinced that real time PCR is a promising tool for diagnosis of PKDL in endemic regions, especially for macular cases. Thus, this method can be deployed as a diagnostic method instead of rK39 dipstick test for PKDL along with clinical diagnosis. In addition to diagnosis, the quantitative ability of this method could be exploited further, for after-treatment prognosis and cure assessment of PKDL cases.Therefore, evaluation of the prognostic value of this method through a longitudinal study is highly appreciated.

C0431 MOLECULAR CHARACTERIZATION OF LEISHMANIA PARASITES IN GIEMSA–STAINED SLIDES FROM CASES OF HUMAN CUTANEOUS AND VISCERAL LEISHMANIASIS, EASTERN ALGERIA.

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1 Background

In Algeria, visceral leishmaniasis (VL) is due to *Leishmania (L.) infantum* while three cutaneous forms (CL) are caused by *L. major*, *L. tropica* and *L. infantum*. In this study, the use of Giemsa–stained slides was evaluated with two PCR techniques, in Eastern Algeria.

2 Methods

A total of 136 samples corresponding to 100 CL smears (skin scrapings) and 36 VL slides (bone marrow aspirates) collected from 2008 to 2014, were tested. Upon DNA extraction, two PCRs were used to amplify the ribosomal Internal Transcribed Spacer 1 (ITS1) and mini–exon genes. Amplified products were digested (PCR–RFLP) and profiles analyzed for *Leishmania* species identification. A statistical analysis was also performed.

3 Results

ITS1–PCR was found significantly more sensitive than mini–exon–PCR (77.95% positives vs. 67.65%; p=0,001). Comparison of PCRs positivity showed statistically significant differences between old and recently prepared slides suggesting a better use of recent slides in PCR analyses. For species identification, PCR–RFLP results of ITS1 and mini–exon were concordant. *L. infantum* was identified from VL cases and *L. infantum*, *L. major* and *L. tropica* from CL ones. According to geographical origin, *L. infantum* was found in North–Eastern provinces while *L. major* was distributed from the North to the Centre–East of Algeria. Interestingly, two *L. tropica* samples were identified in Annaba, located far North–East Algeria.

4 Conclusions

Distribution of leishmaniasis in Eastern parts of Algeria besides finding of *L. tropica* in the far North, is here described for the first time using molecular tools, thus confirming the usefulness of archived slides for PCR identification of *Leishmania* parasites in retrospective epidemiological investigations.

C0490 IN SILICO IDENTIFICATION OF LEISHMANIA SPECIE-SPECIFIC SEQUENCES FOR DIAGNOSIS

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1 Background

Leishmania spp. causes leishmaniasis, responsible for about 1,300,000 human cases and 30 thousand deaths *per* year, with more than 20 species described. *Leishmania* are grouped in two subgenera in South America: *Leishmania* and *Viannia*, which cause visceral and cutaneous or mucocutaneous leishmaniosis, respectively. Currently, *Leishmania* species differentiation is done by multilocus enzyme electrophoresis (MLEE). Since the availability of reference genomes on public databases, DNA sequences have become important research source. We have selected and identified *Leishmania* specie-specific DNA sequences *in silico* and tested them by electronic PCR (e-PCR).

2 Methods

Leishmania coding sequence regions, non-orthologue for *Leishmania*, *Trypanosome* or *Crithidia* were selected from TriTrypDB database. Primers were then designed using BatchPrimer3, as follow: 18-23 bases in length, 55-65ºC melting temperature, 80-250bp product size and 40-60% of GC content. Product sequences *in silico* recovered were aligned using BLASTn (NCBI) against the nucleotide database from NCBI and a personal database containing *Leishmania* spp., *Trypanosome* spp. (TriTrypDB), *Homo sapiens*, *Canis familiaris* (ENSEMBLE) and *Lutzomyia longipalpis* (VECTORBASE) genomes. Alignments containing no gaps or mismatches at primer regions were selected and these primers were tested by NCBI e-PCR tool.

3 Results

Non-orthologue *Leishmania* sequences screening resulted in 5,458 sequences. From this dataset, a total of 251,123 pairs of primers were designed, resulting in 44,693 pairs of primers with no gaps or mismatches after alignments. This set of primers were then tested by e-PCR tool, allowing the identification of 18,314 specie-specific pairs of primers, distributed as follow: 2,072 for *L. aethiopica*, 1,533 for *L. arabica*, 2,536 for *L. braziliensis*, 196 for *L. donovani*, 1,231 for *L. enrietti*, 1,116 for *L. gerbilli*, 44 for *L. infantum*, 2,281 for *L. major*, 943 for *L. mexicana*, 1,492 for *L. panamensis*, 1,881 for *L. tarentolae*, 2,030 for *L. tropica*, and 959 for *L. turanica*.

4 Conclusions

The availability of reference genomes and bioinformatics tools allowed the *in silico* identification of *Leishmania* specie-specific primers that can be useful for differentiation. Next step is to validate these primers by real-time PCR using *Leishmania* reference strains and to develop a specie-specific diagnostic platform for multiple samples.

C0517 A COMPARATIVE ANALYSIS OF THE SERODIAGNOSTIC PROPERTIES OF TOTAL LEISHMANIA EXTRACTS AND INDIVIDUAL ANTIGENS IN BRAZILIAN REGIONS WITH DIFFERENT ENDEMICITY OF CANINE VISCERAL LEISHMANIASIS.

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1 Background

Canine VL patients present high titers of anti-Leishmania circulating antibodies. Serodiagnostic methods based in total parasite antigens can present cross-reactivity with dogs affected by other diseases and detect as false positives animals vaccinated. The existence of zones of high endemicity also complicates the diagnostic by increasing background of the detection methods. We have analyzed the potential diagnostic properties of a collection of recombinant *Leishmania* antigens using sera samples from high and low endemic areas of Brazil: Piauí (PI) in the Northeastern and Santa Catarina (SC), in the South, respectively.

2 Methods

The following *Leishmania* proteins were purified after their expression as recombinant proteins in *Escherichia coli*: KMP11, LiP2a, LiP2b, LiP0, HSP70, HSP83, H2A. For comparison, soluble leishmanial antigens (SLA) were obtained from freeze-thaw *L.infantum* promastigotes. A sera collections were employed as described: from PI - 46 symptomatic VL, 46 asymptomatic VL, 47 *Ehrlichia canis*, 18 vaccinated and 47 negative samples. From SC 52 symptomatic positive and 82 negative samples were studied. Results were processed using Graph Pad software. Serodiagnosis was performed by ELISA. The effectiveness of serodiagnosis was studied by a ROC analysis and reflected as % sensitivity, % specificity a likelihood ratio.

3 Results

In the low endemic area of SC, all samples showed good diagnostic properties. The best values for diagnosis of the canine symptomatic forms were obtained for SLA but comparable values were obtained for the recombinant proteins. ELISA performance decrease substantially when symptomatic samples from the endemic area were studied. In this case the best efficacy was observed for the KMP11, followed by SLA, HSP70 and LiP2a. An analysis performed with the asymptomatic sera revealed that KMP11 and the SLA presented similar characteristics, followed by the P2a protein. Among the preparation tested, LiP2a and LiP0 were the antigens that presented less cross-reactivity when assayed with sera from vaccinated dogs and dogs infected by *E. canis*.

4 Conclusions

Healthy dogs residing in highly endemic areas of canine VL showed higher background values for *Leishmania* proteins than dogs located in low endemic regions of the disease. In the first areas, recombinant expressed single antigens of the parasite can be an interesting alternative to the use of SLA for serodiagnosis of the disease.

C0566 EVALUATION OF POTENTIAL IMMUNODOMINANT PEPTIDES FOR THE SEROLOGICAL DIAGNOSIS OF HUMAN CUTANEOUS LEISHMANIASIS

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1 Background

American Tegumentary Leishmaniasis (ATL) is highly prevalent in the Andean highlands and the Amazon basin of Peru and neighboring countries. Cutaneous leishmaniasis (CL) and mucosal leishmaniasis (ML) occur among rural population or tourists who come in contact with the life transmission cycle in the wild. The need of expensive equipment and experienced personnel to diagnose ATL using the very sensitive and specific nucleic acid amplification makes this method unsuitable for rural areas. An alternative approach is to use the established resources found in endemic areas for new generation of serological tests more rapid and accurate than current conventional methods.

In recent years, through epitope mapping of *Leishmania infantum* histones and ribosomal acid proteins, we have identified two oligopeptides, H2A and P2a, with potential serodiagnostic properties. These oligopeptides were patented last year for the serodiagnosis of ATL (WO 2015001383). We will present both the development, optimization, performance assessment and the evaluation of the Enzyme Linked Immunosorbent Assay (ELISA) diagnostic test with a representative panel of ATL patients from Cusco, one of the two main areas with CL reports.

2 Methods

Epitope mapping of histones and ribosomal acidic proteins resulted in two synthetic oligopeptides with best performance for ATL diagnosis by ELISA. After determining adequate coating and blocking conditions, thorough characterization of positive and negative controls, we evaluated a representative panel of CL samples and compared their performance respect total soluble *Leishmania* antigens. Receiver-Operator Characteristics curve analysis was used to identify the effectiveness of the peptides for diagnosis of CL.

3 Results

Optimum conditions for peptide-ELISA have been determined for P2a and H2A synthetic peptides, resulting in promising positive to negative ratios when coated with buffers near their isoelectric point. We established a high quality and representative panel of positive and negative controls to pursue a correct diagnostic test validation assay. Results of ongoing evaluation of 215 sera samples will be presented that will show positive and negative predictive values, PPV and NPV respectively, of the synthetic peptides.

4 Conclusions

The ELISA test based on oligopeptides from P2a and H2A is a good alternative for ATL diagnosis in rural health posts because its rapidness, simplicity and high PPV and NPV performance.

C0592 REAL TIME PCR FOR RAPID DETECTION AND QUANTITATION OF LEISHMANIA DONOVANI IN CLINICAL SAMPLES AND EFFECTIVE CONTROL OF VISCERAL LEISHMANIASIS

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1 Background

Sustained elimination of Visceral Leishmaniasis (VL) necessitates reduction and control of parasite reservoirs to minimize the transmission of infection. Therefore, a definitive diagnostic procedure is indispensable for early and accurate detection of VL, Post Kala-azar Dermal Leishmaniasis (PKDL), and Relapsed VL (RVL) cases, all of whom are potential reservoirs of *Leishmania* parasites. To overcome the clinical and operational limitations of current diagnostic approaches, a novel real-time PCR method based on Taqman chemistry was devised and evaluated for rapid detection and quantification of *Leishmania donovani* in blood.

2 Methods

The analytical sensitivity of the real-time PCR was measured using a serial dilution of DNA extracted from in vitro cultured *Leishmania donovani* promastigotes. The diagnostic efficacy of the assay was evaluated and compared to a *Leishmania*-specific nested PCR (Ln-PCR) using archived peripheral blood buffy coat DNA from 40 VL, 40 PKDL, 10 RVL, 20 cured VL, and 40 cured PKDL along with 10 tuberculosis (TB) cases and 80 healthy endemic controls.

3 Results

The real time PCR could detect DNA equivalent to 0.1 parasite per reaction. The assay was 100% (95% CI, 91.19-100%) sensitive in detecting parasite genomes in VL and RVL samples and 85.0% (95% CI, 70.16-94.29%) sensitive for PKDL samples. In contrast, the sensitivity of Ln-PCR was 77.5% (95% CI, 61.55-89.16%) for VL samples, 100% (95%CI, 69.15-100%) for RVL samples, and 52.5% (95% CI, 36.13-68.49%) for PKDL samples. The overall sensitivity of real-time PCR was considerably higher than Ln-PCR and discordance between the two methods was significant ($P < 0.0001$). Neither assay detected *L donovani* DNA in buffy coats from cured VL cases, but declining infections were found in three cured PKDL cases by real-time PCR and two cured PKDL cases by Ln-PCR. Both assays were 100% (95% CI, 95.98-100) specific with endemic healthy controls, and none showed cross reactivity with TB samples.

4 Conclusions

This real time PCR assay offers a molecular tool for rapid and accurate detection of VL, PKDL and RVL patients, as well as assessing response to treatment, that can provide a substantial contribution in efforts to eliminate VL.

C0600 A HIGH–RESOLUTION MELT ASSAY TO CHARACTERIZE CUTANENOUS LEISHMANIASIS IN NORTHWEST OF IRAN

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1 Background

Three major forms of human disease including, cutaneous (CL), visceral (VL) and mucocutaneous leishmaniasis (MCL), are caused by several *Leishmania* species which their geographic distribution frequently overlap. These *Leishmania* species have diverse reservoir hosts, sand fly vectors and transmission patterns. In Iran, the main parasite species responsible for CL are *L. tropica*, and *L. major*. Accurate and sensitive methods are crucial for identification of the causative agents, precise prognosis, and also treatment modality. The aim of the present study was to apply a high–resolution melt (HRM) assay for diagnosis and direct identification of *Leishmania* species on Giemsa-stained slides in Sabzevar and Neghab, Iran.

2 Methods

Altogether, 100 Giemsa-stained smear preparations, from the suspected skin lesions were randomly collected from the CL patients in two endemic foci in northwest province of Iran. First, all slides were examined under a light microscope. Second, after DNA extraction, HRM- amplification on 7SLRNA gene was performed with fluorescent SYBR Green to identify the causative species.

3 Results

The pattern of HRM assay showed that all (100%) of isolated samples in Sabsevarwere L.major while in Neghab, 97.5% of isolates were L.major and only in one case (2.5%) L.tropica was indicated.
Among the subjects, 44 (73.3%) and 26(65%) were male and 16 (26.7%) and 14(35%) were female in sabzevar and Neghab, respectively. The most highly infected cases were aged 20-39 years (55% and 45% in sabzevar and Neghab, respectively). Also, the majority of cases occurred in autumn.

4 Conclusions

This study is the first report using of HRM-assay for identification of Leishmania species in Iran. This new technique is useful for rapid identification and quantification of the infecting Leishmania species. This method can be used for diagnostic purposes directly from clinical samples and epidemiological surveys for planning future control strategies.

C0621 CUTANEOUS LEISHMANIASIS CLINICAL CRITERIA ASSOCIATED TO ENDEMIC LEISHMANIA SPECIES IN TUNISIA

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1 Background

Cutaneous leishmaniasis (CL) is a major health problem in Tunisia. It is a seasonal disease transmitted from June to October. Three CL forms due to three distinct *Leishmania* (*L.*) species namely *L. infantum*, *L. major* and *L. tropica* are endemic in the country. Recent spreads of geographical distribution of the three forms have induced the coexistence of more than one species in some foci. The aim of the study was to establish clinical criteria associated with each form because species identification methods are rarely available in rural areas where most cases are observed.

2 Methods

One hundred and fifty one CL confirmed cases were enrolled in the study. Mean age of patients was 28.6 years (range from 8 months to 74 years) and Sex-ratio F/M was 1.16. *Leishmania* species identification was systematically done by ITS1-PCR-RFLP and/or iso-enzyme analysis. For each patient, number, location, clinical aspect and month of onset of lesions were reported.

3 Results

Among the 151 identified isolates, 36 corresponded to *L. infantum*, 97 to *L. major* and 18 to *L. tropica*. *Leishmania infantum* and *L. tropica* lesions emerged later than those caused by *L. major* (61.1% after December *versus* 20.6%, p<0,001). Lesions due to *L. major* were often multiple (73.2%) and limb-situated (63.9%) whereas those caused by *L. infantum*and *L. tropica* were single (91.7% and 83.3% respectively) and face-situated (86.1% and 77.8% respectively). Ulcerative presentation with crust was more frequently observed with *L. major* (73.4%) whereas erythematous presentation was more often with *L. infantum* (71.9%) and *L. tropica* (61.1%) (p<0.001).

4 Conclusions

In addition to the geographical origin of patients, the number, the location, the morphological aspect and the lesions onset month revealed as useful predictive criteria to distinguish the three noso-geographical CL forms prevailing in Tunisia. Such simple clinical characterization is helpful for patients' management and control strategies.

C0624 USING KDNA MINICIRCLE SUBCLASS RELATIVE ABUNDANCE TO DIFFERENTIATE LEISHMANIA (L.) INFANTUM FROM LEISHMANIA (L.) AMAZONENSIS

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1 Background

Leishmaniasis is a neglected disease caused by many *Leishmania* species, belonging to subgenera *Leishmania (Leishmania)* and *Leishmania (Viannia).* Several qPCR-based molecular diagnostic approaches have been reported for detection and quantification of *Leishmania* species. Many of these approaches use the kinetoplast DNA (kDNA) minicircles as target sequence, because they contain conserved regions and are in high-copy number. However, due to sequence similarity between *Leishmania* species, most of the available qPCR assays had potential cross-species amplification. Previous works demonstrated discrimination between *L. (Leishmania)* and *L. (Viannia)* by SYBR green-based qPCR assays designed on kDNA, followed by melting or high resolution melt (HRM) analysis. Importantly, these approaches cannot fully distinguish *L. (L.) infantum* from *L. (L.) amazonensis*, which can coexist in the same geographic area. An accurate diagnostic method for distinction between these two species is necessary to provide prognosis, treatment and monitor disease evolution.

2 Methods

DNA from 18 strains/isolates of *L. (L.) infantum*, *L. (L.) amazonensis*, *L. (V.) braziliensis*, *L. (V.) panamensis*, *L. (V.) guyanensis*, and 62 clinical samples from *L. (L.) infantum*-infected dogs were used in this study. All samples were amplified by a previously developed qPCR (qPCR-ML). Selected PCR products were directly and bidirectionally sequenced using an ABI PRISM 310 Genetic Analyzer. Sequences were manually edited and aligned in MUSCLE. Based on the obtained sequences, a new SYBR-green qPCR assay (qPCR-ama) intended to amplify a minicircle subclass more abundant in *L. (L.) amazonensis* was designed.

3 Results

The novel qPCR-ama assay achieved a detection limit of 0.1 pg of parasite DNA and did not show cross amplification with host DNA or *Trypanosoma cruzi*. The qPCR-ama showed positive results also with *L. (L.) infantum* strains, but the Ct values were dramatically increased compared to qPCR-ML (13-19 cycles). Therefore, combined results of qPCR-ML and qPCR-ama allowed to distinguish *L. (L.) infantum* and *L. (L.) amazonensis* in 100% of tested samples, provided that template DNA was diluted so as to have Ct>25 in qPCR-ML.

4 Conclusions

A new and affordable SYBR-green qPCR-based approach to distinguish between *L. (L.) infantum* and *L. (L.) amazonensis* was developed exploiting the major abundance of a particular minicircle sequence rather than targeting a hypothetical species-specific minicircle sequence.

C0639 MISSED DIAGNOSIS OF CUTANEOUS LEISHMANIASIS

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1 Background

Cutaneous leishmaniasis (CL) is the most common form of the leishmaniasis,disease is cutaneous leishmaniasis (CL).
Diagnosis of cutaneous leishmaniasis (CL) is a crucial issue to initiate the treatment, in endemic areas diagnosis of CL is done based on history of the lesion . Clinical picture of CL is similar to many skin disorders such as fungal, bacterial and viral infection, sarcoiedosis, lupus vulgarious and other skin diseases.
Leishmaniasis is a parasitic disease caused by different species of *Leishmania*.Clinical manifestation of the disease depends on the *Leishmania* species and the host immune response ranges from a self healing skin lesion to a fetal systemic form of visceral leishmaniasis. The most common fom of the disease is cutaneous leishmaniasis (CL).

2 Methods

A 22-year-Afghan patient was referred to the CL clinic of the center . The patient was presented with erythematous plaques lesions on whole body, with no history for atopic dermatitis and other skin disorders, he was diagnosed as CL and had been treated with 20 mg/Kg/day meglumine antimonate intra muscular for 20 days, no response to treatment was seen.. On clinical examination, there was multiple 2 to 3 cm indurated erythematous plaques with surface scaling involving the dorsal surface of the whole body. No palpable lymphatic node was detected. Routine laboratory tests for CL, including direct smear, culture and PCR were negative. The condition was considered as a generalized dermatitis and treatment was started by using less than 0.5mg/kg of oral prednisolone and tapered in 3 weeks to 5mg/d. Emollient and cetirizine also was included in the management. Complete resolution of lesions occurred after 2-weeks of treatment
with history of as a resistant leishmaniasis

3 Conclusions

Laboratory diagnosis of CL needs more attention and diagnosis must be done by trained technicians
Clinical diagnosis of CL must be approved parasitologically. Cutaneous leishmaniasis form depends on responding to treatment and recovery time depends on parasitic species and host immune response.
Eczematoid forms of leishmaniasis is not common

C0646 DEVELOPMENT STUDIES ON A NOVEL LAMP ASSAY FOR THE DIAGNOSIS OF CUTANEOUS AND VISCERAL LEISHMANIASIS

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1 Background

A novel *Pan-Leishmania* LAMP assay was developed for diagnosis of Cutaneous and Visceral Leishmaniasis (CL & VL) which can be used in near-patient settings.

2 Methods

Primers were designed on 18S rDNA and kDNA selected on the bases of high copy number. Prototype LAMP assays were tested for cutaneous leishmaniasis (CL) in a prospective cohort trial of 105 patients with compatible symptoms in South-West Colombia, and for VL on 50 suspect cases in Ethiopia. Swab samples from CL suspects were processed for DNA extraction and tested using LAMP. A composite gold standard comprising of microscopy AND/OR culture positivity was used to calculate the diagnostic accuracy. Samples from 50 VL suspected patients at University of Gondar, Ethiopia were collected including whole blood, peripheral blood mononuclear cells and buffy coat. Diagnostic accuracy was calculated against the reference standard of microscopy of splenic or bone marrow aspirates.

3 Results

The prototype LAMP assay had a sensitivity of 95% (95% CI: 87.2% - 98.5 %) and a specificity of 86% (95% CI: 67.3% -95.9 %) for the diagnosis of CL on lesion swab samples. On VL suspects from Ethiopia, the sensitivity was 92% (95% CI: 74.9 – 99.1%) and specificity of 100% (95% CI: 85.8-100%).

4 Conclusions

For CL, LAMP is a sensitive tool for diagnosis and requires less equipment, time and expertise than alternative CL diagnostics. For VL, LAMP is sensitive and highly specific using a minimally invasive sample type as compared to the gold standard.

C0662 CONCORDANCE BETWEEN DIRECT TRADITIONAL TECHNIQUES AND PCR FOR TEGUMENTARY LEISHMANIASIS

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1 Background

Direct diagnostic for tegumentary leishmaniasis in Paraguay is an unsolved problem, as *Leishmania braziliensis* causes almost the totality of the cases. Scarcity of the parasites for this specie of *Leishmania*, in the lesions, generates low sensitivity for directs assays. Smear from the lesion, stained with Giemsa, and histopathology are used as traditional tests for routine diagnostic. Nevertheless, PCR technique had demonstrated to be useful, as it seems to have better sensitivity to detect parasite DNA.

2 Methods

In order to compare the concordance between traditional techniques and PCR, we took samples from 16 cutaneous and mucocutaneous leishmaniasis cases. Smear of the lesions, stained with Giemsa and, histopathology technique, stained with hematoxylin-eosin, were done. We performed a blind analysis for PCR and traditional direct techniques.

3 Results

We found a high level of discordance between direct traditional techniques, smear and histopathology together, versus PCR (Kappa index = -0.32). The sensitivity for smear and PCR, versus the histopathology, were 50%.

4 Conclusions

The high level of discordance between traditional techniques and PCR, in this work, indicates that it would not be suitable to use routinely the PCR technique as a diagnostic test for tegumentary leishmaniasis. We found also a very low sensitivity for smear and PCR versus histopathology; therefore, these tests are not advisable to be used alone for the detection of tegumentary leishmaniasis.

C0703 NOVELS ANTIGENS OF LEISHMANIA (VIANNIA) BRAZILIENSIS AS CANDIDATES FOR THE INMMUNODIAGNOSIS OF AMERICAN TEGUMENTARY LEISHMANIASIS

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1 Background

American tegumentary leishmaniasis (ATL) is endemic in ten provinces of Argentina. *Leishmania (Viannia) braziliensis* is the main causative agent in this area. Its diagnosis remains as challenge, despite the advances in the development of the molecular methods.

2 Methods

Our main goal is to improve the diagnostic performance of ATL developing ELISA techniques with four recombinant antigens from a local strain of *L. (V.) braziliensis* expressed in bacterial systems: HATLbAg1 (MW: 49.76 kDa), HAT-LbAg2 (MM 94.25), HAT-LbAg3 (MM 40.29), HAT-LbAg4 (MW: 34.9 kDa), or a soluble extract-enriched with membrane proteins of promastigotes of the same strain (ESM). Serum samples from 215 patients diagnosed as *ATL cases* by a combination of parasitological methods, PCR, Montenegro skin test, and clinical-epidemiological criteria were included in this study. On the other hand, the absence of disease was represented by a set of 212 sera, from patients diagnosed as *No ATL cases*, seropositive for Chagas disease (CH), toxoplasmosis or syphilis, and donor subjects from LTA non-endemic areas.

3 Results

The estimated general sensitivities and specificities were 70.71 and 72.88% for HAT-LbAg1; 68.32 and 28.79% for HAT-LbAg2; 68.37 and 47.62% for HAT-LbAg3; 69.9 and 96.97% for HAT-LbAg4, while for the ESM they reached 91.27% and 66.23%, respectively.

4 Conclusions

The specificities increase significantly when patients with Chagas disease are excluded. Importantly, HAT-LbAg4 and ESM ELISAs presented a relatively high diagnostic performance comparing with the parasitological and molecular methods currently used in the ATL diagnosis in the country. These results led us to proposed HAT-LbAg4 and ESM ELISAs as promising techniques for a multi-method diagnosis of this pathology.

C0726 UTILITY OF DIRECT IMMUNOFLUORESCENCE AS A NEW TOOL FOR CUTANEOUS LEISHMANIASIS DIAGNOSIS

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1 Background

Microscopic examination of *Leishmania* amastigote forms, even its limited sensitivity and its needs in expertise, remains the exam of choice in cutaneous leishmaniasis (CL) diagnosis. Thus, the objective of this work is to develop a direct immunofluorescence (DIF) assay, in order to detect the *Leishmania* parasite in dermal lesional samples.

2 Methods

1) *Leishmania major* specific anti-sera was produced by rabbit infection with alive metacyclic promastigotes. Then, specific IgG were purified, fluorescein labeled and tested by DIF on promastigote and amastigote *Leishmania* forms. DIF was applied on dermal smears collected from cutaneous lesions (n=41) among them: a) n=18 were confirmed negative by both microscopic examination and kinetoplastic DNA (kDNA) qPCR; b) n=14 were confirmed positive by microscopic examination; and c) n=9 negative microscopic examination were confirmed positive by kDNA qPCR. The Fluorescence was read in double blind, using a microscope equipped with an UV lamp at 40X magnification. Subsequently, DIF dermal smears slides were washed with absolute ethanol, the material forming the dry smears was scraped, DNA was extracted from the latter and was amplified by a *Leishmania major* internal transcribed spacer 1 (ITS1) PCR.

3 Results

Our results showed that among: a) 18 both negative microscopic examination and kDNA qPCR samples, no one was found DIF positive; b) 14 microscopic examination positive samples, 14 were found DIF positive; c) 9 negative microscopic examination and positive kDNA qPCR samples, 8 were found DIF positive. Moreover, all the DIF positive dermal smears were confirmed ITS1 PCR positive. Compared to the results of microscopic examination supplemented with PCR, the *Leishmania* DIF assay showed good specificity and sensitivity (94% and 96% respectively).

4 Conclusions

Our data suggest that the inclusion of DIF assay could be a useful tool in the CL diagnosis.

C0727 LEISHMANIA INFECTIONS: MOLECULAR TARGETS AND DIAGNOSIS

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1 Background

Leishmaniases are parasitic diseases causing a spectrum of clinical symptoms (notably VL, CL and ML) which affect several millions of people in the world. Despite great progress in diagnostic techniques, leishmaniases cases are extending, constituting an important public health problem. In recent decades, molecular diagnostic tools have been increasingly applied for early *Leishmania* detection. Progress in diagnosis depends on development of effective methods and suitable biomarkers. In this study, we discuss on these molecular markers and tools with suggesting suitable methodologies for each goal of diagnosis and typing.

2 Methods

We propose an update classification of *Leishmania* species. We demonstrate a global map highlighting the geography of pathogenic *Leishmania* species. We summarize a complete list of techniques and discuss on their characteristics and sensitivity/specificity in *Leishmania* detection from subgeneric to subspecies levels. Each method is supplied with a comprehensive description of markers and proposal for their role in detection, identification and quantification of *Leishmania* infection. We outline wide map of genes informative for leishmaniasis diagnosis. We propose a classification of molecular markers for typing 21 pathogenic *Leishmania* species.

3 Results

The implement of molecular applications depends on several criteria including the goal of study, availability, sensibility/specificity, technical skills and the expense. In leishmaniasis diagnosis, several goals are perceptible, classified mainly in two main categories: diagnosis and typing. Based on each goal, the appropriate molecular methods and markers are needed. Several DNA-based systems have been developed which among them, PCR is still considered to be a suitable method. Among different molecular markers, the repetitive sequences such as kDNA minicircle, rRNA genes, miniexons or genomic repeats are considered as the best targets with maximum sensitivity of *Leishmania* detection.

4 Conclusions

There is no test as the comprehensive method for diagnosis of all pathogenic *Leishmania* species. The chosen method often depends on the application. It is necessary to simplify the diagnostic tests interpreted easily with high sensitivity/specificity. Due to lack of this gold standard, more efforts will be required to improve molecular methodologies mostly in diagnostic process. The main goal of future studies can be therefore concentrated on the development of a reliable diagnostic approach which covers these defects.

C0770 DEVELOPMENT OF A GLYCATED CIRCULATORY IMMUNE COMPLEX BASED ASSAY FOR THE EARLY DIAGNOSIS OF PATIENTS WITH MACULAR POST KALA-AZAR DERMAL LEISHMANIASIS

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1 Background

Post Kala-azar Dermal Leishmaniasis (PKDL) a dermal complication is usually caused as a sequel to Visceral Leishmaniasis (VL). PKDL remains a silent parasite pool for transmission of Leishmaniasis and is thus a threat to the ongoing VL elimination program. Available PKDL diagnostics assay including slit smear, biopsy, serological test have low sensitivity and specificity limits. Thus an urgent need of rapid, field-adaptable, cheap, accurate PKDL diagnostics exists.More recently, Glycated Circulatory Immune complex have been demonstrated in the serum of Indian PKDL patients. Accordingly, the present study was carried out to develop a Glycated Circulatory Immune Complex based assay for early diagnosis of patients with macular PKDL.

2 Methods

The study population consisted of (n=50) PKDL patients, endemic healthy controls (n=30), Leprosy (n=30), and Vitiligo (n=30) patients enrolled during different field surveys in different districts of West Bengal 2015-2016, following proper ethical guidelines. Briefly Circulating Immune Complexes (CIC's) were isolated from patient's serum, followed by PEG CIC Index calculation. Patient CIC titers were detected using the newly developed colorimetric assay and compared with conventional Parasite ELISA. The data were statistically evaluated.

3 Results

The diagnostic efficacy indicators like Sensitivity of the Glycated Circulatory Immune Complex Based assay were 78% and Specificity 95% ($p<0.0001$), whereas for the Conventional Parasite ELISA it was 50% and 60% only. Receiver operating characteristic curve analysis revealed that the AUC values were the best for Glycated Circulatory Immune Complex Based assay.

4 Conclusions

Taken together, the Glycated Circulatory Immune Complex Based Assay holds great promise for the early diagnosis of patients with macular PKDL.

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C0814 BAYESIAN ANALYSIS OF DIAGNOSTIC PERFORMANCE OF SEROLOGICAL AND MOLECULAR TESTS FOR LEISHMANIA INFANTUM INFECTION IN CATS IN THE ABSENCE OF GOLD STANDARD

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1 Background

The diagnosis of feline leishmaniosis (FeL) caused by *Leishmania infantum*(*Li*) is based on antibody detection by the immunofluorescence antibody test (IFAT), the enzyme-linked immunosorbent assay (ELISA) or, rarely, by Western Blot (WB). Quantitative real time Polymerase Chain Reaction (PCR) for the detection of *Li* DNA is also frequently used. However, comparative studies of these four diagnostic tests have not been performed in felines. The aim of this study was to compare diagnostic performance of serological and molecular methods to evaluate *Li* infection in cats using a Bayesian analysis in the absence of a gold standard test.

2 Methods

The study included 87 cats from Italian endemic areas, 11 of them with confirmed clinical FeL. Serum antibody detection was performed by IFAT, ELISA and WB and parasite DNA was amplified by PCR on blood and/or lymph node, urine, conjunctival swabs on each sample unit. Cut-off was set at 1:80 dilution for IFAT and 40 ELISA units for ELISA. WB was considered positive in presence of at least a 18 KDa band and PCR when at least 1 tissue tested positive. Statistical analysis was performed through a MATLAB software written routine. We assumed that the test results for a given cat were independent conditionally to their true state (infected or not infected). With 4 tests, for each one of the 2^4 possible realizations the joint probability was computed. The latent data and observations from the joint posterior were simulated in the Bayesian approach by an iterative Markov Chain Monte Carlo technique using the Gibbs sampler for estimating sensitivity (Se) and specificity (Sp) of 4 tests investigating *Li* infection in cats. Once the posterior distributions of all parameters approached equilibrium condition, we estimated the median and the 95% CI from the sample generated from the posterior distribution.

3 Results

The overall rate of *Li* infection was estimated at 17.0% [10%; 26%]. The most accurate test was PCR (Se=95%[75%;100%], Sp=91%[83%;96%]) while WB (Se=96%[74%;100%], Sp=84%[74%;92%]) and IFAT (Se=95%[75%;100%], Sp=84%[75%;91%]) were quite equivalent in discriminating the infected and not infected cats. Conversely, ELISA (Se=88%[64%;99%], Sp=99%[95%;100%]) was the most specific but the less sensitive test.

4 Conclusions

Bayesian analysis in absence of gold standard done on the data obtained in this study shows that PCR performed on more than one tissue offered the best diagnostic performance, followed by both WB and IFAT.

C0816 ANTI-FIXED LEISHMANIA INFANTUM PROMASTIGOTES IGG ANTIBODIES DETECTED BY FLOW CYTOMETRY AS A TOOL FOR SERODIAGNOSIS IN AMERICAN VISCERAL LEISHMANIASIS IN NORTHEASTERN, BRAZIL

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1 Background

Visceral Leishmaniasis (VL) is a severe disease caused by protozoans of the genus *Leishmania* and etiological agent *Leishmania infantum*. It is a zoonosis with an eminently rural characteristic which has been expanding to urban areas. Conventional serological diagnosis for VL cannot accurately allow the differentiation of recent and late infections, not allowing its use to a post-therapeutic cure assessment, and may also present cross-reactions with other diseases. This study aims to evaluate the use of flow cytometry as an alternative methodology in the diagnosis of patients with VL in Pernambuco, Brazil.

2 Methods

Sera of 147 patients diagnosed with visceral leishmaniasis by parasitological (myelogram) and / or immunological tests (rK39 dipstick test, direct agglutination test and urine latex antigen test) were evaluated. For this, the detection of antibodies to the promastigote forms of *L. (L.) infantum* by flow cytometry (AAPF-IgG) was performed by a FACSCalibur (Becton, Dickinson, San Jose, CA) cytometer. Statistical analysis was performed using MedCalc Statistical.

3 Results

From a cutoff point of 20% of the percentage of positive fluorescent parasites (PPFP) and the 1: 512 dilution as the region of serological reactivity, the disease was detected in 82.3% (121/147) of the patients, presenting a sensitivity of 97,6% % and specificity of 100%.

4 Conclusions

Therefore, flow cytometry is a promising alternative for the diagnosis with prospects for its use and the use of IgG subclasses as a biomarker in disease progression, contributing to the clarification of the humoral response in VL.

C0818 ALTERNATIVE PROPOSAL FOR QUALITY CONTROL IN ANTIGEN PRODUCTION PROCESS AND NEW ANTIGEN FORMULATION FOR SKIN TEST IN LEISHMANIASIS DIAGNOSIS

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1 Background

Montenegro skin test (MST) is a widely used diagnostic method to investigate cutaneous leishmaniasis. It consists in an evaluation of delayed hypersensitivity (cellular immune response) in patients with the disease. The actual production of MST possesses some difficulties regarding to the qualitative and quantitative control, and to the antigen production. A hypothesis suggests that the antigen production quality control can be perform in animal models that are capable of reproduce the immune response generate, by this antigen, similar to humans. To overcome the limitations of antigen production is proposed the production of second generation of antigens, obtained through biotechnology pathways with the production of mimetic peptides. These hypotheses are the basis of the study that evaluate the use of experimental model (*Cavia porcellus*) to validate the antigen produced and new antigens formulations for skin test.

2 Methods

Cavia porcellus immunized with *Leishmania amazonensis* or *L. braziliensis* proved to be a suitable *in vivo* model to test antigens for skin test. A positive criteria of induration reactions ≥ 0.5 cm measure between 48 – 72 h after the skin test was establish. Three peptides, previously select by phage display technique by our research group, were tested as skin test antigens aiming to replace the current antigen.

3 Results

The peptides, individually or in a mix promoted induration reactions in *C. porcellus* immunized with *L. amazonensis* or *L. braziliensis.* The indurations varied from 0.5 to 0.7 cm. In the animals immunized with *L. amazonensis* the Peptide3 showed better results than the standard MST antigen. In animals immunized with *L. braziliensis* the Peptide 2, Peptide 3 and the Mix promoted induration reactions higher than standard MST antigen.

4 Conclusions

The results suggest that the peptides can be use as antigens in skin test for cutaneous leihsmaniasis diagnostic.

C0846 QUANTITATIVE REAL TIME PCR TECHNOLOGY FOR LEISHMANIA SPECIES IDENTIFICATION

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1 Background

In Brazil, American Cutaneous Leishmaniasis (ACL) is a disease with a diversity of agents, reservoirs and vectors,and which presents different patterns of transmission. In addition, there is a limited knowledge about some of its aspects, making it a zoonosis of difficult control. Intrinsic features of the parasite stimulate and drivethe host immune response to the infection, being the parasitic species an important factor for the type of clinical manifestation and therapeutic response. Currently, the methods of *Leishmania* spp. identification are costly and laborious, and there is a need to develop technologies for parasite discrimination. In this context, the quantitative real-time PCR (qPCR) has been shown as an alternative. Therefore, the aim of this work is to evaluate the applicability of TaqMan probe technology for identification of *Leishmania* spp.

2 Methods

Using the MEGA 6.0 software, multiple alignments for different targets (HSP70, Cytochrome B, kDNA, G6PD, GP63 and 27 chromosome-rDNA) of *Leishmania* spp. available on GenBank and TriTrypDB were performed.After *in silico* analysis, the most specific target for *Leishmania* (*Viannia*) *braziliensis*, *Leishmania* (*Viannia*) *guyanensis*, *Leishmania* (*Viannia*) *shawi* and *Leishmania* (*Leishmania*) *amazonensis* was used to design primers and probes able of discriminating among species related to ACL in Brazil.Efficiency (*e*), specificity and detection limit (DL) were calculated for each system, and after individual standardization, they were combined to compose to Multiplex-qPCR (MqPCR).

3 Results

ITS1 (rDNA) was the target selected. Two sets of primers and probes were designed, SVS for the subgenus *Viannia* and LaS for *L. amazonensis*. In the individual tests by system, using DNA from *L. braziliensis*, the SVS showed *e*= 91.2%, DL= 50 fg/µL; from *L. guyanensis* DNA *e*= 89.8%, DL= 0.5 pg/µL; and from *L. shawi* DNA *e*= 90.6%, DL= 5 pg/µL. From *L. amazonensis* DNA, LaS showed *e*= 97.43%; LD= 5 fg/µL.After the system combination (SVS + LaS), the MqPCR showed specificity of 100%. Using DNA from *L. braziliensis* and *L. amazonensis* in the same reaction, SVS*e*= 98%, SVSDL= 0.5pg/µL and LaS*e*= 77%, LaSDL= 5fg/µL were observed.

4 Conclusions

The developed MqPCR has been displayed as a tool capable of performing the diagnosis and *Leishmania* spp. identification in the same reaction, as well as it has the ability to provide the parasite load present in the sample under analysis.

C0859 DIAGNOSIS OF CANINE LEISHMANIASIS USING A NEW QUANTITATIVE AND NON-INVASIVE METHOD

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1 Background

Canine Leishmaniasis (CanL) is a severe disease with nonspecific clinical signs. Precise diagnostic methods are necessary for the identification of the parasite. The most commonly used methods for diagnosis are based on immunodiagnostic techniques, however, they may lead to false negatives, in the absence of seroconversion of antibodies after infection, or false positives, when antibodies remain elevated after successful treatment. The objective of the present communication is to develop a quantitative PCR (qPCR) method for the noninvasive detection of L. infantum in dog scraping samples from buccal mucosa. In addition, thanks to this technique we have carried out an epidemiological evaluation of dog samples from different areas of Castilla y León (Spain).

2 Methods

Samples of 48 dogs from different areas of Castilla y León (Spain) were analysed. Samples were collected by passing a sterile swab on the inner surface of the dogs' buccal epithelium, trying to collect as many cells as possible for 1 min. A qPCR method was developed to amplify a 131 bp fragment corresponding to a conserved region of the L. infantum kDNA using a pair of primers designed "ad hoc". The specificity of the technique was assessed using DNA samples from uninfected or infected dogs with other current pathogens like Erhlichia canis.

3 Results

The technique described here presents a high sensitivity, with a detection limit of 35 parasites per buccal scraping, regardless of the number of dog cells collected in the sample. There were no cross-reactions either with DNA isolated dog cells or with DNA from animals infected with E. canis. From the 48 samples provided by veterinary clinics suspected of CanL, 29% were positive. From the animals with negative results, 5 were treated animals against the parasite, thus confirming the success of the treatment. We also evaluated 58 dogs randomly sampled in Castilla y León, where the initial results indicate an unexpected prevalence of 33%. Of the 19 positive animals, 10 had parasitic loads between 1-100 parasites/50,000 dog cells, and 4 had values greater than 10,000 parasites/50,000 dog cells. All the positive dogs lived in rural or peri-urban areas.

4 Conclusions

Through this qPCR, it is possible to perform an early diagnosis of CanL, useful to determine the parasite burden, the efficacy of a treatment or the evolution of a disease, in a painless way for the animal.

C0861 URINE QPCR DIAGNOSIS OVER THE ENTIRE CLINICAL-IMMUNOLOGICAL SPECTRUM OF HUMAN LEISHMANIA (L.) INFANTUM CHAGASI-INFECTION

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1 Background

The clinical-immunological spectrum of human *Leishmania (L.) infantum chagasi*-infection in the Brazilian Amazon was recently reviewed based on the combined use of *Leishmania* skin test (LST) and indirect fluorescence antibody test (IFAT-IgG), associated with clinical evaluation which has provided the identification of five clinical-immunological profiles: three asymptomatic (Asymptomatic Infection – AI; Subclinical Resistant Infection – SRI; and Indeterminate Initial Infection – III), and two symptomatic (Symptomatic Infection – [SI =American Visceral Leishmaniasis – AVL]; and Subclinical Oligosymptomatic Infection – SOI). However, considering the high sensitivity and specificity of the quantitative polymerase chain reaction (qPCR), we have evaluated it as an alternative method for improving the diagnosis of human *L. (L.) i. chagasi*-infection by using the urine of individuals with different clinical-imunological profiles.

2 Methods

The urine deposits were washed in solution of TKM-1 (10mM Tris-HCl pH 7,6; 10mM KCl; 10 mM MgCl2, 2mM EDTA); for DNA extraction was used the resine Instagene Matrix® (Barea et al. 2014). The amplification was carried out by the thermocycler Applied Biosystems 7500 real-Time PCR, using SYBR® Green Taq and the RV1 (5'-CTTTTCTGGTCCCGCGGGTAGG-3') e RV2 (5'-CACCTGGCCTATTTTACACCA-3') (Le Fichoux et al. 1999). The reaction was initially incubated at a denaturation temperature of 95°C/ min., 40 cycles of 95°C by 15 seconds, 55°C by 35 seconds and 70°C by 10 seconds, for a dissociation curve of 95ºC by 15 seconds, 60°C by one minute, and 95°C by 15 seconds.

3 Results

Therewere examined 146 individuals, 91 (62,3%) with prior diagnosis of infection: 13 (14,3%) AI profile, 13 (14,3%) ISR, 43 (47,2%) III, 12 (13,2%) SI= AVL (treated), and 10 (11%) SI= AVL (non-treated), while 55 were negative for LST$^{(-)}$/IFAT$^{(-)}$. qPCR was positive in 61,5% of AI profile, 61,5% of ISR, 65% of III, 50% of treated AVL, 100% of no treated AVL, and 7,3% of negative control group.

4 Conclusions

Urine qPCR analysis confirmed the diagnosis not only of 100% of non-treated AVL cases (=SI profile) as well as a high rate (>60%) of asymptomatic profiles (AI, SRI and III) of human *L. (L.) i. chagasi*-infection, revealing a useful tool for monitoring the clinical evolution of these profiles, principally the III one which, depending on the genetic background of the infected individual, may develop for AVL.

C0862 AN INNOVATIVE, RAPID AND SIMPLE TO USE LAMP SYSTEM FOR MOLECULAR DIAGNOSIS OF LEISHMANIASIS DISEASE

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1 Background

Currently approaches to detect Leishmaniasis diseases consist mainly in parasitological and serological methods. Due to limited usage of such methods, molecular tools are the most suitable methods to detect, with high sensitivity and specificity, parasites of the genus *Leishmania*. As an alternative to current molecular diagnostic tests, that require specialized personnel and the use of sophisticated equipment, an innovative based-LAMP (Loop Mediated Isothermal Amplification) system developed by Enbiotech Srl, provides a suitable form of detecting infectious diseases, with the possibility of performing analysis on the field. This system called ICGENE VET has been used for the specific detection of protozoan parasites of the genus *Leishmania* from lymph-node aspiration samples using a customized kit *Leishmania Screen Glow*, which provides the rapid extraction and amplification of DNA.

2 Methods

The system is composed of a ready-to-use kit *Leishmania Screen Glow* and a portable device (heater and fluorescent reader). It performs rapid DNA extraction using small amounts of biological sample, isothermal genetic amplification and automatic interpretation of results using the ICGENE VET device. DNA was extracted from different lymph-node aspiration samples in 10 minutes using an extraction buffer at room temperature. LAMP amplification was performed successfully within 45 minutes at 60°C. The final volume of the reagents were 50µl, containing 22µl of LAMP mix, 30µl of mineral oil and 3µl of extracted genomic DNA

3 Results

All LAMP amplifications were specific for the investigated targets. Developed reagents are complete with all components necessary for the analysis, from extraction to detection for the following *Leishmania* strains: *L. aethiopica, L. braziliensis*, *L. donovani*, *L. major*, *L. tropica, L. amazonensis,* and *L. infantum.* The amplification and detection system provides a detectable results of up to 10 *Leishmania*/ml.

4 Conclusions

The potential effectiveness of this innovative system as a user-friendly molecular tool is demonstrated with its high efficiency, rapidity and simplicity. One of the most attractive features of this newly developed LAMP assay ICGENE VET is the possibility to obtain rapid molecular results from lymph-node aspiration samples performed by personnel with little or no experience. Other qualities include the possibility of working outside common molecular biology laboratories and without the help of sophisticated equipment.

C0877 SLA-ELISA: A COMPARISON WITH DAT & RK39 ELISA FOR IDENTIFICATION OF SEROCONVERTERS

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1 Background

The most important drawbacks in visceral leishmaniasis (VL) population studies are the difficulty of diagnosing asymptomatic carriers. The aim of this study was to evaluate the performance of serology to identify asymptomatic VL infection in participants selected from a TMRC cohort area, a rural area of Muzaffarpur District Bihar, India.

2 Methods

Blood samples on filter paper were collected from 437 (seroconverters and controls) cohort participants. Samples were tested by ELISA using two different antigens: Soluble Leishmania antigens (SLA), and rK39 recombinant protein and DAT (Direct agglutination Test).

3 Results

When SLA ELISA compared with both rK39 ELISA and DAT showed SLA ELISA was positive in all 139 individuals who were positive with both DAT & rk39 ELISA. SLA ELISA also detects some seropositivity in those who are seronegative with both tests DAT and rk39 ELISA.

4 Conclusions

SLA ELISA having more sensitive method for the diagnosis of asymptomatic infection so we can run only SLA ELISA instead of both serological (DAT and rK39 ELISA) tests. We also found seropositive samples from the control group might be we missed some seropositive samples with these serological (rK39 ELISA and DAT) tests so SLA ELISA could be used as marker of Infection.

C0880 CORRELATION BETWEEN PERIPHERAL BLOOD PARAMETERS AND BONE MARROW FEATURES IN VISCERAL LEISHMANIASIS

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1 Background

Bone marrow of patients with visceral leishmaniasis (VL) is usually hyperplastic and heavily parasitized. In the earlier stages of infection, dysregulation of hematopoiesis and decreased cell production occur. The aim of the study was to describe the quantitative hematological parameters and bone marrow cytomorphological findings in patients with VL, correlating them with each other and to the clinical outcome.

2 Methods

A retrospective cohort study of 118 patients admitted to a referral hospital from December 2006 to August 2007. The blood count was assessed on admission and cytomorphological assessment of bone marrow was performed by two hematologists, in different moments, through the analysis of slides stained with May-Grunwald-Giemsa method.

3 Results

Most patients were male (60.2%), residing in urban areas (72.0%) and were discharged with good clinical conditions (93.2%). Death rate was 6.8%. The mean hemoglobin levels found were 7.4 g/dL (95%CI 7.1 - 7.7). The average white blood cell count was 3,252.0/mm^3 (95%CI 2,877.1 - 3,629.0) whereas the mean platelet counts were 108,900.0/mm^3 (95%CI 96,400.0 - 121,500.0). 21.1% of patients had severe neutropenia, which was not a predictor for patient death. 61.9% of the cases, the bone marrow was normocellular. Most of patients with severe anemia presented erythroid hypercellularity (72.0% of cases). Severe neutropenia was accompanied by myeloid hypocellularity. Figures of dysplasia were frequent and 34.7% of the samples had dyserithropoiesis, 49.1% showed dysgranulopoiesis and 34.7% with dysmegakaryopoiesis. Among patients who had a fatal outcome, 62.5% had hypocellular marrow (p=0,19) and the finding of dysplasia did not contribute to this outcome.

4 Conclusions

Dyserithropoiesis was associated with severe anemia. Findings of medullary dysplasia and reactivity to peripheral cytopenia were also frequent in this group but with no association with the clinical outcome of patients.

C0881 USE OF RECOMBINANT ANTIGENS FOR SENSITIVE SERODIAGNOSIS OF AMERICAN TEGUMENTARY LEISHMANIASIS CAUSED BY DIFFERENT LEISHMANIA SPECIES

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1 Background

American tegumentary leishmaniasis (ATL; also known as cutaneous leishmaniasis (CL)) is caused by various species of protozoa of the genus *Leishmania*. Diagnosis is achieved on a clinical, epidemiological and pathological basis supported with positive parasitological exams and demonstration of Leishmanin delayed-type hypersensitivity. Serological assays are not routinely used in the diagnosis because they have previously been considered to have low sensitivity and the particular *Leishmania* species causing the disease can lead to variable performance.

2 Methods

Antigen-specific antibodies were evaluated in serum samples by enzyme-linked immunosorbent assay (ELISA). Samples were probed with recombinant versions of two highly conserved *Leishmania* proteins, *Leishmania (Viannia) braziliensis* (Lb)-derived Lb8E and Lb6H.

3 Results

rLb6H had better performance than rLb8E, reacting with 100% of ATL and 89.4% of the VL samples. These reactions with rLb6H were highly specific (98.5%) when compared against samples from healthy control individuals. We then assessed rLb6H against sera from ATL patients infected with different species of *Leishmania* prevalent in Brazil (*Leishmania (Leishmania) amazonensis, L. (Viannia) braziliensis* and *L. (V.) guyanensis*) and samples from patients with other infectious diseases. In analyses of 500 sera, ELISA using rLb6H detected all 219 ATL samples (sensitivity of 100.0%) with an overall specificity of 93.9% (considering healthy individuals and other infectious diseases patients). Only a minority of samples from Chagas disease patients possessed antibodies against rLb6H, and all of these responses were low (with a highest reactivity index of 2.2).

4 Conclusions

Our data support further evaluation of rLb6H, and the potential for its routine use, in the serological diagnosis of ATL.

C0883 EXPLOITING THE GLYCOIMMUNOLOGY OF CUTANEOUS LEISHMANIASIS FOR DIAGNOSIS

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1 Background

Cutaneous leishmaniasis (CL) causes skin ulceration with scarring that persists after cure. Increasing cases of Old World CL have been linked to conflict in the Middle East, prompting a need for improved diagnostics that can detect the presence of *Leishmania* and distinguish between *Leishmania* species.
The *Leishmania* glycocalyx is composed of an array of glycan structures conjugated to lipids or proteins, with a composition that differs between parasite species. Some of the *Leishmania* sp. surface glycoconjugates are decorated with terminal α-galactosyl (αGal) sugars, which are highly immunogenic in humans and have been implicated in the host immunity against leishmaniasis and other parasitic diseases. Recent work has found that antibodies against parasite αGal epitopes can serve as biomarkers for Chagas disease and are also elevated in Old World CL patients compared to control individuals.

2 Methods

Serum samples were collected from three patient groups from the Kingdom of Saudi Arabia; Group 1: confirmed *L. major* infection, Group 2: clinically cured and Group 3: patients that have CL-like lesions but where PCR found no *Leishmania* parasite. Chemiluminescent ELISA was used to measure antibody levels to synthetic neoglycoproteins (NGPs), representing a range of αGal-terminating glycan structures conjugated to BSA.

3 Results

Initial findings indicate that several NGPs, containing αGal-terminating glycan structures, have potential to be used as biomarkers for *L. major* infection in KSA patients. Our data show that patients with active CL have significantly higher IgG titres to specific NGPs as compared to the cured and non-CL patients. We found that a combination of four NGPs: Galα(1,3)Galβ(1,4)Glcβ, Galα, Galα(1,6)Galβ, and Galα(1,2)Galβ had a strong diagnostic accuracy (AUC value = 0.89).
Based on these findings we are currently testing whether the same NGPs can also be used to diagnose New World CL. The study is still blinded but preliminary results suggest a potential for these NGPs to also discriminate New World CL infections.

4 Conclusions

Our results so far indicate that several α-Gal NGPs can act as potential biomarkers for CL. Our goal is to create new NGP-based RDTs to screen for disease in areas of most need.

C0896 NEW MARKER FOR THE DIAGNOSIS OF VISCERAL LEISHMANIASIS IN ASYMPTOMATIC DOGS

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1 Background

Leishmania infantum is the etiological agent of zoonotic canine leishmaniasis (CanL) in Mediterranean basin, Asia, Africa and South America. Domestic dog (*Canis familiaris*) is considered to be the major reservoir of the parasite for humans and other mammalian hosts, making CanL not just a veterinary problem but also a public health issue.The prevalence and incidence of CanL are important epidemiological parameters for controlling transmission, which in turn depends on the reliable identification of infected dogs.More than half of the infected dogs do not show any clinical sign but are still capable of transmitting the parasite to the vector. Thus it is necessary to diagnose CanL as early as possible and give special attention to the asymptomatic infected dogs, to avoid the transmission of *Leishmania* from dogs to humans.

2 Methods

ELISA - based techniques were performed for the detection of antibodies against the recombinant protein *Leishmania infantum* cytosolic tryparedoxin peroxidase (*Lic*TXNPx) and a comparison of the results with those employing soluble *Leishmania* antigens from promastigote or amastigote forms and the homologue recombinant protein *L. infantum* mitochondrial TXNPx (*Lim*TXNPx). Moreover, we evaluated the diagnostic potential of *Lic*TXNPx for CanL in the Portuguese canine population and propose an improvement to the existing ELISA-based serological techniques by combining the *Lic*TXNPx and other antigens as a *Leishmania* antigen mixture.

3 Results

The data demonstrated that ELISA based on soluble promastigote or amastigote antigens had generally higher levels of sensitivity (82.9 % and 87.1 % respectively) for detection of antibodies in symptomatic or asymptomatic dogs than for detection of those against isolated recombinant proteins. Nevertheless, the specificities were found to be similar for all target antigens used. Importantly, the protein combinations using ELISA methodology improved the overall sensitivity, maintaining a high overall level of specificity when compare to recombinant antigens using in the diagnosis.

4 Conclusions

Overall, the obtained results showed that the strategy of combining *Lic*TXNPx with other well-defined *Leishmania* antigens, proved to be a sensitive and specific improvement to current serological diagnosis of CanL, being a useful tool for the detection of both clinical and subclinical forms of canine *Leishmania* infection.

C0897 GAMMA-INTERFERON, IL-6, CXCL8 AND IL-10 ARE EARLY MARKERS FOR VISCERAL LEISHMANIASIS EVOLUTION

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1 Background

Cytokines are related to the induction of intense inflammatory response during human VL's acute phase. Aimed to assess the evolution of the inflammatory activity during VL by measuring cytokine levels and to link it with the disease's clinical and laboratory evolution.

2 Methods

A prospective cohort study, composed by 36 patients with symptomatic VL. Clinical and laboratory evaluation were conducted by flow cytometry for INF-γ, IL-1b, IL-6, CXCL8, IL-10, IL-12, and TNF-α pre-treatment levels and at the 7th, 14th, 21st, 30th and 180th days of therapy.

3 Results

IFN-γ, IL-6, IL-10 and CXCL8 levels were early markers for VL therapy response, while IL-1b and TNF-a levels presented a delayed decrease. CXCL8 was related to thrombocytopenia; IL-6, CXCL8 and IL-10 were related to neutropenia ($p \leq 0.05$) and IL-6 was related to the lower albumin levels ($p \leq 0.05$). Higher IFN-γ levels before treatment was related to slower fever dissipation time ($p=0.04$) and higher TNF-a was related to splenomegaly persistence at the 21st therapy day ($p=0.04$).

4 Conclusions

The same TH1 and TH2 cytokine patterns described in severe VL patients is present among homogeneous populations with no severity symptoms. IFN-γ, IL-6, CXCL8 and IL-10 are good markers of the initial response to VL treatment.

C0899 OPTIMIZATION OF THE DNA EXTRACTION AND REAL-TIME PCR METHOD FOR DETECTION OF LOW PARASITEMIA LEISHMANIA INFECTIONS

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1 Background

Detection of low parasite levels can be challenging. In the context of a study on the prevalence of asymptomatic *Leishmania* infections in HIV-infected individuals in North West Ethiopia, we have optimized the DNA extraction and amplication to increase the sensitivity. We evaluated a manual and automated DNA extraction method and compared the sensitivity of two real-time PCRs.

2 Methods

The manual mini spin column-based DNA extraction (Qiagen) and the magnetic bead-based extraction with the LEV DNA kit on the Maxwell16 automate (Promega) were evaluated with two real-time PCRs targeting the small subunit ribosomal DNA (SSU) and the kinetoplast minicircles (kDNA) respectively on the Rotor-Gene Q cycler (Qiagen). The evaluation was performed on 1) serial dilutions of whole blood spiked with *Leishmania donovani* parasites; 2) whole blood and bone marrow samples from visceral leishmaniasis (VL) confirmed patients of the University of Gondar Hospital, Ethiopia (n=20) and of the Institute of Tropical Medicine in Antwerp, Belgium (n=7); and 3) on whole blood of HIV-infected adults living in VL-endemic region in Ethiopia (n=27). A PCR targeting the human Beta globuline (HBB) was used to evaluate the extraction efficiency and PCR inhibition. Cycle threshold (Ct)-values were used to interpret results with lower Ct-values corresponding to higher parasite DNA levels or to a more efficient DNA extraction and/or amplification.

3 Results

DNA extraction with the Maxwell showed better and more reproducible results when starting from 300 µL instead of 1 mL whole blood. The analytical sensitivity was at least 10 times higher with the Maxwell extraction compared to the Qiagen method. VL confirmed cases were more efficiently detected (decrease by 4 Ct-values) when extracted with the Maxwell16 automate than with the Qiagen method. Two of the 27 HIV-infected adults living in a VL endemic region were positive with both PCR methods. Ct-values were significantly lower with the kDNA PCR than with the SSU PCR in all samples tested.

4 Conclusions

These results suggest that the Maxwell DNA extraction in combination with the kDNA PCR showed improved sensitivity to detect low parasitemia, and is the method of choice to diagnose asymptomatic *Leishmania* infections. The automated extraction method is easy to perform, and requires minimal hands-on time, resulting in standardized processing of samples and less risk of contamination.

C0925 EVALUATION OF SKIN MICROBIOPSY AS A NOVEL DIAGNOSTIC TEST TO DETECT INFECTION IN THE SKIN OF DOGS INFECTED BY LEISHMANIA INFANTUM

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1 Background

In urban area, dogs are known to be the main reservoir of *Leishmania infantum* parasites, the etiological agent of visceral leishmaniasis (VL) in the Americas. For the diagnosis of this disease in dogs, molecular techniques are the most sensitive diagnostic tests, such as qPCR of spleen aspirates to detect *Leishmania* kDNA. However, the collection of this sample is invasive and presents risks if not performed by trained veterinarians. It was demonstrated that among the less invasive samples, the skin biopsy (SB) has about the same sensitivity as the spleen aspirate. Nevertheless, SB needs anesthesia and can also be infected by opportunistic bacteria, if not well treated after the procedure. The skin microbiopsy device (SMD) was recently developed for the detection of neoplastic and inflammatory skin diseases. It is even less invasive than conventional SB and the amount of skin sampled can be used for molecular techniques. Thus, the aim of this study was to evaluate the use of SMD as a diagnostic tool for canine VL and compare it with the normal SB.

2 Methods

For the study, 32 naturally infected dogs from an endemic area in Brazil, were selected based on their positivity on at least one diagnostic test, such as spleen culture, qPCR, or serology (by EIE or DPP). These dogs were classified as presenting clinical (22) and subclinical (10) infection. On each dog, SMDs were performed on sites of the skin with lesions (border of ulcers, induration and alopecia) and where the probability of sandflies to bite is high, such as on the muzzle and ears. On 13 of the 32 dogs, SB (3 mm punches) was performed in the scapular region. A qPCR to detect *Leishmania* kDNA was performed in the SMDs and SBs. Results obtained by SMD and SB were compared.

3 Results

Among the 32 dogs tested, 31% was positive by at least one of the SMD, being 90% with clinical infection and 10% with subclinical infection. Our results indicated that the muzzle was the best site for the detection of *L. infantum* kDNA by the SMD, representing 80% of the positive animals, followed by the left (50%) and right (30%) ears. Comparing the performance of SB on the scapular region and SMD on the muzzle, both detected 31% of the animals. The second best sample using SMD in comparison with SB was the left ear (23%), left shoulder (15%) and right ear (8%).

4 Conclusions

These results demonstrate that SMD can be used to diagnose dogs infected by *L. infantum* and the muzzle seems to be the best site in the skin to detect *Leishmania* kDNA.

C0951 INTERNATIONAL WORKSHOP ON THE STANDARDIZATION OF A REAL TIME PCR ASSAY FOR QUANTIFICATION OF PARASITE LOAD FROM LESION SAMPLES OF PATIENTS WITH CUTANEOUS LEISHMANIASIS IN THE AMERICAS

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1 Background

In the Americas, the most prevalent species causing cutaneous leishmaniasis (CL) are *Leishmania braziliensis*, *L. guyanensis, L. amazonensis* and *L. panamensis*. The diagnosis of the disease is performed by the combination of clinical, epidemiological and laboratorial data. Since the beginning of PCR application for diagnosing leishmaniasis, several methodologies were tested without consensus regarding protocols and molecular targets. With the support of PAHO/WHO and DNDi, an one-week international workshop was held in Medellin, Colombia, in December 2016, with the participation of experts from Argentina, Bolivia, Brazil, Colombia, Costa Rica, Mexico, Panama and Peru.

2 Methods

The workshop allowed the comparison between DNA extraction protocols, using a panel of *Leishmania* reference strains and skin lesions samples of golden hamsters and patients with CL. Parasite load were estimated by TaqMan real time PCR multiplex assays, targeting Leishmania 18S rDNA, kDNA and HSP70 genes (FAM/NFQ-MGB), all in multiplex with human RNAse P gene (VIC/TAMRA). Parasite loads were normalized by human DNA amount and results were expressed as parasites/tissue mass equivalents.

3 Results

According to a survey with the participants, *L. braziliensis* was the most prevalent species causing CL (38%), followed by *L. guyanensis* (25%), *L. panamensis* (13%), *L. lainsoni* (8%), *L. amazonensis* (4%), *L. mexicana* (4%) and *L. peruviana* (4%). Furthermore, the molecular target to detect *Leishmania* was 18S rDNA (56%), followed by kDNA (22%), Gp63 (11%) and G6PD (11%), but only 66.7% of the participants have estimated the parasite load by qPCR prior the workshop. Preliminary results with reference strains indicated a reportable range varying from 10^6 to 5 Par. Eq./mL for the 18S and kDNA targets and from 10^6 to 50 Par. Eq./mL for HSP70. For the human RNAse P gene, linearity from 10 to 10^{-3} ng/mL of human DNA was achieved, indicating a wide range to be used to quantify the parasite and normalize data by human DNA amount, an thus correcting the discrepancies between samples.

4 Conclusions

This workshop promoted the comparison of distinct parameters for the molecular detection of *Leishmania* spp. causing CL in the Americas, as DNA extraction methods and parasite targets for PCR. After a complete analysis of the results, it will be possible to establish a consensus qPCR assay, to infer the absolute normalized quantification of parasite load from skin lesion samples of patients with CL.

C0956 NONINVASIVE MOLECULAR DIAGNOSIS OF LEISHMANIASIS IN DOGS

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1 Background

The molecular diagnosis of leishmaniasis by PCR on limph node and bone marrow is accurate, but it is invasive; however, the lymph nodes regression after therapy makes difficult their collection.
Non-invasive sampling for parasite detection and quantitation is crucial for diagnosis and for monitoring *Leishmania* infection in dogs.
The object of the present study was to search for a noninvasive collection methods for detecting and quantitating *Leishmania infantum* DNA in saliva samples from naturally infected dogs living in endemic area, comparison to the performance of IFAT and lymph node aspirates, serum and congiuntival swab by real time PCR.

2 Methods

Leishmania infantum DNA detection was performed in biological sample from twenty dogs of different breeds and age, showing symptoms compatible with the infection, and fifteen leishmaniotic dogs undergoing anti-leishmanial treatments (meglumine antimoniate plus allopurinol/miltefosine plus allopurinol).

3 Results

The detection of *Leishmania* DNA in saliva samples by real time (qPCR) was possible in untreated dogs and after anti-*Leishmania* treatment.
The real-time PCR analysis showed higher levels of *Leishmania* DNA in the lymph node aspirates and saliva samples from all infected dogs than those measured in their congiuntival swab.

4 Conclusions

Overall, the results indicated that real time PCR-saliva is a new sensitive, non-invasive molecular method and could represent a good option for diagnosis of leishmaniasis in dogs and for monitoring infection in drug-treated dogs.

C0957 MULTICENTRIC STUDY OF DIPSTICK TEST FOR SERODIAGNOSIS OF VISCERAL LEISHMANIASIS AND DEVELOPMENT OF A DEFINED ANTI-LEISHMANIAL VACCINE FORMULATION FOR LONG LASTING PROPHYLACTIC IMMUNITY

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1 Background

Combating visceral leishmaniasis (VL) includes early diagnosis and devising safe and effective vaccination strategies. Diagnosis of VL relies largely on invasive sampling of tissue aspirates. Commercially available rK39-antigen based strip tests performs better in the Indian subcontinent but poor in East Africa and moderate in Latin America. After treatment, VL may also emerge as skin lesions called post kala-azar dermal leishmaniasis (PKDL). Development of an easy and reliable diagnostic tool and a preventive vaccine formulation for durable protection against VL is endeavored.

2 Methods

Enzyme-based dipstick test were developed for detection of leishmania specific antibodies from serum samples.Dipsticks were prepared for multicentric study in India, Nepal, Sri Lanka, Brazil and Spain. Serum samples were collected from VL patients, cutaneous leishmaniasis (CL) patients, endemic and non-endemic healthy controls and from diseases other than VL from each region. Cysteine protease C, a known virulent factor was cloned in mammalian expression vector and formulated in intra-lamellar MPLA bearing cationic liposome. Following determination of toxicity of the formulation, in vitro immune-stimulatory property of the formulation was assayed. In vivo vaccine potential of MPLA liposomal CPC was evaluated in BALB/c mice against virulent *Leishmania donovani* challenge infection and the immune correlates of protection were analyzed.

3 Results

In multicentric study, dipstick is 99.22% sensitive and 98.64% specific collectively for the diagnosis of VL. It does not cross react with CL samples. It is equally effective for PKDL diagnosis in the Indian subcontinent. Validation of dipstick in Sudan, Ethiopia and Iran is under process.
MPLA liposomal CPC was determined to be non-toxic, and reduced parasitic load in both liver and spleen of BALB/c mice through induction of a Th1 biased immune responses. Multiparametric analysis of splenocytes indicate that MPLA-cationic liposome facilitate expansion of both CD4 as well as CD8 polyfunctional T cells.

4 Conclusions

Dipstick is field adaptable, simple and affordable test for the serodiagnosis of VL and PKDL without having cross reactivity with CL and other diseases. MPLA- cationic liposome mediated delivery of CPC induces long lasting protective immunity against VL.

C0981 EFFICACY OF A REAL TIME PCR FOR LEISHMANIA DETECTION AND IDENTIFICATION BY MELTING CURVE ANALYSIS

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1 Background

Leishmaniasis is an emergent disease, endemic in 98 countries worldwide. There are more than twenty infective *Leishmania* species, which can cause various clinical forms of the disease including asymptomatic cases. The knowledge of the infecting specie is important to manage the treatment and prognosis of disease. Many molecular methods have been useful to detect and discriminate the *Leishmania* species. The aim of this study was evaluate the capacity to discriminate *Leishmania* subgenera or species using a SYBR green-based qPCR assay followed by melting temperature analysis.

2 Methods

The LRIB-F and LRIB-R primer pair targeting 167 bp of *Leishmania* small subunit rRNA gene was designed according to GenBank sequences. Sensitivity and efficiency of the qPCR assay using the designed primers were performed with DNA from *Leishmania* and *Viannia* subgenera reference strains. After standardization, melting curve analysis of the amplified fragment was evaluated at specie and subgenus level. DNA samples from cutaneous, mucocutaneous and visceral leishmaniasis were tested.

3 Results

qPCR assay with the new primers had an optimal efficiency and sensitivity equal to 0.03 pg of DNA/reaction tube for all *Leishmania* strains tested. Melting analyses of qPCR product showed a significant difference between melting temperature (Tm) of the two subgenera *Leishmania (Viannia)* (average Tm: 87.93±0.03 ºC) and *Leishmania (Leishmania)* (average Tm: 86.64 ± 0.24 ºC) ($p < 0.01$). The Tm obtained with the analyses of all strains was plotted on a scale, in which the strains were grouped according to the form of leishmaniasis. Were found significant difference between new world visceral (NW-VL) and cutaneous (NW-CL)/mucocutaneous (NW-ML) leishmaniasis ($p < 0.01$); NW-CL and old world visceral (OW-VL)/cutaneous (OW-CL) leishmaniasis ($p < 0.01$); and NW-ML and OW-VL/OW-CL ($p < 0.01$). The average Tm of cutaneous, mucocutaneous and visceral leishmanisis samples tested were 87.83, 87.81 and 86.53 ºC, respectively. These Tm were plotted on the scale and the samples were recognized as NW-CL, NW-ML and NW-VL.

4 Conclusions

The assay showed high efficiency and sensitivity for detection of *Leishmania*, introducing a new idea for species identification, considering the form of the disease and a scale of melting temperature.

C0996 BIOTECHNOLOGICAL ADVANCES IN THE DIAGNOSIS OF CANINE VISCERAL LEISHMANIASIS: A MULTIPLEX SEROLOGY BY FLOW CYTOMETRY

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1 Background

Canine Visceral Leishmaniasis (CVL) due to *Leishmania infantum* is a major global zoonosis. An accurate diagnosis of CVL is an essential control measure, and it is often made by the detection of specific serum antibodies through several serological techniques. Herein, we describe an innovative flow cytometry serological test based on the use of functional microspheres from Cytometric Bead Array (CBA) Flex Set System, which were coated with recombinant antigens.

2 Methods

The recombinant antigens rLci1A and rLci2B were selected due to their potential in diagnostic testing for CVL. The microspheres A4 and E4 were chosen in the Cytometric Bead Array (CBA) Flex Set configurable kit, and were respectively coated with rLci1A and rLci2B antigens, forming the microspheres–protein antigenic systems A4–rLci1A and E4–rLci2B. An individual and a combined multiplex serological assay were performed, and the accuracy was assessed in a set of 150 canine serum samples.

3 Results

The A4–rLci1A microspheres–protein antigenic system showed sensitivity of 85.0% in *L. infantum*-infected asymptomatic dogs, and 95.0% in oligosymptomatic and symptomatic. The specificity in dogs infected by *L. braziliensis* was 100.0%, while in dogs infected with *Ehrlichia canis* it was 30.0%, and 20.0% in *Babesia canis* samples. Vaccination by Leishmune® as well by Leish-Tec® leads to a specificity of 70.0%, and regarding LBSap vaccine, it was 80.0%. The E4–rLci2B showed sensitivity of 85.0% in asymptomatic dogs, and 100.0% in oligosymptomatic and symptomatic. The specificity was 80.0% in dogs infected with *L. braziliensis* as well as *E. canis*, and 60.0% considering *B. canis*. In the vaccinated dogs, the specificity was 80.0% regarding Leishmune® and Leish-Tec®, and 60.0% for LBSap. The multiplex serology through the combination of A4–rLci1A and E4–rLci2B showed sensitivity of 85.0% in asymptomatic dogs, and 100.0% in the oligosymptomatic and symptomatic. The specificity was 100.0% in dogs infected with *L. braziliensis*, 70.0% in *E. canis*, and 60.0% regarding *B. canis*. Considering the vaccinated dogs, the specificity was 100.0% to Leishmune®, Leish-Tec®, and LBSap.

4 Conclusions

The multiplex serology resulted in greater accuracy. It was marked by high sensitivity in subclinical and clinical forms of CVL, and with specificity characterized by an absence of false-positives in *L. braziliensis* serum samples as well as in the animals that underwent vaccination.

C1004 THE POTENTIAL IMPACT ON TRANSMISSION OF EARLIER DIAGNOSIS FOR VISCERAL LEISHMANIASIS IN THE INDIAN SUB-CONTINENT.

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1 Background

Timing of use and accuracy of diagnostics are key in visceral leishmaniasis (VL) control and elimination. Due to the low specificity of rK39 and the complexity of delivering treatment, diagnosis of VL requires the presence of more than 2 weeks fever, an enlarged spleen and a positive rK39 test. This intrinsic 14 day delay in diagnosis means that a large reservoir is not appropriately treated and continues to propagate transmission.

2 Methods

We used empirical data on health-seeking behaviour and health-system performance from the Indian state of Bihar, Bangladesh and Nepal to parameterize a mathematical model. Diagnosis of cases is key to case management, control and surveillance. Treatment of cases prevents onward transmission, and we show that the differences in time to diagnosis in these three settings explain the observed differences in incidence.

3 Results

Shortening the time from health-care seeking to diagnosis is likely to lead to dramatic reductions in incidence in Bihar, bringing the incidence down to the levels seen in Bangladesh and Nepal. This will be especially important as prevalence decreases because the immunity of the population to VL is likely to reduce, and large epidemics may ensue if control is not maintained. We explored the possibility of diagnosing patients before the onset of clinical VL, and show that this could have a marked impact on incidence, however, limited spec¬ificity is a major barrier to such a strategy.

4 Conclusions

Our modelling shows that a VL test with high specificity that enables treating patients early, i.e. before 14 days of fever, would reduce transmission of disease by eliminating a pool of infectious patients. Even a test with a sensitivity of just 30%, would have a dramatic effect of the transmission of KA and prevent its resurgence when paired with the passive health-care system and tests of high sensitivity, such as the test for rK39 antibody response. We will review recent published data on early diagnosis and treatment delays.

C1047 N-GAL AS AN EARLY BIOMARKER OF CHRONIC KIDNEY DISEASE IN DOGS INFECTED WITH LEISHMANIA INFANTUM

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1 Background

Canine visceral leishmaniasis is a protozoan disease endemic in the Mediterranean area. It is caused by *Leishmania infantum* and transmitted by female phlebotomine sand flies. The disease causes glomerulonephritis and chronic kidney disease (CKD) by immune complex deposition that may lead to end-stage renal failure. Kidney disease is usually investigated by determination of BUN (blood urea nitrogen) and creatinine levels in serum; however, they are not sensitive enough for identifying early renal failure. New markers able to detect initial renal failure in dogs could avoid the progression to irreversible renal damage.
In human, the lipocalin protein associated with neutrophil gelatinase (N-GAL) is described as an early marker of acute kidney injury and its levels are related to the severity of the injury. In dogs, urinary NGAL concentrations seem to be well correlated to serum creatinine level, glomerular filtration rate and proteinuria. Therefore, its measure might be a good marker of renal injury, nephrotoxicity and other nephropathies.
The aim of this work was to evaluate the association between urinary N-GAL concentrations in dogs with leishmaniasis and the different stages of the renal disease caused by the parasite.

2 Methods

For this purpose, 21 dogs experimentally infected with *Leishmania infantum* were classified into four groups on the basis of the results of urinary protein/creatinine ratio (UPC) and serum creatinine as follow: Group 1: non proteinuric, nonazotemic (n=5); Group 2: borderline proteinuric, nonazotemic (n=5); Group 3: proteinuric, nonazotemic (n=11); Group 4: proteinuric and azotemic (n=0), according to the guidelines of the International Renal Interest Society (IRIS).
Urinary N-GAL concentration was measured with a dog N-GAL ELISA kit (BioPorto Diagnostics). Then, urinary N-GAL/creatinine ratio (UN-GAL/Cr) was calculated.

3 Results

This study shows that urinary N-GAL concentration in dogs infected with *Leishmania infantum* increased proportionality to renal damage. Furthermore, UN-GAL/Cr presented statistically significant differences between groups, detecting the higher N-GAL concentration and UN-GAL/Cr in dogs of Group 2 (borderline proteinuric and non-azotemic) and Group 3 (proteinuric and non-azotemic).

4 Conclusions

According to the results, urinary N-GAL protein is able to detect renal damage in dogs caused by leishmaniasis. Moreover, UN-GAL/Cr seems to demonstrate renal injury earlier than the UPC. So, it could be recommended as an early biomarker of CKD.

C1052 DEVELOPMENT AND EVALUATION OF TWO URINE-BASED IMMUNOLOGICAL ASSAYS AND IDENTIFICATION OF URINE REACTIVE PEPTIDES THROUGH COMPARATIVE PROTEOMICS APPROACH AND EPITOPE MAPPING: STEPS TOWARD NONINVASIVE DIAGNOSIS OF VISCERAL LEISHMANIASIS

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1 Background

Visceral Leishmaniasis (VL), a relentless parasitic infection which comes under neglected tropical diseases, could be fatal if the delayed occurred in diagnosis. Post kala-azar dermal leishmaniasis (PKDL) a skin related manifestation possibly acts as reservoir of VL transmission. Routine diagnosis of VL and PKDL such as tissue aspiration and serological tests are mostly invasive and incapable of assessing treatment response. Therefore, a noninvasive diagnostic test and identification of a single peptide or epitope for VL diagnosis additionally, to determine cure would be an advantage in disease management and control.

2 Methods

We developed two immunological assays, ELISA and dipstick test for the detection of *Leishmania* promastigote membrane antigen (LAg)-specific antibodies in human urine samples. Reactivity of different polypeptides of LAg with the urine was determined through immunoblot assay and electroeluted for ELISA and identified through mass spectrometry. Immunoreactive polypeptides were subjected to clone for expression of recombinant antigens and synthetic peptides were generated after B cell epitope mapping.

3 Results

The respective sensitivities and specificities found were 97.94% and 100% in urine ELISA, and 100% and 100%, in dipstick assay for VL diagnosis. PKDL cases were 100% positive in both the tests. ELISA and dipstick test illustrated treatment efficacy in 90% of total VL cases after at least six months of treatment. Polypeptides present in LAg with relative molecular masses of 34, 36, 51, 55, 63 and 72 kDa showed reactivity specific to active VL urine. Reactive polypeptides of LAg were identified as LACK, Cystein protease, Elongation factor 1α, α-tubulin, Glycoprotein and HSP 70 and cloned for expression. Reactivity of cloned antigens and their B cell epitope mapped synthetic peptides was compared with infected urine samples.

4 Conclusions

Urine-basedELISA and dipstick test perform immensely effective for diagnosis of VL and PKDL. Utility of these indirect methods of monitoring parasite clearance after therapy can define infected verses cured. Identification and characterization of urine reactive leishmanial antigens, their clones and synthetic epitopes lead to achieve an ideal antigen for urine based noninvasive diagnosis of VL. It can be implemented for interventions in disease management of VL particularly in poor resource outskirts.

C1072 EVALUATION OF A NOVEL RECOMBINANT T-CELL MHC-II LINEAR MULTIEPITOPE PROTEIN FOR THE DIAGNOSIS OF HUMAN CUTANEOUS LEISHMANIASIS

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1 Background

Cutaneous leishmaniasis (CL) is an important endemic disease in Latin America. Parasitological methods for CL diagnosis are insufficiently sensitive, while the highly sensitive PCR-based methods are not readily applicable in the field. Traditional serological diagnosis using total crude leishmanial antigen (TSLA) is hampered by variable sensitivity and cross-reactivity. Recently, the use of synthetic peptides or multiepitope proteins showed potential for improved immunodiagnosis of leishmaniasis, with most studies focusing on human or canine visceral disease. Here, we produced and tested a recombinant multiepitope protein comprising linear B-cell epitopes, as a means to improve the serological diagnosis of CL.

2 Methods

Epitopes were predicted by bioinformatics tools from the genome database of *Leishmania braziliensis*, as linear T-cell MHC-II epitopes located on the surface of convex conformational domains exposed in secreted proteins. From 19 predicted epitopes, 9 were selected based on their surface exposure. DNA sequence conservation analysis within regions encompassing the selected epitopes was performed in 117 *L.* (*Viannia*) strains. The synthetic gene encoding the multiepitope protein (Multi2) was expressed in *E. coli*. This antigen was tested in an indirect enzyme-linked immunosorbent assay (ELISA) format and its performance compared with TSLA. Thirty-six serum samples from CL patients, 47 from healthy volunteers, and 10 from patients with Chagas disease were tested. Receiver-Operator Characteristics (ROC) curve analysis was used to identify the effectiveness of the two antigens for diagnosis of CL.

3 Results

There was good epitope DNA sequence conservation across *L.* (*Viannia*) strains analyzed (few indels and 4 synonymous SNPs were predicted in 2 and 4 epitopes respectively). The Multi2-ELISA showed higher sensitivity (94.4%, [95% confidence interval (CI), 90.7%-100%]) than TSLA-ELISA (88.9%, [95% CI, 85.2%-99.5%]). Comparable specificities were obtained for Multi2-ELISA (95.7%, [95% CI, 91.1%-100%]) and TSLA-ELISA (97.9%, [95% CI, 93.2%-100%]). There were no cross-reactions with chagasic patients' sera by Multi2-ELISA (0%, [95% CI, 0%-19.5%]), as compared to TSLA-ELISA (100%, [95% CI, 89.9%-100%]). The area under the ROC curve was 99.2% with Multi2-ELISA and 97.3% with TSLA-ELISA.

4 Conclusions

This multiepitope approach offers a promising alternative option for human CL diagnosis, with the potential to circumvent the drawbacks of TSLA-based assays.

C1073 UTILITY OF ANTIGEN-SPECIFIC SERUM ANTIBODIES FOR THE DETECTION OF ASYMPTOMATIC L. INFANTUM INFECTION AND SYMPTOMATIC VISCERAL LEISHMANIASIS AMONG DOGS IN TERESINA, BRAZIL.

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1 Background

Leishmania infantum can establish either an asymptomatic infection in dogs or can manifest as canine visceral leishmaniasis (CaVL). Parameters that readily detect infected but clinically healthy (asymptomatic) dogs, while simultaneously distinguishing them from diseased, symptomatic dogs would be invaluable for use in control programs and for providing appropriate guidance for dog owners. In an attempt to identify such markers, we evaluated serum samples using serum bank of Piaui, Brazil prepared from dogs with known infection status for the presence of antibodies against the previously defined recombinant antigens.

2 Methods

Serodiagnosis was performed by ELISA and the recombinant antigens used were rK28, TR18 and KR95, kindly provided by IDRI. Sera samples from high endemic area in the Northeastern of Brazil (Piaui), with different clinic status were used in this study as describe: 77 symptomatic CaVL, 56 asymptomatic CaVL and 252 negative samples. Results were processed using Graph Pad software.

3 Results

As expected, our data indicate that, unlike uninfected dogs, the vast majority of dogs with symptomatic CaVL have circulating antibodies against these antigens. The majority of infected but asymptomatic dogs also showed a high levels of antigen-specific antibodies, and these were detected at levels similar to those observed in the diseased dogs.

4 Conclusions

Thus, while these particular antigen-specific antibody responses are useful for detecting *L. infantum* infection in dogs, they do not provide the discrimination required to differentiate infection from disease and cannot therefore be used as prognostic indicators for the progression of infection to disease.

C1089 LANCET SCRAPING IS BETTER THAN FILTER PAPERS AND BIOPSIES FOR THE DETECTION OF LEISHMANIA (VIANNIA) SPECIES

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1 Background

Molecular tools for identification of human cutaneous leishmaniasis caused by *Leishmania (Viannia)* subgenus have different accuracies depending principally of the type of sample. Our aim was to compare the performance of three different collection methods: punch biopsies (Bx), lancet scrapings (L) and filter paper imprints (FP) on two molecular assays: 1) kinetoplast DNA-based polymerase chain reaction (kDNA-PCR) for parasite detection and 2) FRET probes-based Nested Real Time PCR (Nd-RT-PCR) for determination of the infecting species, in order to establish the most reliable procedure to apply in the point of care.

2 Methods

A minimum sample size was determined in the R platform (assuming 95% of sensitivity with 0.05 of significance and 80% of test power). All samples were collected from the Amazonian rainforest of Peru. In order to make a reliable assessment of accuracy, each result was classified as positive if any of the samples were positive by kDNA-PCR. In addition, we defined a positive result by Nd-RT-PCR when two markers were amplified (mannose phosphate isomerase and 6-phosphogluconate dehydrogenase). The best sampling method for each assay was determined by the McNemar test. Finally, sensitivity and specificity of both molecular approaches per sample type were estimated on the Caret package.

3 Results

The analysis of 78 subjects (234 samples) found evidence that the sampling method has a direct influence on species-specific Nd-RT-PCR diagnosis. In this sense, we observed a better sensitivity for L (83.3%, p-value=0.017) compared with FP (70.0%) and Bx (66.7%). In contrast, we did not find significant differences in the genus based kDNA-PCR in any of the methodologies used (p-values greater than 0.05 for Bx and FP versus L).

4 Conclusions

Our results indicate that lancet scraping is the best method for *Leishmania* species identification by Nd-RT-PCR over Bx and FP and emphazise the importance of the sampling method in the performance of molecular genotyping of *Leishmania*. The difference among sampling methods needs to be addressed at the point of care to ensure proper diagnosis, especially in endemic areas with multiple circulating species.

C1099 COMPARATIVE EVALUATION OF SEROLOGICAL AND MOLECULAR TECHNIQUES FOR EARLY DETECTION OF CANINE VISCERAL LEISHMANIASIS INFECTION

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1 Background

Canine visceral leishmaniasis (CVL), an endemic disease in Spain, is caused by *Leishmania infantum*. It is transmitted by vectors, and affects mainly dogs causing progressive lesions and even death.
The aim of this study was to validate and to compare molecular diagnosis techniques (Real time PCR or qPCR) from four different samples and three serological techniques: a commercial ELISA kit, an *in house* Indirect Immunofluorescence (IFAT), and Direct Agglutination Test (DAT).

2 Methods

A total of 29, six months old, pathogen free *Beagles*, were infected with 10^8 promastigotes in sterile PBS injected through the cephalic vein. Serum samples were used for serology and whole blood EDTA, bone marrow, popliteal lymph nodes samples and conjunctival cotton swabs for qPCR using the SYBR Green dye premix. Primers were designed based on the minicircles of kinetoplast for *L. infantum*. For the EDTA blood samples, 200 µl were used for DNA extraction, and from the tissues a 20 mg were used for extraction from the bone marrow and from the lymph node. Two cotton swabs were used for DNA extraction.

3 Results

Serology results indicated that the commercial ELISA test has the highest sensitivity (82.8%) compared to DAT (51.7%) and IFA (65.5%) tests. IFAT and ELISA showed a moderate degree of concordance (k=0.592). Real Time PCR gave positive results from each type of samples from the 29 animals. Interestingly, the optical density values from the ELISA test and the CT values from qPCR from swab samples showed an inverse correlation: a lower CT value corresponded to a higher OD value (R^2=0.524, $p<0.001$). Medium CT values from bone marrow and lymph nodes were significantly similar (Student's t-test <0.77), media 25,69±3.7 and 25,39±4.25, respectively. They significantly differ from the CT from blood samples (33.36±1.5) (Student's t-test <0.001) and from cotton swabs 30.84±2.66 (Student's t-test <0.01).

4 Conclusions

qPCR detects kDNA of *L. infantum* from different samples before the animal had developed a detectable antibody response. It is reasonable to use blood or swab sample for early detection of *Leishmania* for epidemiological studies.

C1118 MOLECULAR ANALYSIS BASED ON DNA DISSOCIATION TEMPERATURE TO DETERMINE LEISHMANIA SPECIES

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1 Background

Leishmaniasis is a widely distributed disease present in Old and New World, and is caused by different species of *Leishmania* parasites. These species are associated with distinct transmission cycles, clinical manifestations and therapeutic responses. Consequently, the correct identification of infecting species is of utmost importance. The diagnostic of leishmaniasis is based on the combination of clinical, epidemiological and laboratory data, but mainly through the cultivation of the parasites, which can be followed by the isoenzyme assay for species identification. Isolation of the parasite, however, provides low efficiency and requires time and resources that can be minimized by using a molecular approach directly in clinical material, allowing even the detection of mixed infections and hybrid strains. Several studies focusing on the diagnostic and *Leishmania* species identification were performed, but this goal has not been properly achieved yet. In this context, molecular techniques such as *High Resolution Melting* (HRM) analysis has a great potential for this purpose, since it allows investigation of the entire region covered by primers, sensitive to even one polymorphism. Thereby, the aim of this work is to establish a methodology based on the HRM that exempts parasite isolation and cultivation, as well as allows the detection and identification of Leishmania species in different clinical materials.

2 Methods

For this, initially, to the standardization of the technique, the DNA of 16 *Leishmania'* strains, representing 14 species, was submitted to HRM reactions with different targets, such as *HSP70* and *COI*. All experiments were performed in duplicate, with technical triplicates for each strain. Furthermore, this 16 strains were also submitted to conventional PCR and sequencing.

3 Results

HSP70 and *COI* reactions were standardized and the results showed that *COI* primers were more effective in distinguishing species, such as *L. panamensis* from *L. guyanensis* and *L. shawi,* as well as *L. infantum* from *L. donovani*.

4 Conclusions

HRM assay has shown great potential to differentiate main *Leishmania* species, which may become a potential tool for molecular direct diagnosis of leishmaniasis. However, the use of other targets is still needed, in order to supplement the obtained data, as well as the accomplishment of other tests; such activities represent the next steps of this project, with the final aim of developing an algorithm for molecular typing of *Leishmania* species with different targets.

C1124 L. (V.) PANAMENSIS AND L. (V.) GUYANENSIS DISCRIMINATION BASED ON HSP70 NESTED PCR-RFLP OF SAMPLES FROM CUTANEOUS AND MUCOSAL LEISHMANIASIS PATIENTS

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1 Background

Amplification of *hsp70* gene has been widely used to identify New World Leishmania species and allows discrimination between *L. (V.) panamensis* and *L. (V.) guyanensis*. However, the positivity of the PCR in clinical samples with confirmed diagnosis of mucosal and cutaneous leishmaniasis, is influenced by the number of *hsp70* gene copies and the parasite load. Nested PCR allows to improve the amplification of *hsp70* gene in clinical samples.

2 Methods

In this study, we included thirty samples from patients with confirmed diagnosis of mucosal (biopsy) and cutaneous (non-invasive samples)leishmaniasis identified as *L. (V.) panamesis / L. (V.) guyanensis* by PCR-RFLP miniexon gene. Amplification of *hsp70* gene by conventional PCR was not detected in these samples. *hsp70* conventional PCR was performed on all samples using Fw: (5 'GACGGTGCCTGCCTACTTCAA 3') and Rv: (5 'CCGCCCATGCTCTGGTACATC 3') primers. Amplification products were purified and the following internal primers were used for the second round of amplification: Fw (5'-ACTTCAACGACTCGCA GCGCCA-3') and Rv (5'-ATCGGGTTGCATGTGCTCTCCA- 3'); an aliquot of PCR products of this second round were sequenced and another digested with *BccI*. DNA from reference strains of Viannia and Leishmania subgenus were used as positive controls. DNA from clinical samples with suggested diagnosis of mucosal leishmaniasis, but another different confirmed diagnosis was used as negative control.

3 Results

Amplification of *hsp70* gene was not observed in samples at the first round of PCR. This result confirmed the finding described above. *hsp70* gene amplification was obtained in all samples with nested PCR. RFLP allowed the identification of *L.(V.) panamensis* and *L. (V.) guyanensis* in 83.3% and 16.7 % of the evaluated samples respectively. PCR-RFLP and sequencing were concordant in all samples included (Cohen's kappa coefficient of 1).

4 Conclusions

The nested PCR-RFLP for hsp70 amplification is a useful test for diagnosis of leishmaniasis and typing of *L. (V.) panamensis* and *L. (V.) guyanensis* species from non-invasive clinical samples and mucosal biopsies.

C1161 EARLY PRODUCTION OF GROWTH FACTORS AS BIOMARKERS OF POSITIVE RESPONSE TO TREATMENT IN HUMAN CUTANEOUS LEISHMANIASIS.

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1 Background

The efficacy of an antileishmanial drug is established based on clinical features of the lesion being cure determined by wound healing and re-epithelialization, a process that is controlled by growth factors, mainly epidermal growth factor (EGF), fibroblast growth factor (FGF), connective tissue growth factor (TCGF), vascular endothelial growth factor (VEGF), transforming growth factor-β (TGF β) and transforming growth factor α (TGF α). Because we observed previously in the cutaneous leishmaniasis model in hamsters (*Mesocricetus auratus)* that in the week two of treatment the profile of expression of the different factors and the levels of expression can predict the therapeutic response to the treatment, in this work we focused in validating these results in samples from human cases of cutaneous leishmaniasis.

2 Methods

Ten patients with positive diagnosis of cutaneous leishmaniasis were included. A scrape was taken from the ulcer before starting the treatment and a second samples was obtained in the day 15 of the treatment. RNA was purified using High pure RNA isolation kit (Roche®) and the levels of expression of each factor were measured by qRT-PCR using Verso one step probe (Thermo Scientific®) and factor induction level was calculated using the delta delta CT method.

3 Results

Before treatment, all patients expressed only connective tissue growth factor and for the week two of the treatment they expressed FGF, TCGF and EGF, the level of induction was only calculated for TCGF and the level of induction was 2.034 ± 0.3 because other factors were not detected at the start of the treatment.

4 Conclusions

The profile of production of the growth factors is associated to the outcome of the treatment and this profile may be used as an alternative for early prediction of the result of the treatment. However the validation in cohort studies is needed.

C1175 IMPROVEMENTS ON THE DETECTION OF LEISHMANIA SP. IN NATURALLY INFECTED DOGS BY REAL TIME PCR: A DUPLEX AND READY-TO-USE REACTION FORMAT

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1 Background

Canine visceral leishmaniasis is usually diagnosed by serological tests, but these methods present limitations in accuracy. Improvement in assessing *Leishmania infantum* infection in dogs is needed. Quantitative PCR (qPCR) is an accurate diagnostic technique which can also quantify parasite load. qPCR, however, presents limitations that hinder its implementation in diagnostic routines. An absolute requirement for freezer storage of qPCR reagents is one of them. The need for a second reaction assessing internal controls, such as constitutive genes, to avoid false-negative results, can also raise the reaction cost. We aimed to develop a gelified duplex qPCR for the concomitant identification of a canine constitutive gene and *Leishmania* DNA in dog's samples.

2 Methods

A new reaction for detection of the 18S canine gene was combined to an established qPCR that amplifies a conserved sequence of *L. infantum* kinetoplast DNA (kDNA) to create a duplex qPCR. This reaction was tested using blood, spleen or skin samples from 82 dogs from an endemic area. The duplex reaction performance was compared to the traditional singleplex format. Additionally, a gelified, freezer-free duplex format was also produced and compared to the regular, liquid/frozen format. For qPCR accuracy comparison, samples were characterized by standard parasitological and serological protocols.

3 Results

The new duplex qPCR exhibited the same detection limit of 0.1 parasites/reaction as the singleplex reaction format. Such limit of *L. infantum* kDNA detection was observed for samples from all three tissues evaluated. The gelified format showed a limit of detection of 1 parasite/reaction without affecting the reaction efficiency. Our results showed that spleen aspirates samples provided the higher positivity rate (92.9%), followed by skin (50%) and blood samples (35.7%). Our results demonstrated that amplification of *Leishmania* and canine DNA in a single reaction exhibited the same sensitivity and specificity as the amplification of *Leishmania* kDNA alone.

4 Conclusions

Detection of host genes strengthens qPCR results by confirming DNA presence in the samples and the absence of polymerase inhibitors. We also presented the gelified qPCR format that allows reagents to be stored on the plate. The gelified duplex qPCR showed similar performance as the regular format and presents advantages as the reduction of the hand-on-time to set up the test while reducing the chance of operator's mistakes.

C1180 PRODUCTION OF ANTIBODIES AGAINST SALIVARY PROTEINS OF LUTZOMYIA LONGIPALPIS IN DOGS FROM AN ENDEMIC AREA, A LONGITUDINAL STUDY

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1 Background

Sand fly salivary proteins play a major role in *Leishmania* transmission in endemic areas. Results from studies on human and canine antibody response against sand fly saliva demonstrated high immunogenicity of salivary proteins. Previous findings from a cross-sectional study of our group showed no association between reactivity to salivary proteins and severity of canine visceral leishmaniasis (CVL). However, no data are linking the development of vector's anti-salivary antibodies with development of CVL in dogs in a longitudinal study. The aim of this study was to evaluate the reactivity of dog sera to rLJM11 and rLJM17, that can act as markers for exposure to biting sand flies, in a canine cohort study of one year.

2 Methods

For the study, 72 dogs from an endemic area in Brazil, were selected based on their seronegativity to sand fly saliva antigens evaluated by ELISA. Antibodies were measured by ELISA using two recombinant proteins (rLJM11 and rLJM17) from *Lutzomyia longipalpis* saliva. The 72 dogs were followed up by one year. Every six months, they were clinically evaluated, and classified as dogs with CVL clinical signs and those without clinical presentation, using a clinical score ranging from 0 to 24. CVL diagnosis was performed by splenic aspirate culture and qPCR or serological tests. The median of the clinical score was calculated considering dogs with CVL positive results, stratifying them by reactivity to salivary proteins.

3 Results

Among the dogs followed, 78 % became reactive to rLJM11 and rLJM17 after six months, and 23% remained negative. After 12 months, 93% became positive for the salivary proteins, while 7% remained negative. During the follow-up of the dogs, some dogs presented reactivity against rLJM11 and rLJM17 and a negative result for LVC, and in the following evaluations they maintained the positivity for the vector saliva proteins, and they became positive for LVC. Our results reinforce our previous report that there were not association between salivary proteins reactivity and CVL severity. The median of the clinical score did not differ among dogs exposed and non-exposed during the follow-up.

4 Conclusions

Utilization of these two recombinant proteins, as biomarkers, would be beneficial as epidemiological tools for screening canine sera in endemic areas of visceral leishmaniases for vector exposure that can aid in evaluating the efficacy of vector control measures and for estimating the risk of *L. infantum* transmission in dogs.

C1187 ASSESSMENT OF CROSS-REACTIVITY FOR TRYPANOSOMA CRUZI CHIMERIC PROTEINS IN DIAGNOSIS OF CHAGAS DISEASE IN INDIVIDUALS WITH CUTANEOUS LEISHMANIASIS FROM A CO-ENDEMIC AREA FROM BRAZIL

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1 Background

A common pitfall of serological diagnose of Chagas disease is the potential cross-reactivity with antibodies from patients infected with *Leishmania* spp, which are co-endemic throughout large regions in tropical countries. A strategy to overcome this limitation and increase the sensibility involve the construction of new molecules using repetitive fragments of *T. cruzi* conserved proteins. Our objective was to assess the cross-reactivity with sera from individuals previously diagnosed as positive for cutaneous leishmaniasis (CL).

2 Methods

T. cruzi sequences were selected, and four chimeric constructs (IBMP-8.1, IBMP-8.2, IBMP-8.3 and IBMP-8.4) were optimized and synthesized for recombinant production in *Escherichia coli*. IBMP cross-reactivity was evaluated using in-house ELISA with 400 positive individuals for CL. The sera were also screened by three commercial *T. cruzi* EIA and by one indirect immunofluorescence test (IIF) and their performance compared to those obtained for IBMP chimeras.

3 Results

Based on the total number of samples, IBMP-8.1, IBMP-8.2, IBMP-8.3 and IBMP-8.4 chimeras cross-reacted with 2.25%, 3.25%, 1.75% and 1.00%, respectively. Inversely, for ELISA Chagas III (BIOSChile, Ingeniería Genética S.A., Chile), which uses whole extracts of *T. cruzi* strains Mn and Tulahuen as antigens, 265 (66.25%) samples were found to be the cross-reactivity. Gold Elisa Chagas (Rem, Brazil), which uses both recombinant antigens and purified lysates from Brazilian strains of *T. cruzi* epimastigotes, cross-reacted with 31 sera (7.75%). However, the Imuno-ELISA Chagas (Wama Diagnóstica, Brazil), which is based on recombinant antigens, yielded 0.75% of cross-reactivity. Interestingly, from 400 samples only 144 (36.00%) cross-reacted by IgG IIF (>1:40) (Immunocruzi, bioMerieux Diagnostics SA, Brazil). Considering that this methodology employs lyophilized *T. cruzi* epimastigotes as antigenic matrix, we expected similar results those found by ELISA Chagas III. Serological cross-reactivity for IBMP proteins was not surprising. Indeed, these molecules are composed of specific *T. cruzi* fragments, and a modest similarity to human nonpathogenic microorganisms was observed when the amino acid sequences were screened in the NCBI database using the protein BLAST software.

4 Conclusions

Our data support the idea that IBMP chimeras may be used in areas of co-endemicity between *T. cruzi* and cutaneous leishmaniasis. The next step is to assess cross-reactivity with visceral leishmaniasis.

C1196 ACCURACY DIAGNOSTIC STUDY OF BONE MARROW ASPIRATE AND RK39-ANTIGEN SEROLOGY FOR VISCERAL LEISHMANIAIS, IN PARAGUAY

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1 Background

Visceral leishmaniosis is an endemic disease in Paraguay. Correct diagnostic must be the goal to confirm the case, to lead to an appropriate treatment. Smears stained with Giemsa of bone marrow aspirate are used commonly to diagnostic visceral leishmaniasis cases. The bone marrow aspirate procedure do not involve great danger for the patient, but it requires an expert to take it. Nevertheless serological test, preformed with rk39 antigen, is a very practical and useful tool to make a quick and easy test.

2 Methods

We evaluate the accuracy of the diagnostic made by smears stained with Giemsa of bone marrow aspirate and serological rk39 test. We include 1,492 visceral leishmaniasis suspected patients.

3 Results

Sensitivity of bone marrow aspirate for all the patients was 88,4 % (86.8-90.0%) and specificity 99.8% (99.6-100.0%). However, rk39 test had a sensitivity of 91.1% (88.9-93.3%) and a specificity of 83.7% (80.5-86.6%). Likelihood positive ratio for bone marrow aspirate was 626 and for rk39 test, 5.59. Bone marrow aspirate had a sensitivity for HIV/AIDS patients (94.7-98.7%) and for children smaller than one year (85.5-97.9%), meanwhile rk39 test had lower sensitivity for both populations (75.2-90.4% and 58.5-81.7% respectively).

4 Conclusions

Smears stained with Giemsa of bone marrow aspirate is an excellent method to diagnostic accurately visceral leishmaniasis cases as it had a complete accuracy performance. Rk39 serological test had a lower sensitivity, especially for HIV/AIDS and little children, so it can be used routinely as a diagnostic complement in not immunocompromised patients.

C1198 SELECTION THROUGH PHAGE DISPLAY OF A SINGLE DOMAIN ANTIBODY AGAINST HISTONE H2B FROM LEISHMANIA BRAZILIENSIS

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1 Background

Cutaneous leishmaniasis is a frequent cause of chronic skin ulcers in tropical regions of Peru. Among the diagnostic methods, antigen detection should be preferred over either costly nucleic acid detection methods or those serologic conventional methods that are not able to differentiate between active and cured infections. New biotechnological tools, such as recombinant antibodies, are a hopeful alternative for parasite detection and diagnosis. Among these, the single-domain (SD) antibodies have numerous advantages over classical antibodies, as they are easily produced in *E. coli* expression systems and they are highly stable over time. Here, we used a phage library expressing a high diversity of synthetic SD antibodies to select, through phage display, those who reacted against the long isoform of the H2B histone from *Leishmania braziliensis*. We selected this target because of H2B considerable abundance within the cell, thus increasing the chance to obtain a very sensitive detection test.

2 Methods

The long isoform of the H2B histone of *L. braziliensis* was produced in *E. coli* and purified via recombinant technology. The PREDATOR phage library designed by the Department of Engineering-Aarhus University was then used to select phage clones that reacted with the purified protein. The selected clones were individually amplified and analyzed by phage-ELISA. The most reactive clones were sequenced and cloned to a bacterial plasmid for SD antibody production. The SD reactivity with the H2B antigen was evaluated by ELISA and Western Blot assays. Furthermore, Indirect Immunofluorescence (IFI) assays was also tested against *L. braziliensis* promastigotes.

3 Results

One SD antibody, AC10EX, was selected because of its high reactivity against H2B in ELISA. Moreover, this SD was able to detect corresponding histones using Western Blot. Reactions with the products encoded by H2B fragments demonstrated that target sequence is on the amino terminal region. IFI assays revealed a binding of the SD antibody to the nucleus and cytoplasm of *L. braziliensis* promastigotes. In the model of ear´s mice infected with *L. amazonensis* the western blot assay with AC10EX show reactivity compared with no reactivity in non-infected mice control.

4 Conclusions

AC10EX, an SD antibody selected using phage display, was capable of recognizing histone H2B from *L. braziliensis* and proved to be a useful tool for the detection of *Leishmania* parasites.

C1202 SINGLE DOMAIN ANTIBODY DEFINED BY PHAGE DISPLAY AS A POTENTIAL TOOL FOR THE DIAGNOSIS OF CUTANEOUS LEISHMANIASIS DEVELOPED AGAINST AMASTIGOTE´S ANTIGENS

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1 Background

American tegumentary leishmaniasis is a public health problem in the Americas, where Brazil and the Andean countries, including Peru, are the most affected. Detection of nucleic acids offer highly sensitive and specific diagnostic methods but due to its high costs and the need for specialized infrastructure and personnel presents implementation problems in rural areas. Like promising alternative is the single-domain (SD) antibodies technology where recombinant antibodies can be used with remarkable features like very high specificity, solubility, heat-stability and the possibility for further improvement through recombinant technology. In the present study, we used a SD antibody synthetic library fused to the gene III protein of M13 filamentous bacteriophage called PREDATOR to select an SD antibody against whole cell *Leishmania* amastigotes.

2 Methods

The synthetic SD antibody library PREDATOR was screened against whole cells of axenic amastigotes produced in vitro of *Leishmania.* After performing three different biopanning processes, a stringent selection with a subtractive and amplification process was developed. The clones obtained by this biopanning process were grown individually and then were assessed by phage-ELISA. The optimal phage-SD antibodies selected were sequenced and produced as soluble peptides. The reactivity of the candidate SD antibodies was evaluated by indirect ELISA and indirect immunofluorescence assays against axenic amastigotes and promastigotes, amastigotes of *L. amazonensis* from ear´s mice BALB/c infected as well as cross-reactivity against PBMC and ear´s lysate of non-infected mice.

3 Results

One SD antibody denominated 6BIC was selected as specific detection reagent. ELISA assays discriminated between ear´s mice infected with *L. amazonensis* and corresponding non infected control as well as promastigotes. No cross reactivity was observed when PBMC where analyzed. This fact is important to screen for blood circulating parasites. Using 6BIC for indirect immunofluorescence detection assays, the SD antibody showed a granulated pattern on the axenic amastigotes surface membrane although it rendered a slight and diffuse reactivity with PBMC.

4 Conclusions

To the best of our knowledge, this is the first report documenting a SD antibody, 6BIC, that is specific for *Leishmania* whole cell amastigotes. Further work on it using directed mutagenesis to improve their affinity to optimize sensitivity and specificity is envisaged.

C1204 HIGH ACCURACY OF AN ELISA TEST USING A RECOMBINANT PROTEIN OF LEISHMANIA IN SERODIAGNOSIS OF CANINE VISCERAL LEISHMANIASIS

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1 Background

Visceral leishmaniasis (VL) is a serious public health problem, being dogs the main urban reservoir of the disease. Thus, accurate and rapid canine diagnosis is essential for VL control. 12 recombinant antigens were selected from a cDNA library of *Leishmania infantum* due to their high potential as candidates in diagnostic testing. From the 12 recombinant antigens, 4 were selected using MAPIA as a trial technique.

2 Methods

The present study aimed to compare the accuracy of the commercial ELISA (EIE®CVL), the confirmatory test actually recommended by the Ministry of Health in Brazil, and an ELISA using the four selected antigens (rLci1, rLci2, rLci5, and rLci12). Initially, the sensitivity was evaluated using sera obtained from 26 symptomatic and 21 asymptomatic infected dogs, and the specificity was evaluated using 15 sera samples from healthy dogs, or dogs with other infections. In a second evaluation, the accuracy of an ELISA using the best recognized recombinant protein, Lci5, the DPP®CVL and EIE®CVL were compared using sera from 153 infected dogs (108 symptomatic and 45 asymptomatic), and from 117 negative dogs. All samples were classified as positive according to the results of culture and qPCR from splenic aspirate.

3 Results

Sensitivity obtained in the detection of symptomatic dogs were 86% for rLci1, 81% for rLci2, 96% for rLci5 and 67% for rLci12, and for detection of asymptomatic dogs was 33%, 43%, 67% and 43%, respectively for Lci1, Lci2, Lci5 and Lci12. The EIE®CVL detected 79% of symptomatic animals and 52% of asymptomatic dogs. The specificity was 94%, 81%, 88%, 100% and 100% for Lci1, Lci2, Lci5, Lci12, and EIE®CVL, respectively. The Lci5 presented the best results when were compared with the other recombinant antigens and with the commercial test EIE®CVL. For this reason, Lci5 was selected to the second evaluation with a greater number of samples. Compared to EIE®CVL and DPP®CVL, the ELISA using Lci5 offered a higher accuracy to CVL diagnosis: presenting sensitivity of 80%, compared to 73%, and 64%, for DPP®CVL, and EIE®CVL, respectively. The specificity was 92% for ELISA using Lci5, 96% for DPP®CVL and 90% for EIE®CVL. The accuracy was 85%, 83%, and 75%, respectively for ELISA Lci5, DPP®CVL, and EIE®CVL, respectively.

4 Conclusions

We concluded that the ELISA using rLci5 is a promising test that could replace the EIE®CVL, as CVL confirmatory test and improve the diagnosis of CVL in Brazil.

C1206 EVALUATION OF THE FTA® ELUTE CARD IN ARCHIVING AND DETECTING PARASITE DNA IN BLOOD OF INDIVIDUALS INFECTED WITH LEISHMANIA INFANTUM

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1 Background

Filter paper is a practical vehicle to collect samples in remote areas and to transport the clinical material to the place of analysis, but it is not adequate for a long term storage, since it did not protect proteins and DNA from degradation. A special filter paper- FTA® elute card- (Whatman(®) was developed to overcome such problems, but it was never used to collect, transport and store blood of individuals infected with *Leishmania infantum.* Here we evaluated the purity, quantity and the rescue of *L. infantum* DNA in blood of infected dogs after 06 years of storage in FTA® elute card at room temperature. For comparisons, we also determined in those dogs the same parameters in blood stored in microtubes and kept at -20ºC for the same period.

2 Methods

We analyzed 38 dogs, all of them with proven infection with *L. infantum* in blood. DNA extraction from FTA® elute card was performed with 30 uL of distilled water at 95 ° C for 30 minutes under stirring. The extraction of blood from microtubes was performed in silica columns with commercial kits. Quantity (ng / uL) and purity (A260 nm / A280 nm) were determined by spectrophotometry. Sample positivity for *Leishmania* DNA was evaluated by standard PCR, using primers targeting the kinetoplast sequences. We analyzed the same parameters and used the same methods in 2010 and 2016.

3 Results

The quantity of blood DNA from the FTA® elute card was equivalent after six years of storage at room temperature (43.0 ± 32.3 in 2010 and 35.5 ± 16.4 in 2016), and also the purity (1.8 ± 0.3, and 1.8 ± 0.2, respectively) ($p > 0.05$). Similar results were found in blood stored in microtubes. A slight decay in *Leishmania* DNA positivity was observed in FTA® elute card (89%,34/38), but positivity was comparable with that obtained with blood stored in microtubes at -20ºC (92%, 35/38) ($p > 0.05$).

4 Conclusions

The long period of blood storage in FTA® elute card at room temperature did not affect the quantity and the purity of DNA. In addition, this vehicle showed a diagnostic performance comparable to the blood stored in microtubes and kept at -20 °C for the same period of 6 years. According to our results, FTA® elute card is a suitable vehicle for a long term archiving of blood from individuals infected with Leishmania infantum in biobanks and for detecting parasite DNA.

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C1270 BASELINE PARASITE LOAD IS NOT AN ASSOCIATED FACTOR WITH TREATMENT OUTCOME IN CUTANEOUS LEISHMANIASIS

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1 Background

Beside clinical judgment, currently there is no a qualitative or quantitative objetive biomarker predictive of treatment outcome in cutaneous Leishmaniasis (CL). Althoug infecting strain has been priorly associated with treatment outcome, regional genotipic variations in new world regions as well as inhability to implement strain identification in endemic regions makes strain identification an inviable alternative to be implemented as consensual criteria. Since Peru harbors almost 7 different strains and their behaviour and therapy susceptibidity has reported to be polarly different to other countries, an standardize, comparable and widely acceptable biomarker is urgently required.

2 Methods

As a part of prior studies in which Parasite load was quantify by qPCR, a secondary analysis study was performed in 49 subjects with confirmed CL in whom supervised therapy was applied at our center and follow-up was performed for at least six months or until treatment failure was confirmed.

3 Results

Patients were mainly males (89%) with a median age of 33 years-old. They acquired the infection both in jungle and highland regions and the predominant strain were L. braziliensis (40%) and L. guyanensis (26%). Individuals harbored mainly two or less lesions (87%) with a median duration of 2 months (1-18). Lesions were mainly ulcerative (93%) with a median area of 10 mm2 (0.7-39). All were treatment naive and they received systemic pentavalent antimonials (S-Sb5+, 64%) or S-Sb5+ combined with oral pentoxifylline for 20 days in our center. Parasite load had a median value of 5x104 in the cured group, 11x104 in the failure group and 8.7x104 in the unknown outcome group, although these results were not statistically significant (p=0.4). Only the infecting strain (L. braziliensis, p=0.013) and prolongued duration of disease (p=0.03) were associated with treatment failure.

4 Conclusions

We could not identify that baseline parasite load is predictive of treatment outcome in peruvian patients with CL. Although elevated parasite loads can be hypothetically associated with a slow clearance of amastigotes in CL lesions, other biomarkers must be explored as for example dinamics of PL during therapy as well as other host-related determinants in further controlled and prospective studies.

C1278 DEVELOPMENT OF A HIGH RESOLUTION MELTING ANALYSIS (HRM) ASSAY FOR DETECTION AND IDENTIFICATION OF OLD WORLD LEISHMANIA SPECIES

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1 Background

In principle, PCR-High Resolution Melting analysis (HRM) allows rapid result delivery and accurate differentiation of DNA molecules (without electrophoresis) based on nucleotide differences, making it suitable for accurate, sensitive and specific diagnosis. In case of cutaneous leishmaniasis (CL), highly endemic worldwide, particularly in Africa and Mediterranean region, is caused by different parasite species resulting in different eco-epidemiological leishmaniasis forms. Parasite identification, which requests molecular tools, is central to diagnosis, treatment and control strategies of the disease. We aimed at developing a PCR-HRM tool using a 120bp *Leishmania* sequence (SC120) that contains taxonomically informative nucleotide divergence, and at applying it to identify new and already typed parasites and parasites within CL patients' samples.

2 Methods

PCR primers were designed and selected for consistent DNA amplification and differentiation by HRM of *Leishmania* DNAs. Conditions established were tested on a set of 72 DNAs purified from strains isolated from diverse hosts, clinical presentations or geographical origins, already typed or newly isolated. Sensitivity of detection was evaluated. It was also applied to detection and identification of parasites in archived material on Giemsa-stained smears (N=33) or frozen- aspirates in saline buffer (N=20) from Tunisian CL patients. Results were compared to another technique: ITS1-PCR-RFLP (HaeIII).

3 Results

SC120-PCR-HRM analysis was able to differentiate between *L. donovani/ L.infantum, L.major*,*L.tropica*, *L.aethiopica*, *L.turanica* and *L.arabica*. Differentiation could be observed with as little as one nucleotide difference between or within species. Results were congruent to PCR-RFLP ITS1 or SC120 DNA sequencing. This assay detected 62,5 – 6,25 haploid genomes. It could detect and identify *L. major* or *L. infantum* as the causal agent in the 20 frozen samples. However, it amplified DNA only from one stained smear (vs 10/33 by double ITS1-PCR) for which input DNA was estimated to contain 77 haploid *Leishmania* genomes by second derivative maximum.

4 Conclusions

This new molecular assay is useful for detection and identification of Old World *Leishmania* species. It is rapid, sensitive, specific, simple, and can be used with good quality DNA for epidemiological studies and surveys or to support etiological diagnosis.

The study received financial support from MESRS-Tunisia (LR00SP04 /LR11IPT04).

C1378 EVALUATION OF RAPID DIAGNOSTIC TEST KITS FOR CANINE LEISHMANIASIS

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1 Background

Canine leishmaniasis (CanL) is a zoonotic vector-borne disease caused by *Leishmania infantum*. It is prevalent in Mediterranean countries, South America, parts of Asia and Africa. Diagnosis of CanL is generally supported by detection of *L. infantum* antibodies either by indirect fluorescent antibody test (IFAT) or enzyme-linked immunosorbent assay (ELISA). Rapid in-clinic tests are also utilized and can provide an important first step in diagnostic algorithms. An in-clinic ELISA, SNAP® Leishmania Antibody Test kit, has been available for many years and has been shown to have high diagnostic accuracy (Ferroglio et al. 2007, Marcondes et al. 2011). Recently, a rapid lateral flow test, Leishmania Ab Test kit, produced by BioNote (Gyeonggi-do, South Korea) has been made available in some global markets under the BioNote or private label brand names. However, its accuracy has not been reported. The aim of this study was to evaluate the diagnostic accuracy of the BioNote and the SNAP tests with a clinical sample set.

2 Methods

Serum samples were sourced from IDEXX Reference Laboratory in Barcelona, Spain. They were part of routine samples being submitted by attending veterinarians for detection of anti-*Leishmania* antibodies using LEISCAN® *Leishmania* ELISA Test. Positive samples were defined for this study as those with ≥1/320 equivalent titer (indicative of disease per OIE guidelines "Leishmaniosis. OIE Terrestrial Manual 2014, Chapter 2.1.8"), negatives with ≤ 1/20 equivalent titer. A total of 72 positive and 60 negative samples were included in this study.

3 Results

With this clinical sample set from Spain, the SNAP *Leishmania* test demonstrated 93.1% sensitivity and 98.3% specificity, similar to previously published results from Italy and Brazil (Ferroglio et al. 2007, Marcondes et al. 2011). The sensitivity and specificity for the BioNote test were 80.6% and 95.0%, respectively.

4 Conclusions

Consistent with previously published studies, SNAP Leishmania Antibody Test kit, based on ELISA technology, continued to demonstrate a high accuracy for detection of anti-*Leishmania* antibodies in suspected CanL cases. The new BioNote test was found to be less sensitive.

C1406 AMERICAN TEGUMENTARY LEISHMANIASIS DIAGNOSIS: COMPARISON BETWEEN FLOW CYTOMETRY AND CONVENTIONAL SEROLOGICAL METHODS

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1 Background

American Tegumentary Leishmaniasis (ATL) is a neglected and severe disease which is a major public health issue in Brazil. Its diagnosis presents difficulties because it needs to associate different aspects such as clinical, epidemiological and laboratorial characteristics to get to a definitive result. The aim of this work was to compare the performances of flow cytometry, enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescence (IFA) with ATL patients before treatment (BT) and 1, 2 and 5 years after treatment (AT) and also patients with spontaneous cure.

2 Methods

IFA and ELISA assays were performed in house; flow cytometry was performed following a previously established protocol.

3 Results

For the IFA assay, patients with sera dilution above 1:20 were considered. From the evaluated sera, 92.85% (13/14) were positive BT. One year AT, 61.54% (8/13); two years AT, 70% (7/10) and five years AT, 50% (5/10). For the ELISA test patients with serum dilutions above 1:40 were considered positive. 92.8% (13/14) patients BT were positive, 1 year AT 53.8% (7/13); 2 years AT 88.8% (8/9) and five years AT, 100% (5/5). On flow cytometry, it was possible to identify 86% (12/14) patients BT and 77% (10/13), 80% (8/10) and 70% (7/10) of negativity in patients 1, 2 and 5 years AT respectively. Comparing the ROC curves, the performances of IFA and flow cytometry, it was observed that the area under the curve (AUC) of flow cytometry was slightly higher than IFA (AUC IFA = 0.879 – AUC flow cytometry = 0.890). Afterwards, it was compared the performances of flow cytometry and ELISA, where once again, flow cytometry stood out (AUC ELISA = 0.808 – AUC flow cytometry = 0.896). Regarding the spontaneously cured patients, the analysis of the mean percentage of positive fluorescent parasites (PPFP) along the titration curves of anti-fixed promastigote *L. (V.) braziliensis* IgG confirmed the applicability of this method for monitoring spontaneous cure of ATL with outstanding co-positivity (100%) and co-negativity (100%) performance indexes. In a complementary approach this study intended to evaluate the performance of the test for ATL diagnosis in a population including other infectious/parasitic diseases.

4 Conclusions

With these results, it is possible to conclude that flow cytometry can be applied as an alternative diagnostic tool for ATL since it was able to detect the disease in patients and it was negative in cured patients.

C1414 AN ALTERNATIVE IMMUNOHISTOCHEMICAL METHOD FOR DETECTION OF LEISHMANIA SPP. IN FELINE SKIN BIOPSIES

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1 Background

Feline leishmaniosis (FeL) has been reported in several countries where zoonotic visceral leishmaniosis is endemic, such as Portugal, Spain, Italy, France, Greece, Israel and Brazil, being frequently associated with skin lesions. Although dogs have been classically incriminated in the transmission of the disease to man, cats are also being suggested as alternative secondary reservoir hosts. Given the fact that cutaneous lesions of FeL may be easily overlooked in endemic regions, the present study aimed to investigate the presence of amastigote forms in feline skin biopsies by using an immunohistochemical method alternative to the conventional ones.

2 Methods

A streptavidin–biotin immunohistochemical method was used, with a canine hyperimmune serum as the primary antibody, in 21 feline skin biopsy samples, previously submitted for histopathological analysis. Cats included in this study were originated from geographical areas in Portugal where canine leishmaniosis is endemic and presented nodular, ulcerative or crustous skin lesions. A skin sample from a dog with cutaneous lesions of leishmaniosis was used as a positive control and a normal feline skin sample was used as a negative control.

3 Results

The average age of cats was 5 years (range: 1-12 years); most of the cats included were female (n = 14; 66.7%) and from European breed (n = 12; 57.1%). Skin lesions were observed on the head (face, nasal plannum and/or pinnae) (n = 7; 33.3%), abdomen (n = 6; 28.6%) or in a generalized distribution (n = 6; 28.6%). On routine histopathology, granulomatous or pyogranulomatous dermatitis was observed in most cases. Amastigote forms were observed in one (n = 1; 4.8%) cat with a diffuse granulomatous dermatitis. The presence of *Leishmania* within macrophages was easily confirmed after immunohistochemical analysis. The positive case corresponded to a 10-year old male cat, with generalized skin lesions, associated with dermatophytosis. All other samples (n = 20; 95.2%) were negative.

4 Conclusions

This alternative immunohistochemical technique represents a potentially useful tool to confirm or rule out *Leishmania* spp. infection in feline tissues, especially in endemic regions.

C1417 CRITERIA USED IN LEISHMANIA SPECIMEN COLLECTION FOR DIAGNOSIS

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Walter Reed Army Institute of Research USA

1 Background

Walter Reed Army Institute of Research (WRAIR) is the only College of American Pathology (CAP) and Clinical Laboratory Improvements Program (CLIP) certified Leishmania Laboratory in the world. All laboratory procedures and quality control measures are executed to meet with CAP and CLIP standards. The laboratory is inspected every two years to retain CAP certification, ensuring that all standards are met.

2 Methods

Instructions for proper biopsy collection are provided to each site requesting Leishmania diagnosis. Training and education of technical personnel and physicians involved in Leishmania diagnosis and research are provided to ensure that proper specimens are received and that physicians can interpret test results.The practice of continuous improvement is employed in our operations to include collaborations and consultation of experts in the Leishmania /parasitology fields. Proper documentation is provided to support the patient diagnosis and to determine the best routes for maximizing Leishmania diagnostic capabilities.

3 Results

Leishmania diagnosis is obtained through Microscopy of touch/spread smears, rK39 in serum, PCR of biopsy and blood samples, and tissue culture. Cellulose Acetate Electrophoresis (CAE) allows for speciation of parasites. In cases where the parasites are difficult to culture, diagnosis is obtained by xenodiagnoses where laboratory animal subjects are injected with a sample to obtain the parasites.

4 Conclusions

Samples and cultured parasites are stored using cryogenic techniques for use in future research procedures and assay development.

C1420 TOOLS USED IN LEISHMANIA DIAGNOSTIC LABORATORY TO DIAGNOSE PATIENTS FOR LEISHMANIASIS

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1 Background

Leishmaniasis is a disease complex caused by 42+ species of protozoan parasites belonging to the genus Leishmania, of which 22 species affect humans. According to the CDC report, estimates show that the number of cases ranges from 700,000 to over 1 million for cutaneous leishmaniasis, and ranges from 200,000 to 400,000 for visceral leishmaniasis.

2 Methods

Successful culturing of Leishmania parasites requires several techniques to ensure proper identification of the species involved in the disease manifestation. An accurate and verifiable final diagnosis is the most important tool in finding the correct treatment for the affected individuals. A series of laboratory techniques such as culturing and expanding the parasites for species identification through molecular techniques is required for proper diagnosis. Basic laboratory techniques include clinical, parasitological and immunological diagnosis.

3 Results

It is very important to understand the progress of the disease in order to discover, develop, implement and validate all possible methodologies to expand and share the acquired knowledge in dealing with this neglected disease.

4 Conclusions

The LDL (Leishmania Diagnostic Laboratory) is the only College of American Pathologist certified laboratory that is also approved by the Clinical Laboratory Improvement Program for high complexity testing of Leishmania disease under the Department of Defense (DoD).

C1426 PERFORMANCE OF L. (L.) INFANTUM AMASTIGOTES ANTIGENS COMPARED TO PROMASTIGOTES FOR SEROLOGICAL DIAGNOSIS OF CANINE LEISHMANIASIS

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1 Background

Serological diagnosis of canine visceral leishmaniasis (CVL) is still a challenge since cross reactivity with sera of dogs with other diseases and low sensitivity for detecting asymptomatic dogs have been observed. Based on a previous study that obtained high sensitivity and 100 % of specificity in the Immunofluorescent Assay Test (IFAT) using imprint of spleen from hamsters chronically infected by *Leishmania infantum* rich in amastigotes, the main objective of this study was to evaluate and compare the performance of the immunoassay ELISA with amastigotes and promastigotes of *L. infantum* as source of antigens for CVL diagnosis.

2 Methods

Total crude antigens was prepared by freezing and thawing axenic amastigotes from culture, purified amastigotes from spleen of chronically infected hamster, and promastigotes from culture. We used 155 sera of dogs from an endemic area for CVL with positive parasitological diagnosis by PCR in lymph node.The animals were assorted into symptomatic (n = 67) and asymptomatic (n = 48) dogs, in accordance with the presence/absence of clinical signs and laboratory tests. Eighty-one sera of dogs from non-endemic area for CVL with PCR negative result for *Leishmania* were used as negative controls.

3 Results

Good correlation and agreement were observed among tests using the three antigens ($p<0.001$). Sensitivity of ELISA with axenic amastigotes was 0.82, for purified amastigotes was 0.79 and for promastigotes was 0.85 ($p>0.05$), and specificity was 0.84, 0.86 and 0.91, respectively ($p>0.05$). Respective with the clinical groups, symptomatic dogs presented higher antibody titers and frequency of positive results ($p\leq0.05$) in the three tests. ELISA with axenic amastigotes reached high sensitivity for diagnosing symptomatic dogs (0.91), while ELISA using promastigotes was more sensitive for diagnosing asymptomatic dogs (0.79).

4 Conclusions

According with our results, we suggest the use of ELISA with crude antigens of promastigote since it detected higher number of asymptomatic dogs, the major population found in endemic areas, and showed comparable results for diagnosing the symptomatic dogs. In addition, it presented lower number of false positive results compared to ELISA with axenic amastigotes and that with purified amastigotes.

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C1436 PREVALENCE OF LEISHMANIA RNA VIRUS-1 IN SEVERE AND NON-SEVERE AMERICAN TEGUMENTARY LEISHMANIASIS

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1 Background

Leishmania RNA virus-1 (LRV-1) is a double stranded RNA virus identified in 20-25% of the *Viannia* complex endemic to Latin America, and is believed to be a predictive biomarker of severe American tegumentary leishmaniasis (ATL). Our objective was to determine the baseline prevalence of LRV-1 in patients with confirmed severe and non-severe ATL.

2 Methods

Patient specimens were recruited from Public Health Ontario Laboratory and 2 sites in Peru between 2012 and 2016. Demographic and clinical data including age, sex, country of acquisition, and disease severity were simultaneously recorded in addition to specimen collection. DNA and RNA were extracted for species identification and detection and quantification of LRV-1. Species identification was performed using end-point PCR and restriction fragment length polymorphism (RFLP) analysis followed by confirmatory Sanger sequencing where needed. Patients were stratified according to clinical phenotype. We defined non-severe ATL as localized CL, whereas severe ATL was defined as having either: mucosal involvement; lymphatic involvement; ≥4 lesions in ≥2 anatomic sites; or inflammatory (ie., purulent or necrotic) ulcers.

3 Results

Of 135 patients enrolled, 31 (29.8%) were excluded due to insufficient clinical or demographic data, specimen volume, or undetectable human reference gene. Twenty-nine (27.8%) of 104 total ATL cases were positive for LRV-1. Nineteen (35.2%) of 54 severe ATL cases and 10 (20%) of 50 non-severe ATL cases were positive for LRV-1, respectively (p=0.07). Causative species identified included: *L. V. braziliensis* (n=32), *L. V. guyanensis* (n=23), *L. V. panamensis* (n=15), *L.V. peruviana* (n=14), *L. V. lainsoni* (n=3), *L. V. guyanensis/L. V. panamensis* hybrid (n=1), and unknown (n=16). There was no difference observed in disease severity by sex (p=0.25), causative species (p=0.61), or country of acquisition (p=0.43).

4 Conclusions

Our findings suggest a weak possible association between LRV-1 status and the severity of ATL. Given the likelihood of relapse in patients with severe ATL and associated risk of treatment failure, further elucidation of LRV-1's contribution to disease severity in ATL warrants exploration.

C1438 LEISHMANIA (VIANNIA) BRAZILIENSIS ISOLATED FROM SALIVA OF PATIENT WITH CUTANEOUS LEISHMANIASIS.

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1 Background

American cutaneous leishmaniasis (ACL) is an important disease in Brazil and in several other countries. The identification and characterization of *Leishmania* species involved in infection is performed with samples from patients. In this study we report the first isolation of *Leishmania* (*Viannia*) *braziliensis* in saliva from one patient with cutaneous leishmaniasis without lesions on mucosa.

2 Methods

The saliva flow was seeded into a Base Blood Agar (Difco 45) and inoculated into a peritoneum of hamsters. The culture was examined after five days of inoculation to observe the parasites and the infected hamsters were observed for three months and euthanized under anesthesia (Tiopental 60-100mg/kg). Then, some fragments of viscera (liver and spleen) were removed and inoculated in a medium for culture. The promastigotes were inoculated in Schneider's medium supplemented. Conventional tests were performed (*in vitro* culture, in *vivo* smear and PCR) with other samples obtained from the same patient.

3 Results

The parasites were obtained by isolation of the sample by saliva *in vivo* culture. This sample was characterized by ' multilocus enzyme electrophoresis ' (MLEE) and confirmed by PCR specific for the subgenus *Viannia*.Conventional tests with tissue fragment and swabs were positive to confirming the diagnosis of ACL. The patient responded well to treatment with N-methyl glucamine at a dose of 15mg / kg / day, with only one cycle of medication. He was monitored for seven months and throughout this period did not developed mucous lesion.

4 Conclusions

The isolation of *L. braziliensis* from saliva of the patient reported here suggest that this clinical sampling is non-invasive and with easy collection method. Future studies are necessary to evaluate the performance of the various collection methods to obtain clinical samples from individuals with ACL. The saliva demonstrated to be effectiveness, fast and well accepted by patients. Therefore, saliva has potential to be used in the field and with pediatric populations.

C1540 SLA-CELL PROLIFERATION TEST COMPARATION IN ACTIVE LEISHMANIASIS VS CURED IN DIFFERENT CLINICAL LEISHMANIASIS FORMS

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1 Background

SLA-cell proliferation test has been confirmed as marker of specific cellular immunity for visceral leishmaniasis. Largest leishmaniasis outbreak in Europe was declared since June 2009 in Fuenlabrada (Madrid, Spain). In this setting, our aim was to compare SLA-cell proliferation test in active vs cured leishmaniasis in patients with visceral, cutaneous, ganglionar and mucosal leishmaniasis

2 Methods

From January 2013, blood samples from patients with visceral leishmaniasis (VL), cutaneous leishmaniasis (CL), leishmanial localized lymphadenopathy (LLL) and mucosal leishmaniasis (ML) were collected and analyzed by Leishmania-specific PCR, serology (IFAT), SLA-cell proliferation test and cytokines production after stimulation with SLA, to compare cell immunity in active leishmaniasis vs cured patients.

3 Results

139 patients were included in the study: 64 VL, 49 CL, 23 LLL and 3 ML.

	PCR positive (%)		IFAT >1/80 (%)		SLA, mean SI, (SD)		SLA positive (%)		IFNg producti
	AL*	C*	AL*	C*	AL*	C*	AL*	C*	AL*
VL	66	4	93	74	1.49 (1.01)	5.65 (3.64)	16	90	234
CL	3	1	0	1	3.18 (2.11)	3.48 (2.87)	48	59	557
LLL	0	5	20	16	4.35 (3.36)	4.14 (2.23)	67	81	1373
ML	0	0	0	33	2.10 (1.96)	2.32 (1.80)	50	22	353

* AL: active leishmaniasis, C: cured

Only comparative between VL active and cured resulted statistically significant: $p<0.001$ for PCR, SLA %, SLA mean SI and IFNgamma %, $p=0.04$ for IFNgamma mean, $p=0.022$ for IFAT

4 Conclusions

CL, LLL and ML groups of patients demonstrated no significant variation in any test between active and cured cases. Many CL and LLL patients had positive SLA-cell proliferation test before treatment. VL patients had negative SLA-cell proliferation test before treatment, reflecting failure of cellular immune response, that became positive when cured. Therefore, it can be useful as marker of clinical curation in VL

C1543 DETECTION AND IDENTIFICATION OF CAUSATIVE AGENT OF CUTANEOUS LEISHMANIASIS IN REFERRED PATIENTS TO THE HEALTH CENTER OF GONBAD-E-QABUS FROM GOLESTAN PROVINCE USING SPECIFIC PCR

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1 Background

Golestan Province is one of endemic foci of cutaneous leishmaniasis (CL). The aim of the present study was to assess specific-PCR on Giemsa's stained slides for diagnosis of CL as well as to detect species of Leishmania spp in referred patients to health center laboratory of Gonbad-e-Qabus.

2 Methods

This cross-sectional study was carried out in during 2010. To diagnosis of CL, direct smear (DS) from skin lesions and staining with Giemsa was performed. In addition, if DS was negative, specific PCR on kinetoplast DNA (kDNA) extracted from direct smear to genus and species identification was done. The data were registered and then analyzed by SPSS software.

3 Results

Of 303 suspected patients, 238 cases (78.5%) infected to CL by direct smear examination. The rest of them (n=65) were negative. The PCR result was positive in 34(52.3 %) out of 65 smears among whom direct examination did not reveal Leishmania amastigotes. Also Leishmania species isolated from all patients were Leishmania major by species-specific primers.

4 Conclusions

According to the fact in some cases the DS is reported negative as well as this method has a low sensitivity versus high sensitivity of PCR. Thus we suggest use of PCR method to high accurate of diagnosis as well for ruling out the CL in suspected patients particularly from endemic regions.

C1574 SEQUENCING OF LEISHMANIA CAUSATIVE AGENT OF CL IN IRAQ

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1 Background

Human leishmaniasis is a vector-borne disease caused by more than 20 *Leishmania* species in98 countries on five continents [1].The genus *Leishmania* belongs to the class of Kinetoplastida [2] and to the order ofTrypanosomatida, which diverged very early in the eukaryotic lineage [3].

The differentiation at the genus level based on the amplification of the kinetoplastminicircle DNA (kDNA, about 10000 copies per cell) or the variable sequences of the small subunit ribosomal DNA genes (SSU rDNA, 40–200 copies per cell) [6-9]. They allow identification of Leishmania parasites only at the generic and/ or subgeneric level. Both targets have also been usedfor the development of real-time PCR assays in order to determine parasite burden in clinicalsamples. The ability to distinguish between *Leishmania*species is crucial for a correct diagnosis of the disease as well as for making decisions regarding treatment and control measures. This is especially useful in areas where several Leishmania species co-exist[10,11].

2 Methods

Samples collections

Skin lesion samples were collected from patients in Iraqi's hospitals and placed in sterile test tubes, then transported to laboratory and stored in -20°C until genomic DNA extraction step.

Sequence analysis

Phylogenetic tree analysis based on the Kinetoplast DNA partial sequence that used for *Leishmania* sp. confirmative detection. The phylogenetic tree was constructed using Unweighted Pair Group method with Arithmetic Mean (UPGMA tree) in (MEGA 6.0 version) [20,21].

3 Results

A total of 44 (M=25, F=19) suspected patients with cutaneous leishmaniasis were re-cruited and parasitologically examined. The results showed that 30 (68%) of the patients gave positive results by Giemsa-smeared . Parasite DNA was extracted from Giemsa-stained smears positive for *Leishmania*. A nested polymerase chain reaction (PCR) was then used to amplify the ITS1 region. Our results showed that patients with lesion(s) induced by *L. major* = 21 (M=12, 57.1%, F=9, 42.2%) and lesion(s) induced by *L. tropica* = 9 (M=5, 55.6%, F=4, 44.4%). There were no significant difference between male and female.

4 Conclusions

The present study reveals that both *L. major* and *L. tropica* are causativeagents of CL in Iraq. Therefore,both anthroponotic and zoonotic epidemiological patterns of transmission could not be ruled out. For effective control, determination of possible animal reservoir hosts should be explored using molecular methods.

C1582 ISOLATION OF LEISHMANIA SPECIES CAUSATIVE AGENT OF CUTANEOUS LEISHMANIASIS FROM PATIENTS, RESERVOIRS AND VECTORS IN ENDEMIC AREAS OF IRAQ: IDENTIFICATION AND GENOTYPING USING MOLECULAR METHODS AND COMPARISON WITH IRANIAN ISOLATES

Abdulsadah A.Rahi[1], Magda Al-Rubaiee Ali[2], Ali Khamesipour[3], Mehdi Mohebali[4], Amir Akhavan Ahmad[5], Alireza Firoozi[3], Alireza Khatami[3], Valian Keshavarz[5]

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1 Background

Cutaneous leishmaniasis is a parasitic disease transmitted by biting insects (sandflies) .The disease is endemic in Iraq and in other neighbouring countries such as Kuwait, Saudi Arabia and the Islamic Republic of Iran .In Iraq there are 2 main species of the genus *Leishmania* causing the infection: *Leishmania major* and *Leishmania tropica.* CL is a self-healing disease. If left to heal on their own without treatment, the lesions can cause significant scarring. Untreated healing times vary from 2 to 15 months depending on which species of leishmaniasis has infected a patient, rapid diagnostic and treatment remains important to avoid unattractive scars and parasite dissemination.

2 Methods

A retrospective analysis of routinely collected demographic data was performed. Parasites were isolated from the lesions of these patients before treatment. This study was performed in the dermatology clinics of hospitals in eight provinces of Iraq **(Wasit**, **Baghdad**, **Basrah**, **Diyala**, **Kirkuk**, **Najaf, Anbar and Salah-Adin)** for a period from October, 2012 to December,2013. A total of 786 cases of suspected CL were included in this study and diagnosed by Giemsa stain,culture and PCR.

3 Results

Giemsa-stained smear revealed amastigotes in 18.6% and in vitro cultivation gave positive results in 11.96%, whereas Nested- PCR detected *Leishmania* in 100% of CL samples ; 82.5% typed as *L.major* and 17.5 % typed as *L.tropica*. The epidemiological analysis show the highest infection (39/146) was recorded in the age group(21-30) years oldand the lowest (19/146) was in the age group (10 and less) years old. Our study revealed that CL patients in Iraq were younger than the general population (median age 21*vs.* 30 years) and the male was predominant 63% than female 37%. Children were more likely to have lesions on the upper extremities 53.7% and wet type of ulcer was predominated (78.1%) than dry type (21.9 %) . The results of the study also indicated that CL have been concentrated in Najaf 25% and Wasit 22.5% provinces.

4 Conclusions

Molecular methods have revolutionised the diagnosis of leishmaniasis.PCR based assays, they were found to be rapid, sensitive and discriminative at species or even strain level. However the diagnosis of leishmaniasis remains a scientific challenge.

C1592 PREDICTING RELAPSE AND PROGRESSION OF VISCERAL LEISHMANIASIS USING SCALABLE RAPID DIAGNOSTIC SEROLOGY

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1 Background

Visceral leishmaniasis (VL) is fatal if not treated and is targeted for elimination in India, Nepal and Bangladesh. Cure is currently defined only by an absence of symptoms (fever, splenomegaly) at 6 months post-treatment. Ten to 20% of those deemed cured at 6 months have been reported to subsequently relapse with VL and require re-diagnosis and re-treatment. Additionally, asymptomatic infections of *L. donovani* outnumber VL cases and asymptomatics may progress to clinical disease. Standard serology detecting total IgG cannot distinguish cure from relapse, or progressors from non-progressors, thus hindering disese control efforts. We have previously identified IgG1 anti *Leishmania* as a potential marker of both VL relapse, and progression to symptomatic disease. We have adapted this assay to a rapid diagnostic test (RDT) format. If IgG1 remains raised at 6 months post-treatment, the person is at risk of relapse. Likewise, if IgG1 is raised in an asymptomatic person, they are at risk of progressing to VL. Here we present results of further validation of the RDT using Indian, Nepalese and Sudanese sera.

3 Methods

A lateral flow immunoassay RDT was produced using *L. donovani* whole cell lysate as the antigen and with anti IgG1 detection. RDTs were carried out retrospectively on serum from India of VL relapse, cured patients, progressors and non-progressors; Sudanese treated individuals and relapse; relapse cases from Nepal. RDTs were visually scored as positive or negative.

4 Results

The RDT to detect IgG1 anti *Leishmania* correctly identified people who were deemed to have relapsed with VL following apparently successful treatment and was negative or reduced for those deemed cured at 6 months. The asymptomatic non-progressors were negative by RDT and the progressors were positive and went on to develop VL from 6 days to 6 months after serum collection.

5 Conclusions

We have demonstrated that an RDT detecting IgG1 anti *Leishmania* can predict relapse and progression to VL. We can now manufacture the RDT in bulk and at low cost, circa €1 per test. Ongoing research aims to identify individual parasite antigens to replace the cell lysate for better standardisation. This RDT provides a minimally-invasive, low cost means of following up treatment and predicting relapse, thus reducing transmission, morbidity and mortality.

C1603 SERO-DIAGNOSIS OF VISCERAL LEISHMANIASIS AND HUMAN IMMUNODEFICIENCY VIRUS IN WESTERN AND COASTAL PARTS OF KENYA

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1 Background

Visceral Leishmaniasis (VL) and Human Immunodeficiency Virus infection (HIV) are caused by leishmania *donovani* and human immunodeficiency virus. If left untreated, the case fatality is almost 100% and drug resistance has been reported for HIV. This makes these diseases a public health threat, hence proper diagnosis is important. Sero-diagnosis of these diseases is essential especially in surveillance and epidemiological studies. This study aimed at using developed recombinant antigens for both *L. donovani* and HIV in sero-epidemiological studies using a multiplex bead based platform.

2 Methods

We prepared gag, gp41, gp120 and KRP42 recombinant antigens and coupled them onto microspheres; then evaluated them in multiple assays and set a cut-off value for each assay. The antigens were purified and cut-off values for the assay were calculated using a mean MFI value plus three (3) times standard deviation (SD) to each antigen.

3 Results

The assay system was applied for 3411 blood samples collected from the general population randomly selected from two health and demographic surveillance system (HDSS) cohorts in the coastal and western regions of Kenya. The immunoassay values distribution for each antigen was mathematically defined by a finite mixture model, and cut-off values were optimized. Sensitivities and specificities for each antigen ranged between 71 and 100%. Sero-prevalences for each pathogen from the Kwale and Mbita HDSS sites (respectively) were as follows: *L. donovani* (KRP42), 12.6% and 17.3% and HIV 3.0% and 20.1%.

4 Conclusions

We succeeded in providing a proof of concept in the development of a multiplex assay system for leishmania and HIV sero-diagnosis. The results of the study could lead to the possibility of a comprehensive surveillance system for leishmania, HIV and other infectious diseases in Africa

C1606 ACCURACY OF A RAPID DIAGNOSTIC TEST FOR CUTANEOUS LEISHMANIASIS IN MOROCCO

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1 Background

In Morocco, cutaneous leishmaniasis (CL) is caused by *L. tropica*, *L. major* or *L. infantum*. The diagnosis in patients with skin lesions is often based on clinical grounds. This clinical diagnosis can be confirmed through microscopic identification of parasites after Giemsa coloration, but this test is only available in the provincial laboratories.
A rapid diagnostic test (RDT) for CL has recently become available. This test is easy to perform and gives a result in less than an hour. The aim of this study is to estimate the accuracy of the CL RDT on endemic CL areas.

2 Methods

The study is ongoing since December 2016. The required sample size is 240. Study participants are recruited in nine health centres located in five endemic provinces in Morocco.
The RDT under evaluation (index test) is the CL Detect™ Rapid Test (InBios International, USA); a membrane-based amastigote antigen (peroxidoxin) detection test. The reference standard (RS) consists of two tests: direct microscopic examination of skin smears and PCR ITS1. If at least one is positive, the RS is considered positive.
One dental broach and four skin smear samples are taken from a single lesion. The dental broach sample is used for the RDT. The skin smear samples are sent to provincial laboratories for microscopy testing and to reference laboratories in Morocco and Belgium for PCR, genotyping and parasite load determination.
Data analysis was performed using Stata 13 (Stata Corporation, Texas, USA). We estimate the sensitivity (Se), specificity (Sp), positive and negative predictive values (PPV, NPV) with their confidence intervals. Registration in clinical trials.gov: NCT02979002

3 Results

Until March 2017, 186 patients who meet the eligibility criteria were included, mostly male (n = 96/186, 52%) and 93 was under 18 years old. The median age was 23 years.
Of all participants, 50% had a new lesion with an evolution less than 45 days. Lesion size mean was 0.7±0.6 in *L.tropica* area and 1.6±1.1 in *L.major* area.
No adverse events from performing the RDT were reported by all health professionals even if the use of Emla* cream or injectable Lidocaine was not systematic.
For all areas (n=186) the test accuracy is Se=72[95% CI, 66-79]; Sp=92%[95%CI, 86-96], PPV=93%[95%CI, 89-97]; NPV=70%[95%CI, 64-77]
The room temperature seems influencing this accuracy. Between 08 and 13°C (n=91) the Se=62%[95%CI, 52-72] and the Sp=90%[95%CI, 84-96]. While between 14 and 22°C (n=95) the Se=86%[95%CI, 79-93] and the Sp=93%[95%CI, 89-98].

4 Conclusions

Preliminary data show CL RDT is a useful addition to the CL clinical case management especially in isolated localities far from provincial laboratories; as a positive RDT has a very good PPV and warrants treatment.
We noted a dependence of sensitivity on room temperature. The manufacturer recommends doing the test between 20-30°C which is hard to achieve in field conditions.

The sensitivity of this RDT could be improved.

C1610 COMPARATIVE ANALYSIS AMONG THE DIFFERENT ALGORITHMS USED FOR DIAGNOSIS OF VISCERAL CANINE LEISHMANIOSIS.

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1 Background

The routine diagnosis of visceral leishmaniasis (VL) canine is commonly based on clinical and epidemiological parameters associated with laboratory tests. Given that the disease in dogs in Brazil by the public there is no treatment, the search for more effective diagnostic methods that are easy to perform, fast, simple, affordable and less invasive, it is necessary, reducing the time to obtain results. The aim of the study to conduct comparative analysis between the different algorithms used for the diagnosis of canine VL (CVL), in the region of São José do Rio Preto, from 2008 to 2012.

2 Methods

We studied the different algorithms used in the diagnosis of CVL from seroepidemiological surveys canines. We evaluated the total of tests processed in each algorithm, the average time to examine all the samples collected in different periods and algorithms, samples considered inadequate with new sample request for each algorithm and overall concordance analysis between the methods.

3 Results

During that time we used four different algorithms, and 12,871 (28.4%) examined only by indirect immunofluorescence assay (IFA); 632 (1.4%) by enzyme immunoassay (EIA) and IFAT, both groups from blood eluate collected on filter paper; 22,387 (49.4%) by EIE and IFAT and 9,453 (20.8%) for the Dual Path Platform (TR-DPP®) and IEE, these two groups examined with blood serum. In the analysis of samples considered inadequate for the diagnosis of CVL, it was observed that the blood samples collected in dry tubes for IEE algorithm as a screening test and IFT as confirmation were the most rejection and new sample request (11.2%). The linearity indicates that the current algorithm using TR-DPP and EIE demand less time (days) in relation to the others. Regarding the assessment of the overall concordance rate between the CVL of diagnostic methods used in serological and confirmatory test screening, TR-DPP® / EIE performed better 58.5%.

4 Conclusions

The study noted that the algorithm each new features were added favorable for its implementation and a reduction in reject rates of inadequate specimens. The overall concordance between the methods revealed that although the new TR-DPP® algorithm (screening) and IEE (confirmatory) has contributed to the decrease in current disagreement, the IEE test confirms less than 60% of TR-DPP® reagents. The disagreement in results between the screening test and confirmatory indicates the need to search for new alternatives for the canine diagnosis.

C1620 NON-INVASIVE DIAGNOSIS AND TEST-OF-CURE OF VISCERAL LEISHMANIAISIS: MASS SPECTROMETRY IDENTIFICATION OF PARASITE ANTIGENS DETECTED IN URINE BY A COMMERCIAL CAPTURE ELISA

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1 Background

Visceral leishmaniasis (VL) diagnosis is based on clinical signs and serology, and is confirmed with an invasive biopsy. Test-of-cure after treatment is not definitive using these methods. An ideal diagnostic would be non-invasive, rapid, point-of-care and able to determine cure. Detection of parasite antigens in urine could satisfy these criteria. Currently, the only urine antigen test in use is a latex agglutination test that suffers from several drawbacks.

The VL urine assay *Leishmania* Antigen Detect™ (InBios International Inc., Seattle) uses a pair of polyclonal antibodies against *Leishmania donovani* whole cell lysate. In previous testing, the ELISA had high sensitivity for VL from different geographic regions and specificity of 100%. It was also able to indicate cure; showing a reduction in urine antigen by Day 30 post treatment and negative results for all samples after 6 months. We sought to identify the parasite antigens that are captured by the rabbit antibody in this ELISA.

2 Methods

A polyclonal affinity-purified rabbit antibody against *L. donovani* whole cell lysate, as used in the *Leishmania* Antigen Detect™ ELISA, was coated onto polystyrene dishes. Total protein was precipitated from 130 ml of urine from 31 Indian VL patients and incubated in the dishes. Unbound material was washed away. Specifically-bound material was eluted and submitted to mass spectrometry. Data were screened to exclude human proteins and parasite proteins were identified.

3 Results

Several putative *Leishmania* proteins were identified from urine by mass spectrometry. The nature of these antigens will be presented along with an assessment of their potential in the development of a non-invasive diagnostic.

4 Conclusions

Identification of the specific *Leishmania* antigens that are detected in VL human urine by a polyclonal antibody would enable development of highly sensitive and specific polyclonal or monoclonal antibodies and subsequent adaptation to a low-cost and scalable rapid diagnostic test.

Such a test would negate dangerous and unpleasant biopsies, enable cure to be determined after successful treatment, be suitable for HIV co-infected patients and assist with drug development; all of which would assist with disease control efforts.

C1632 MOLECULAR AND SEROLOGICAL TESTS FOR THE DIAGNOSIS OF VISCERAL LEISHMANIASIS IN DOGS IN AN ENDEMIC AREA IN BRAZIL: ABOUT 1 IN 5 SEROLOGICALLY NEGATIVE ANIMALS ARE INFECTED.

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1 Background

Visceral leishmaniasis (LV) has been occurring in increasing incidence in urban areas in Brazil. Euthanasia of infected dogs is one of the legal instruments adopted to control LV in Brazil and the identification of infected dogs in official LV control programs is done using serological methods. However, due to the low accuracy of the serological methods, alternatives techniques have been widely investigated. The objective of this study was to evaluate the PCR for the diagnosis of VL in domiciled dogs of urban area where the disease is endemic.

2 Methods

Real-time PCR applied in conjunctival (CS), blood (BL) and lymph node (LN) samples was evaluated. In conjunctival swab samples, the performance of conventional PCR (cCS) was also evaluated. The serum samples were tested with the two official tests used in Brazil, the rapid test DPP® Visceral Canine Leishmaniasis, Biomanguinhos, FIOCRUZ for detection of antibodies against K26 / K39 fusion antigens of amastigote of the agent (DPP); and the immunoenzymatic Leishmaniose Visceral Canina, Biomanguinhos, FIOCRUZ for the detection of antibodies against soluble antigens of promastigote of the parasite (ELISA). Two samplings were studied, one containing 974 cases and another, 115.

3 Results

In the first sampling, the highest number of positives was obtained with LN, followed by ELISA, DPP and BL, with the following values: 229 (95% CI 200.3-260.7), 213 (95% CI 185.3-243.6), 87 (95% CI 69.7-107.3) and 37 (95% CI 26.1-51.0), respectively. With the second sampling, the number of positive dogs were: 54 for CS (95% CI 40-70.5), 42 for ELISA and LN (95% CI 30.3-56.8), 36 for DPP (95% 25.2-49.8), 22 for cCS (95% CI 13.8-33.3), 11 for BL (95% CI, 5.5-19.7). Concordance between tests were low and differences between tests positivity were significant in most pairwise comparisons. About one in five seronegative dog is classified as positive by molecular tests.

4 Conclusions

The serological tests fail to accuse an expressive number of infected animals in endemic areas, although the potential of infected but serologically negative animals as sources of infection is unknown. The superiority of real-time PCR over the conventional PCR and the indication that whole blood samples are not suitable targets for LV diagnosis in mass surveys are emphasized. The low agreement between the different diagnostic tests shows that the detection of infected animals should be done with multiple tests combined in parallel.

C1635 COMPARISON OF PCR-RFLP WITH DIRECT MICROSCOPY AND NNN CULTURE METHODS IN THE DIAGNOSIS OF CUTANEOUS LEISHMANIASIS

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1 Background

Leishmaniasis is a parasitic infectious disease with a wide clinical spectrum in tropical and subtropical areas.The aim of the study is to assess the PCR-RFLP method using two primers, ITSRL and L5.8S, compared with direct microscopy and NNN culture methods for the diagnosis of cutaneous leishmaniasisis.

2 Methods

Among 60 CL suspected patients who referred to the Center for Research in Skin Disease and Leishmaniasis, Isfahan University of Medical Sciences.the three methods, direct microscopy, NNN culture and PCR-RFLP were used to detect cutaneous leishmaniasis.

3 Results

The results showed that 60%, 62% and 90% of specimens from suspected cases were positive using direct microscopy, NNN culture,and.PCR RFLP methods respectively.

4 Conclusions

Due to Higher sensitivity of the PCR-RFLP method for detection and speciation of aethiologic agent of CL in medical centers it is suggested the method is utilised along with NNN culture and direct microscopy techniques to establish an accurate and rapid method for detection of *Leishmania* parasites.

C1644 COMPARATIVE EVALUATION OF DIAGNOSIS OF POST KALA AZAR DERMAL LEISHMANIASIS BY QPCR AND MICROSCOPY IN A COHORT OF KALA AZAR PATIENTS TREATED WITH THREE NEW TREATMENT REGIMENS

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1 Background

In India, Post Kala-Azar Dermal Leishmaniasis (PKDL) cases are a reservoir of the leishmania parasite and have a major role in anthroponotic transmission of Visceral Leishmaniasis (VL). Early diagnosis and management of PKDL cases is essential for attaining the 2017 VL elimination program target in the Indian sub-continent and for its long-term sustainability. This study was conceptualized to provide evidence to the Indian National Program about the occurrence of PKDL in VL patients treated with new regimens. In India, microscopic examination and molecular tests (PCR/qPCR) are performed only at the VL referral hospital RMRI in Bihar. This study compared the results obtained from qPCR and microscopy for confirmatory diagnosis of PKDL.

2 Methods

This cohort observational study is conducted in two district hospitals of Bihar and at the RMRI in Patna. All VL patients (n=1761) who had previously been treated in the pilot implementation study in 2012-15 with new treatment regimens, single dose Ambisome, miltefosine and paromomycin combination for 10 days, or Ambisome and miltefosine for 8 days, are invited to participate in the study.
Ethical clearance was obtained from RMRI Ethical Committee.
After obtaining informed consent, the subjects are assessed for history and clinical manifestation of PKDL at a follow-up visit at least 24 months after VL treatment. All clinically suspected cases (skin lesions with past history of VL treatment) are screened by rK39 rapid diagnostic test. Probable PKDL patients (skin lesions and rK39 positive test) are referred to RMRI for parasitological confirmation by microscopy and qPCR in skin slit tissue.

3 Results

Up to 30 Oct 2016, at total of 556 VL treated patients were screened for PKDL. There were 16 (2.9%) suspected cases identified, from whom 13 had rK39 positive test and were referred to RMRI for confirmatory diagnosis. 2 cases had been treated earlier for PKDL based on clinical symptoms. 5 cases did not demonstrate parasites. Diagnosis was confirmed by qPCR for 5/10 patients who had qPCR done (50%), whereas microscopy was positive for 3/11 (27%) who had microscopy performed. PCR was always positive when parasites were seen by microscopy. All confirmed PKDL cases presented macular lesions.

4 Conclusions

Preliminary results indicate that qPCR is highly sensitive for PKDL patients, particularly in macular lesions with low parasite density. Results from a larger sample will be presented at the conference.

C1672 USE OF HIGH-RESOLUTION QUANTITATIVE PROTEOMIC ANALYSIS TO IDENTIFY NEW POTENTIAL BIOMARKERS FOR THE TREATMENT MONITORING OF CANINE LEISHMANIOSIS

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1 Background

Proteomics allows identification of new biomarkers for diagnosis, therapeutic intervention of disease and treatment monitoring, Quantitative gel-free proteomic approach using isobaric labeling reagents such as Tandem Mass Tags (TMT®; Proteome Sciences) enables concurrent identification and quantification of proteins originating from up to ten different samples in a single experiment. Moreover, the use of high resolution mass spectrometry instrumentation for the analysis of differentially TMT-labeled peptide moieties results in high quality data with good sensitivity, excellent signal-to-noise ratio and a broad dynamic range. However, TMT technology has not been previously applied to detect novel biomarkers for treatment monitoring in canine leishmaniosis. So, the objective of this study was to use the TMT labeling approach to generate comparative protein profiles of serum samples from dogs with leishmaniosis before and after treatment in order to identify new potential biomarkers.

2 Methods

Serum samples from 5 clinically diseased dogs collected before and 1 month after the treatment with meglumine antimoniate (100 mg/kg, SC, q24h) and allopurinol (15 mg/kg, PO, q 12h) were used for the proteomic study. Non-depleted serum samples were subjected to reduction, alkylation, digestion and labeling with the 6-plex TMT reagents according to manufacturer procedure (Thermo Scientific) with some modifications. To obtain the information about protein identities and relative quantification, LC–MS analysis of TMT-labeled peptides was performed. Thermo raw files were used for Mascot search against NCBInr database mining *Canis lupus familiaris* proteins.

3 Results

Gel-free label-based proteomic approach enabled identification of 117 canine proteins. Among these, 23 showed significant difference ($p<0.05$) in expression (two downregulated and 21 upregulated ranging from 1.25x – 2.5x). Most of these proteins are involved in two main physiopathological mechanisms: (1) immune response, such as IgA, inter-alpha-trypsin inhibitor heavy chain H4, serotransferrin, histidin rich glycoprotein, alpha 2 macroglobulin, paraoxonase and fibronectin; and (2) coagulation cascade, such as kininogen-1 isoform X1 and X2, plasminogen precursor and beta-2-glycoprotein 1 precursor.

4 Conclusions

TMT-based proteomic approach allowed identification of novel serum proteins that could be potentially suitable biomarkers for treatment monitoring of canine leishmaniosis.

C1674 SERUM N-GLYCOME AS POTENTIAL BIOMARKER IN VISCERAL LEISHMANIASIS.

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1 Background

Re-emergence and spread of visceral leishmaniasis (VL) is a global fact nowadays. Relapse rate poses a major challenge for the leishmaniasis control programs worldwide. Potential biomarkers of recovery after treatment are a priority in both immunocompetent and immunodepressed patients. N-Glycosylation is the most abundant and highly dynamic post-translational modification, and hence, well suited as biomarker as it is quite sensitive to pathophysiological changes of diverse etiology. The potential as biomarker in diagnosis and patient stratification in cancer and chronic inflammation has been reported elsewhere over the last five years, revealing strong associations with different conditions. The serum N-glycome is the full complement of N-glycan structures present on the serum glycoproteins. As the majority of serum proteins are secreted glycoproteins, alterations in the tissue and organ physiology should be reflected in the glycan composition of serum. Monitoring glycosylation changes could be the starting point for the discovery of biomarkers that relate alterations in glycoprotein processing to disease.

2 Methods

Analysis of N-glycome of VL patients before and after chemotherapy (compared to healthy controls), could provide valuable information on the pathogenesis of leishmaniasis. The aim of this study was to investigate whether changes occur in N-glycosylation upon infection with VL and whether a change occur upon treatment. For this, we have obtained the serum N-glycome profile from clinically defined populations including 21 patients with VL before and 3 months after treatment with liposomal amphotericin, 12 samples from the follow-up of 3 VL-HIV patients and 19 endemic controls in a *L. infantum* postoutbreak area (Fuenlabrada, Madrid). In addition, 43 non endemic controls were studied. The N-glycome was analyzed by HILIC-UPLC of PNGase released, fluorescently labeled glycans from individual serum samples and resolved in 41 chromatographic peaks and quantified.

3 Results

Multivariate (Discriminant) analysis of the 41 variables was able to cluster the samples according to the clinical groups, with no false assignments, providing solid evidence of N-glycome changes upon disease and then upon treatment. The test was able to discriminate those non immunodepressed VL patients from those non-immunodepressed. No differences were found in control healthy non-VL populations based on geographic origin.

4 Conclusions

Detailed structural analysis of GPs allowed to identify some glycans with biomarker of therapeutic efficacy.

C1683 CYTOKINE PROFILES IN ORAL FLUID IN AMERICAN CUTANEOUS LEISHMANIASIS PATIENTS: AN USEFUL TOOL IN DIAGNOSIS AND PROGNOSIS OF THIS DISEASE?

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1 Background

The cytokine responses are involved in the progression and resolution of American cutaneous leishmaniasis (ACL). T-helper 1-like cytokine response (Th1) mediates *Leishmania* infection resolution in localized cutaneous leishmaniasis (LCL) patients. Whereas an up-regulation of Th1 and Th17 responses have been involved in the pathogenesis of mucocutaneous (MCL) form of the disease. Most of this information comes from studies in sera, lesions and after *in vitro* stimulation of lymphocytes with Leishmanial antigens. Nevertheless, those responses have not been investigated in oral fluid. In this study, we evaluated the variation of pro-inflammatory Th1, Th17 and regulatory cytokines (IFN-g, IL-22, IL-15 and TNF-α, IL-10) levels in the secretion of the oral mucosa (saliva) and sera, as potential biomarkers that characterize the immune response in patients with ACL.

2 Methods

It was conducted a cross-sectional study. IFN-g, IL-22, IL-15, TNF-α and regulatory IL-10 cytokine levels were assessed by ELISA, in serum and saliva from healthy volunteers, (C, n=29) and patients with LCL (n=16) and MCL (n=17). They were diagnosed at the Institute of Biomedicine Dr. Jacinto Convit, Caracas, Venezuela. For analysis of the data, the Mann-Whitney, and Spearman test were used.

3 Results

We found that LCM patients showed the highest serum levels of proinflammatory and regulatory cytokines. In the oral fluid, MCL patients continue to show an increase levels of IL-15, TNF-α, and IL-10 compared to LCL patients and healthy volunteers (p=0,022-0,0034). However, similar IFN-g levels were observed between groups. IL-22 was decreased in salivary samples of MCL patients but increased in LCL patients. Interestingly, the IL22 salivary levels were positively correlated with IL15 for LCL patients (p<0,02), and with TNF-α for MCL patients. Finally, the values of the salivary and the serum cytokine levels were positively correlated for TNF-α (p = 0.006) and IL-10 (p = 0.0002) in both groups of patients.

4 Conclusions

IFN-g, IL-22, IL-15 and TNF-α, IL-10 were successfully evaluated in oral fluid samples from ACL patients. Variations in their levels could be used as indicative of the immunological status of patients. Therefore, cytokine detection in oral fluid is a useful technique with the advantage of being a minimally invasive method for diagnosis and prognosis in this disease, particularly for TNF-α, IL-10.

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C1724 TARGET PRODUCT PROFILE FOR A POINT-OF-CARE TEST FOR DIAGNOSIS OF DERMAL LEISHMANIASIS

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1 Background

Dermal leishmaniasis comprises of a group of infections caused by *Leishmania* parasites. Between 0.7 and 1.2 million cases of cutaneous leishmaniasis (CL) are reported worldwide per year. Although not lethal, CL causes chronic and disfiguring skin lesions, and is an important cause of morbidity and social stigma. Post-kala-azar dermal leishmaniasis (PKDL), a complication of visceral leishmaniasis (VL) that is common in areas where *L. donovani* is endemic, could also have a potential role in the transmission of VL. Microscopy of Giemsa stained samples, including skin scrapings, fine needle aspirates, or slit-skin smears, remains the reference test for diagnosis of the different forms of dermal leishmaniasis. However, microscopy has a low and variable sensitivity and requires trained and experienced personnel. The technical complexity of the more sensitive molecular techniques (e.g. PCR) limits their application as routine diagnostic tools in endemic areas. Thus new point-of-care (POC) tests are needed. The use of these tests should improve access to treatment, which will benefit patients and communities by reducing the risk of both sequelae and *Leishmania* transmission. To ensure that patient, control programs, and market needs are met, the requirements of such a test should be specified in a target product profile (TPP).

2 Methods

To identify minimal/optimal characteristics for a POC test for dermal leishmaniasis, a draft TPP was first reviewed and discussed with experts from academia and international organizations with expertise in CL diagnosis and management, during a RedLeish meeting (DNDi-Latin America) focused on clinical research. Consensus characteristics were defined in a second step with a Delphi method, in which CL experts and stakeholders from different *Leishmania* endemic regions were invited to participate.

3 Results

We present a TPP for a POC test to diagnose the different forms of dermal leishmaniasis, including localized CL, mucocutaneous leishmaniasis, diffuse CL, CL recidivans and PKDL. This TPP includes 29 features of the test, which refer to specific requirements or specifications of the diagnostic tool to be developed.

4 Conclusions

This TPP identifies the requirements for a POC test for dermal leishmaniasis diagnosis, and will guide efforts by the academic institutions and product developers to achieve the ideal test, which will have a strong impact on patient management and disease control.

C1729 ANALYSIS OF THE REACTIVITY OF ANTINUCLEAR AUTOANTIBODIES AGAINST THE LEISHMANIA INFANTUM ANTIGENS

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1 Background

Visceral leishmaniasis (VL) is a severe parasitic infectious disease caused by *Leishmania (L.) infantum* (syn *L. chagasi* in South America). VL has been associated with hyper-gammaglobulinemia and antinuclear antibodies (ANA) and may simulate systemic lupus erythematosus. In this study, we evaluated the specificity of the ELISA tests commonly used in the diagnosis of VL.

2 Methods

Serum samples from healthy volunteer adults (n = 80) as well as adult patients (n = 80) with evidence of autoimmune disease and positive ANA (ANA +) were analyzed.
Sera were used as follows:

- Control 1: (n = 42) healthy volunteers adult at the National Center for Blood Transfusion CNTS.
- Control 2: (n = 42) healthy pregnant women in screening for Toxoplasmosis at the laboratory of medical parasitology, Pasteur institute.

Several antigens have been prepared in the laboratory from promastigotes of L. infantum, namely: soluble Leishmania antigen (SLA), crude Leishmania histone (CLH). The recombinant kinesin antigen 39 (rK39) of L. infantum were kindly provided by Dr Manuel Soto (Centro de Biologia Molecular "Severo Ochoa", Facultad de Ciencias, Universidad Autonoma de Madrid, ESP) and Dr. Steven G. Reed, Infectious Disease Research Institute, Seattle, USA). The specific antibodies anti-*L. Infantum* antigen (SLA, CLH, rK39) were performed using a developed ELISA tests. AANs were searched for Hep-2 cells and then antigenic specificity was determined using the ANA screen and ANA Profile 1 Euroline kits (Euroimmun, Lubeck, De), anti-histone autoantibodies were searched using the kit Anti-histones ELISA IgG (Euroimmun).

3 Results

Among the AAN serums, 32 recognize the SLA antigens and of (AAN +), 15 had anti-rK39 antibodies, and 29 exhibited anti-THC antibody.There was a positive and significant correlation between the absorbance of the anti-rK39 IgG ELISA and the anti-CLH IgG ELISA (r = 0.239 and P = 0.032). There is a cross-reaction between the AANs towards Leishmania antigens, especially histones.

4 Conclusions

In light of these results, we recommend taking precautions when interpreting the results of the serodiagnosis of VL in adult subjects with positive ANA.

C1741 QUANTIFICATION OF ANTI-LEISHMANIA ANTIBODIES IN SALIVA OF DOGS

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1 Background

Traditionally, the detection of specific serum anti-*Leishmania* antibodies by quantitative or qualitative serological techniques has been the most used method for the diagnosis of Canine Leishmaniosis (CanL). Nevertheless, saliva may represent an alternative to blood due to it is a sample easy to collect, no painful, minimally invasive and with more economical sampling, shipping and storage in comparison with serum. In this study, two time resolved-immunofluorometric assays (TR-IFMAs) for quantification of anti-*Leishmania* IgG2 and IgA antibodies in saliva were developed and validated and the ability to determine whether the assays were able to distinguish between *Leishmania*-seronegative and seropositive dogs was evaluated.

2 Methods

The analytical validation of the assays was performed by evaluation of assay precision, sensitivity and accuracy. For the clinical validation serum from 50 naturally exposed dogs to phlebotomine sand flies were tested by ELISA and, as a result, dogs were classified as *Leishmania*-seropositive or seronegative. Saliva samples from these dogs were analyzed by TR-IFMAs and results were expressed as Units of Fluorometry for *Leishmania* (UFL).

3 Results

Analytical validation indicated that the assays were precise with an intra- and inter-assay coefficients of variation lower than 11% and the assays also demonstrated a high level of accuracy, as determined by linearity under dilution ($R^2 = 0.99$) and recovery tests (> 88.60 %). Clinical validation showed that anti-*Leishmania* IgG2 in saliva were significantly higher in the seropositive group compared with the seronegative ($p < 0.0001$), whereas no significant differences for anti-*Leishmania* IgA between these groups were observed. In addition, TR-IFMA for quantification of anti-*Leishmania* IgG2 in saliva showed a wide difference in the concentration of that antibody between seropositive and seronegative dogs.

4 Conclusions

It can be concluded that the assays developed may be used to quantify anti-*Leishmania* IgG2 and IgA antibodies in canine saliva with an adequate precision, analytical sensitivity and accuracy. In addition, the quantification of anti-*Leishmania* IgG2 antibodies in saliva could be a practical tool, simple and minimally invasive to evaluate the humoral response in CanL.

C1748 OPTIMIZING AND IMPROVING OF TAQMAN REAL TIME PCR TECHNIQUES TO FIRMLY IDENTIFICATION OF LEISHMANIASPECIES BY TARGETING COII GENE

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1 Background

Genetic recombination within strains in *Leishmania* species cause genetic exchanges with hybrid progeny in next generation and chromosomal anoploied.To recognize variation regions of COII gene, specific primers and probes designing were done and qPCR was used to accurate identification of *Leishmania* species , subspecies and co-infection.

2 Methods

DNA of *Leishmania was* extracted. COII gene was amplified by PCR and a recognized region was cloned. Specific probes and primers were designed in different channels to identify *Leishmania*species.

3 Results

Ten samples of standard *Leishmania*species were amplified. Specific primers and probes were designed for *L.major*and *L.tropica*. These primers and probes separated these two important species and subspecies.The primer and probe could separate all *Leishmania*species. different channels of TaqMan were Considered, these new molecular tools were able to separate two coinfections of *L. major*and *L. tropica*and their subspecies.

4 Conclusions

The designed primers and probes could firmly identify not only all the *Leishmania*species but also subspecies and strains by Considering different channels of TaqMan.

C1772 DIRECT SEARCH OF THE PARASITE IN PERIPHERAL BLOOD, BONE MARROW AND LYMPH NODE FOR THE DIAGNOSIS OF CANINE VISCERAL LEISHMANIASIS IN ENDEMIC AREA OF BRAZIL

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1 Background

Leishmaniasis is a serious zoonosis, widely distributed in Brazil, which has the domestic dog as the main reservoir in the urban area. The presence of the dog is not a determining factor for infection in humans, but it can be observed that the canine infection usually precedes the human. The symptoms manifested by the infected animal are diverse and the diagnosis is performed by serological methods, however the direct search of the parasite is the gold test to confirm the infection. The objective of the study was to investigate Leishmania amastigotes (direct search) in peripheral blood (ear), bone marrow (iliac crest puncture) and lymph node (aspiration puncture) for the diagnosis of Canine Visceral Leishmaniasis.

2 Methods

A total of 236 dogs from Campo Grande (Mato Grosso do Sul), positive for leishmaniasis in the ELISA and/or DPP rapid test serological, were referred to the Zoonoses Control Center for euthanasia from May to July/2016. Performed smears on slides, stained by Giemsa and examined under an optical microscope (1000x).

3 Results

A total of 93 male and 143 female dogs were analyzed. Of these, 175 samples of peripheral blood (74.2%), 185 samples (78.4%) of bone marrow and 234 samples (99.2%) of lymph node were positive. 141 dogs showed positive results in the three biological samples and all dogs had at least one positive sample. The combination of the two serological tests showed 100% positivity.

4 Conclusions

To confirm the diagnosis of Canine Visceral Leishmaniasis a combination of serological tests (DPP and/or ELISA), clinical examination and direct search from lymph node puncture is recommended. Acknowledgments to FUNDECT for financial support.

C1794 LOOP-MEDIATED ISOTHERMAL AMPLIFICATION AS A RAPID AND ACCURATE DIAGNOSTIC METHOD FOR HUMAN LEISHMANIASIS

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1 Background

The current common approach for molecular diagnosis of human leishmaniasis relies on PCR or real-time PCR, these are technically demanding and require cold chain for reagents transport and storage, limiting their use in peripheral settings. In addition to this, there are no standardized methods across the different regions and diagnostic laboratories, where the use of *in house* protocols is the rule. Loop-mediated isothermal amplification (LAMP) overcomes the dependence on expensive equipment and allows rapid, sensitive and specific amplification of the target DNA. The LoopAmp™ *Leishmania* Detection Kit (Eiken Chemical Co., Japan) has the additional advantage of using lyophilized reagents, which guarantees stability in the absence of cold chain. The aim of this study was to evaluate the accuracy of this test for the diagnosis of human cutaneous and visceral leishmaniasis.

2 Methods

An assembly of 191 DNA samples from visceral and cutaneous leishmaniasis suspect patients of *Leishmania infantum* endemic area (Fuenlabrada, Spain) was used in this evaluation. This was analyzed by three different molecular tests: i) LoopAmp™ *Leishmania* Detection Kit run in a Genie III fluorimeter (Optigene); ii) *Leishmania* nested PCR (LnPCR) run in a conventional thermal cycler (Applied Biosystems 3200); and iii) real-time quantitative PCR (qPCR), run in a LightCycler 2.0 (Roche) using SYBR Green I to detect the amplification.

3 Results

Using qPCR as reference test, LAMP presented 98.6% [CI 95% 94.97% to 99.83%] sensitivity and 96.0% [CI 95% 86.29% to 99.51%] specificity either reading the result by fluorimetry or blue light illumination and visual inspection. LnPCR presented 99.3% [CI 95% 96.11% to 99.98%] sensitivity and 96% [CI 95% 86.29% to 99.51%] specificity. The concordance between qPCR and LAMP was excellent ($\kappa = 0.9458$), and we also found a strong correlation between the time of amplification plot (*Tp*) of LAMP and the threshold cycle (*Ct)* value of qPCR ($r^2=0.7551$; $p<0.0001$).

4 Conclusions

In conclusion, the LoopAmp™ *Leishmania* Detection Kit presented a diagnostic performance comparable to qPCR and LnPCR. Its simplicity, speed, sensitivity and specificity allows rapid and accurate diagnosis of human leishmaniasis, making this a suitable tool for confirmatory diagnosis of leishmaniasis in decentralized settings. The excellent correlation found between *Tp* and *Ct* value deserves further studies in order to investigate the accuracy of LAMP to quantify the parasite load.

C1798 ELEVATED IP-10 PLASMA LEVELS AS A MARKER OF ACTIVE DISEASE IN PATIENTS WITH VISCERAL LEISHMANIASIS CAUSED BY LEISHMANIA INFANTUM INFECTION

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1 Background

Chemokines are an integral part of the host defense against pathogens. Interferon-γ–inducible protein 10 (IP-10) and IL-8 are two important chemokines that play a key role in the recruitment of immune cells. Activated neutrophils (PMNs) and macrophages are the first cells to arrive at the site of *Leishmania* infection. In humans, PMNs containing *Leishmania* start secreting chemokines such as IL-8 that are essential in attracting more PMNs to the site of infection. Furthermore, IP-10 is a potential candidate for immunotherapy to cure visceral leishmaniasis because it promotes recruitment and activation of Th1-polarized cells. The role of these two chemokines in the context of *L. infantum* infection needs further elucidation.

2 Methods

In the present work, we have evaluated the plasma secretion of IP-10, IL-8, IFN-γ and IL-10 of patients with active visceral leishmaniasis caused by *Leishmanial infantum* and same patients after cure (N = 17) in order to study their potential as markers of disease/cure of visceral leishmaniasis caused by *L. infantum* infection.

3 Results

The results showed that in all patients the plasma concentrations of IP-10, IL-10 and IFN-γ were high during active disease and these levels significantly decreased after treatment. In the acute phase, IP-10 showed higher levels regarding to IFN-γ and IL-10 when means were compared (1357,78 pg/mL regarding to 14,73 pg/mL and 16,35 pg/mL, respectively). Same was observed for cure status (331,45 pg/mL regarding to 0,85 pg/mL 0,73 pg/mL, respectively). Further, we have found a strong correlation between the levels of IP-10 and IFN-γ in both active visceral leishmaniasis patients (r^2=0.9386, $p<0.0001$) and cured patients (r^2=0.9140, $p<0.0001$). The plasma IL-8 levels were not related with the active or cure status of the patients.

4 Conclusions

In conclusion, the decrease in plasma levels of IP-10, IL-10 and IFN-γ concentrations was related with cure after treatment. The high plasma levels of IP-10 postulates this chemokine as a sensitive marker of active disease in *L. infantum* infection, as already described for *L. donovani* and other infectious diseases like tuberculosis. Further studies need to be done to evaluate IP-10 levels as a marker to follow-up patients and to prevent relapses.

C1799 DEVELOPMENT OF AN ANTIGEN DETECTION TEST IN URINE FOR THE DIAGNOSIS OF ACTIVE VISCERAL LEISHMANIASIS

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1 Background

Visceral leishmaniasis (VL) diagnosis is routinely performed by invasive splenic, bone marrow, or lymph node aspirates, followed by microscopic identification of the parasites. Due to the limitations of this invasive approach, the presence of anti-parasite antibodies in serum is also routinely used to diagnose VL. Antibody tests have variable sensitivity in different endemic regions, and do not discriminate active disease from cured individuals. An antigen detection test, detecting leishmanial carbohydrate antigens in urine of VL patients with active disease was developed 10 years ago. The sensitivity/specificity of the test varied widely, probably due to the heterogeneity of the carbohydrate antigens. As an alternative, we have previously shown that an assay that uses three defined *Leishmania infantum* protein antigens circumvented these restrictions for the diagnosis of New World VL. However, these antigens or biomarkers were detected only in ~60-70% of patients with VL caused by *Leishmania donovani* (Old World VL). Here we report the identification of four unique *L. donovani* biomarkers that will help increase the sensitivity of the assay.

2 Methods

In a similar approach that allowed the identification of *L. infantum* proteins in urines of New World VL patients, Mass Spectroscopywas used to identify *L. donovani* protein biomarkers in the urine of Old World VL patients from Kenya and India. The following biomarkers were unambiguously identified: *Ld-mad1* (malate dehydrogenase), *Ld-clp1* (clp protease), *Ld-mao1* (MaoC dehydratase) and *Ld-ppi1* (peptidyl-prolyl isomerase). *L. donovani* genes encoding these four proteins were cloned, recombinant proteins produced and rabbit antibodies were obtained to generate reagents for the development of a capture ELISA.

3 Results

Thus far, antigen detection ELISA has been developed and evaluated in pre-clinical studies for *Ld-clp1* (limit of detection ~40 pg/ml) and for *Ld-mao*1 (limit of detection ~10 pg/ml). Sensitivities of individual assays were 52% and 44% respectively. However, combination of the results of the two assays increased the sensitivity to 72% for the diagnosis of VL caused by *L. donovani*.

4 Conclusions

The results are very encouraging in that they suggest that an expanded multiplexed assay composed of the four newly discovered VL biomarkers +/- the former *L. infantum* biomarkers should dramatically increase the sensitivity of a final test for the diagnosis of both Old and New World VL.

C1805 NEW APPROACH FOR CONFIRMING CURE IN LEISHMANIA INFANTUM INFECTED PATIENTS UNDER IMMUNOSUPRESSOR TREATMENTS

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1 Background

Immunosuppression is a major risk factor in the appearance of overt clinical leishmaniasis; it can also alter the presentation of the disease and response to the treatment. Nowadays, the prevalence of immunosuppresion conditions not associated with HIV virus is increasing. The treatments with glucocorticoids affect T cytotoxic lymphocytes by blocking cytokine expression, which cause an increase in the susceptibility to infections, particularly to intracellular infections as *Leishmania* parasites. Moreover prolonged use of steroids has been associated with decrease production of IL-2, IFN-γ y TNF-α, key cytokines to resolve leishmaniasis.

2 Methods

In this work, we evaluated the lymphoproliferative response (CPA) to soluble *Leishmania* antigen (SLA) and the cytokine (IFN-γ, TNF-α, IL-10, IL-17) and granzyme B secretion in the supernatants of PBMCs stimulated with SLA in five immunosuppressed patients (glucocorticoids and steroids treatments) with active visceral leishmaniasis cause by *L. infantum* and eight months after cure. In addition, CD4, CD8 and CD3 levels were measured in flow cytometer by selecting the lymphocyte gate. The immunosuppressive treatment was withdrawn during the treatment of leishmaniasis and reintroduced after establishing clinical cure at three months.

3 Results

In the active moment, the patients did not present specific secretion of any of the cytokines studied, and this was related with negative CPA and lymphocyte number below the normal range. However, after cure the patients presented a positive CPA and lymphocyte number within the normal range, which contributes to a specific production of IFN-γ, TNF-α and granzyme B. These differences found in CPA, cytokine secretion levels and lymphocyte number were statistically significant between cure and active status.

4 Conclusions

The combined use of CPA and cytokine secretion allows the assessment of the specific immunity developed against *Leishmania* in immunosuppressed patients. Further, we have described that these patients, after recovery of visceral leishmaniasis, developed a specific Th1 immune response that remains patent five months after re-introducing immunosupresor treatments. Further studies are needed to evaluate the accuracy of this approach for the follow-up of cured visceral leishmaniasis patients under immunosupressor treatments, and even for preventing relapses.

C1808 CONTRIBUTION OF QUANTITATIVE REAL TIME POLYMERASE CHAIN REACTION TO THE DIAGNOSIS OF CUTANEOUS LEISHMANIASIS IN TUNISIA.

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1 Background

Cutaneous leishmaniasis (CL) is a major health problem in Tunisia because of the high incidence of cases mainly in the center south of country. Diagnosis is suspected on evocative clinical presentation in patients living in or coming from endemic areas. Conventional laboratory methods, including direct examination of dermal smears and culture isolation of *Leishmania (L.)*, although highly specific, lack of sensitivity. Our aim of this study was to appraise the contribution of quantitative real time Polymerase Chain Reaction (qPCR) to the diagnosis of cutaneous leishmaniasis.

2 Methods

The study was conducted on 156 patients referring to the Department of Medical Parasitology of Pasteur Institute for CL suspicion. Epidemiological and clinical data were recorded for each patient and sampling of lesions was made by dermal scraping. Laboratory diagnosis was based on direct examination of Giemsa stained dermal smears and culturing on NNN medium. qPCR TaqMAN® targeting the kinetoplast DNA, was completed for some patients with negative direct examination, after DNA extraction using Qiamp DNA Blood Mini Kit® (Qiagen, Hilden, Germany).

3 Results

In total CL diagnosis was confirmed in 78 cases (50%). The mean of age was 33,6 years and sex ratio 1,6. Patients were mainly contaminated in the Centre-South of Tunisia, where zoonotic CL due to *L. major* is endemic (73,1%).The diagnosis was made by direct examination and/or culture in 66 cases. qPCR was performed on 32 among the 90 negative samples by direct examination. DNA amplification was obtained in 11 out of 32 (34,3%). Corresponding lesions were highly suspected on epidemiological and clinical criteria. Negative qPCR ruled out the diagnosis of CL in 15 non evocative lesions.

4 Conclusions

In conclusion, regarding its better sensitivity compared to conventional methods, qPCR revealed an interesting alternative for CL laboratory diagnosis and highly useful for the physicians to state on CL suspected cases.

C1826 USE OF AN INTERFERON GAMMA RELEASE ASSAY (IGRA) TO TEST T-CELL RESPONSIVENESS TO SOLUBLE LEISHMANIA INFANTUM ANTIGEN IN WHOLE BLOOD OF DOGS FROM ENDEMIC AREAS

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1 Background

Previous studies on dogs' peripheral blood mononuclear cells indicated thatquantifying IFN-γ secretion is a promising tool to follow the clinical progression of Canine Leishmaniasis (CanL).However using PBMC is difficult technique needing a lot of blood, and is more adapted to research than to routine diagnosis. Interferon-Gamma (IFN-γ) Release Assays (IGRAs) are rapid, sensitive and easy tests using whole blood that allow rapid screening of cellular immunity. The aim of this study was to use IGRA to investigate *Leishmania infantum*-specific IFN-γ release in dogs living in endemic area of leishmaniasis and to interpret the results in light of clinical status, specific anti-*Leishmania* humoral response and presence of *Leishmania* DNA in blood.

2 Methods

Fifty seven dogs living in greater Tunis area were classified according to the results of clinical examination, serological and molecular analysis. The dogs were screened for *Leishmania* infection by ELISA and IFAT then by PCRs on blood. All DNA samples were screened for *Kinetoplastidae* infectionby kinetoplast DNA (kDNA) real-time PCR and all positive kDNA qPCR specimens were systematically amplified by ITS1-PCR and sequenced for *Leishmania* species identification.After classifying the dogs with conventional techniques, we used IGRA to test IFN-γ release in whole blood after 20-hours challenge with soluble *Leishmania* antigen. PBS and mitogen stimulation were used as negative and positive control.

3 Results

IGRA results were valid in 43 dogs according to controls' results. All "healthy non-infected dogs" were negative by IGRA whereas all "asymptomatic and oligosymptomatic infected dogs" were positive by IGRA.The highest concentrations of *Leishmania* specific IFN-γ measured by IGRA were produced by "asymptomatic and oligosymptomatic infected dogs", whereas CanL dogs showed a significant IFN-γ production decrease ($p<0.05$). Furthermore, in seropositive dogs, the highest IFN-γ concentrations were observed in samples with the lowest specific antibodies titers ($\rho= -0.58$; $p =0.01$).

4 Conclusions

IGRA seems to be a useful new tool that permits the easy evaluation of cellular immune-response in naturally infected dogs

C1832 SHORT TERM FOLLOW-UP OF ACUTE PHASE PROTEINS IN DOGS WITH CLINICAL LEISHMANIOSIS GIVEN TWO DIFERENT CONVENTIONAL TREATMENTS

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1 Background

In dogs, *Leishmania infantum* infection can manifest as subclinical, self-limiting or even life-threatening disease.Disease progression is determined by the type of immune response produced in the animal and levels of anti-*L. infantum* antibodies correlate well with clinical disease. Serum concentrations of acute phase proteins (APPs) could provide valuable information for the diagnosis, monitoring and prognosis of CanL (canine *Leishmaniosis*). The objective of the present study was tocompare the change in the acute phase proteins (APPs) in response to two treatments in patients with CanL.

2 Methods

Twenty dogs with clinical leishmaniosis were randomly assigned to two treatment groups: antimonials plus allopurinol or miltefosine plus allopurinol. Serum levels of APPs including C reactive protein (CRP), haptoglobin (Hp), ferritin (Ft), paraoxonase-1 (PON-1) and albumin (Alb) were monitored over three months of treatment (days 0, 30 and 90).

3 Results

At the study onset, most of the dogs showed some APP abnormalities. Over the study period, a trend was observed towards normalization of the proteins examined with the exception of Hp levels recorded in the antimonials group and Ft levels in both treatment groups. Although no differences were detected between groups in the first or subsequent visits, significantdifferences in serum Ft and PON-1 concentrations were noted between treatments from days 0 to 30 and 0 to 90 . No differences in CRP, Hp, Alb were observed.

4 Conclusions

Significant differences in Ft andPON-1 concentrations were detected from the beginning until the end of treatment period between two treatment groups. These findings suggest that certain APPs could be useful biomarkers for the follow-up of canine leishmaniosis treatment.

C1833 USE OF THE QPCR FOR DETECTION OF LEISHMANIA INFANTUM AND LEISHMANIA BRAZILIENSIS IN NATURALLY INFECTED DOGS

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1 Background

The leishmaniasis is a parasitic zoonosis caused by the *Leishmania* genus. The *Leishmania infantum* species cause a severe form of the disease, and it has infected millions of people in the world, resulting in death when untreated. The dogs shelters countless parasites in their skin, therefore it is considered important in the transmission cycle. However, the role of dogs in *Leishmania braziliensis* cycle was not defined. Despite this, the dogs can develop signs that may hinder clinical diagnosis. Hence, the serological diagnosis is not specific and cross reactivity between *Leishmania* species is common. The real-time quantitative (qPCR) is an excellent alternative for differentiation of *Leishmania* species. The objective of this work was to evaluate the prevalence of the *Leishmania* species for dogs.

2 Methods

We collected blood samples from 138 dogs of the city of Marechal Deodoro -AL, and stored in tubes containing EDTA for the realization of qPCR for the detection of *L. infantum* using LINF1B system and *L. braziliensis* using kDNA1 system.

3 Results

A total of 14.49% (20/138), was positive for *L. infantum* and 34.78% (48/138)for *L. braziliensis*. The co-infected dogs represented 7.97% (11/138) from the total samples.

4 Conclusions

The prevalence of *L. braziliensis* brings back the need to review the role of dogs as reservoirs, as well as the infection to phlebotomines. It is also necessary distinguish among *Leishmania* species to help veterinary and human clinical analysis, reducing euthanasia and confirming the clinical form, respectively. These results make to think about dogs that are erroneously diagnosed as positive for *L. infantum* due to cross-reactions in serological methods.

C1836 EVALUATION OF DIAGNOSTICS METHODS APPLIED TO CANINE VISCERAL LEISHMANIASIS

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1 Background

The visceral leishmaniasis (VL) is an important zoonosis on public health; it is caused by *Leishmania infantum*, a protozoan parasite. The dogs have a relevant role in the epidemiology of the disease, since it is the main source of infection. The diagnosis is a problem for the advancement of studies in countries whereTrypanosomiasis are endemic, due to the possibility of cross-reactivity among different Families of the parasites. Studies for the evaluation of diagnostic methods to detect *Leishmania infantum* in Brazil are very important, because false positive results in serological tests applied for dogs are common, and these errors combine for an unnecessary euthanasia. The objective of this study was to evaluate the diagnostic methods applied in canine visceral leishmaniasis (CVL).

2 Methods

Blood samples were collected from 138 dogs of the city of Marechal Deodoro –AL/Brazil. These samples were submitted to the gold standard methods: immunochromatographic test (DPP®) as a screening, and Enzyme Linked Immunosorbent Assay (ELISA) as confirmatory test. Then, molecular test was carried out for the detection of kDNA from *L. infantum* through real-time quantitative PCR (qPCR).

3 Results

A total of 18.84% (26/138) were positive to the screening test DPP®, and of these, 50% (13/26) were positive using ELISA, and 30.76% (4/13) for qPCR.

4 Conclusions

The results demonstrated the need to define a set of criteria for confirmation of CVL, since the existence of overlapping areas with trypanosomatids from different species carry to cross reactions in serology. We obtained misleading results in serological methods when compared with the qPCR. We suggest that, in positive cases in screening techniques, before the animal is referred for euthanasia, it should be confirmed by qPCR in association with clinical evaluation.

C1845 IDENTIFICATION OF NEW ANTIGENS OF LEISHMANIA INFANTUM WITH POTENTIAL USE IN THE SEROLOGICAL DIAGNOSIS OF CANINE VISCERAL LEISHMANIASIS

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1 Background

Leishmaniasis is a complex group of diseases caused by intracellular protozoan of the genus *Leishmania* that infect macrophages of a variety of mammals. The visceral form of the disease is fatal if untreated. Although the continuous development of immunodiagnostic tests over the last decades, intrinsic limitations of each technique result in an unsure diagnosis. A high sensitivity tests are desired for screening and early cases detection. Moreover a diagnostic test with high specificity is determinat for the prognosis and treatment of cases. During the last years, our group has been developing immunogenomic studies to identify new antigens with potential use in improving the diagnosis of canine visceral leishmaniasis (CVL) through the production of peptides and recombinant proteins.

2 Methods

In the present study proposed to identify and characterize, by using data mining methodologies in the area of ??immunogenomics. Two proteins of *L. infantum* that presents similarity with proteins present in the database ImmunomeBase (comprising proteins of *Homo sapiens* with biological function associated to host defense processes) were selected. The genes encoding the MLi and ELi proteins were expressed in *Escherichia coli* strain BL21-Star using the pET28a-TEV vector. After purification by affinity chromatography both proteins were used in ELISA tests for CVL. The antigenic properties of the proteins were evaluated and linear B cell epitopes were identified and validated for potential use in the CVL serodiagnosis.

3 Results

Significant differences ($p < 0.0001$) were observed when MLi and ELi antigens were used to discriminate infected from control canine sera (negative animals and infected with *Babesia* only), achieving a specificity of 96% and 84% and sensitivity of 90% and 89%, respectively.

4 Conclusions

Our results suggest that the use of genomic approaches can open perspectives of intervention and identification of new targets for CVL diagnosis.

C1857 THE FIRST PCR DETECTION AND SEQUENCING OF ISOLATES FROM CUTANEOUS LEISHMANIASIS CASES FROM NIGERIA

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1 Background

For the first time we show molecular evidences of Cutaneous Leishmaniaisis (CL) in Nigeria with a sequence confirmation of *Leishmania major* in one of the isolates from classical CL lesions. Leishmaniasis has been a neglected disease in Nigeria despite the first diagnosis in 1924. Clinical diagnoses of the disease are mostly wrong because of poor knowledge of the disease. Scanty published works exist containing basic serological surveys. To our knowledge, there are no PCR nor sequence evidences from Nigeria, raising a doubt as to whether CL recently exists in Nigeria. We performed direct PCR based diagnosis on exudates and biopsies from non-healing chronic skin ulcers presented to hospitals in Plateau State, Nigeria between August, 2015 to November, 2016.The ulcerations were between 1-3 cm in diameter, occurring at exposed body parts. The lesions were wrongly diagnosed as ordinary ulcerations or due to allergic reactions, hence, they persisted.

2 Methods

Sequel to local anaesthesia, impression smears were made for microscopy, skin biopsies were obtained and DNA extracted for PCR as described by the Tropical Institute, Antwerp, Belgium by amplifying the 'N' and 'F' fragments from the heat shock protein (hsp70) gene sequence. The sequence analysis of the 1,286 base pairs amplicon from the 'F' fragment of the first positive sample was done at the Institute of Tropical Medicine Antwerp, Belgium.

3 Results

Microscopy showed extracellular *Leishmania* Amastigotes while the fragment 'N' PCR electrophoresis showed the expected amplicon band size of 593 base pairs confirming the presence of *Leishmania* DNA in positive samples. Out of thirty-one individuals, eleven ulcers were positive for *Leishmania* Amastigotes and DNA. The 'F' PCR fragment sequences of the first isolate showed 100 percent similarity with the 'F' fragment of *Leishmania major* sequences from the gene bank.

4 Conclusions

While our sequencing of the PCR product of recent isolates will follow, our preliminary results underscore the need for large scale molecular epidemiology of Leishmaniasis in Nigeria. Secondly, research into the sandfly mappings and animal host reservoirs is necessary. Thirdly, awareness among health workers including PCR screening of suspected cases in Nigeria should be encouraged to limit wrong diagnosis. Finally, there is a strong indication that Leishmaniasis is under reported in Nigeria and there is an urgent need for sustained interventions.

C1876 SEROLOGIC DIAGNOSIS OF LEISHMANIASIS IN COMMUNITY CATS FROM AN URBAN AREA IN BRAZIL – PRELIMINARY RESULTS.

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1 Background

Dogs are widely recognized as main domestic reservoir of *Leishmania infantum*, former etiologic agent of visceral leishmaniasis in Brazil. These parasites can be found in other mammals with no significant importance in surveillance and control. However, case reports in cats have increased in the past years, as the number of domestic cats also raised in urban areas. In addition, the importance of domestic cats as reservoirs in urban areas is yet to be determined.

2 Methods

A transversal study was made using sera of 106 stray cats from an urban park of Belo Horizonte city, state of Minas Gerais, Brazil. One single sample was collected from each cat from December (2015) to September (2016). Samples were tested by two different methods of ELISA using a crude antigen or a recombinant repetitive antigen of *Leishmania infantum* (kDDR). Biopsies from ear skin were collected for histology and conventional PCR. Additionally sera of 20 and 33 domestic cats, respectively, from a veterinary hospital and a non-endemic city were used as negative controls.

3 Results

The optic density (O.D.) of the cat's population in both ELISA started to increase from May to July and gradually decreased until September. From December to April, lower levels of specific anti-Leishmania IgG were detected. Ear skin sample of 36 cats were negative in PCR. Interestingly, samples from cats of the veterinary hospital had lower levels of specific IgG in both ELISA. However, cats from a non-endemic area had a high dispersion of O.D. values, some of them higher than the positive control.

4 Conclusions

These results suggests a seasonal distribution of seroreactivity in the cat's population. However, the lack of parasites in the skin suggests a different pattern of the infection, comparing to infected dogs. In addition, this can suggest a lesser importance of cats in the natural transmission of leishmaniasis. Besides that, kDDR-ELISA apparently showed better results than conventional ELISA, in detecting infected cats, however more data is needed to better understand the fate of the infection in cats.

C1882 ANTI-LEISHMANIA ANTIBODIES IN BLOOD DONORS FROM ENDEMIC AREAS OF BRAZIL

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1 Background

In Brazil, incidence of visceral leishmaniasis (VL) is 3,289 cases annually (2015). In endemic areas, around 95% of *Leishmania* (*L.*) *infantum*-infected individuals remain asymptomatic and likely be undetected and accepted as blood donors. The first report of transfusion-transmitted VL (TTVL) came from China in 1948, where two children became infected. TTVL reports are rare, may be because it is not easy to differentiate it from vector-mediated transmission, since most cases of transfusion transmission occur in endemic areas and do not necessarily, result in symptomatic LV. TTVL is reported worldwide but there are no data in Brazil. The aim of this study was to assess the seroprevalence of anti-*Leishmania* rK39 antibody in blood donors of blood banks localized in endemic areas for VL.

2 Methods

The study was carried out with 5,914 blood donors from 10 Brazilian states, which were surveyed for IgG antibodies by enzyme-linked immunosorbent assay (ELISA) using recombinant K39 antigen (ELISA-rK39). From these samples, 620 were also assayed in ELISA-rK28, ELISA-*L. major* like promatigote total extract and Indirect fluorescent assay (IFA) with *L. major*-like promatigotes. Infectious Disease Research Institute, USA, provided recombinant K39 and K28 antigens.

3 Results

Anti-rK39 antibodies were detected in 147/5,914 samples (2.5%) and 24.4%, considering 620 samples. Anti-rK28 antibodies were detected in 65/620 samples (10.8%). Using *L. major*-like antigens, 182/620 samples (30.2%) were positive by ELISA and 24/620 (4.0%) by IFA. Considering ELISA with rK39, rK28, *L. major*-like and IFA, 20/620 (3.2%) blood donor candidates were positive in at least three tests.

4 Conclusions

From these results, it seems that rK39 is more sensitive than rK28 to detect asymptomatic infections. Sensitivity broadly varied among antigens and assay formats. Anyhow, the great prevalence of IgG antibodies achieved in blood samples from asymptomatic donors points out to the risk of transfusional leishmaniasis in endemic areas. As in those areas, it is almost indistinguishable transfusion- or vector-mediated transmission the occurrence of transmission by transfusion is probably underestimated. Our data raise concerns on blood transfusion safety.

C1890 PROGNOSTIC IGG PATTERNS BY WESTERN BLOTTING IMMUNODETECTION IN DOGS INFECTED WITH LEISHMANIA INFANTUM

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1 Background

Leishmaniasis in infected dogs can progress differently depending on the answer of dog's immune system.. IgG1 and IgG2 are the two main immunoglobulin that have been related to host Th1/Th2 response and disease evolution. Here we used Western Blotting (WB) to analyze the idiotype expression of total IgG, IgG1 and IgG2 in dogs naturally infected with *L. infantum* and treated with N-methil meglumine antimoniate in order to detect specific immunoglobulins that can be used as markers of a favorable or unfavorable prognosis.

2 Methods

Seventy dogs naturally infected with *L. infantum* were selected for this study and chemotherapeutically treated with N-methil meglumine antimoniate following standard protocols and periodically examined by WB for a period of 30 months.

3 Results

Twenty-seven polypeptide fractions of the *L. infantum* crude antigen (with molecular weight ranging from 10 to 286 kDa) positively reacted at WB. Significant correlation emerged between eight *L. infantum* antigens (169, 99, 66, 64, 49, 33, 31 e 14 KDa) and total IgG in dogs with favorable prognosis. In dogs which did not recover after treatment with N-methil meglumine antimoniate, immune reaction against antigen polypeptides with molecular weight: 31, 49, 66 and 115 kDa were statistically more frequent. No significant IgG1 or IgG2 patterns could be identified in either group.

4 Conclusions

The association between WB immune-dominant bands and successful or unsuccessful recovery in treated dogs can be used as prognostic marker to evaluate the outcome of clinical therapy. Likewise, information on immune-dominant polypeptides in recovering dogs could be used to implement novel vaccines targeting these specific antigenic fractions of *L. infantum.*

C1893 COMPARATIVE STUDY OF DIAGNOSTIC METHODS OF VISCERAL LEISHMANIASIS IN ADULT PATIENTS WITH AND WITHOUT AIDS

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1 Background

Diagnostic methods for visceral leishmaniasis (VL) are well known in immunocompetent patients, but poorly studied in HIV/ AIDS patients. The aim of the study was to compare the diagnostic methods of VL in patients with and without HIV/AIDS.

2 Methods

We studied 127 adult patients with VL diagnosed consecutively at an infectious disease service in an endemic area to VL, in the Center-West region of Brazil. Forty-eight (37,8%) had HIV/ AIDS. In the healthy control group, 430 blood samples from blood donors were used. Four diagnostic methods were evaluated: direct parasitological examination (DP), indirect fluorescent antibody test (IFAT), immunochromatographic or rapid test (RT) and polymerase chain reaction (PCR). Not all participants were submitted to the four tests. In the control group it was not performed DP.

3 Results

The sensitivity of DP in HIV/AIDS patients was higher than in immunocompetent patients (91,3% vs 67,6%, p = 0,002), whereas RT was lower in this group (47,2% vs 94,7%, p <0,0001). The PCR in peripheral blood showed good sensitivity in both groups (97,8% vs 97,3% p = 0,681). The DP presented sensitivity similar to PCR in the HIV/SIDA group. The comparison of the methods in each group revealed that in the group with HIV/AIDS the sensitivity of RT was much lower than that of parasitological (91,3 vs 47,2, p = 0.006, k = -0,29) and similar to PCR (9,.3 vs 97,8, p = 0,37, k = 0,39). It was also observed that in the immunocompetent group RT and PCR presented similar sensitivity (94,7% vs 97,3%, p = 1,00, k = -0,04). The predictive positive value (PPV) in non-HIV patients was high when compared to HIV patients. (58,1% vs 24,6%). Regarding the likelihood ratio (LR) for positive test result (PLR), the best performance tests were TR in non-HIV patients compared to HIV patients (7.834% vs 3.9049%) and LR for negative test result in non-HIV patients performance was better than those with HIV (0.0599% vs. 0.6004%).

4 Conclusions

In adult patients without prior immunosuppression, RT is a good diagnostic method for VL, however, for patients with HIV / AIDS, DP in bone marrow aspirate, although it is an invasive and painful procedure, remains indispensable, whereas molecular biology are not available in the diagnostic routine for these patients, due to the emergence of this disease in Brazil.

C1897 COMPARISON OF INTRADERMAL SKIN TEST AND QPCR TO DETECT AMERICAN CUTANEOUS LEISHMANIASIS IN ENDEMIC REGION OF BRAZIL.

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1 Background

American cutaneous leishmaniasis (ACL) is a public health problem across the World and remain endemic in several regions of Latin America. WHO estimates that more than 350million of people are under risk to Leishmanisis. The disease can cause single, localized and diffuse cutaneous or mucosal lesions. The common etiological agent for ACL in Brazil is *Leishmania (Viannia) braziliensis*. The intradermal skin test (LST) has high sensitivity to detect leishmaniasis, however, it does not distinguish disease than infection. It is normally negative on cutaneous diffuse forms and immunosuppressive patients and it is positive around 4months later than first lesion. Early detection of *L.(Viannia) braziliensis* by real time PCR(qPCR)can help to confirm infection. The study compared positivity of LST and qPCR in blood.

2 Methods

The study was carried out in an endemic municipality of Pernambuco (Brazil): Amaraji (Mata Norte). The cases were recruited by active search. A survey by LST was applied and 5mL of blood were collected from patients. In order to differentiate new cases (incidence) from old (prevalence) and etect disease in immunosuppression patients, qPCR was performed in blood samples. The qPCR used the target kDNA *L.(Viannia)* spp.and set of primers B1/B2. DNA were extracted using a commercial kit(Qiagen®). The positivity of both tests were compared

3 Results

In Amaraji, 70 patients were investigated to leishmaniais, the median age was 23 years old 0-67). Incidence of ACL in 2014 was 10% and prevalence, 20%,the majority of patients were male (40%). Positivity of LSTin the epidemiological survey in the municipality was30%. The positivity of qPCR in blood was 74,5%. The mean, mode and median values of LST results and quantification of DNA using qPCR are described on table 1.

Table 1:Comparison qPCR x LST

		Quantitative qPCR(fg)	LST(mm)
	Valid	59	70
	Missing	11	0
Mean		33,7	2,91
Median		20,37	0
Std. Deviation		77,55	5,04
Min.		0	0
Max.		594,57	20

4 Conclusions

Positivity of qPCR was greater than LST. It could mean that qPCR can detect DNA of *L.* (*Viannia*) *braziliensis* even when the disease was not clinical stablished or in patients immunosuppressive with no response of LTS. The preliminary qPCR results were not compared to clinical form of disease nor infection. The use of this

technology can be even more precise than LST. Therefore, early diagnosis and appropriate treatment is extremely important to control disease and prevent transmition.

C1901 MINIATURE ELECTROCHEMICAL CHRONOAMPEROMETRIC BIOSENSOR FOR LEISHMANIA PARASITE DETECTION AND QUANTIFICATION USING GOLD NANOPARTICULES/CASEIN CONJUGATES

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1 Background

Leishmaniasis, a complex vector-borne disease caused by obligate intra-macrophage protozoa that affect animals and incidentally humans. Several *Leishmania* surface molecules facilitate survival in these diverse host environments. Both amastigote and promastigote forms of Leishmania spp. express a major glycosyl-phosphatidylinositol (GPI)-anchored glycoprotein of 63 kDa named gp63 or leishmanolysin. It is an enzyme capable of degrading many protein substrates including casein, azocasein, gelatin, albumin, hemoglobin, and fibrinogen. This glycoprotein is one of the molecules involved in the infectivity of *Leishmania* and in parasite uptake by macrophage receptors in mammalian cells. Leishmaniasis still remains a parasitic disease of major importance that no vaccine is available against it. Currently, the detection and diagnosis of *Leishmania* infections rely on several laboratory methods in addition to clinical symptoms, clinical history, travel history, and geographic location of patient. Recent developments in new diagnostic tools have opened new avenues for a vast improvement in parasite and *Leishmania* detection. Nano materials particularly Gold nanoparticles (AuNPs) due to their good biocompatibility with peptides and proteins and their electro-catalytic propriety, play an important role in current sensing and bio-sensing technologies. Their use as carriers of biomolecules and their application in biomedical and nutritional technologies, between others, is an emerging research field.

2 Methods

In this work, AuNps and AuNPs/casein conjugates were firstly characterized and used for the design of an electrochemical biosensor for *Leishmania* parasite detection through the chronoamperometric measurement method where, at an applied fixed potential, AuNPs catalyze the hydrogen evolution reaction on screen-printed carbon electrodes .

3 Results

Obtained results show that gold nanoparticles can be easily loaded with casein micelles, driven toward *Leishmania* surface and electrochemically detected by chrono-amperometric method. The results indicate that, using electrochemical chronoamperometric methode and AuNPs/casein Conjugates, *Leishmania* parasites can be quantitatively detected with a limit less than 1 parasite/ml.

4 Conclusions

A simple, praticable and rapid biosensing methode was developped for leishmania parasite detection and quantification, a useful tool for leishmaniasis diagnostic.

Friday, May 19th

Control programs. National and international funding organizations

C0068 REGIONAL LEISHMANIASIS CONTROL CENTER (RLCC)

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1 Background

Leishmaniasis, which has been neglected for decades, is an serious endemic disease, but the true epidemiological data were underestimated. There was a huge disconnect between physicians and patients in the knowledge of leishmaniasis, and it was difficult to find a doctor who could properly diagnose or treat leishmaniasis. Yemeni leishmaniasis patients had been suffering from neglect, improper diagnosis, maltreatment, lack of affordability, and the shortage of appropriate medicine. Once afflicted, patients suffered from serious complications, including death without a recorded cause. The situation is similar to that of leprosy decades ago. Accordingly, I realized the importance for an initiative to confront the dire need for action to implement effective leishmaniasis surveillance and control.

2 Methods

In January 2013, I organized my ISD-sponsored community dermatology project Eradication of Leishmaniasis from Yemen Project (ELYP) as the first and most effective community movement against leishmaniasis in the history of the disease in Yemen. ELYP had strategies to address and eradicate leishmaniasis from Yemen, support needy patients, and improve patients' lives. Bringing global attention to the burden of leishmaniasis in Yemen and the issues of women and children were its main objectives. The first phase of ELYP included the foundation of the Regional Leishmaniasis Control Center (RLCC) in Sana'a in April 2013, with a grant from the American Academy of Dermatology. Subsequently, three more units were established at Al Baydaa, Ibb, and Hajjah governorates, and more than 100 volunteers were involved. The second phase of ELYP included the announcement of RLCC's community educational, surveillance, detection, treatment, control and research programs and campaigns to o execute ELYP's strategies to implement effective leishmaniasis surveillance and control.

3 Results

As an outcome, RLCC has addressed the magnitude of leishmaniasis in Yemen, eradicated it from some areas, dealt with and improved its alarming prevalence

among children and women who are the neglected and highest risk groups, solved some issues associated with poor access to proper drugs and significantly raised community awareness of the disease that was near to zero among most community sectors. Medicine donation through Medicine-For-All Program has enabled patients with leishmaniasis to freely receive medicine they otherwise would not have been able to afford, reduced their mortality and morbidity and minimized the extensive impact leishmaniasis stigmata have on their lives.

4 Conclusions

RLCC has made a big difference in the leishmaniasis situation in Yemen, achieved spectacular success and received worldwide critical acclaim. In addition to those sensible achievements, it attracted local and global attention to these problematic issues.

C0113 A REVIEW ON THE CONTROL OF PHLEBOTOMINE SAND FLIES IN IRAN

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1 Background

Leishmaniasis has long been known as a significant public health challenge in many parts of Iran. *Phlebotomus papatasi* and *P.sergenti* are the vectors of Zoonotic Cutaneous Leishmaniasis and Anthroponotic Cutaneous Leishmaniasis respectively,and 5 species of sand flies including *P.kandelakii*, *p.neglectus*, *P.perfiliewi*, *P.keshishiani* and *P.alexandri* are considered as probable vectors of Zoonotic Visceral Leishmaniasis.

2 Methods

A literature search was performed of the relevant multiple databases from 1966 to 2013 to include studies on sand flies,vector control,leishmaniasis,*Phlebotomus.*

3 Results

Sand fly control in Iran began in 1966 ,and long- term evaluation of its effects was completed in the study areas of the country. A review of vector control strategies in Iran to combat leishmaniasis including indoor residual spraying,application of chemicals in rodent burrows,impregnation of bed nets and curtains with insecticides,the use of insect repellents,impregnation of dog collars and the susceptibility of sand fly vectors to various insecticides has been summarized thus far.The investigation of the behavioral patterns of the adults of different sand fly species,introduction of biological insecticide agents,the use of insecticidal plants and other novel strategies for the control of sand fly have received much attention in the areas of studies,hence should be recommended and improved since they provide optimistic results.

4 Conclusions

Whether integrated vector management is needed, IRS remains the main support for vector control in epidemics which provides a cheaper solution to prevent leishmaniasis. House spraying with insecticides,although being able to combat an outbreak,can not be used on a long term basis in urban areas to maintain transmission at a low level. Well organised town planning expansions based on environmental protection measures such as a lack of waste management or open sewage,the presence of idle land among the new construction homes,inadequate garbage collection and sanitation,crowded suburban areas with residents of low socio-economic levels and with existence with domestic animals are the key to prevention of leishmaniasis in urban areas. Impregnated bed nets and curtains provide the best way out in rural areas where transmission is largely occurs outdoors.Increasing the awareness of physicians and medical staff on the ecology and control of sand flies and close collaboration between heath authorities and researchers are required to achieve the best control measures.

C0118 ONLINE TRAINING FOR CUTANEOUS LEISHMANIASIS: LESSONS LEARNT FROM AN INTERACTIVE COURSE

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1 Background

Universitat Oberta de Catalunya (UOC) together with the World Health Organization (WHO) have implemented training of health professionals in cutaneous leishmaniasis (CL) highly endemic countries through an interactive on-line course. Three editions were conducted; two in 2014-2015 and one in 2016. This online approach was intended to reduce the administrative burden compared to classical training workshops.

2 Methods

The specialization course consists of 6 ECTS (European Credit Transfer System) (150 study hours) targeted to laboratory technologists, clinicians, nurses or policy makers working in CL endemic areas. It lasts 16 weeks (March – June).

Students receive up-to-date information on specific CL topics such as: natural history of the disease, epidemiology, diagnosis, treatment and surveillance, with focus on the Old World forms.

Teaching strategies are on-line, asynchronous and participatory, interacting with a dermatologist expert in the field. It is based on scientific articles and WHO manuals, as well as the study of real cases and the sharing of the different field experiences. The student's achievements are measured through five continuous assessment activities together with a final multiple choice test which is compared to pre-test at the beginning of the course.

3 Results

To date, three editions have already been made, two in 2014-15 (English and French) and one in French in 2016. The total number of people who enrolled for the courses was 47 and originated from seven countries: Afghanistan, Algeria, Chad, Morocco, the Syrian Arab Republic, Tunisia and Yemen. The drop-out rate was 47% (21/47) and students who abandoned the course did so at the beginning of the training period. The 92% (24/26) of the students who did the full course had a successful final assessment. The final evaluation of the students was very positive or positive for most of the questions, despite the initial difficulties of familiarization with distance learning.

4 Conclusions

On-line training courses on clinical management of CL are a useful tool to train health professionals working in endemic countries. A close follow-up of the students enrolled is needed in order to reduce the dropout rate.

C0121 ASSESSMENT OF A LEISHMANIASIS REPORTING SYSTEM IN THE TROPIC OF BOLIVIA USING A CAPTURE-RECAPTURE METHOD

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1 Background

Leishmaniasis surveillance depends on local and national health authorities. These data are necessary to estimate the disease burden in each country and to establish priorities among policy-makers and funding organisations. However, the scarce and poor quality of national registers of leishmaniasis are constant barriers to designing adequate intervention plans.

From the total of 98 countries where leishmaniasis is endemic, only two-thirds have presented incidence reports from the last five years. Furthermore, it is well known that registers have an significant level of under-reporting. In Bolivia, leishmaniasis is endemic in more than 70% of the country and there are not studies focused on the assesment of completness of national reports. This study has two aims: first, to describe the incidence, distribution and characteristics of leishmaniasis in communities of Cochabamba Bolivia, and the second, to evaluate the extent of under-reporting by the NPLC during the period 2011 to 2014 in these communities.

2 Methods

Active case detection during medical attention campaigns was used to detect all patients with current or past cutaneous and mucocutaneous leishmaniasis living in three communities in the tropics of Cochabamba. A capture-recapture method was used to assess the completeness of the National Programme of Leishmaniasis Control reports during the period 2011 to 2014.

3 Results

An annual incidence rate of 1105.3 per 100 000 was found for 2012. From 712 participants examined in these communities, 214 suffered cutaneous leishmaniasis during their lives (30%). Active lesions were found in four patients (4%). Reinfection with cutaneous leishmaniasis was found in three patients (1%). Mucocutaneous leishmaniasis was found in ten patients (5%). The pattern of transmission is zoonotic. Leishmaniasis was more frequent in men, the age group more affected was from 20 to 39 years old. People living in communities located deep in the forest were the most affected. The extent of under-reporting for the period of 2011 to 2014 was 53%. There were big differences among communities reaching 66% of under-reporting in the most remote community. There was no difference among years.

4 Conclusions

Leishmaniasis produces a high burden of morbidity in tropical communities of Cochabamba. There is a high level of under-reporting in national reports. More research is needed to evaluate the extent of under-reporting at national level and determine the associated factors.

C0130 SECTIONAL STUDY FOR CUTANEOUS LEISHMANIASIS CROSS CAUSES IN DERMATOLOGY HOSPITAL BEAR ASTA MELID, TRIPOLI

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1 Background

Leishmaniasis is a disease caused by infection with the protozoan parasite *Leishmania* and is responsible for significant mortality and morbidity in tropical and subtropical countries.There is currently no vaccine available to protect people against infection, therefore the main methods used to control it is vector control, drug treatment of clinical cases or control of reservoir hosts.

2 Methods

This study is designed to collect data from files of leishmaniasis patients follow in Dermatology Hospital Bear Asta Melid, Tripoli, Some of extra data were taken from medical staff when required.The total of 258 samples was obtained from patient files during the period from the January 2014 to December 2015. The questionnaire was prepared and modified from WHO leishmaniasis questionnaire in leishmaniasis center.

3 Results

The result was indicated that the frequency of infection was high in 2015 than 2014 , increased by rate 1.5. The high frequency of infection was in Autumn than other season , that in October was higher infection rate. The high infection frequency in younger age group between 0-25 years. The conmen infection area were Trahouna, Al kamous, Kabow and Al heria. There was poor social awareness of the disease and of the availability of treatment in this sampled population. Most of the community considered sand fly biting as a nuisance of moderate intensity and dusk as the peak biting time.

4 Conclusions

In conclusion, we recommended that more effective surveillance, case reporting, and control measures be implemented in high-risk areas.

C0199 UNDERSTANDING EFFICACIES OF PREVENT AND CONTROL MEASURES IN LEISHMANIASIS OUTBREAK IN SPAIN USING MATHEMATICAL MODEL

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1 Background

Leishmaniasis is a vector born disease of worldwide distribution, occurring in 98 countries. Since late 2010, an unusual increase of human visceral and cutaneous leishmaniasis cases has been observed in the south-western Madrid region, totaling more than 600 cases until 2015. In that region it is caused by *Leishmania infantum*, which is transmitted mainly by the bite of sand fly *Phlebotomus perniciosus.* Some hosts, such as human, domestics dog and cat, rabbit (*Oryctolagus cuniculus*), and hare (*Lepus granatensis*), were found infected by the parasite of this disease in the area. Hares were considered the most important reservoir in the outbreak. Various measures were adopted to prevent and control the disease which presented results in reduce human cases since 2013.

2 Methods

We used a mathematical model to evaluate the success of the strategie, by evaluating the efficacy of each measure in reducing infected hosts. There were considered all hosts in the disease dynamic and measures as culling hares and rabbits, insecticide-impregnated collar for dand insecticide of residual action.

3 Results

Culling both hares and rabbits, without immediate reposition of the animals, is the best measure adopted, decreasing the proportion of all infected hosts. Singly, culling hares was more efficacious than culling rabbits to reduce the proportion of infected individuals of all hosts, as well as, reducing vector contact with hares resulted in a higher influence in reduce them. The reduction of the vector density per host of the park near from the outbreak decreased the leishmaniasis incidence of hosts of the park and urban area, otherwise the reduction of the vector density per hosts of urban area (humans, dogs and cats) decreased only the infected population of this area, although at higher proportion. The use of insecticide-impregnated collar and vaccination in dogs reduced only infected dogs. The parameters related to the vector contact with dog, cat or human do not present a high impact on the other hosts infected by the agent of leishmaniasis.

4 Conclusions

Culling hares and reducing the density of vector in the Park were the more efficacious measures. Otherwise, some measures did not present important impact on reduce infected hosts. Our results, contribute to direct future actions in this outbreak. The present mathematical model was able to reproduce this zoonotic leishmaniasis dynamic, so it can be applied in other areas with the same disease scenario where the disease is stablished.

C0269 PRODUCTION OF CUTANEOUS LEISHMANIASIS IN PARAUAPEBAS – PARÁ/BRAZIL

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1 Background

Historically, Brazil is one of the countries with the highest number of cutaneous leishmaniasis (CL) cases, and the State of Pará (PA) is a disease hotspot. This work aimed to understand the occurrence of CL in Parauapebas (PBS)/PA, between 2005 and 2007, in agreement with the economy activities and employment situation installed there. This study was carried out for the Ministry of Health by the Department of Endemic Diseases of the Oswaldo Cruz Foundation, aiming to contribute to the understanding of the local epidemiological status of CL in one of the most endemic municipalities in PA.

2 Methods

The methodology used was bibliographical surveys of the historical occupation of the region; Quantitative analysis of the national database of case registration and fieldwork was applied to investigate notification forms and conduct interviews with local cases and leaders.

3 Results

293 cases were recorded in PBS during the study period, of which 53% were autochthonous and 47% were imported. 82% were male adults and only 18% were women and children. 60% of the cases were affected in the rural zone and 18% were affected at the Serra dos Carajás (SCJ). Three case profiles were identified: (1) Resident cases of the urban zone with probable infection at SCJ; (2) Cases residing in rural areas with probable infection in the rural locality; (3) Resident cases of urban areas with probable infection in rural areas.

4 Conclusions

The high number of imported cases is explained by the population attraction exerted by the SCJ, the largest iron deposit in the world, and the urban growth suffered by PBS. Also, the municipality has a better health infrastructure, attracting patients from neighboring municipalities. There is little involvement of the cases at the peridomicile and a probable relation between the occupation and the risk of becoming ill. Profile (1) corresponds to men who work in mining activities with few safety measures and are exposed to infection. In profiles (2) and (3) the cases are infected while performing agricultural activities. In profile (3), despite living in the urban area, their work activities occur in the agricultural area. This intense flow of people between the urban area, rural area and the SC, and the population's lack of knowledge about CL prevention reflect in the dynamics of the disease in PBS.

C0304 ADDRESSING THE EMERGENCE AND SPREAD OF LEISHMANIASES IN THE BORDERS OF ARGENTINA, BRAZIL AND PARAGUAY + URUGUAY + BOLIVIA: ARGENTINA NODE, SOCIAL SCIENCES COMPONENT

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1 Background

The region reported steady cutaneous leishmaniasis cases, but with outbreaks in the last 20 years associated with employment access or dwelling in forest borders with high abundance of *Nysomyia whitmani*. Canine visceral leishmaniasis (VL) and urban 'hot spots' of *Lutzomyia longipalpis* were reported since 2010 with human VL since 2013.

2 Methods

The center of the study area was Iguazú Department (ID), the Argentinean side of the three-country border. Land use and coverage were cross-checked between field observations and secondary sources (satellite imagery and statistics on conservation, cultivated and urban areas). We performed in-depth and semi-structured interviews with key informants and community, and participant observation of inter-specific relationship human-reservoir-vector for VL.

3 Results

Spatial analysis revealed overlapping in the land use categories (20% of ID surface), so there are an increased exposure of human population to forest borders and forestry hinterlands. This scenario implies poor urban planning, dwelling aside forest with transmission of CL and high mobility of humans and its dogs. In four municipalities canine VL management showed great heterogeneity of practices among social actors with the same role (sanitary authorities, public health personnel, private practitioners, owners and breeders) about dogs culling, vaccination, treatment and vector bite prevention.
Inter-specific social distance is smaller between host and reservoir, than between host and vector. Dog – human relationship generates diverse social relations: anthropomorphism (animal shelters), commodity (breeders) and a relation with a non-social being (Mbyá aboriginal people).

4 Conclusions

Land management is associated with CL control. The diversity in the human-dog bond makes the 'responsible ownership' universal discourse insufficient as a surveillance strategy for VL reservoirs. As foundation for an effective health program is required the agreement of public and private practitioners on canine VL management. Further, we suggest incorporating euthanasia and animal rights debate as a cross cultural instance within the OneHealth-EcoHealth approach.
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C0358 NEW INSIGHTS ON BIOLOGICAL AND CLINICAL ASPECTS OF CUTANEOUS LEISHMANIASIS (CL) FROM SURINAME

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1 Background

In the Republic of Suriname (South America), Cutaneous Leishmaniasis (CL) is so far the principal form of leishmaniasis. For a long time it was believed that *Leishmania (Viannia) guyanensis* was the only *Leishmania* species causing CL in the country. Furthermore, the vectors involved in its transmission were not exactly known, and animal reservoirs had not been identified. Our studied aimed to provide new insights in the biological and clinical aspects of the CL in Suriname

2 Methods

Important new insights have been obtained in the fundamental biological and clinical aspects of CL in Suriname. This was achieved by conducting a multi-disciplinary study (5 years) on the epidemiological and etiological aspects of this disease and on research towards improved diagnosis of CL and available forms of treatment of this condition.

3 Results

: *L. (V.) guyanensis* remains the principle cause of CL in Suriname (90%), but other species (*L. (V.) lainsoni*, and *L. (V.) n*aiffi, have also now been identified, including muco-cutaneous leishmaniasis (MCL) causing *L. (V.) braziliensis* and *L. (L.) ama*zonensis. The only routine treatment option in Suriname is pentamidine isethionate (PI), but treatment failures are increasing and this drug is not effective against *L. (V.) braziliensis*. The entomological studies revealed four new records for Suriname (*Lu. aragaoi*, *Lu. damascenoi*, *Lu. ayrozai*, and *Lu. sordellii*, as well as six proven vectors for leishmaniasis (*Lu. squamiventris sensu lato*, *Lu. umbratilis*, *Lu. flaviscutellata*, *Lu. whitmani*, *Lu. ayrozai*, and *Lu. ubiquitalis*. Assessing *Leishmania*-specific antibodies and *Leishmania*-specific DNA in blood samples of dogs (shelter animals, guard dogs, and hunting dogs that frequently visit the interior of the country) using the direct agglutination test (DAT) and qPCR , respectively, revealed that five dogs were sero-positive for canine leishmaniasis, and *Leishmania* DNA was detected in one dog, albeit at relatively low quantities.

4 Conclusions

CL remains an important public health problem in Suriname, and physicians should also be aware of increasing cases of MCL. There is a need for alternative treatment options as PI is losing its effectiveness. The sand fly reservoir is abundant. Although not proven conclusively, the dog may well represent a reservoir for the *Leishmania* parasites in Suriname. An integrated leishmaniasis control program for the country is needed, including vector and reservoir control, improved surveillance and new treatment modalities.

C0405 GEOSPATIAL ANALYSIS OF ANTHROPONOTIC CUTANEOUS LEISHMANIASIS (ACL) IN THE HERAT PROVINCE, WESTERN AFGHANISTAN BASED ON GEOGRAPHICAL INFORMATION SYSTEMS

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1 Background

Anthroponotic cutaneous leishmaniasis (ACL) caused by *Leishmania tropica* was the prominent form of CL in Herat province, Western Afghanistan.

2 Methods

In the present study, distribution pattern of ACL and the role of environmental factors on the occurrence of disease were investigated in this focus based on geographical information systems(GIS).

3 Results

Close proximity to Harirud river, elevation range 700-1200 m, Intensive and intermittent irrigated cultivation and haplocalcids with torriortents and torrifluents soils types were the most effective environmental risk factors for the occurrence of ACL in West of Afghanistan. Rocky lands and bare soil was also considered as the next effective landcover but in which most polluted points were in the close proximity of irrigated areas.

4 Conclusions

Difference between two populations of *L. tropica* in Western Afghanistan and neighboring foci may be originated from the water flow rate of Harirud and consequent humidity in comparison to rivers in other endemic foci where following conditions may affect on the vector strains.

C0482 ADDRESSING THE EMERGENCE AND SPREAD OF LEISHMANIASIS IN THE BORDERS OF ARGENTINA, BRAZIL AND PARAGUAY + URUGUAY + BOLIVIA: THE BRAZILIAN SIDE

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1 Background

Visceral (VL) and cutaneous leishmaniasis (CL) were registered on the triplice border of Brazil - Argentina and Paraguay. In the Brazil side CL is endemic in Foz do Iguaçu (Foz) and Santa Terezinha de Itaipu (STI). The VL form has been signaled more recently. To find out if VL is emerging or is being recognized only now and if two species are simpatricaly distributed, we studied biological, environmental and socio-cultural variables.

2 Methods

The biological variables studied were: seroprevalence in dogs (A), vectors species (B), and human cases (C). The environmental variables studied were: micro, meso and macro scale (D). Social variables: In-depth and semi-structured interviews to key informants and community (E). The sites collection samples was based on grids of 400x400m in urban and periurban houses, and periurban-rural-forest transects. Three nights mini-light trapping and samples from the five closer dogs were taken in a transversal survey, and a seasonal sub-sample.

3 Results

A) in dogs from Foz do Iguaçu *Leishmania infantum* and *L. braziliensis* are the main parasites and the rate of infection was 16.91% and 3.5% respectively. Old dogs have a high sero-prevalence indicating that the VL is present for a longer time in Parana's furthest western region. In the transects only *L. braziliensis* is authocthonous. B) Vectors: *Lutzomyia longipalpis* was present in urban peridomestic (28/93 sites) examinated and spreading to rural-urban and rural areas (29.6 and 33.34% respectively). *Ny. whitmani* remains in sylvatic-rural stratum, and 'green urban patches', persistent in colder seasons. C- in humans the two *Leishmania* species were present: *L. braziliensis* (15 annual cases) and *L. infantum* (7 cases with 03 fatalities). D) Transmission scenarios: i) urban distribution of *Lu longipalpis* with prevalence rates of 28.91% increasing along time, the clustering of *Lu longipalpis* and VLC could be not overlapped. However, in the hot spots *Lu. longipalis* and VLC restricted to small overlapped clusters. E) It is observed a disparate perceptions of public and private practitioners (human and animal health).

4 Conclusions

Leishmania braziliensis and *L. infantum* surround sympathetically in the tríplice border area. *L. braziliensis is* endemic with low human infection rate. The *L. infantum* dog's infection rate is high; *Lu. longipalpis* is dispersed throughout the city of Foz showing elevated risk of human VL occurance.
FS IRDC, CNPq. MS, SESA CCZ Foz

C0492 PERFORMANCE OF BIOCAN®, DPP® IMMUNOCHROMATOGRAPHIC TESTS AND ELISA (BIOMANGUINHOS) IN THE DIAGNOSIS OF CANINE VISCERAL LEISHMANIASIS IN BRAZIL.

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1 Background

Dogs are the main reservoirs of *Leishmania infantum* and an accurate diagnosis in this species is essential for the control of Visceral Leishmaniasis (VL). Here, we evaluate the performance of 3 tests utilized in the serological diagnosis of canine VL (CVL). BIOCAN® (T1) and DPP® (T2) are imunochromatographic rapid tests (ICT) antigen-based in rK39 and rK28, respectively. ELISA-BioManguinhos (T3) uses crude soluble extracts from *Leishmania major* as antigen.

2 Methods

This study used a panel of dogs from which splenic aspirates had been previously characterized, by culture and real time PCR (qPCR) for *Leishmania* sp., to estimate the performance characteristics of the 3 tests. This panel had no serological screening, to avoid selection bias in the study sample. T1, T2 and T3 were challenged with this serum panel of 55 CVL negative and 90 CVL positive dogs, from Camaçari, Bahia (Brazil), an endemic area with about 20% prevalence of CVL. The presence of *Babesia canis* and *Ehrlichia canis* were also investigated in negative dogs by qPCR, detecting 30 positive dogs for *E. canis* and 3 positive for *B. canis*

3 Results

T3 obtained a high specificity (SPE) 1 (0.93-1), while for T1 and T2 the SPE was slightly lower 0.96 (CI 95% 0.88-0.99). In contrast, all tests had low sensitivity (SEN): T3 0.49 (0.39 - 0.59); T1 0.52 (0.42-0.62); and T2 0.61 (0.5-0.70). The presence of babesiosis or ehrlichiosis in the sampled dogs did not interfere with the results of this evaluation. The diagnostic accuracy (SPE and SEN combined) was: T3 0.68 (0.60-0.75); T1 0.69 (0.61-0.76); and T2 0.74 (0.67-0.81). The best positive predictive value (PPV) was obtained for T3, PPV 0.84 (0.48-0.94), followed by T2 0.81 (0.52-0.94) and T1 0.78 (0.48-0.93). According to Cohen's kappa (k), the level of agreement between T1 and T3 was almost perfect (k 0.86 $p<0.001$); while for T1 with T2 and T2 with T3 were substantial (k 0,77 $p<0.001$, and k 0,78 $p<0.001$ respectively).

4 Conclusions

In this study, T2 reached a slightly higher diagnostic accuracy than T1 and T3which achieved similar results. All analyzed tests have potential to be used in the diagnosis of CVL. Nevertheless, the low SEN may underestimate the real prevalence of CVL; thus, there is a need for tests with greater SEN that would allow a better detection of infected dogs enabling more efficiently measures of control of the cycle of *L. infantum* in endemic areas.

C0493 COMPOSITE INDICATOR FOR CUTANEOUS LEISHMANIASIS IN THE AMERICAS. A GUIDANCE APPROACH TO SURVEILLANCE AND CONTROL ACTIONS.

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1 Background

In the Americas, cutaneous leishmaniasis (CL) is a vector born disease and is considered a public health problem. It is present in 20 countries distributed from the United States to Argentina, being endemic in 18 countries. Generally, two indicators (cases and incidence) are used by surveillance and control programs to analyze the CL data. However, when individually analyzed and stratified, each indicator shows different results in different metrics, which complicates the decision-making across Control Programs. The objective of this study is to present the CL composite indicator (CI), which has been used in the Americas to prioritize, define and direct actions towards surveillance and control of this disease.

2 Methods

Three main raw indicators (RI) for CL occurrence were selected as they were readily available in the Regional Information System of Leishmaniasis (SisLeish): "Total number of cases", "Incidence" and "Density". In order to standardize and combine all indicators, the single scores were transformed into z-scores by subtracting the mean and dividing it by the standard deviation. The standardized results obtained for each indicator were added together leading to a CI. This indicator was validated by external collaborators, experts, the Pan American Health Organization/World Health Organization (PAHO/WHO), and representatives from several countries.

3 Results

The CI of each analyzed territorial unit was classified by natural break points, which allowed the generation of five risk of transmission strata: low, medium, high, intense and very intense. The CI is presented together with calculated percentages indicating the contribution of each RI to the final value of the CI. These analyses are included in the SisLeish.

4 Conclusions

The CI was created to combine and standardize all indicators into one single metric, expressing the relevance of CL occurrence. It demonstrated to be sensitive to highlight areas where the occurrence of CL was important and was undetected by just looking at RI separately. This methodology permits the schematic visualization of the severity status of a specific region into categories based on the result of the CI, and contributes to National Programs in the guidance towards CL surveillance and control actions. In short, the CI defined the Countries and Regional epidemiological status of CL in the Americas, and allowed the Regional Program of PAHO/WHO to identify and define CL priority endemic countries.

C0495 LEISHMANIASES DIAGNOSES AND TREATMENT SELF-LEARNING ON-LINE COURSE: A VALUABLE TOOL FOR HEALTH CARE PROFESSIONALS AND THE EXPERIENCE OF ITS USE IN THE AMERICAS.

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1 Background

In the Americas (AM), Cutaneous and Visceral Leishmaniasis (LC) (LV) are endemic in 18 and 12 countries, respectively. Early diagnosis and adequate treatment (DT) are essential for the control of this disease. The Pan-American Health Organization/World Health Organization (PAHO/WHO) created on-line self-learning (SL) courses to promote capacity building of health care professionals (HCP) in DT of leishmaniasis. This study describes and evaluates these courses.

2 Methods

The courses were developed by Contextual Instructional Design (CID) stages, which is a SL planning process with strategies and activities for the instructional design. Its content and methodology are divided in 5 modules, and are based on scientific evidence, experts' opinion, clinical cases, and evaluations, allowing the development of capabilities and problem solving. Currently, there are 3 "Diagnosis and Treatment" courses, available at the Public Health Virtual Campus (PHVC) in 2 languages: CL (Spanish (Span), since 2013), VL (Span, since 2015; Portuguese (Port), since 2014). Initially, it was only available in Abode Flash, being updated to HTML5, which permitted viewing in both desktop computers and mobile devices. The courses are a result of the effort from the internal areas of PAHO/WHO, experts, collaborators and National Programs of Brazil.

3 Results

Thus far, the CL course had 6,356 participants with an approval rate (AR) of 44.5% from 32 countries (22 from the AM, 5 from Europe, 3 from Africa and 2 from Asia), the VL Span course had 773 participants, 54.4% AR from 22 countries (20 from the AM, 1 from Europe and 1 from Oceania), and the VL Port course had 1,950 participants with a 27% AR. According to our on-line survey, 82% of the participants had internet access through a mobile device. The Span CL and VL course had 94.7% and 93% of acceptance, and 97.9% and 99% of the participants stated that the course was relevant to their professional development and improvement in their field of work, respectively.

4 Conclusions

The courses had a great acceptance, dissemination and relevance to HCP, and 68.7% and 90% of the participants were from the AM for the CL and VL course, respectively. Even though there was a low AR, probably due to lack of time to dedicate in the completion of the extensive content of the modules, the SL courses have proven to be an effective opportunity for HCP, to enhance their knowledge and to reach new abilities and decision-making actions.

C0496 REGIONAL EXTERNAL ASSESSMENT PROGRAM OF THE PERFORMANCE (PEED) FOR MICROSCOPIC DIAGNOSIS: STRATEGY FOR QUALITY CONTROL OF CUTANEOUS LEISHMANIASIS IN THE AMERICAS

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1 Background

In the Americas, cutaneous leishmaniasis (CL) is endemic in 18 countries with an annual average of 57,000 cases. The parasitological diagnosis remains the standard in diagnosis of CL due of its high specificity, being recommended the microscopic examination (ME). In 2015, the Pan American Health Organization/World Health Organization (PAHO/WHO) created the Regional External Assessment Program of the Performance (PEED) for Microscopic Diagnosis of CL, to establish the technical procedure, design and assess National Reference Laboratories (NRL) from the Region, with the goal of maintaining an efficient quality management system, strengthening and monitoring of the diagnosis.

2 Methods

The PEED is a voluntary and confidential Program, with a qualitative, continuous, and simultaneous inter-laboratory comparison testing scheme for the ME of CL, and is led by the Parasitology Group of the Colombian National Institute of Health and PAHO/WHO. There is an annual cycle with a single dispatch of a test panel with 10 slides of direct smears, each with three appositions, and colored with Romanowsky derived pigments. The panel should be analyzed by the same diagnostic routine, and the results must be reported in terms of presence or absence of *Leishmania* amastigotes. The statistical methodology is the Kappa's index of agreement resulting in 6 levels of concordance (no agreement, poor, weak, moderate, good and very good).

3 Results

In 2015,17 NRL, from 14 countries, received the test panel of the PEED's first cycle; however, 14 NRL reported the results. 4 (28.5%) had a moderate concordance (CD), 3 (21.5%) had a good CD and 7 (50%) had maximum CD (very good). The general average of the Kappa's index was 0.84 which corresponds to the very good CD. The second cycle of the PEED is still in progress with and adhesion of 22 NRL from 18 countries.

4 Conclusions

The enrolled NRL of the first cycle had an overall very good performance and there was an increase in the enrollment for the second cycle, suggesting a good adhesion of the program and strengthening of the diagnosis. Furthermore, it supported PAHO/WHO in the planning and selecting of priority technical cooperation. The microscopic examination is an easy, cheap, fast, and is often the only available method at all health care levels in endemic areas, which is why, is important to maintain its internal and external quality control to achieve reliable diagnosis and adequate election of CL specific treatment.

C0586 COMMUNITY KNOWLEDGE AND PREVENTIVE PRACTICES REGARDING KALA-AZAR IN AN ENDEMIC AREA IN BANGLADESH

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1 Background

Vector control is an important part of controlling arthropod-transmitted diseases such as leishmaniasis. Although the current vector control strategy for kala-azar is based on indoor residual spraying in Bangladesh, long-lasting insecticidal nets (LLINs) have been proposed as an alternative measure to IRS. The knowledge, attitude and practice of people live in endemic area about kala-azar are essential for successful vector control strategies.

2 Methods

As part of a cluster-randomized trial to evaluate permethrin treatedLLINs for kala-azar control in Pabna district, Bangladesh, the questionnaire-based surveys were done to assess whether the vector control using LLINs is sustainable application for the people lived in endemic are or not. A total of 1393 respondents (one respondent from each household) were interviewed by a trained staffs by house-to-house survey.

3 Results

Mean age of respondents was 39 and half of the respondents (49.5%) were no history of education. The majority of the respondents (99%) had heard about kala-azar and believed that kala-azar is a severe diseases (93%). A noticeable proportion of respondents (72%) did not know about a transmission way of kala-azar, only one-fifth of the respondents stated the bite of insect (mosquito or fly) for kala-azar transmission. Approximately 95% of the respondents did not know about post kala-azar dermal leishmaniasis. Despite the limited knowledge of kala-azar transmission and prevention, the majority of households (93%) slept under bed nets the night before the survey. In 85% of households, all family members slept under bed net, and about 66% of households used bed nets all year round.

4 Conclusions

Vector control using LLINs can be one of an acceptable tool for people live in endemic area in Bangladesh.In order to maximize the benefits of kala-azar control strategies, appropriate educational intervention should be implemented. This research was supported by JST/JICA, SATREPS (Science and Technology Research Partnership for Sustainable Development).

C0587 OUTPUT OF KALACORE PROJECT TO SUPPORT NATIONAL KALA-AZAR ELIMINATION PROGRAMME IN BANGLADESH

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1 Background

National Kala-azar Elimination Program of Bangladesh has been facing challenges for strengthening its disease surveillance, logistic and supply change management, developing materials for BCC/IEC activities and their implementation. Also active participation of frontline public health worker (FPHW), formal and informal private health providers, school teachers and clinicians in the tertiary health facilities in the VL endemic areas have been missing in the program. Outbreak investigation and response guideline and its implementation is needed particularly for the maintenance and consolidation phases of the program. KalaCORE, a UKaid supported project aims to support the national program to fill up above mentioned gaps and icddr,b is its implementation partner. Here we are reporting the achievement of the project by September, 2016.

2 Methods

The project has been started in October, 2015. Methodological pillars of the project were training of hired staff, series of workshops with national experts, program personnel and major stakeholders of the program, training of Upazila health complex (UHC) program personnel by project staff, training of FPHW, formal and informal providers and senior-junior clinicians of tertiary health facilities. Independent international monitors evaluated the project activities and its outputs.

3 Results

The program has been strengthen with human resources which resulted in better VL and PKDL case management in Surja Kanta Kala-azar Research Centre as modern diagnosis using qPCR is taking place and time to make diagnosis of PKDL patients has brought down to 2 days compared to weeks before; better surveillance as under reporting is close to zero, patients' follow up went up to 100%, improved patient referrals by UHC and web-based reporting of cases has been scale up in 100 VL endemic sub-districts. BCC / IEC materials such as leaflets, flip chart, posters and stickers are developed and approved by the MoH, GoB and handed over to the program. 512 teachers, 2741 informal and 85formal care providers were trained. 1187 frontline care providers were trained and they reached 47594 community people for increase knowledge about VL. Outbreak management guideline is finalized and is taken by the program; 225 people have been trained in Outbreak response.

4 Conclusions

Independent reviewer found a substantial volume of support delivered by KalaCORE that taken up NKEP. This is a replicable project that will fit in a developing country set up to support VL elimination.

C0633 MEASURING THE EFFECTIVENESS OF A WHO MANUAL FOR CASE MANAGEMENT OF CUTANEOUS LEISHMANIASIS IMPLEMENTED IN THE AZILAL AND OUARZAZATE PROVINCES OF MOROCCO.

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1 Background

With the aii of standardizing the treatment of cutaneous leishmaniasis (CL) in the Eastern Mediterranean Region of the World Health Organization (WHO), WHO published in 2014 a manual for CL case management, including variables for monitoring and evaluation. This paper analyses the effectiveness of the implementation of the WHO manual in two provinces, Azilal (*L. tropica*) and Ouarzazate (*L. major*), Morocco.

2 Methods

A quasi-experimental management case study was set up in 12 endemic sites. An online questionnaire was created for individual patient data collection at province level. In 2014, individual patient data were collected retrospectively for 2013 as baseline data (Baseline data 2011-2012). Data were collected prospectively in 2014 after the publication of the WHO manual up to 2016. The data were compared between three periods of time: 2 years before, one-year after and second-year after the implementation of the WHO manual in terms of completeness, quality and compliance of treatment and initial cure rate. A total of 10 indicators were used.

3 Results

751 CL cases was collected in the witness period 2011-2012, 323 and 393 CL cases were respectively registered in the first and second study phases after the introduction of the WHO manual. Baseline data collected enable to calculate 9 out of the 10 indicators with a completeness >90% in Azilal, but only 2 indicators in Ouarzazate. After 2014, completeness rate was 100% in both provinces. The completeness required to apply the WHO treatment algorithm (number, size and site of lesions, parasitological diagnosis, immunological status, diabetes) increased after 2014 but the immunological status remained reported <1% in Azilal. In both provinces, the compliance with the WHO treatment algorithm is very high when the patients require intralesional antimonials, but poorer if the patients require only lesion dressings or systemic antimonials (this is a change versus the national guidelines). Compliance is 95%, 84%, 90% in Azilal and 24%, 42%, 39% in Ouarzazate respectively for the three periods. Initial cure rate is 99.8%, 100%, 99% in Azilal and 18%, 96%, 90% in Ouarzazate respectively for the three periods.

4 Conclusions

The introduction of the WHO manual has improved the completeness and the quality of the data collected. Nonetheless, the treatment algorithm recommended by WHO has apparently not been fully adopted. An evaluation should be carried out to find out the reasons behind the discrepancy.

C0653 HIGHLY INCREASED DETECTION OF CUTANEOUS LEISHMANIASIS IN ENDEMIC AREAS OF NICARAGUA DURING 2016

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1 Background

The Damien Foundation, a Belgian non-governmental (NGO), is running a joint project with the National Leishmaniasis Program in Nicaragua that started in 1995. The project implement control activities in 11 municipalities where notification of cutaneous leishmaniasis cases is greater than 10 per year. The main activities include training and refresher sessions of health-workers, integrated leishmaniasis and other health problems detection campaigns, community sensitization, supervision of diagnosis and treatment of patients on treatment, monitoring and evaluation meetings and provision of equipment and laboratory consumables. Nicaragua government provides the Leishmaniasis drugs. During the last five years, the same activities have been implemented. We report a highly increased number of mucocutaneous leishmaniasis cases during the first nine months of 2016.

2 Methods

A cohort study including all cutaneous leishmaniasis cases notified during the period of January to September 2015 and during the same period in 2016. Most of the cutaneous leishmaniasis cases are diagnosed by skin smear. In case of negative results, PCR is used for diagnosis. Few cases are diagnosed based on clinical and epidemiological features. Cases confirmed start treatment with Glucantime (antimonial pentavalent drug) following standards from the National Leishmaniasis Program. The treatment is administered by health-workers at health facilities or a community worker at patient's domicile depending on the distance. When a patient missed one appointment a community performs a domiciliary visit. All diagnosis and treatment care are provided is free.

3 Results

The number of cutaneous leishmaniasis was 1391 in 2015 versus 3601 in 2016, three times higher ($P<0.01$). The number of mucocutaneous leishmaniasis was 20 in 2015 versus 16 in 2016 ($P=0.8$). The total number of patients not treated were 44 in 2015 versus 160 in 2016 ($P<0.01$). Reasons for not treatment start are lost-to-follow-up and drugs availability. Cure rate in 2015 was 92% (1284/1391) versus 72% (2683/3601) in 2016 ($P<0.01$).

4 Conclusions

We report a sharp increased number of total numbers of cutaneous leishmaniasis during 2016. This unexpected rise of cases has hampered leishmaniasis program performance. Further investigation is needed to find the possible causes of this observation.

C0682 EVALUATION OF THE EFFECTIVENESS OF CANINE SOROLOGICAL SURVEYS AS A MONITORING ACTION AND CONTROL OF VISCERAL LEISHMANIOSIS IN THE REGION OF SÃO JOSÉ DO RIO PRETO.

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1 Background

Surveillance and Control Program of Visceral Leishmaniasis in the state São Paulo (VCPVL/ESP) recommends annual serologic surveys canine census and sample, aiming to know the epidemiological situation of the disease in areas with active transmission or potential transmission and at the same time, identify serologically positive dogs for disposal. The objective was to evaluate the seropositivity, periodicity and effectiveness of canine serological investigations used to control visceral leishmaniasis (VL) in the region of São José do Rio Preto from 2008 to 2012.

2 Methods

This is a descriptive study based on secondary data obtained from the state reference laboratory São José do Rio Preto. the results of 45,343 blood samples from dogs were studied, including the canine serologic census surveys and / or sampling, for the diagnosis of canine VL (CVL), from 2008 to 2012. The data relating to dog home, the date of collection, results of laboratory tests, type of serological survey and sample type, were obtained from laboratory tests collection of papers used in the canine survey. Were assessed positivity rate, total serological surveys and start / end of activities.

3 Results

The region of São José do Rio Preto is composed of 102 municipalities, these 25 municipalities presented canine transmission and / or human and / or vector presence 12 conducted sample survey, eight (8) census survey, two (2) with at least one sample survey and other census and five (5) municipalities there was no record of conducting any survey. The highest positivity rates in census surveys were observed in Urania 25.4%, 20.1% and Votuporanga Palmeira D'Oeste 19.0%. There was the difficulty of municipalities to completion of canine serological surveys within a year, as recommended by VCPVL, and some municipalities, the time taken for completion of the annual canine serological survey was up to two (2) years.

4 Conclusions

The region has high CVL positivity rates. Most municipalities did not conduct the census and / or sample surveys, as recommended by VCPVL. As for the effectiveness of the CVL control actions, the study reveals that most municipalities do not meet the VCPVL/ESP, in compliance with the control shares through census or sample surveys canines provided annually.

C0746 EVALUATION OF THE EFFECT OF A DEDICATED EDUCATIONAL COMIC BOOK ON SCHOOLCHILDREN'S KNOWLEDGE, ATTITUDES AND BEHAVIORAL INTENTIONS TOWARD CUTANEOUS LEISHMANIASIS

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1 Background

Comics combine 2 very rich forms, literature and visual art, that make them effective educational tools particularly in children. Considering this potential for comics, Sanofi's Access to Medicines Department in collaboration with the Pasteur Institute of Tunis developed an educational comic book called "Yamina's secret". Health messages expressed by the story were entirely adapted to the epidemiological realities of Zoonotic Cutaneous Leishmaniasis (ZCL) in North Africa within the context of transmission, management and control of the disease in Maghreb countries.The comic book translated in Arabic was pilot tested in Tunisia for acceptability among 12-13 years old schoolchildren, in 2015. This study aims to assess its impact on ZCL-related Knowledge, Attitude and Practice (KAP).

2 Methods

The survey was done in an endemic region of ZCL (Kairouan governorate, Central Tunisia) with the collaboration of Regional authorities of Health and Education.Eleven primary schools were randomly sampled. Study was conducted in classroom sitting and concerned 240 pupils from year 6 in the public education system. The effect of reading the story on ZCL-related KAP was evaluated in a pre-/post-reading approach by means of KAP multiple-choice questionnaire survey. Overall scores were compared using Wilcoxon test. Specific differences in KAP items were assessed by Mac-Nemar test.Comparisons were done for the overall study population and stratified by schools.

3 Results

Nine out of ten questions were evaluated. The pre-reading median overall score (IQR) was 55% (33-66%). A significant increase in score to 77% (55-88%) ($p<0.0001$) with significant raises in all ZCL-related KAP items were shown after reading the comic book. Baseline knowledge of ZCL frequency, symptoms, modality of transmission to humans, severity and potential sequellae was variable according to schools and significantly increased in case of low or medium level. Baseline knowledge of incubation period was globally low and increased in all schools. Positive behavioral intentions toward doctor's visit, individual protection and management of CL lesion were initially at middle to high level and improvement was statistically significant but low. Baseline positive attitude toward CL patient was low to middle and showed the least increase after reading the comic book.

4 Conclusions

This comic book improved knowledge of CL and produced more positive attitude and behavioral intentions toward CL patients and disease.

C0841 SOCIO-DEMOGRAPHIC AND HEALTH-SYSTEM RELATED DETERMINANTS OF MORTALITY AMONG THE VISCERAL LEISHMANIASIS PATIENTS IN A HIGH ENDEMICITY REGION IN INDIA

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1 Background

In India, 70% - 90% visceral leishmaniasis (VL) cases are estimated to occur in the state of Bihar. However, no studies conducted thus far attempted to enumerate the actual burden of symptomatic VL cases and associated mortalities in this state. To address this knowledge gap, a state-wide VL situation assessment study was undertaken by CARE India in 2015. We present the mortality estimates and the epidemiologic determinants of mortality obtained from this study.

2 Methods

Using a cross-sectional design, we identified all symptomatic VL cases that got diagnosed between June'13 and December'14 in all 33 VL-endemic districts of Bihar. A three-pronged approach was adopted to ensure completeness of the case capture mechanism – i) tracing of all VL cases registered with the state-run health facilities; ii) scanning all private treatment/diagnosis providers to identify the VL patients that did not seek care from the state-run facilities; and iii) snowballing with the identified cases and key informants for detection of additional cases. The identified VL cases were subjected to interview and medical record review to collect information on socio-demographic characteristics, onset of disease symptoms, diagnosis of VL, pre- and post-diagnosis treatment history, and type and duration of drugs received. For death cases, interviews were conducted with the next-of-kin. Kaplan-Meier and Cox Proportional Hazards analyses were conducted to identify the determinants of mortality.

3 Results

Overall, we identified 7,897 VL cases and 253 deaths. The annual incidence proportion of symptomatic VL cases was 0.71/10,000 population. The incidence rate of mortality was 3.3/100 person-years. Cumulative survival probabilities of VL patients were found to be lower among the older individuals, males, those diagnosed at the public laboratories and the treatment defaulters. Hazards analysis revealed that obtaining diagnosis from a private laboratory [Adjusted Relative Hazard: 0.65 (95% CI: 0.48 - 0.89)], treatment with amphotericin-B [0.40 (0.16 - 0.96)] and completion of treatment course [0.08 (0.06 - 0.10)] were associated with lower risk of mortality among VL patients.

4 Conclusions

For reducing mortality, the existing VL program should focus on increasing access to quality diagnosis and treatment, improving treatment adherence and ensuring widespread availability of liposomal amphotericin-B in Bihar.

C0886 PERFORMANCE OF THE CONTROL ACTIONS OF VISCERAL LEISHMANIASIS IN A BRAZILIAN ENDEMIC REGION

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1 Background

The municipality of Teresina, capital of the state of Piauí, located in the northeastern region of Brazil, is one of the cities endemic for visceral leishmaniasis (LV), where the first epidemic of the disease was registered in the early 1980s. LV control in Brazil is based on three basic measures: 1) detection and treatment of human cases; 2) control of reservoirs; 3) control of the vectors. The last two measures are carried out by Teresina Zoonosis Control Center (CCZ) in areas with transmission of human disease. This study evaluated the performance of control actions performed in the period from 2002 to 2013 in the city of Teresina.

2 Methods

We compared the programmed actions performed annually, using data provided by the ZCC. Regarding the control of the reservoir, the variables studied were: 1) blood samples for canine diagnosis; 2) dogs slaughtered after laboratory diagnosis; 3) correlation between the slaughter of dogs and the prevalence of canine VL in later years. The control of the vectors was analyzed based on home spraying with residual power insecticide.

3 Results

Only 52% of the blood samples for canine VL diagnosis were collected during the analyzed period. Only 60% of the dogs diagnosed were slaughtered. The prevalence of canine infection was 19.2%, and it increased in the years after the dogs were sacrificed. Household spraying to control the vectors was performed in only 38% of the programmed households. The difficulties for the execution of the control actions of LV in Teresina include: 1) reduced number and poor performance of the people involved in the execution of control actions; 2) refusal of the population to deliver the dogs diagnosed with VL for slaughter, as well as to accept the home spraying; 3) irregularity in the supply of insecticide and kits for diagnosis by the Ministry of Health.

4 Conclusions

Due to the difficulties and poor performance in the execution of the LV control actions, as well as the lack of scientific evidence for the maintenance of this control model, we suggest an urgent reformulation of these actions, under penalty of maintaining or worsening the disease current situation at the municipality.

C1001 RISK FACTORS FOR CUTANEOUS LEISHMANIASIS IN EL BOROUJ (MOROCCO)

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1 Background

The first cutaneous leishmaniasis (CL) case in El Borouj (Morocco) was detected in 2007, preceding an epidemic outbreak that is currently ongoing endemic. There are many potential risk factors for CL in El Borouj, and the aim of this study was to identify them correctly and assess their relevance in order to design control programs.

2 Methods

1. CL cases were georeferenced in order to perform a spatial and temporal analysis. 2. Epidemiological data of families with and without CL cases were collected. 3. The etiological agent was identified through PCR. 4. Sandflies were captured using CDC traps. 5. The *Leishmania* infection rate in the vector was assessed by PCR. 6. The presence of potential animal reservoirs was evaluated through the analysis of *Leishmania* spp. in animal hair and the analysis of sandfly blood meals. 7. The identification of the risk factors implied was evaluated through logistic and lineal regression using the statistical package IBM SPSS Statistics 20.

3 Results

CL cases are mainly concentrated in the most densely populated neighborhoods. The vectors of the three *Leishmania* species responsible for CL were captured. *Phlebotomus sergenti* density was 4.2 sandflies/trap/night and 2.7% of specimens were found infected by *L. tropica* during September/October. In blood-fed *P. sergenti* females, human, cat and rabbit blood was detected and 1 out of 33 dogs was infected by *L. tropica*. Using an univariate analysis, risk factors associated to CL were the presence of animals, the proximity to a ruined house and the *P. sergenti* density. In the multivariate model, only *P. sergenti* density was identified as a risk factor. In addition, *P. sergenti* density showed positive association to some city districts, the presence of a water well, the accumulation of organic material as fertilizer and the existence of dogs.

4 Conclusions

L. tropica was confirmed as the main etiological agent of CL in El Borouj and *P. sergenti* as its vector. The main transmission session was September/October. The results support the anthroponotic nature of CL in El Borouj, even though dogs, cats or rabbits might play a role in the maintenance of the parasite in this focus. Exposure to *P. sergenti* is the only risk factor for CL and the reduction of its density could be achieved through control measures such as the management of water wells, manure use and dogs presence.
This study was funded by the University of Granada (CICODE, 2013)

C1016 CANINE VISCERAL LEISHMANIOSIS IN PARAGUAY, MANAGEMENT BY THE NATIONAL PROGRAM OF ZOONOSES CONTROL AND RABIES NATIONAL CENTER, YEAR 2016

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1 Background

Visceral leishmaniosis is a chronic parasitic disease caused in the Americas by *Leishmania infantum*, transmitted by the bite of infected phlebotominae female sandflies *Lutzomyia longipalpis*, that affects humans and its urban reservoirs dogs. It is an endemic tropical disease in Paraguay with approximately 100 human visceral leishmaniosis cases per year.

2 Methods

The purpose of this work was to determine the seroprevalence of canine leishmaniosis by immunochromatographic rK39 test in serum samples obtained for routine exam requested by dog`s owners and veterinarians, active surveillance in areas of silence transmissions and control of human cases notified for the National Service of Eradication of vector-borne diseases (SENEPA) to the National Program of Zoonoses Control and Rabies National Center (PNCZyCAN) in 2016.

3 Results

A total of 6772 blood samples were analyzed by immunochromatographic rK39 (Biocan Diagnostics Inc, Canada), in the laboratory of Leishmaniosis of the PNCZyCAN, from 11/17 departments of the country. The results were analyzed by Epiinfo 7 statistical program. The 3358 canine blood samples proceeding for routine exam from Asunción (capital city), San Pedro, Cordillera, Central, Paraguarí, Guairá, Caaguazú, Misiones, Presidente Hayes and Boquerón departments, showed 1259 positive serum samples with a prevalence of (37.5%) of canine leishmaniosis. The active surveillance performed in Asunción, Central, Alto Paraná and Canindeyú departments showed that 113 out of 1834 samples had a positive result with a prevalence of (6.2%) of canine leishmaniosis. From the 27 focus of human visceral leishmaniosis performed in Asunción, Central, Cordillera, and Paraguarí departments was observed that 214 out of 2030 samples were positive with a prevalence of (10.5%). It was observed a global seroprevalence of 23.4% of canine leishmaniosis. Euthanasia procedures were performed in 1038 positive dogs (65.4%) proceeding from routine exam, focus of human visceral leishmaniosis and active surveillance in areas of silence transmission of visceral leishmaniosis.

4 Conclusions

The high canine visceral leishmaniosis shows the urgent need to continue a strict epidemiological surveillance, sanitary education and community participation by the Ministry of Public Health and Social Welfare in the control of this disease in Paraguay.

C1105 IMPORTANCE OF LARVAE CONTROL IN INSECTICIDE TRETAMENTS OF PERIDOMESTIC AREAS INFESTED WITH LUTZOMIA LONGIPALPIS

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1 Background

Leishmania infantum is the etiologic agent of visceral leishmaniasis in Argentina, and the phlebotomine fly *Lutzomyia longipalpis* is its main vector. Hens and chickens are refractory to *Leishmania infantum* infection but they are a food source that attracts adult *Lu. Longipalpis*, representing a risk factor for human health when henhouses are in human peridomiciles.
The principal strategy of *Lu. longipalpis* control has been based on the use of pyrethroid insecticides. Recently we evaluated the application of an emulsifiable concentrate (EC) formulation containig the larvicide pyriproxyfen and the adulticide permethrin against *Lu. Longipalpis* (Juan L. et al, *J Am Mosq Control Assoc.*2016;32(3):224-229). Our results suggested that the use of pyriproxyfen contributed to control *Lu. longipalpis* larvae, complementing the effect of permethrin on adults.
The aim of this new study is the comparison of the effectiveness on *Lu longipalpis* of the peridomestic application of the adulticide-larvicide formulation with a similar formulation containing only the adulticide.

2 Methods

The work was conducted in the city of Clorinda (Formosa, Argentina). Based on the previous trapping results obtained with Centers for Disease Control and Prevention (CDC) light traps, 10 households positive for phlebotomines during 3 consecutive years were chosen for further testing. 5 of them (group A) were treated with a CE formulation of permethrinand pyriproxyfen and the other 5 (group B) with a EC formulation containing only permethrin. The spray covered henhouses and surrounding areas, including floor, with 100 mg/m2 of permethrin (groups A and B) and 20 mg/m2 of pyriproxyfen (group A).

3 Results

Results of average number of *Lu longipalpis* captured with CDC light traps 1, 3 and 5 weeks after the treatment showed values of 0,2; 0,4 and 0 respectively for group A and 4,0; 22,6 and 11,4 respectively for group B. The treatment with the formulation containing pyriproxyfen (group A) resulted in a significant greater control until 5 weeks with respect to the formulation without the larvicide.

4 Conclusions

Results of this study suggest that the inclusion of pyriproxyfen in tretaments to peridomestic *Lu. Longipalpis* control introduce a promisory efficacy improvement by its larvicide effect on terrestrial larvae

C1130 EPIDEMIOLOGICAL BEHAVIOR AND TREATMENT OF VISCERAL LEISHMANIASIS IN THE LAST YEARS IN PARAGUAY.

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1 Background

Visceral Leishmaniasis (LV) is a protozoan disease caused by Leishmania infantum, with a wide tropical distribution. In our country it is considered an endemic disease and a public health problem. The presentation ranges from an asymptomatic infection to severe and complicated form. The treatment of choice remains meglumine antimoniate, but there are other second-choice treatments such as amphotericin with good clinical response in our environment despite the toxicity of the same, depending on the conditions of the host.

2 Methods

Retrospective, descriptive study in patients diagnosed with visceral leishmaniasis in the period from 2006 to 2015 reported by means of epidemiological records to the national program for the control of leishmaniasis

3 Results

A total of 932 patients were analyzed: children under the age of 10 years, 313 (34%) patients, of whom were less than 2 years old 283 children aged 10 to 20 years 150 (16%) patients, aged 20-40 years 198 (21%) patients, more than 40 years (29%) patients, with a minimum age of 2 months and a maximum of 87 years. It corresponds to the masculine sex the 68% female the 32%. Among the departments most affected, the central department accounts for 70%, Coordillera 9%, Paraguarí 5%, Itapúa 4%, other 12%. They received treatment with 11% liposomal amphotericin b, amphotericin b dexoxicolate 37%, meglumine antimoniate 46%, sodium stylogluconate 6%. Deaths 9% coinfection HIV 12%

4 Conclusions

In our country, this disease remains a serious public health problem, despite the efforts made through the national leishmaniasis control program. As the literature clearly describes it is predominant and with high lethality ages included in the extremes of life, preponderating the male sex. The central department is still the most affected with host-associated factors such as HIV infection. Treatment remains a challenge, due to the importance of timely diagnosis and the choice of the best therapy according to individual conditions.

C1134 EVALUATION OF THE APPLICATION OF LIME ON THE NATURAL BREEDING SITES OF LUTZOMYIA EVANSI IN THE MUNICIPALITY OF OVEJAS, SUCRE-COLOMBIA.

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1 Background

In Colombia, leishmaniasis outbreaks have been reported in different urban areas, mainly related with the development of human settlements. Measures of vector control to reduce the burden of disease are focused on the adult form of the vector through the application of insecticides, repellents and impregnation of mosquito nets. Therefore, in order to explore the potential of an alternative measure for the control of *Leishmania* vectors, this study proposes to evaluate the activity of the lime on natural breeding sites of *Lutzomyia evansi* in the peri-urban area of a municipality of the Colombian Caribbean coast.

2 Methods

Two groups were selected: treated area and control group every one with 28 trees (breeding site of larvae). In the treated area, six treatments with 250 grams of lime diluted in one liter of water were applied to the 28 trees every two months for 1 year. In addition, 250 g of lime were used to paint each tree at a height of 50 cm. 1 kg of soil was taken from each tree every two months before and after application of lime. 400 gr of each sample were used to determine changes on physiochemical properties of soil as pH, organic matter, Ca, P, Na, K, Mg and texture. Others 600 gr were used to search of sand fly larvae under the stereoscope. Sand flies were captured on the base of the trees in both the intervened and the control area.

3 Results

Since we are dealing with longitudinal data, linear mixed models were adjusted for some endpoints (or responses): pH, Mg, K, P and Organic Matter. The explanatory variable in every case was treatment with five levels. Least square means were compared having as a reference level the value of the mean, for each endpoint, at baseline.So far, four treatments were analyzed. In the treated area, significant differences ($p < 0.001$) were observed with an increase in the averages of the pH, Mg, and Ca variables of each lime treated breeding site compared to the averages of these variables at baseline. No differences were observed for Organic Matter. 147 and 44 sandflies adults were captured on the basis of the trees in the control zone and treated area respectively. Likewise, 37 larvae in the control area and 20 in the treated area have been recovered.

4 Conclusions

Increases in pH, Ca and Mg values at each breeding site of the treated area were recorded. There was a decrease in the number of recovered larvae and sand flies captured resting on the base of the trees of the treated area

C1146 TEGUMENTARY LEISHMANIASIS IN ENDEMIC AREAS OF PARAGUAY.

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1 Background

Leishmaniasis is a zoonosis, with a wide tropical distribution. In our country it is considered an endemic disease and a public health problem. In addition, the main risk factors, resulting from social, economic and environmental processes, favor their transmission and hinder their control.

2 Methods

Prospective, descriptive study in patients suspected of tegumentary leishmaniasis coming from endemic areas in Paraguay. The study was carried out through the active search of cases in the period of one month.

3 Results

18 patients were found, of which: 3 patients with cutaneous lesions, 8 mucous and cutaneous and mucous : 7, average age 49 years, male predominance, mostly farmer, from the department of San Pedro , Canindeyú, Concepción, where wooded areas predominate and deforestation. The mean lesion time was 13 years for the mucous lesion. Smears were performed in 5 patients with cutaneous lesions, with direct observation of the parasite in 3 cases. Histological examination was positive in 8 cases. Molecular biology technique (PCR) was performed in all patients, being positive 8/16 for leishmania of genus braziliensis

4 Conclusions

Cutaneous leishmaniasis is still an endemic disease in our country, through an active search for one month, 18 patients were enrolled, who agreed to the diagnosis for a subsequent treatment. The evolution time was mostly extended. Smears were performed in patients with skin lesions, 3/5 positive, which should be implemented in the country due to its low cost and simple execution. The technique of molecular biology is a method, although useful, it is not 100% sensitive, which demonstrates the importance of the epidemiological and clinical analysis, supplemented to the auxiliary diagnostic methods, in addition to an early detection of the disease, to avoid its Evolution to mucosal forms.

C1217 VISCERAL LEISHMANIASIS IN SOMALIA: WHAT DO WE KNOW OF ITS EPIDEMIOLOGY AND CONTROL?

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1 Background

Somalia, in the Horn of Africa, has areas endemic of visceral leishmaniasis (VL). It affects the rural poor, internally displaced, and nomadic populations in the southern zone. Due to its protracted conflict since 1991, access to health care is limited, while VL control mainly relies on diagnosis and treatment. We reviewed evidence about VL epidemiology and appraised access to care for VL within the context of this fragile state.

2 Methods

We searched the MEDLINE (via Pubmed) online database for articles published up to 31 March 2016, with no date limit nor language restriction. Additional information was obtained from grey literature and hand search of references. A retrospective data analysis from a VL control project managed by Médecins Sans Frontières (MSF) from 2002-2008 was described with updated information.

3 Results

The literature search yielded 12 papers. VL has been reported in Somalia since 1934, and has persisted ever since in foci in the southern parts of the country (lower Juba, Middle Shebelle, Bakool and Bay regions). The parasite species, *Leishmania donovani*, was confirmed in Somalia in 2000-2001. In Bakool, *P. martini* and *P. vansomerenae* were identified as potential vectors, associated with the ubiquitous termite mounds. There have been no studies on local determinants, risk factors for VL, transmission dynamics, or vector control in Somalia, but anthroponotic transmission is likely. Thus the main VL control option is early diagnosis and treatment, currently mostly provided by non-state actors. The availability of VL care in Somalia is limited and insufficient at best, both in coverage and quality. Precarious security remains a major obstacle to reach VL patients in the endemic areas, and the true VL burden and its impact remain unknown. The MSF project in Xuddur, Bakool region treated a total of 3449 cases from 2002-2008. From 2013, a range of 300-800 cases per year were reported.

4 Conclusions

VL in Somalia should not be left as just another neglected disease in a neglected conflict. Locally adjusted, innovative approaches in VL care provision should be explored, without undermining the ongoing health system development in Somalia. As VL outbreaks may occur in this vulnerable population, improved coordination amongst various stakeholders (WHO, NGOs, community) and improved surveillance is key.

C1287 STRUCTURE, ACHIEVEMENTS AND CHALLENGES IN LEISHMANIASIS CONTROL IN THE WHO AFRICAN REGION

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1 Background

Leishmaniasis remains a major NTD in this region. About 14% of the global burden is found in 27 countries. Visceral Leishmaniasis (VL) is endemic in 21 countries mainly East African (EA) countries. Cutaneous leishmaniasis (CL) is endemic in 24 countries and 15 countries are endemic for both VL and CL (with Cameroon, CAR and the Gambia requiring confirmation). There is no structured regional programme for leishmaniasis in the WHO African Region. Very little is known about CL in this region. The epidemiology of VL is better known especially in East Africa. There is major overlap in the endemicity of leishmaniasis with other case-management neglected tropical disease (NTDs) in the African region which calls for integrated interventions.

2 Methods

The sources of data are reports from the national control programmes, personal communication to national programme managers and literature review.

3 Results

The WHO Regional Committee adopted in 2013 the resolution AFR/RC63.R6 endorsing the *"Regional NTD Strategy" and the "Regional Strategic Plan for NTDs, 2014–2020"*, as means of implementing the WHO Global NTD Roadmap. Most East African countries established national programme and updated their VL treatment manuals. From 2004-2013 more than 60,000 VL cases (in Ethiopia, Kenya, South Sudan and Uganda) and over 125,000 CL cases (in Algeria) were diagnosed/treated. The rollout of combination therapy is successful in Ethiopia and South Sudan. The VL case fatality rate in the past 3 years is below 4% in Ethiopia, South Sudan and Kenya.

The Regional Office developed an "Integrated strategy for the control of Case Management NTDs". Some countries in the region included leishmaniasis in their multi-year NTD strategic plan. Major challenges include: lack of complete epidemiological information on leishmaniasis, poor performance of rapid diagnostic tests, suboptimal efficacy of antileishmanial medicines, inadequate access to treatment and lack of adequate evidence for appropriate vector control measures.

4 Conclusions

The national leishmaniasis programmes in the WHO African Region need to be strengthened for the implementation of the WHO Road map for the control of the disease (prompt case management and IVM). It requires partnership and coordination with improved national ownership. The new agreement: WHO- GILEAD gives new hope to strengthen the VL control programme in the region.

C1291 ACCESS TO ANTILEISHMANIAL MEDICINES: THE GLOBAL DEMAND

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1 Background

Early diagnosis and treatment is essential in the control of leishmaniasis, a multifaceted disease that possesses an uncommon complexity with varying treatment needs for different forms. The virulence of some *Leishmania* strains, causing up to 30 000 deaths annually, amplifies the concern of access to quality-assured medicines.
A limited number of antileishmanial medicines remain the only option for treatment despite their shortcomings such as an increasing relapse rate, limited administration routes and toxicity. Most of the medicines originate from single suppliers, who need yearly forecasts or a minimum order quantity for production and sometimes cannot provide affordable prices for endemic countries or international organizations. Irregular requests often lead to insufficient treatment coverage for certain medicines and/or countries. With a deficient number of studies evaluating the global demand of antileishmanial, this study aims to analyse the current global and country-specific needs of antileishmanial medicines and proposes mechanisms to improve access to quality-assured medicines.

2 Methods

A questionnaire with 19 to 46 variables, depending on country endemicity for visceral, cutaneous and/or post-kala-azar dermal leishmaniasis, was sent to national programme managers of 30 high-burden countries, inquiring about the drugs veritably used. Procurement quantities were obtained from the World Health Organization, Médecins Sans Frontières, Drugs for Neglected Diseases initiative, IDA, Kalacore and suppliers to analyse the global distribution. The demand for each of the antileishmanial medicines was analysed for each of the endemic countries, comparing recommended treatment regimens against those currently implemented.

3 Results

For the total number of cases reported in 2013, the total global quantity of sodium stibogluconate, meglumine antimoniate, liposomal amphotericin B, paromomycin and miltefosine needed was 83 763 vials, 3 025 833 vials, 180 138 vials, 98 394 vials and 125 160 tablets respectively.

4 Conclusions

The demand for medicines is evident and a global estimate of needs facilitates sustainable and timely supply between manufacturers and recipients. Different strategies need to be developed to facilitate allocation of existing drugs efficiently and help reinforce control efforts. An overview of the demand market can also promote the entry of new manufacturers to enhance existing drugs or even the development of new products.

C1297 CUTANEOUS LEISHMANIASIS CONTROL IN AFGHANISTAN: CHALLENGES AND OPPORTUNITIES

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1 Background

Cutaneous leishmaniasis (CL) is a major public health problem in Afghanistan.
Current control strategies stand on the treatment of active cases with pentavalent antimonials that should be provided free of charge through public health centers.
Since 2008, WHO has provided technical and financial support to elaborate a new national CL control strategy, new case management guidelines, a revised surveillance system, training of health staff, educational activities to enhance the public awareness and to procure quality-assured medicines.
Reduction of donors support during 2013-2014, especially with anti-leishmanial medicines, resulted into collapse of service delivery system with reduction of detected cases and treatment.

2 Methods

Analysis of the reported data of the country for the period of 2010-2015.

3 Results

Total reported CL cases increased from around 10 000 cases / year in the period 2003-2005, to an average of 32 000 cases / year in the period 2007-2012, but decreased to an average 20,000 cases /year in 2013 and 2014 (37.5% reduction).
Cases discharged with initial cure in four provinces decreased from 11462 in 2012 to 5549 (52% reduction) in 2013 and 4288 (63% reduction) in 2014, while the defaulter cases in those same four provinces increased from less than 2000 in 2012 to over 4000 in 2013 (100% increase).
Improvement of services in 2015 resulted in increase of total reported cases to 29 767. The cases discharged with initial cure increased to 6883. Defaulter cases decreased to around 1800.

4 Conclusions

The CL control program should be fully supported to expand its service delivery coverage and ensure the availability and accessibility to the quality services in all endemic areas.

C1302 COMPLEXITY OF SYSTEM AND EFFECTIVE WAY OF TREATMENT OF INDIAN VISCERAL LEISHMANIASIS PATIENT

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1 Background

The effective control of Visceral Leishmaniasis (VL) involves many factors. India has well developed health control systems however, due to unawareness of patient, lack of monitoring, clinical complications in various infectious diseases, makes the final treatment more complex. Our understanding of effective way of treatment has been largely skewed towards other infectious disease, imparting less attention to neglected tropical disease. To answer this, we have conducted a field based study, where our data shed light on various important parameters through which patients crossed before the final and effective treatment.

2 Methods

This study was conducted over 50 square kilometer area in endemic region in healthy subjects, earlier affected with VL disease. Total 126 subjects were enrolled and subjected to a structured interview for full description of number of medical practioners and their categories (MBBS and other government degree), various kind of expenditures (travel, medicines, diagnosis), hospitals (government and private) and social factors (reference person of final treatment) using a predesigned program.

3 Results

We found that 71% subjects got final treatment was completed under private hospitals while rest under government hospitals. Data shows that before final and last treatment, total 32% subjects, were visited three times, 42% visited twice, 12% single time and rest patients visited 4 or more than 4 medical practioners. Cost of complete treatment is also variable as expenditure data shows that total 46% person have more expenditure on medicines while 32% have more on various diagnosis test. Data also shows that 16% person have more expenditure on travel while 6% have more expenditure on medical practioner fees. Data on total time period of complete duration (days) of treatment shows that 36% patients have undergone thrice and 32 % on twice the time duration, in compare to last final treatment time.Based on our data we have developed an algorithm of effective treatment of VL disease.

4 Conclusions

We found complex distributions of various parameters before the final and last treatment of VL diseases. This study identifies effective parameters (MBBS medical practioner and VL treated persons as referee) in the way of proper treatment. Our data revealed complex clustering and suggest that identification of important expenditure factors (medicine) were used for making appropriate management decision in effective control of VL in India.

C1516 A NEW WEBSITE FOCUSED ON THE CONTROL AND PREVENTION OF LEISHMANIOSIS: STOPLEISHMANIA.ORG

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1 Background

Leishmaniosis prevention and control should include novel communication strategies that must be easily accessible and would include new advances in the knowledge of the disease. Recently, other animals different than dogs have been described as competent reservoirs of *Leishmania*, playing an important epidemiological role in recent outbreaks in Europe and Asia. At the moment, most of the information available is fundamentally devoted to humans and dogs, and there is no a global approach to the disease, taking into account all possible reservoirs.

2 Methods

Stopleishmania.org is an initiative of the VISAVET Health Surveillance Centre (UCM), the National Centre of Microbiology (ISCIII), the Animal Protection Centre (Madrid City Council) and the Directorate of Public Health (Regional Government of Madrid). This project launches the possibility of collaboration with other research centres, feeding the site with updated information on this disease. The website uses an accessible format and complies with the international Web standards, and is optimised to maximize its visibility (php XHTML CSS / W3C / WAI AA / SEO). The contents are available in Spanish and English, and come from the knowledge and experience in the disease of the above mentioned organisms, including research and information outreach.

3 Results

The result is a website mainly focused on dissemination of the fundamental aspects for the control and prevention of the disease, taking into account that leishmaniosis due to its zoonotic condition, requires a multidisciplinary vision in accordance with the "One Health" principles. The web is complemented by a collaborative tool where the registered research groups can present themselves and disseminate their research results, therefore providing updated information on the scientific advances in the disease control.

4 Conclusions

Stopleishmania.org is a new website devoted to leishmaniosis for the dissemination of scientific advances related to this disease, which would bring to the society key guidelines for the prevention and control of the disease. The website fulfils an important educational role. Moreover, this initiative will facilitate the creation of multidisciplinary working groups to face zoonotic diseases. Although the project is initially proposed at national level, an international dimension is defined as a future objective in order tostrength international collaboration between countries that share common health threats like leishmaniosis.

C1522 PLAY, LEARN AND PREVENT. A STRATEGY SOCIO-EDUCATIONAL FOR THE PREVENTION OF THE CUTANEOUS LEISHMANIASIS AND THE DENGUE IN ENDEMIC COMMUNITIES FROM THE EAST-CALDAS AREA, COLOMBIA.

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1 Background

In Colombia Leishmaniasis and Dengue are considered public health problems. Several control programs focused in fumigations, awnings and technical training that, usually, do not consider cultural and sociodemographic features. This study report part of the eco-epidemiological evaluation for the cutaneous leishmaniasis and dengue control in areas influenced by the hydroelectric power station Miel I: meaning Norcasia, Victoria, Samana and Marquetalia (Caldas), supported by ISAGEN.

2 Methods

Suported by anthropologic studies, we used a participative methodology where games and new technologies were the main axis. First we created a didactic tool adapted culturally named "*Bolsa Viajera para la prevencion de la Leishmaniasis y el Dengue*" (Traveller bag for the prevention of the Leishmaniasis and Dengue), which, contains 5 games (puzzle, maze, tales, deck of cards and stairs) plus an instructive. Once established the traveller bag we performed the intervention with teachers of schools of 39 rural towns: We performed a pre and post-evaluation focused in the knowledge of the diseases, theoretical-practical workshops and real time accompaniment, through E-mail and Whatsapp groups in the practical phase with the students.

3 Results

50 teachers participated in the social-educational intervention; who, with pictures, classroom diaries and videos shared the experience with the students. The evaluation revealed a significant change in the knowledge about both diseases, mainly: features and behavior of the vectors and the transmission cycle. During the intervention there was an evident change in the conceptions, attitudes and practices concerning the diseases, highlighting an increase in the use of the awning between students and teachers. The teachers did not limited the participation of the students, they implemented as well the strategy with student of high schools and caretakers, impacting 1275 inhabitants.

4 Conclusions

We successfully implemented control strategies that started from the specific necessities of the communities, build with and for them, probing that such strategies can be used as efficient and effective transversal actions inside the local vector control programs. We managed to sensitize the community with the traveller bag, a tool created in context, that more than information offers formation. The socio-educational process where teachers worked together with researchers and health care personal, made an intervention with high impact in the health of the population.

C1542 AGGREGATION OF PATIENT LEVEL DATA FOR ROUTINE REPORTING USING DHIS2 TRACKER MODULE FOR VL SURVEILLANCE AND CONTROL PROGRAM

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1 Background

DHIS2, a tool for collection, validation, analysis, and presentation of individual (tracker) and aggregated data, can be tailored to integrated health information management activities for different contexts. While the Tracker module of DHIS2 enables one to collect, manage and analyze transactional, case-based data records, information about individuals can be tracked for patients over time using a flexible set of identifiers. The WHO uses DHIS2 Aggregate data capture module as the system for Worldwide disease surveillance with a central database with all the selected tropical diseases. Drugs for Neglected Diseases initiative (DNDi) in collaboration with WHO adopted DHIS2 to aggregate available Visceral Leishmaniasis (VL) data from clinical trials and general patient records to a format compatible with national database in order to share data and better conduct VL surveillance. It is critical to maintain patient confidentiality throughout the process.

2 Methods

We piloted a DHIS2 tracker module to capture patient data remotely from DNDi sponsored VL treatment and clinical sites into a central database in Nairobi. The data indicators were created based on logical computations and calculations from the variables built to automatically aggregate the data. Aggregate format were used for analysis, visualization and generation of reports just as the data for other studies in the WHO's surveillance database. The system was programmed to display the aggregated data reports on the main Dashboard, which was interpreted, presented and used for analysis at different levels for example facility levels, district level and national levels.

3 Results

Use of indicators, enabled the creation of an aggregate data from individual patient data as well as analyze, visualize and generate reports in a format compatible with the WHO's surveillance database from the clinical research data of VL.

4 Conclusions

The pilot using indicators in DHIS2 has made it easy and possible to aggregate the patient tracker data and avail it in a format that is analyzable and easy to interpret making it also possible to visualize in charts and graphs which are displayed directly on the systems Dashboard. The data from clinical trials which are individual patient data can now be in aggregated data format for surveillance purposes. Therefore, DHIS2 Tracker module is an essential tool for making this process possible. The aggregated data can be shared while maintaining confidentiality.

C1599 ANTI-PHLEBOTOMINE SANDFLY EFFICACY OF SYSTEMIC INSECTICIDES ADMINISTERED TO DOGS

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1 Background

Zoonotic Visceral Leishmaniasis (ZVL) is a vector borne disease transmitted from dogs to humans throughout the bite of phlebotomine sand flies. Systemic insecticides in dogs could be used in control programs of ZVL in endemic countries like Brazil. Four systemic insecticides approved for their use in dogs were previously selected based on their potential to be used as a new tool to control human cases of ZVL. These insecticides are proved to be safe and effective against the on-label insects and parasites. However, to be used in a ZVL control program their anti-phlebotomine activity should be proven.

2 Methods

The anti-phlebotomine activity of four systemic insecticides in dogs was evaluated using a randomized clinical trial. Thirtydogs were randomly allocated into five groups, four treatments and one control, of equal size. The treatments evaluated were: Guardian®SR, Elanco (moxidectin); Comfortis®, Elanco (spinosad); Bravecto®, Merck Animal Health (fluralaner); NexGard®, Merial (afoxolaner). All the treatments were administered to the dogs following the label indications. Blood from dogs was taken at days 2, 4, 12 and 31 post-treatment. Membrane feeding bioassays using *Phlebotomus papatasi* were used to evaluate the anti-phlebotomine efficacy of the different treatments. Sand fly mortality was observed 24 and 48 hours following the membrane feeding.

3 Results

Results from day 2 after treatment have been analysed. Fluralaner was the only compound that showed sand fly mortality 24 hours after membrane feeding (mean: 57%, 95% CIs: [47%, 67%]) significantly higher than mortality from the control group (mean: 30%, 95% CIs: [20%, 40%]).

4 Conclusions

Fluralaner showed a rapid and significant killing effect on blood-fed sand flies. Results from days 4, 12 and 31 post-treatment should confirm this anti-phlebotomine activity. The use of fluralaner as a control tool for ZVL in endemic areas will be discussed.

C1600 CAN SYSTEMIC INSECTICIDES IN DOGS BE USED TO CONTROL ZOONOTIC VISCERAL LEISHMANIASIS?

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1 Background

Zoonotic Visceral Leishmaniasis (ZVL) is a public health problem in some endemic countries like Brazil. ZVL is caused by *Leishmania infantum* which transmitted is from infected dogs to humans throughout phlebotomine sand flies bites. The current control measures, in particular culling infected dogs, have not been able to decrease ZVL incidence in humans. The use of systemic insecticides in dogs may be an alternative or complement to current control methods. Mass administration of systemic insecticides to dogs in endemic villages may interfere in the dynamics of *L. infantum* transmission to humans.

2 Methods

We used the British Small Animal Veterinary Association (BSAVA) Small Animal Formulary (2016), Vademecum Guíavet (2016), and the website Parasitipedia to list all systemic insecticides currently being used in dogs. The systemic insecticides had to be administered orally (PO) or subcutaneously (SC) and registered for their use in dogs in Europe, North, Central and South America. The pharmacological characteristics of the drugs identified were gathered from the web pages of the European Medicines Agency (EMA) (2016) and the U.S. Food and Drug Administration (FDA) (2016). The following properties were recorded for each insecticide: mode of action, pharmacokinetics, safety, and effectiveness against targeted insects.

3 Results

We selected the systemic insecticides with higher potential for controlling ZVL transmission based on: (1) capability to kill blood-fed sand flies; (2) optimal plasma concentration; (3) long-lasting action and; (4) ease of administration. From eleven possible candidates we selected five systemic insecticides: spinosad, fluralaner, afoxolaner, sarolaner, and moxidectin.

4 Conclusions

If the anti-phlebotomine capacity of these compounds is confirmed in experimental studies the use of these systemic insecticides in ZVL control programs is very promising. We discuss the limitations and benefits of using this new control tool compared to current practices.

C1611 ANALYSIS OF A DECADE OF RESEARCH IN THE FIELD OF LEISHMANIA IN THE INTERNATIONAL ARENA

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1 Background

Bibliometric studies have encompassed to a large extent of the scientific and research communities and they can frequently be seen in many journals in different fields. To date, the high volume of bibliometric studies are performed in the medical field and the researchers of this field attempt to analyze the process in collaboration with medical professionals and finally perform the management of knowledge in the studies of the medical field. One of the most important topics in the field of the medical research is related to the field of Leishmania.

2 Methods

This research was conducted using scientometrics technique. The population for this study comprises all the articles registered with the subject of *Leishmania* in the period of 2006 to 2015 in Web of Science. To do the analyses of this research in combination, Pajek and VOSviewer were used.

3 Results

This ten-years study period (2006-2015) showed that 13658 records were indexed in the field of Leishmania in the Web of Science database. The findings indicate that during the past ten years, the studies of *Leishmania* had enjoyed the relative growth so that the number of researches from 1071 in 2006 to 1537 cases in 2015 had reached in this area indicating more emphasis of the researchers to the topics of this study in this area. Sundar S is the most active researcher in the field of Leishmania by compiling and participation in 232 Articles. Brazil has the first ranking by performing 3315 studies of Leishmania compared with other countries in the world. The United States and two countries of the United Kingdom and Australia are higher than other countries that they have the most collaboration in performing the studies of Leishmania with each other. Also, the journal of Plos Neglected Tropical diseases has had the most collaboration in the development of the studies of Leishmania by printing 483 papers.

4 Conclusions

Due to the fact that for any planning and policy-making, having knowledge about the cost-scientific capabilities is necessary. On the one hand, content analysis of scientific production and on the other hand, the study of the impact of the researchers of this field will state the policy makers that how the researchers are become mighty to compete in the global arena. Certainly, improvement of scientific status in the field of Leishmania over time will result in developing in the prevention, treatment and reduction of mortality.

C1736 HEALTH EDUCATION AS A STRATEGY FOR EMPOWERMENT AND SOCIAL MOBILIZATION TO PREVENT LEISHMANIASIS IN THE DEPARTMENT OF VICHADA, COLOMBIA

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1 Background

The department of Vichada (Colombia), represents one of the largest territorial extensions in the country, with environmental, population and social conditions that allow the development of different events of public health importance. In Vichada cutaneous leishmaniasis has a prevalence of 50 cases per 100,000 inhabitants (Sivigila 2007-2015), justifying the development of a project whose objective is the integral three-pronged approach to tackle the disease: the clinical care of patients (diagnosis and treatment), an ecoepidemiological study to determine the risk factors and the development of a social component through anthropology and teaching (pedagogical component).

2 Methods

The pedagogical component is approached from institutionalism developing a School-Health articulation in which the Vichada departments of education and health participate. It includes sensitization of the various teaching institution directors, socialization of the subjects related to leishmaniasis with teachers of the natural sciences and students of eighth and ninth grades, who initially performed a previous knowledge survey (as input behavior). Later there was feedback to the rest of the student population and the community in general, through the development of recreational activities and local strategies focused on local risk factors.

3 Results

In the development of the strategy 12 educational institutions of the department participated (85%). The students who conducted the previous survey showed limited knowledge regarding leishmaniasis, based on which they were trained and participated in the development of activities of social mobilization, cultural activities and physical intervention. The teaching of measures to prevent leishmaniasis was successfully included in the curriculum of the natural sciences.

4 Conclusions

Continuity in these processes is enabled by the inclusion of the topics in the curriculum grid, as well as the participation of directors, teachers and students in awareness-raising and training activities using different pedagogical methods. This also guarantees social empowerment and community participation in the prevention of infectious diseases such as leishmaniasis.

C1749 COMMUNITY-BASED HEALTH PROMOTION APPROACHES IN AFGHANISTAN AND LESSONS TO LEARN FOR PREVENTION, HEALTH COMMUNICATION, EDUCATION, AND PROMOTION IN CUTANEOUS LEISHMANIASIS.

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1 Background

The Afghan Ministry of Public Health (MoPH) stresses the general need for community-based prevention programs and health promotion (CBPaHP) activities. The purpose of this study was to investigate CBPaHP approaches in Afghanistan and to identify practices. The lessons learned will help to develop evidence-based prevention, health communication, education, and promotion in cutaneous leishmaniasis for the National Malaria and Leishmaniasis Control Program (NMLCP).

2 Methods

An explorative case study was conducted, including desk research, review on healthcare providers, website analysis, questionnaires and semi-structured interviews. 25 organizations responded to the questionnaire and 27 interviews with 23 key health care providers were conducted via Skype, recorded, and transcribed. Data were analyzed using qualitative content analysis according to Mayring. The results were validated by feedback of the interviewees.

3 Results

Good examples of practices for comprehensive disease prevention could be identified. The most decisive factors were (1) in-depth understanding of health concepts and common health practices, (2) cooperating with respected leaders (e.g. mullahs), (3) addressing key opinion leaders and decision makers (e.g. mothers in law) and (4) supporting health messages with Koran verses. On the national level, sustainable prevention strategies are integrated in the MoPH standard package of health services, supported by awareness rising media and mass campaign. Providing information by the medical staff during consultation or in health education sessions in the waiting room, showed no convincing efficacy for health promotion. On the community level, interactive dissemination of health related messages, modeling of behavior change as well as stimulating dialogue to address cultural taboos was a crucial component in the training of health nurses, health councils as well as peer trainers. Pictorial materials, timed and targeted counseling, assessment of and change in environment were recommended.

4 Conclusions

Based on the collected empirical evidence important practices for CL prevention,health communication, education, and promotion in CL were identified to initiated discussion and develop further research.

C1757 MULTI-CENTRE TECHNICAL CASE STUDY IN AFGHANISTAN WITH THE SMARTPHONE APPLICATION ECULEIDO FOR CLINICAL DOCUMENTATION AND SURVEILLANCE OF CUTANEOUS LEISHMANIASIS PATIENTS

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1 Background

There is no appropriate early warning and response system at the moment for Cutaneous Leishmaniasis (CL) control in Afghanistan. The present multi-centre study was to conducted as pilot study to investigate whether a smartphone application was a reliable alternative for CL case documentation. The ultimate goal was to gather evidence that an electronic clinical case report form in a smartphone application can be used for CL clinical documentation and public health surveillance.

2 Methods

The study began in December 2012 at the national the Leihsmaniasis and Malaria Centre (LMC) in Kabul, in January 2013 the LMC in Herat was included, and in February 2013 the LMC Mazar-e-Sharif followed. The smartphone application was assessed using criteria refering to technical reliability, usability and acceptance by the health care staff.

3 Results

On average, 10 patients were documented per day. In 4 months a total of 1,510 new patients were documented, representing a total of 5,049 visits. The mean number of visits were 3 visits per patient. The health care staff needed on average 3 minutes to register a new patient and less than one minute to document a new visit.

4 Conclusions

The study showed technical means to improve with a smartphone application the clinical cases management and real-time monitoring of CL and to develop a public health management system on a facility, regional and national level on the basis of clinical and other disease relevant health, economic, geographic and environmental indicators.

C1849 EXPERIENCE ON INTRODUCTION AND ROLL OUT OF LIPOSOMAL AMPHOTERICIN B FOR THE TREATMENT OF VISCERAL LEISHMANIASIS IN NEPAL

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1 Background

Nepal's Visceral Leishmaniasis (VL) elimination programme revised the national treatment protocol in 2014 to introduce new treatment regimens including Liposomal Amphotericin B (L-AmB) as single drug regimen or in combination for the treatment of VL. VL treatment is provided free of cost in Nepal.

2 Methods

L-AmB was received and introduced in September 2015 with some delay on arrival and introduction experienced due to the devastating earthquake of April 2015. Trainings to physicians and nurses from major hospitals of endemic districts and referral centres were organized in December 2015. The trainings, also attended by two participants each from Bhutan and Thailand and facilitated by national facilitators and two trainers from India, were later scaled up covering all major public and private hospitals. Orientations to programme managers and focal persons from all endemic districts were organized. L-AmB was delivered to treatment sites and district public health offices, prior to which storage capacity with availability of appropriate cold chain maintenance was assessed. The treatment initially started in 3 sites is now available in more than 20 sites across the country. Periodic monitoring & evaluation was conducted to facilitate and assess implementation.

3 Results

Over 300 medical doctors and nurses and over 60 programme managers and focal persons were trained or oriented on new treatment regimens. Almost all cases diagnosed thereafter are being treated with L-AmB either in single or combination regimens. The new regimens have significantly reduced treatment duration and hospital stay as have improved treatment compliance and thus has been well accepted and welcomed by treating doctors, nurses and programme persons. We have observed that patients and their care takers are also satisfied with the free new treatment although it is available only in designated hospitals. Recently, the government has made free provision of laboratory and radiological investigations equivalent to 50 USD per VL case available in major hospitals.

4 Conclusions

The introduction and roll out of new treatment regimens, especially L-AmB for VL treatment, has been welcomed and well accepted by medical community, programme and patients. However, its limited availability at district and above level hospitals and the emergence of VL in new areas and districts has prompted for further capacity building and roll out.

C1851 ROLE OF KALACORE IN SUPPORTING CONTROL AND ELIMINATION OF VISCERAL LEISHMANIASIS IN NEPAL

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1 Background

Since 2005, the government of Nepal participates in aregional collaborative effort along with Bangladesh and India to control and eliminate visceral leishmaniasis (VL). Thetarget is to reduce the incidence rate of VL below 1/10 000 by 2015at district level which has was achieved in 2013 and been sustained since then. Despite the achievement, several important challenges still remain to sustain the control and elimination.KalaCORE funded by the UK Department for International Development (DFID) has started to implementthe capacity building activities to support ongoing control and elimination of VL in Nepal.

2 Methods

KalaCORE is a 2.5 years programme (May 2016 – October 2018); has started the implementation of activities since June 2016 in Nepal. The programme focuses on five specific activities(i) training & capacity building ofhealth workersat different health care levels (from volunteers, vector control officers to physicians) to strengthen the case detection strategies, disease surveillance,early recognition of clinical VL and PKDL for effective treatment in a context of reduced incidence, (ii) the development and strengthening of VL outbreak investigation and response, (iii) development of a targeted IEC/BCC strategy for VL control (iv) sustain medical entomology capacity and (v) support of Pharmacovigilance activities with regards to safety of VL drugs.

3 Results

So far, 62 vector control officers includingpublic health officers working in the VL endemic districts were trained on case-based surveillance and response to VL/PKDL. An appropriate IEC/BCC strategy was developed and is under implementation to increase awareness among health workers and the public for VL control. A total of 1435 female community health volunteers including health facility in-charge working in VL endemic districts were trained on targeted IEC/BCC for VL/PKLD identification at community level. An evidence-based outbreak investigation and response guideline was developed for emerging VL outbreaks. Several other activities have been planned and will be implemented soon.

4 Conclusions

KalaCORE has been taking lead role to strengthen the capacity of healthcare workers on early diagnosis, treatment, effective disease surveillance and outbreak response which in turn contributes to support VL elimination in Nepal.

C1853 COMBINED ACTIVE CASE DETECTION OF VISCERAL LEISHMANIASIS, POST KALA-AZAR DERMAL LEISHMANIASIS AND LEPROSY IN THREE DISTRICTS OF NEPAL

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1 Background

Visceral leishmaniasis (VL), also known as Kala-azar in Indian sub-continent, is endemic mostly in districts along southern plain belt of the country bordered with India. VL, post kala-azar dermal leishmaniasis (PKDL) and leprosy share some common control strategies such as intensified disease management, early case detection and treatment and active case search approach. Being co-endemic NTDs in these districts, a combined VL, PKDL and leprosy active case search was carried out to accelerate VL and leprosy elimination efforts.

2 Methods

The combined active case search was conducted in five village development committees (VDCs) each from three VL and leprosy co-endemic districts during February-May, 2013. Orientations at district and health facility levels and one-day training to female community health volunteers (FCHVs) at health facility level were organized followed by house- to-house search for suspected VL, PKDL and leprosy cases by mobilizing FCHVs equipped with pictorial case definition and search cards. Suspected VL and PKDL cases were then examined and confirmed by performing rK39 tests and non PKDL skin lesions were further examined for leprosy at health facilities by trained and experienced health workers. All the cases were put on appropriate treatments.

3 Results

Al total of 712 suspected VL cases were screened out of which 14 were diagnosed as VL. Similarly, 8 cases of PKDL and 7 cases of leprosy were detected during this combined active search. No cases of PKDL and leprosy were detected in Sarlahi district. These seven newly detected leprosy cases would have been missed or detected later had this active search initiated by VL programme not been combined with leprosy. This activity also provided VL and leprosy services to the door steps of at risk populations thereby minimizing the travel related and other costs incurred in quest of services; and building the trust between the communities and health care services.

4 Conclusions

Active case detection is an important activity that detects VL, PKDL and leprosy cases early providing opportunity to start early treatment thereby contributing to reduce the transmission. Combined active case search are cost effective and have collaborative and collateral benefits to programmes targeted and involved.

C1855 PROGRESS TOWARDS VISCERAL LEISHMANIASIS ELIMINATION IN NEPAL

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1 Background

Visceral leishmaniasis (VL) is a vector-borne neglected tropical disease. In Nepal it is endemic mostly in southern plain districts with emergence observed more recently in other parts of the country. Acceleration of VL control was initiated back in 2005 by Epidemiology and Disease Control Division/Department of Health Services by formulating a national plan targeting elimination of VL by reducing annual incidence to less than 1 case per 10,000 populations at district level by 2015. National strategic guideline on kala-azar elimination introduced Liposomal Amphotericin B (L-AmB) and combination treatment regimens in 2014.

2 Methods

The national programme has adopted early diagnosis and complete treatment, vector control, surveillance, capacity building and community & social mobilization as major control strategies. The programme provides rK 39 rapid test kits as standard diagnostic method. Parasitological tests and PCR are also performed at higher and referral level hospitals and centres. BP Koirala Institute of Health Sciences, Dharan and Sukra Raj Tropical & Infectious Disease Hospital, Kathmandu are designated as referral centres.

3 Results

There has been significant reduction in incident cases over the years with just few hundred new cases detected annually translating into incidence rate well below 1 case per 10, 000 populations since 2013 in all endemic districts and many possible deaths averted. Treatment regimens have been updated, with the introduction and expansion of L-AmB and combination regimens as a major shift. Change in epidemiological pattern has been observed with narrowing hotspots in traditionally endemic districts while new foci are emerging in other districts. Additionally the VL elimination programme has provided collateral benefits to health systems through trained health work force, increased access to free diagnosis and treatment services, stronger monitoring and surveillance, VL focused pharmacovigilance, operational researches and increased resource mobilization.

4 Conclusions

Despite limitations, the programme has made significant progress and is comfortably on track to achieve elimination of VL as public health problem. However, sustaining the gains made so far and the emergence of new foci remain as major challenges. Further efforts and investment are required to consolidate the gains and prevent rollback in the future. At this stage, the programme would immensely benefit by an independent appraisal.

C1856 EVALUATION OF THE EFFECTIVENESS OF THE USE OF INSECTICIDE-IMPREGNATED COLLARS FOR THE CONTROL OF CANINE VISCERAL LEISHMANIASIS IN BRAZIL.

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1 Background

Visceral Leishmaniasis is widely distributed worldwide and it still persists as an important and global public health problem. In Brazil, the control measures currently adopted have not been able to prevent the spread of the disease.

2 Methods

A community intervention trial was carried out in the city of Montes Claros, state of Minas Gerais, area with high transmission. Two control strategies were randomly allocated: (1) the control measures usually recommended by the Control Program of the Brazilian Ministry of Health (sacrifice of infected dogs and vector control) and (2) use of deltamethrin-impregnated dog collars in addition to the control measures recommended by Control Program (intervention area). At the beginning of the study, a census survey was carried out among domiciled dogs to detect the prevalence of *L.infantum* infection. Monitoring of canine infection (control and intervention areas) and replacement of collars (intervention area) occurred through visit cycles at 12, 18 and 24 months after the initial survey. At each visit the dog was tested and the owner answered a questionnaire about the general characteristics of the animal. Multilevel logistic regression models were used to test the effect of collars on the risk of canine infection, with households as second level units (clusters).

3 Results

17.409 dogs participated in the study, 9.658 in the intervention area e 7.751 in the control area. The prevalence of infection in the initial survey was similar between the areas (p=0.632). The risk of infection was 51% lower in the intervention area. For each additional dog at home, the risk of infection increased by about 9% (OR = 1.09, 95% CI 1.06-1.12). Of the dogs that participated in the second cycle, almost 48% were new to the study, which shows the high canine replacement. The loss of collar between the first and second cycle was 56%.

4 Conclusions

The use of collars as a control measure was effective, however, it is necessary to consider the key issues for its implementation, such as canine renewal and replacement of lost devices. Studies on the cost-effectiveness of use of collars as a control measure are recommended.

C1858 LABORATORY BIOASSAY ABOUT THE LETHAL AND SUB-LETHAL EFFECT OF AZADIRACHTIN (NEEM) ON LARVAE OF PHLEBOTOMUS PERNICIOSUS DIPTERA: PYCHODIDAE: PHLEBOTOMINAE

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1 Background

It is well documented that neem tree extract (azadirachtin) has insecticide properties and act as an insect growth regulator (IGR) for some insect's larvae while being innocuous for mammals and birds. It is also well-known that phlebotomine sand flies (e.g *Phlebotomus perniciosus*) are vectors of important diseases including leishmaniasis. This study evaluates the lethal and sub-lethal effects of azadirachtin on larvae and pupae of *Ph. perniciosus.*

2 Methods

The study used latest instars larvae of an autochthonous laboratory colony of *Phlebotomus perniciosus*. Different dilutions of a commercial emulsion of 3.2% azadirachtin (Laboratorios Flower S.A) were mixed with larval food. Polyethylene containers were used to perform the bioassays. Each container included 30 larvae and 1 gr of larval food. The experiment included 6 treatment groups and one control, with three replicates per group. The treatment groups included azadirachtin concentrations of: 6,4% (twice the recommended dose), 3.2% (the recommended dose), and sub-lethal doses of 1.6%, 0.8%, 0.4% and 0.2%. Larvae were observed daily until death or development to pupae stage.

3 Results

In control group 95% of the larvae developed into pupae before day 8 after treatment. However, no larvae developed into pupae in any of the treatment groups. Differences statistically significant in larvae mortality 9 days after treatment were found comparing control with treatment groups. For example treatment groups with azadirachtin concentrations of 6.4% and 3.2% showed 100% (95% CIs: 99.98%, 99.99%), and 80% (95% CIs: 80%, 90%) mortality respectively. Meanwhile sub-lethal concentration of 1.6% and 0.2% showed 70% (95% CIs: 60%, 80%) and 35% (95% CIs: 24%, 45%) mortality respectively. Larvae stage duration time was prolonged in sub-lethal concentrations.

4 Conclusions

Azadirachtin showed to be very effective against *Ph. perniciosus* larvae. Sub-lethal doses increased the time period in larvae stage and consequently induced death before development into pupae. Lethal doses induced larvae death much faster. Compounds including azadirachtin at different concentrations could be used as a vector control measure in endemic areas of leishmaniasis.

Diagnostic methodology in practice and under development. Biomarkers in leishmaniasis

C0371 IDENTIFICATION OF COMMON CUTANOUSE LIESHMANIASIS SPECIES IN HERAT CITY OF AFGHANISTAN USING MOLECULAR METHODS (PCR _ RLFP)

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1 Background

Cutaneous leishmaniasis (CL) is one of the main public health problems in Afghanistan that has across the board increase, particularly in Herat city. Parasitological diagnosis of cutaneous leishmaniasis is of vital importance before medical care.

2 Methods

In the present work, the polymerase chain reaction-restriction fragment length polymorphism technique was applied to amplify internal transcribed spacer one (ITS1) gene and identification of Leishmania species from CL patients in Herat. The Giemsa-stained skin scrapings by direct microscopy with that of ITS1-PCR tichnique was compared in diagnosis of cutanouse leishmaniasis. The samples were collected from 64 patients (41 male and 23 female) that have been referred to Leishmaniasis and Malaria center of Herat Regional Hospital. The patients had not recieved any kind of treatment before sampling. Slash skin smear were taken on DNA banking cards and slides. Epidemiolpgical analysis was carried out on obtained data.

3 Results

The PCR result was positive in 50 cases from 64 CL cases (78.12%).RFLP results was 48 cases (96%) for *L. tropica* and 2 cases (4%) for *L.major*. Out of the 64 slides scanned for amastigotes using 100x oil-immersion light microscopy, 37 (57.81%) gave usable results. Twenty seven (42.19%) slides could not be microscopically scanned. A majority of the patients showed ''dry''-type single lesions on the face. Size of lesions varied from 0.5 to 10 cm. The lesions were located on the head in 26 patients, hand in 23 patients, foot 5 patients, head and hand in 7 patients, head and foot in 2 patients and one patient with 15 lisions in head, hand and foot.

4 Conclusions

CL appears to be endemic in Herat, clinically presenting as 'dry' type, caused mainly by L tropica (96% of cases) and occasionally by L major (4%). There is a need for a multicenter study to evaluate the extent of the disease and diffusion of each Leishmania responsible species in Afghanistan.

C0444 REVISITING OPTICAL MICROSCOPY AS A DIAGNOSTIC TOOL IN EPIDEMIOLOGICAL STUDIES OF ASYMPTOMATIC CANINE LEISHMANIOSIS

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1 Background

We investigated the validity and scope of optical microscopy (OM) in diagnosing asymptomatic canine leishmaniosis (CanL) in an epidemiological study in Southeast Spain. OM examination has been traditionally considered to have low sensitivity and high specificity and unsuitable for epidemiological screening. In a previous study in healthy dogs in Murcia, the percentage of *Leishmania* OM, PCR and ELISA-positive animals were 2%, 67% and 27%, respectively (Risueño et al., 2012). However, some authors claim that OM sensitivity can improve if more than one sample from lymphoid tissues per dog are examined and enough time is dedicated to each smear (Saridomichelakis et al., 2005). This study was performed to evaluate this hypothesis in a similar group of dogs to those used by Risueño et al (2012).

2 Methods

Forty three stray dogs were clinically examined and blood, spleen, bone marrow (BM) and lymph node (LN) samples were taken for CanL diagnosis by OM, tissue culture, PCR and antibody-ELISA. Tissue imprints were stained with the panoptic method and examined at x1000 magnification for 20 minutes each.

3 Results

Seven of the 42 dogs had clinical signs compatible with CanL. The percentage of positive PCR, ELISA, culture and OM among symptomatic and asymptomatic dogs were 100%, 100%, 86%, 86% and 56%, 28%, 5%, 28%, respectively. Whilst PCR and ELISA prevalence was similar to that reported by Risueño et al (2012), the percentage of OM positive asymptomatic dogs was much greater. OM sensitivity relative to PCR was 75% when combining results from more than one tissue and was 40%, 50% and 60% for BM, LN and spleen samples alone.

4 Conclusions

Detailed OM examination of spleen, LN and BM samples from the same animal can greatly improve the sensitivity of this method to diagnose latent CanL and can be almost as sensitive as the PCR in symptomatic dogs. These findings have important practical implications for CanL diagnosis in veterinary practices where other diagnostic methods are unavailable. Although spleen samples provided greatest sensitivity for OM examination, they are more difficult to take than BM and LN. However, the latter two combined provide a higher sensitivity than spleen alone.

C1003 EVALUATION OF A RAPID DEVICE FOR THE SEROLOGICAL DIAGNOSIS OF LEISHMANIA INFANTUM INFECTION IN CATS

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1 Background

Feline leishmaniosis (FeL) caused by *Leishmania infantum* (Li) is increasingly diagnosed in endemic areas and the immunofluorescence antibody test (IFAT) is the most used serologic test for FeL (cut off set at 1:80). Rapid tests are on the market for qualitative detection of canine anti-Li antibodies and their use in cats is theoretically possible as serum IgG are bound by protein A coupled with reporter molecules (enzymes or colloidal gold). In fact, protein A is able to bind the Fc portion of IgG of different animal species, included cats. Accurate rapid tests would be useful for facilitating the diagnosis of FeL in suspected clinical cases. The aim of this study is to compare for the first time a rapid immunochromatographic test (Speed Leish K™ [SLK], BVT Group Virbac, France) with IFAT for the detection of Li antibodies in cats.

2 Methods

Ninety-eight serum samples of cats from Sicily (endemic area) were retrospectively selected according to Li IFAT titer: n.70 < 1:80; n. 28 ≥ 1:80. Additional n.27 cat serum samples obtained from North of France (not endemic area) were evaluated. Any sample was tested by both IFAT (cut off 1:80) and SLK the same day and always by the same blind operators. Specificity (Sp), sensitivity (Se), negative (NPV) and positive (PPV) predictive values, and optimal reading time (ORT) between readings performed after 10', 15', and 20' were computed.

3 Results

Overall n.30 samples had IFAT titer >1:80 (range 1:80-1:10240) including 2 cats from France (one with titer 1:80 and one 1:160) and they tested positive by SLK as follows: n.15 after 10', n.18 after 15', and n.20 after 20'. Unfortunately, no further investigations were possible to confirm Li infection in the 2 positive cats from North of France. Ninety-one samples were IFAT negative at 1:80 serum dilution and they tested negative by SLK as follows: n.83 after 10', n.63 after 15', and n.57 after 20'. As a consequence, Sp and PPV of SLK progressively decreased as the reading time increased (10': Sp 91.2%, PPV 65.2%; 15': Sp 69.2%, PPV 39.1%; 20': Sp 62.6%, PPV 37%) and at the same time Se and NPV slightly improved (10': Se 50%, NPV 84.7%; 15': Se 60%; NPV 84%; 20': Se 66.7%, NPV 85.1%).

4 Conclusions

Compared to IFAT (cut off 1:80) SLK Se and Sp values obtained testing feline sera were lower than those reported for canine samples and far from allowing accurate ruling in or ruling out Li infection in cats.

C1007 USE OF THE ORAL SWAB FOR THE PARASITOLOGICAL CONFIRMATION OF HUMAN SUBCLINICAL INFECTION BY LEISHMANIA INFANTUM

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1 Background

Serology and/or the leishmanin skin test (LST) have been the basis to define asymptomatic infection, the most common outcome after *Leishmania infantum* inoculation. Although recommended, parasitological confirmation of human subclinical infection has been neglected due to the difficulty to collect invasive samples in large scale. Here, we used the oral swab, a sample that could be easily collected in field settings for searching *Leishmania* in individuals living in regions where the parasite is transmitted.

2 Methods

This cross-sectional study was developed in an endemic area for visceral leishmaniasis (VL) in North Brazil (Pará state) and was conducted in two phases. First, we used LST and the indirect fluorescent antibody test (IFAT) for the immunological diagnosis of infection. Accordingly with our previous studies, we staged the infection of 75 individuals without signs and symptoms of VL as follows: indeterminate initial infection (III, n=23, LST− and IFAT+/++), asymptomatic infection (AI, n=31, LST+/++++ and IFAT−), and subclinical resistant infection (SRI, n=21, LST+/++ and IFAT+/++). Further, we investigated the presence and quantity of *Leishmania* DNA in their oral swabs by conventional PCR with primers targeting the heat-shock protein 70 that also permit the identification of *L. infantum* after digestion with *Hae* III.

3 Results

We demonstrated *L. infantum* DNA in 78% (18 out of 23) of III, 74% (23 out of 31) of IA, and in 76% (16 out of 21) of ISR individuals. Parasite burden ranged between 0.3 to 61 parasites per reaction, and was higher in ISR group (27.3 ± 22.0) compared to IA (3.0± 5.5) and III (6.0± 7.3) ($p \leq 0.05$).

4 Conclusions

Identification of unapparent infection in foci of *L. infantum* transmission would allow new perspectives in VL control. Immunological reactivity (IFAT/LST) may not be indicative of infection, but of exposing to the parasite. Thus, the parasitological diagnosis should still be the major component in diagnosis. With the use of oral swab we could detect *L. infantum* DNA in high proportion of residents from an endemic areas for VL who was experiencing different stages of subclinical infection, including those individuals with seronegative or LST results. In summary, oral swab is a good sample to be used in epidemiological studies for confirming parasitological diagnosis of human cases with unapparent *L. infantum* infection.

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C1163 ENZYME LINKED IMMUNOSORBENT ASSAY (CELISA) AS A SCREENING METHOD DIAGNOSIS AND EPIDEMIOLOGICAL STUDIES OF CANINE VISCERAL LEISHMANIASIS IN BRAZIL.

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1 Background

According to the Brazilian Ministry of Health (MS), DPP® test is the standard screening method for canine visceral leishmaniasis (CVL), while ELISA is the standard method for diagnostic confirmation of the disease. The DPP® test is frequently used only for field work performed by MS and is not commercial available in Brazil. As an alternative, the aim of this study was evaluate the diagnostic efficiency of a cELISA compared to DPP® and to identification of the parasite by isolation and culturing.

2 Methods

In a first moment, serum samples and lymph nodes biopsy from 109 dogs living in Foz do Iguaçu (Paraná state – Brazil) were collected and used to perform the standardization of the cELISA based on the DPP® and parasite culturing (in NNN media). *Leishmania infantum* crude extract was used as antigen and the cut off of the assay was determined considering two (2SD) and three (3SD) standard deviation. In a second moment, the standardized cELISA was performed using serum samples from other 1,770 dogs. The samples were classified into three groups: Group D – 628 animals with clinical suspicion for CVL; Group PV – 161 animals prior to vaccination; and Group RC – 981 random animals. All of those animals were also classified into the following groups, according to clinical presentation for CVL: Group A - 1,183 asymptomatic animals; Group O - 203 oligosymptomatic animals; and Group S - 384 symptomatic animals. Sensitivity, specificity, correlation and clinical classification analyses were performed by GraphPadPrism 6.0, Medcalc 16.8.4 and EpiInfo 7.1.5.2.

3 Results

The values of sensitivity, specificity and correlation for cELISA were 0.90, 0.22 and 0.1787, respectively, when considering 2SD and 0.89, 0.26 and 0.204, respectively, when considering 3SD. The parameter 2SD was accepted for cu off between positive and negative. While for DPP®, the sensitivity, specificity and correlation values were 0.89, 0.22 and 0.153, respectively. Among groups, the positivity rates were 64.33% (D), 50.31% (PV), 31.09% (RC) for cELISA and 48.73% (D), 22.18% (PV), 20.18% (RC) for DPP®. Based on clinical classification, the rates of positivity were 37.87% (A), 50.31% (O), 64.33% (S) for cELISA and 21.39% (A), 38.92% (O), 54.31% (S) for DPP®.

4 Conclusions

cELISA can be useful as a screening method for CVL detection, as well as for epidemiological studies by detecting antibodies against *L. infantumt,* even in sera from clinical healthy animals.

C1235 IDENTIFICATION OF LEISHMANIA BY MALDI-TOF MASS SPECTROMETRY: VALIDATION OF A NOVEL AND FREE WEB-APPLICATION.

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1 Background

Molecular methods allow *Leishmania* typing directly from clinical samples or cultured parasites. Based on parasite culture, Multilocus Enzyme Electrophoresis is considered by the World Health Organization to be the gold standard in parasite typing. PCR-Based methods such as RFLP or amplicon sequence analysis are simple, rapid and currently available in most laboratories. Matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) MS is integrated in the laboratory workflow and has accelerated pathogen identification to improve patient care. However, the use of MALDI-TOF MS for identification of protozoa is limited in scope and lacks databases and standardized protocols. With respect to the protozoon *Leishmania*, since no commercial solution of reference mass spectra library (MSL) was available, the aim of this work was to construct a reference MSL backed up by the international collection of *Leishmania* strains located in Montpellier (BRC-Leish). The MSL is accessible *via* a web application. The online system allows a rapid identification of a *Leishmania* isolate using Bruker Microflex system whatever the geographical location of the laboratory.

2 Methods

MSL construction. 131 strains were selected from the Biological Resources Center of *Leishmania* at the Academic Hospital of Montpellier (France), based on MLEE identification data and geographic origin. **MSL Validation.** A panel of 230 *Leishmania* strains, representing 22 taxons, was used to test the MSL.

3 Results

4 subgenera, 8 complexes, and 33 *Leishmania* species were included in the MSL library. A potential incorrect identification was observed at the 'species complex' level (e.g. *guyanensis* versus *panamensis*; or *braziliensis* versus *peruviana*) when identification scores were lower than 50, and particularly when <35. When the threshold was set at 35, 97,4% of the 230 strains tested were correctly identified at the complex level, and 84,8% at the taxon level. The identification at the level of the taxa is less efficient for 3 complexes: *donovani*, *braziliensis* and *guyanensis*.

4 Conclusions

This is the first online database that allows a direct identification of *Leishmania* by MALDI-TOF. This system relies in the creation and validation of a robust database freely available free on a website. It would be interesting to further develop this tool by the participation of the users community. This would allow the database to be progressively enriched and updated by the data of all users, particularly those from new or rare taxa.

C1850 COMBINATION OF MOLECULAR AND ISOENZYMATIC METHODS IN THE IDENTIFICATION OF LEISHMANIA IN CUTANEOUS AND VISCERAL LEISHMANIASIS IN TUNISIA

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1 Background

Three species of *Leishmania* are in cause of leishmnaniasis in Tunisia: *L. major (zymodeme MON-25), L. killicki (MON-8),* and *L. infantum (MON-1, MON-24 and MON-80). While L. major* is widely endemic in the center and the south of Tunisia, *L. infantum* is endemic in the north. In contrast to *L. killicki* witch is located in a micro-focus in the south. In the last decades, many repetitive sporadic cases are observed out site known foci. Therefore, the identification of *Leishmania* species is necessary when leishmaniasis is caused by different species especially for epidemiological purpose especially to survey existing foci and to detect new ones. Thus, the aim of our work is to identify *Leishmania* in positive cases of Cutaneous (CL), visceral leishmaniasis (VL) and canine leishmaniasis (CanL) in the center of the country using molecular and isoenzymatic methods.

2 Methods

CL and VL cases are sent to laboratory for diagnosis purpose. If positive, PCR ITS is applied first for molecular identification. If *L. infantum* than isoenzymatic method is used to identify zymodeme.

3 Results

By molecular method we found that *L. major* is the most predominant species in the center of Tunisia (88%). *L. infantum* species is also identified suggesting again the extension of this species from the north to the south of the country. Within *L. infantum* species, MON-1 is still the most zymodeme identified by isoenzymatic method. This result can be due to culture contamination and also to the fact that *L infantum* are difficult to isolate *in vitro* as noted in our samples. All samples have been identified by molecular methods. In the opposite, only 30% of positive cases were isolated by *in vitro* culture and identified by isoenzymatic method.

4 Conclusions

Isoenzymatic method is still the gold standard method in the identification of *Leishmania*. However, it needs the isolation of *Leishmania* not always possible. Molecular method is more sensitive at species level. The combination of both methods is useful in the identification of *Leishmania*. In the center of Tunisia *L. major* is the most frequent. However, the other species are found suggesting a permanent updating of the geographic distribution of *Leishmania* in Tunisia

Epidemiology. Asymptomatic infection

C0069 LEISHMANIASIS IN YEMEN: A CLINICOEPIDEMIOLOGICAL STUDY OF LEISHMANIASIS IN CENTRAL YEMEN

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1 Background

Leishmaniasis is a serious public health problem in Yemen. This study was designed to identify clinical and epidemiological features of leishmaniasis in Yemen.

2 Methods

The study was conducted at the Regional Leishmaniasis Control Center in central Yemen. Data sourced from the medical records of 152 patients with confirmed active leishmaniasis, managed during April–August 2013, were analyzed.

3 Results

A total of 94.1% of patients were rural residents. Al Bayda was the most endemic governorate (59.9%). Children represented the group at highest risk (57.2%), followed by adult females (32.9%); together these groups accounted for 90.1% of all patients. Mucocutaneous leishmaniasis was the most prevalent form (49.3%), followed by cutaneous leishmaniasis (47.4%), and visceral leishmaniasis (3.3%). The wet ulcer was the most common type of lesion (49.7%) and the single lesion (69.4%) represented the most common presentation. All patients were ignorant of the nature of the disease, and 55.9% had a history of using “popular” treatments.

4 Conclusions

Cutaneous, mucocutaneous, and visceral leishmaniases have significant endemicity in Yemen, especially in central areas. Al Bayda is the governorate with the highest endemicity, and rural children and women represent the populations at highest risk. Mucocutaneous leishmaniasis seems to be the most prevalent form and a single wet ulcer is the most common presentation. Infected refugees may represent new foci for imported Leishmania species. Ecology, geography, climate change, cultural gender- and age-specific duties, urban night activities, and use of popular treatments are among proven risk factors.

C0070 STIGMATA IN CUTANEOUS LEISHMANIASIS: HISTORICAL AND NEW EVIDENCE-BASED CONCEPTS

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1 Background

This report is a part of many articles and studies published by the Regional Leishmaniasis Control Center (RLCC), Yemen.

2 Methods

Willing that this review might enrich understanding of the global epidemiological profile of CL, the author relied on research evidence, his experience and a short survey concurrently conducted at the RLCC to highlight the historical and current concepts of cutaneous leishmaniasis (CL), as the globally most prevalent and highly stigmatizing form of leishmaniasis disease, and propose new ideologies upon CL stigmata (CLS). He introduced a new classification of CLS, discussed its common implications and determinants and reviewed a successful community-led action to reduce the burden of CL and its related stigmata in Yemen.

3 Results

The author has identified and classified CL related stigmata into three types: (1) CL Social Stigma (CLSS), which might be perceived, enacted or internalized; (2) CL Aesthetic Stigma (CLAS), which might be might be perceived or enacted; (3) CL Psychological Stigma (CLPS). Almost, every patient with CL may has at least one of the three CL-related stigmata. CLAS is the differential feature that cause stigmatization in CL to look more challengeable, spectral and different from that of all other comparable neglected tropical diseases, such as leprosy. Social and aesthetic stigmata are synonyms of CL in some countries, such as Yemen, and young women are the most affected sex group. The current survey confirmed some concepts, implications and determinants of CLS discussed in this review. It concluded that the majority of Yemeni patients with active CL lesions or those carrying CL-related scars and deformities experience at least one type of CL-related stigmata considering them worse than the disease itself. Psychological type (CLPS) is the most prevalent form of CLS, followed by social (CLSS) and aesthetic (CLAS) types.

4 Conclusions

Apart from its physical impact, it is evident that CL poses severe and spectral stigmata with considerable implications upon the physical, moral, social, economic and public health contexts that may kill stigmatized individuals, both socially and literally. Although, CL is stigmatizing in all affected societies that affects all sex and

age groups, the type and severity of CL stigmata vary, however, and rely on several personal, sociopolitical and geopolitical determinant factors that may be common or different across societies. This report has introduced new ideologies upon stigmatizing process in CL and highlighted further types of CL-related stigmata, which might increase the understanding of that hidden dimension and enrich the global epidemiological profile of CL.

C0091 A STUDY ON VISCERAL LEISHMANIASIS IN ASYMPTOMATIC STRAY DOGS IN MASHHAD AREA, KHORASAN RAZVAI PROVINCE OF IRAN

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1 Background

Visceral leishmaniosis is divided into zoonotic, in which dogs are reservoirs of the disease for people and anthroponotic, in which man is the reservoir of infection for other humans (Baneth, et al., 2005). *L.infantum* and *L. chagasi* are agents of zoonotoc visceral leishmaniosis, while anthroponitic infection is caused by *L.donovani* in India and East Africa. In the Middle East, zoonotoc visceral leishmaniosis is caused by *L.infantum* that domestic dogs are the major reservoir host (Baneth, et al., 2005). The prevalence rates of canine leishmaniosis is are varied in endemic areas and depended on the climatic condition. The prevalence rate of disease was reported between 10 and 70% in the mediterranean area. (Kirmse et al. 1987; Manciati et al. 1986). So far, visceral leishmaniosis have been reported in the dogs and wild carnivores as the main reservoirs from different parts of Iran (Nadim et al., 1978; Hamidi et al., 1982; Mohebali et al. 2001; Bokai et al. 1998). Canine leishmaniasis is a chronic disease and characterized clinically by lymphadenopathy, cutaneous signs, weight loss and anemia. In addition, many infected dogs are asymptomatic and serve as reservoir host of diseases in endemic areas (Dantatores et al., 2007; Moshfee et al., 2009; Hosseininejad et al., 2012).

2 Methods

IFAT

In the present study, the indirect immunofluresecent antibody test (IFAT) was used as sero-diagnostic tool. The *L. infantum* antigen (Megacor Co, Austria MegaScreen FLUOLEISH) has been used for this study.

PCR assay

An assay based on the seminested PCR was used for amplification of variable area of the minicircle kDNA (with a slight modification) as described elsewhere (Aransay, et al 2000).

3 Results

A total of 94 sera tested, 11.7 % (11/94) were shown *leishmania* specific antibodies with titers 1:50.The PCR positivity rate was 63.6% (7/14) at least one of the tissue samples of stray dogs. The *Leishmania infantum* kDNA was detected in 28.5% (3/14) liver, 37.5 %(5/14) spleen and 50% (7/14) skin samples in asymptomatic stray dogs

4 Conclusions

The results of the present study showed that many of asymptomatic stray dogs were infected by *L. infantum* and the skin sample was better than other tissue samples for detection of *Leishmania kDNA* by PCR, so we conclude that the PCR and serological data alone are not enough to make correct diagnoses of leishmaniasis and need to use a combination of diagnostic methods

C0100 VISCERAL LEISHMANIASIS IN NORTHERN ETHIOPIAN VILLAGES: A FINE SCALE ECO-EPIDEMIOLOGICAL ANALYSIS

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1 Background

In Sudan and Northern Ethiopia, visceral leishmaniasis (VL) caused by *Leishmania donovani*, is transmitted by *Phlebotomus orientalis.* The epidemiology of VLis the result of a complex matrix of interactions between the vectors, the pathogens and the hosts with the environment. This study aimed to characterize the factors contributing to the transmission of VL to humans employing a set of tools for spatial and temporal analyses: Landscape ecology and landscape epidemiology.

2 Methods

During 2010 - 2013 a transverse cohort study was conductedin 18 villages in the Tahtay Adiabo district of northern Ethiopia based on a census comprising over 11,000 individuals. Around 4,900 individuals were screened and tested for infection with *L. donovani*. Based on the incidence of VL reported during the census, the *L. donovani* infection rates determined by PCR during the cohort samplings and the environmental and geographical variables, three villages were selected for an in-depth eco-epidemiological study including two years of extensive collection of entomological data. In 2014 a nested case-cohort study of ~400 individuals was conducted in the three selected villages, confounder information available in the previous cohort data-base was used to select controls that closely match cases in term of sex, age and geographical location.

3 Results

The distribution of the vector, *Phlebotomus orientalis* showed a correlation with specific ecotopes. Specifically, there appeared to be a correlation between the vegetation cover and the distribution of sand flies [and VL]. By analyzing satellite images taken during the dry season, it was possible to identify that the majority of villages affected by VL during recent years are inside an area characterized by vertisols (radius 28 Km). Entomological studies identified that *P. orientalis* is closely associated with vertisol ecosystems rather than specific micro-environments, being most abundant in cracked black cotton soils. Spatial analysis of the incidence of VL, show a clustered distribution within populated areas and micro-clusters of more than one case per house or nearby house. Houses of patients were most frequently within a few meters of vertisol fields.

4 Conclusions

These observations may support the hypothesis that the infection with *L. donovani* occurs in domestic settings and farming environments set in vertisols rather than in other soil-types. Extensive data sets are currently being analyzed and these will validate or otherwise affect the results presented here.

C0101 THE ROLE OF HUMAN CARRIERS OF LEISHMANIA DONOVANI IN THE EPIDEMIOLOGY OF VL IN NORTHERN ETHIOPIA

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1 Background

Leishmania donovani in east Africa, is generally considered to be transmitted anthroponotically, although evidence exists for zoonotic reservoirs. Annual VL incidence averages 4,000 cases in Ethiopia, where 3.2 million people are at risk. Previous studies in East Africa have suggested that those at greatest risk for VL include dog and cattle owners, males, and children under 15 years, particularly those exhibiting malnutrition due to poor dietary intake or infection with intestinal parasites. Additionally, the movement of previously unexposed individuals into endemic areas has exacerbated VL prevalence among migrant agricultural populations in northern Ethiopia. As part of a study aimed at elucidating the role of symptomatic and asymptomatic *L. donovani* infected persons in the epidemiology of Kala Azar, we conducted a thorough study of persons living in the endemic district of Tahtay Adiabo in Northern Ethiopia. An important component of the project was the identification of putative parasite reservoirs in VL and PKDL patients as well as asymptomatic (sub-clinical) carriers.

2 Methods

Some 4,900 individuals living in 18 villages were screened for infection or exposure to *L. donovani*, of these, 600 individuals were selected for a nested matched case control study in three of the villages and followed for 2 years. Physical and laboratory tests were performed; Leishmanin Skin Test (LST), Direct Agglutination Test (DAT), ELISA, Western blot and kDNA / RT-PCR.

3 Results

Initially, of the 4,757 dried blood samples tested by RT-PCR, 680 samples (14.3%) were found positive for *Leishmania* k-DNA. A relatively high number of asymptomatic individuates was recorded by ELISA, DAT and by Skin/Blood Microbiopsies and correlated with specific anti-leishmanial antibodies evidenced by Western blotting as typical 14- and/or 18-kDa bands.

4 Conclusions

Asymptomatic infections shown to be common in Ethiopia may serve as parasite reservoirs for biting sand flies, contribution to anthroponotic transmission of visceral leishmaniasis. However, blood parasitemia may be misleading in that *Leishmania spp* are not “true” blood parasites and may potentially be more abundant in the skin and internal organs. A better understanding of parasite transmission, dissemination, and survival in the human host is needed.

C0104 RISK FACTORS OF CUTANEOUS LEISHMANIASIS (CL) CAUSED BY LEISHMANIA TROPICA: A CASE-CONTROL STUDY IN TUBAS DISTRICT IN PALESTINE

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1 Background

Leishmaniasis is still considered as a neglected disease by the World Health Organization. This study applies an eco-health approach that integrates public health measures, epidemiology, and disease ecology to study CL caused by *Leishmania tropica* in the West Bank.

2 Methods

The study was conducted in Tubas district (northeast of the West Bank). The district was divided to three different sites according to their urbanization and socio-economic status. A matched Case-Control study was conducted in the endemic sites. Questionnaires were used to determine the epidemiological risk factors.

3 Results

There was a significant difference in age distribution (**p**= 0.022) of cases between the three sites. The city and villages cases were males in majority, while Bedouin cases were mostly females. The peak temporal distribution of cases was in December-January months. In the urban setting controls tend to use insect repellents (**p**= 0.044), and spraying inside their houses (**p**= 0.022) more than cases. Houses of cases were significantly closer to the village's edge than controls (**p**= 0.0001). Other risk factors include: living closer to farms (**p**= 0.0001), raising domestic animals (**p**= 0.017), seeing hyraxes in vicinity of houses (**p**= 0.0001), and the distance of hyrax colony from houses (**p**= 0.0001). In the rural setting risk factors include living closer to the village edge (**p**= 0.05). In the Bedouin area risk factor includes living closer to hyrax colonies (**p**= 0.037).

4 Conclusions

Conclusions: In more urbanized sites, the effect of living next to the site's edges, facing the wadies and open green areas, increase the exposure to vector (sand-fly), and proximity to the reservoir (hyrax) colonies. In addition high density of reservoir, proximity to human houses, as well as high density of vector, with the absence of personal protection against the vector, increased the risk of disease transmission. These risk factors must be included in future interventions to reduce CL incidence.

C0106 EPIDEMIOLOGIC CONSIDERATION ABOUT VISCERAL LEISHMANIASIS IN ALBANIA.

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1 Background

The aim of this study is to analyze some epidemiologic data of visceral leishmaniasis in Albanian children.

2 Methods

We studied the new cases each year and the distribution of the disease according to age, gender and living area.

3 Results

Year	New	Gender		Living Area		Age (in years)			
	Cases	Female	Male	Rural	Urban	0-1	1-4	4-10	10-14
1995	76	32(42%)	44(58%)	25(33%)	51(67%)	11(14%)	52(68%)	10(13%)	3(5%)
1996	108	43(40%)	65(60%)	30(28%)	78(72%)	16(15%)	60(56%)	20(19%)	12(10
1997	75	43(57%)	32(43%)	12(16%)	63(84%)	18(24%)	37(49%)	14(19%)	6(8%)
1998	83	30(36%)	53(64%)	32(39%)	51(61%)	13(16%)	52(61%)	10(12%)	8(10%
1999	146	54(37%)	92(63%)	46(32%)	100(68%)	20(14%)	82(56%)	34(23%)	10(7%
2000	88	42(48%)	46(52%)	25(28%)	63(72%)	16(18%)	58(66%)	10(11%)	4(5%)
2001	110	53(48%)	57(53%)	23(21%)	87(79%)	14(13%)	69(63%)	17(16%)	10(8%
2002	134	51(38%)	83(62%)	32(24%)	102(76%)	20(15%)	88(66%)	24(18%)	2(1%)
2003	80	37(46%)	43(54%)	10(13%)	70(87%)	12(15%)	50(63%)	10(12%)	8(10%
2004	73	30(41%)	43(59%)	28(38%)	45(62%)	14(19%)	46(63%)	12(16%)	1(2%)
2005	101	41(40%)	60(69%)	23(23%)	78(77%)	18(18%)	63(62%)	17(17%)	3(3%)
2006	63	27(43%)	36(57%)	15(24%)	48(76%)	11(17%)	35(56%)	15(24%)	2(3%)
2007	73	30(41%)	43(59%)	13(18%)	60(82%)	12(16%)	51(70%)	8(11%)	2(3%)
2008	68	31(46%)	37(54%)	19(28%)	49(72%)	14(21%)	32(47%)	16(23%)	6(9%)
2009	51	20(39%)	31(61%)	14(27%)	37(73%)	7(14%)	34(66%)	9(18%)	1(2%)
2010	53	27(51%)	26(49%)	18(34%)	35(66%)	4(8%)	30(56%)	13(25%)	6(11%
2011	32	16(50%)	16(50%)	12(38%)	20(62%)	1(3%)	23(72%)	5(16%)	3(9%)
2012	31	14(45%)	17(55%)	16(52%)	15(48%)	3(10%)	17(55%)	10(32%)	1(3%)
2013	38	18(47%)	20(53%)	13(34%)	25(66%)	4(10%)	22(58%)	10(27%)	2(5%)
2014	29	10(35%)	19(65%)	5(17%)	24(83%)	3(10%)	18(62%)	6(21%)	2(7%)
2015	19	7(36%)	12(64%)	3(16%)	16(84%)	3(16%)	10(52%)	4(21%)	2(11%
Total	**1531**	**656(43%)**	**875(57%)**	**414(27%)**	**1117(73%)**	**234(15%)**	**929(61%)**	**274(18%)**	**94(6%**

4 Conclusions

Most of the cases are from 1 to 4 years old. Children form urban areas and males are more infected.

C0111 DESCRIPTIVE ANALYSIS OF LETHALITY AND DEATHS PATIENTS WITH CUTANEOUS LEISHMANIASIS IN BRAZIL, 2007-2014

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1 Background

The cutaneous leishmaniasis is a low mortality disease, however, can cause severe deformities that impact the self-esteem of patients with psychological involvement due to social stigma. Although rarely fatal, there are reports of deaths in patients with the disease. The disease lethality rate in Brazil ranges from 0.5% to 1.1%. The objective of the research is to describe the deaths of patients with cutaneous leishmaniasis and assess the lethality of these cases in Brazil, from 2007 to 2014.

2 Methods

A descriptive ecological study of cases of cutaneous leishmaniasis registered in the Information System of Notifiable Diseases that evolved to death. The frequencies of deaths and the total lethality rates, the lethality rates by tegumentary leishmaniasis and the lethality rates due to other causes were compared, according to some variables contained in the information system.

3 Results

Were registered 996 deaths of patients with the disease and total lethality of 0.55% in the period. The women, the elderly and the mucosal form had the highest lethality rates. Half of the country's deaths occurred in Minas Gerais, Bahia, Mato Grosso e Maranhão.

4 Conclusions

Although cutaneous leishmaniasis has a low lethality in Brazil, a relevant number of patients with a disease have evolved to death. The differences between the mortality rates between the sexes, age and the clinical form of the disease suggest a possible association of the female sex, age greater than 50 years and the mucosal form with death

C0179 CUTANEOUS LEISHMANIASIS AMONG REFUGEES IN TURKEY: CLINICAL FINDINGS, DIAGNOSIS AND GENOTYPES

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1 Background

In Turkey, CL is mainly reported from South-east Anatolia, Mediterranean, Middle Anatolia and Aegean regions. At the same time, due to mass population movements driven by internal conflicts and wars among our country's neighbouring countries and Turkey acting as a safe haven for these displaced people for long periods of time because of its suitable location, the importance of CL is increasing every day in Turkey. This study is aimed at determining the clinical aspects, diagnosis and genotyping of CL patients coming to Turkey from abroad.

2 Methods

In our study, the clinical materials obtained from the patients or was sent for diagnostic purposes from other centers to Celal Bayar University Medical School Parasitology laboratory between years 2012-2015 were assessed retrospectively. In total there were 34 patients with the mean age of 23,10±14,75 from different countries (Syria, Iraq, Afghanistan, Iran, Turkmenistan).

3 Results

By light microscopy 25 (%81) of giemsa stained slides prepared from the skin lesions of the patients were positive for *Leishmania* amastigotes. For culture methods, 24 (%77) and 29 (%93) samples were positive with NNN and enriched medium respectively. By molecular methods utilizing ITS-1 gene region, 27 (%87) of the cases were positive. 21 of the cases were found positive by all three methods while 3 were negative. 31 of the refugees were diagnosed as CL. Infecting strains determined by genotyping (Real-Time ITS-1 PCR, HeatShock Protein 70 PCR and Real-Time CysteineProtease B Gene PCR) were as follows; 17 *L. tropica*, 8 *L. major,* 3 *L. infantum*, 2 *L. donovani* and 1 *L. aethopica.*
The clinical types of the CL cases were 4 CL recidivans, 1 lupoid CL, 8 wet-type CL and 18 dry-type CL. 9 cases had a history of treatment with meglumine antimoniate (MA) and 6 of the cases were not cured after the second treatment regiment with MA.

4 Conclusions

This study is thus aimed at guiding the clinicians working in the field and giving base ground for future studies on the clinical presentations, diagnosis, genotyping of CL for the import CL cases which entered our country and the refugees living in Turkey.
Turkish Society of Microbiology Study Group for Infections Related to Migration and Travel contributed to this study.
We would like to thank Dr. Albert Bart and Prof. Dr. Tom van Gool from Amsterdam University Medical Center and Celal Bayar University Parasite Bank and Air Liquide company.

C0187 PREVALANCE OF LEISHMANIASIS AMONG PATIENTS IN A RENAL UNIT IN AN AREA WHERE CUTANEOUS LEISHMANIASIS AND CHRONIC KIDNEY DISEASE OVERLAP IN SRI LANKA

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1 Background

Visceral leishmaniasis (VL) complicating kidney transplantation (KT) is reported globally. Patients with chronic kidney disease (CKD) and KT are frequently placed on immunosuppressive therapy and subjected to repeated blood transfusions to manage renal complications. Cutaneous leishmaniasis (CL) due to *Leishmania donovani* is an established disease in Sri Lanka. We also report increasing numbers of CKD and KT patients from same geographic areas. The aim of this study was to assess the prevalence of anti-*Leishmania* antibodies and amastigotes in blood among diagnosed patients with CKD and patients who had undergone KT, and to assess the correlation of blood transfusion and immunosuppressive therapy in the same group.

2 Methods

Patients (n=170) were enrolled from renal clinics and renal ward, Teaching Hospital, Anuradhapura. These patients were diagnosed of having CKD based on the Kidney Disease | Improving Global Outcomes (KIDGO) 2012 guidelines. Patients with KT had recorded history. History and examination relevant to VL and blood samples were taken with consent.
Blood samples were assessed for presence of anti- *Leishmania* antibodies in serum using rK-39 rapid diagnostic test strip (rK-39 RDT), rK-39 ELISA and Direct Agglutination Test (DAT). Buffy coat films stained with Giemsa's were examined for presence of amastigotes.

3 Results

None of the patients had past or current history suggestive of CL or VL. All 170 patients were negative for *Leishmania* amastigotes in buffy coat smears and rK-39 RDT. Two (1.2%) Patients showed marginal positivity (Mean OD values layed between the cut off positive value and positive control) with rK-39 ELISA and another 02 (1.2%) gave positive titres at 1: 6,400 and 1: 3,200 dilutions with DAT. Patients those who showed positivity with ELISA did not show positive results with DAT and vice versa. This could be due to known poor correlation between rK-39 ELISA and DAT when antibody titres are low. All 4 of these patients had CKD and had a history of receiving blood transfusions. None of the patients on immunosuppresive therapy were positive.

4 Conclusions

The weak positivity of anti-*Leishmania* antibodies in 4 patients (2.4%) would suggest that they were most likely to be asymptomatic VL careers. Possible risk factors could be unscreened blood transfusions or infective sandfly bites. Since CKD patients with a history of CL were not encountered in this study population, the possibility of CL visceralization with immunosuppression could also not be ignored.

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C0191 VISCERAL LEISHMANIASIS AMONG THE INDIGENOUS PEOPLE IN BANGLADESH: A RETROSPECTIVE CROSS SECTIONAL STUDY

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1 Background

Visceral leishmaniasis (VL) is one of the major public health problems in Bangladesh with an estimated annual incidence of 12,400 to 24,990 cases. Our previous study showed that VL case fatality rate in the indigenous community of VL endemic areas in the country was comparatively high. In this study, we aimed to investigate the burden of VL and its risk factors in the indigenous population in Bangladesh.

2 Methods

We conducted a retrospective cross sectional study in a randomly selected Union (Rishikul) of Godagari sub-district of Rajshahi district, Bangladesh from August, 2009 to December, 2011. The trained Field Research Assistant (FRA) screened all indigenous households in the study area and enrolled into the study. The FRAs also recorded past VL patients during the household screening. Household head with and without VL in the past two years were interviewed to determine their socioeconomic status, knowledge about VL, and treatment seeking behavior. The exact logistic regression model was used to find the independent risk factor for VL.

3 Results

Average age of the study population was 26.0 years (SD, 18.3) and 50.0% were females (N=4342). VL prevalence was about 11 per 10,000 indigenous people in Rajshahi district. The VL prevalence among children (16/1,000) was significantly higher than the adults (7/1,000) (P=0.009). Household with VL had better knowledge about VL than the household without VL. However, household with VL had poor practice of bed-net use and health seeking behavior compared to household without VL. Irregular use of bed-net (OR=3.85, 95% CI, 1.10-13.80) and higher ratio of family members per bed-net (OR=1.85, 95% CI, 1.10-3.25) were found as significant independent risk factors for VL in this community.

4 Conclusions

Prevalence of VL among indigenous population in Bangladesh is high. Our study findings will help National Kala-azar Elimination Programme to develop a specific strategy for protecting vulnerable population such as indigenous people from VL in Bangladesh.

C0201 ASYMPTOMATIC VISCERAL LEISHMANIASIS IN A NONENDEMIC POPULATION AFTER DEPLOYMENT TO IRAQ ?,-?

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1 Background

Visceral leishmaniasis (VL) is a chronic parasitic infection transmitted by sand fly bites and endemic in Iraq. The case rate in the Iraqi population is reported as 4.8 VL cases/100,000 persons. Active VL was confirmed in 22 cases in U.S. personnel who deployed between 2001-13. Studies have found asymptomatic VL in endemic countries; the number of asymptomatic VL infections in the U.S. military after deployment is unknown.

2 Methods

200 asymptomatic service members who deployed to Iraq and 50 controls (no travel lifelong to VL areas) will be recruited. Subjects completed a risk factor survey and gave blood samples. We assessed the humoral immune response using rK39 serology, *Leishmania (L.) infantum* enzyme-linked immunosorbent assay (ELISA) and western blot. Quantitative real time polymerase chain reaction (PCR) testing was used to assess for active parasitemia. An interferon gamma release assay (IGRA) tested for the cellular immune response to *L. infantum*. Additionally, matched peri-deployment serum samples permit evaluation of vector exposure and *Leishmania* serology close to the time of potential infection.

3 Results

We report results of current blood samples obtained from 96 cases and 25 controls. Antibody testing by rK39 immunochromatographic assay was uniformly negative, ELISA 4% positive. 2/56 (3.6%) *Leishmania* PCR positive samples were identified. IGRA testing identified 20/96 (21%) had elevated interferon gamma (IFNγ) after stimulation with soluble *Leishmania* antigen. Risk factors for infection (defined as any positive test result) included deployment during 2003-5, male gender, and exposure in Ninewa, Salahaddin, Diyala/Babylon governorates.

4 Conclusions

To date, our testing shows that 24/96 (25%) participants have evidence of asymptomatic visceral leishmaniasis after deployment to Iraq. Most have a cellular immune response to *Leishmania* which would be the anticipated finding more than a decade later.

C0216 EPIDEMIOLOGICAL CHARACTERISTICS OF VISCERAL LEISHMANIASIS IN MOROCCO (1990-2014): AN UPDATE.

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1 Background

Describe the spatio-temporal distribution of VL in Morocco between 1990 and 2014 period in order to highlight important features and trends of VL.

2 Methods

This is a retrospective study collecting data of incidence VL annual reports of parasitic diseases from the Epidemiological direction of fight against diseases of the Ministry of Health from 1990 to 2014. The data are the result of passive monitoring, based on the notifications registered by all medical staff of University Hospitals (UH) of Morocco that declared each VL cases to the epidemiology department of the Ministry of Health. Database contains all patients data including gender, age, residence and time of hospitalisation. A standard form was used in this study to extract epidemiological data between January 1990 to February 2015.

3 Results

2421 cases were reported in Morocco between 1990-2014 with an average annual reported incidence rate of 0.4 cases per 100.000 inhabitants. Before 1996 the average annual incidence of VL was 50 cases on average. After this date the number of cases increased and then remained stable with around 100-150 cases per year. Children whose age varies between 1 and 4 years old are the most affected with 1,327 (74%) of total cases; nevertheless the adult starts to be affected by the disease. In 2000, 65% of positive cases of VL are concentrated at both northern regions: Taza-Al Hoceima-Taounate with 45% of cases, Tanger-Tetouan mainly represented by Chefchaoun with 20% of cases. The Fez-Boulemane region located in the center recorded 12% of cases. Throughout the years the map VL distribution has been progressively changed and spatial spread of the disease to the center is noted in 2007. 2014 has been marked by an even greater extension of the disease to the center and south of Morocco. Nationally in 2014, 34 of 75 provinces and prefectures are affected compared to 2000, when 22 out of 82 provinces and prefectures were affected. Leishmania infantum was identified the causative agent based on speciesspecific PCR-Lei70 assay.

4 Conclusions

Two thousand four hundred and twenty one cases of VL have been reported since 1990 until 2014. Before 1996, 50 cases was the average annual incidence of VL. This number increased and remained stable (100-150 cases per year). An even greater extension of the disease to the center and south of Morocco has been marked in 2014. The result of the molecular identification by Lei70-PCR with RsaI enzyme shows that *L. infantum* is responsible for VL.

C0217 EVALUATION OF LEISHMANIA AND ITS IMPLICATIONS IN PUBLIC HEALTH IN THE REGION OF BAURU, SÃO PAULO STATE, BRAZIL

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1 Background

Leishmaniases are diseases of interest in public health and have shown vast geographical expansion and increasing urbanization, affecting particularly humans and dogs. Domestic dogs are considered the major reservoirs of leishmaniases, and enzootic diseases generally precede the occurrence in humans, constituting an important parameter in the estimate of human cases. Identification of the circulating parasite in a certain transmission focus of the disease is rather fundamental to the establishment of prophylactic and control measures. The aim of the present study was to evaluate *Leishmania* occurrence in order to learn the ones that circulate in a determined disease transmission focus.

2 Methods

Using serological and molecular biology techniques, the present study evaluated 37 human samples and 2324 canine samples from cities located in the region of Bauru, São Paulo State, Brazil. Human samples were analyzed by employing indirect immunofluorescence assay (IFA) and canine samples by adopting immunochromatographic assay (TR-DPP®) and ELISA. For the molecular evaluation, DNA was extracted with the kit Illustra™Tissue & Cells Genomic Prep Mini Spin (GE Healthcare), followed by quantitative PCR (qPCR) amplification with the primers LITSR/L5.8S and Lch14/Lch15 to identify the genus and the species, respectively. In addition, sequencing of positive samples was performed for human cases and for the canine samples that could not have the species identified.

3 Results

Using IFA and PCR,75.7% (28/37) of human cases could be confirmed, and the parasite was identified as *Leishmania infantum* (syn. *chagasi*) by means of sequencing. As regards canine samples, 54.5% (1267/2324) were confirmed by serology and 54.1% (1258/2324) by molecular biology. In all three samples with amplifications corresponding to the genus, the species *Leishmania braziliensis* was identified according to the sequencing. Such data of serological and molecular diagnosis comparison provided important information about the distribution of leishmaniasis cases in the region and allowed the detection of the first case of canine autochthonous transmission in Pederneiras Municipality and the first autochthonous case in humans in Mineiros do Tietê Municipality.

4 Conclusions

The use of serological and molecular techniques for epidemiological surveillance in the municipalities can better guide the currently adopted control strategies and locate infected hosts.

C0242 PSYCHOSOCIAL IMPACT OF CUTANEOUS LEISHMANIASIS AMONG HIGH SCHOOL STUDENTS IN ERRACHIDIA PROVINCE - MOROCCO

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1 Background

Cutaneous leishmaniasis (CL) is usually a slowly healing localised skin disease in Morocco, but in some cases it leads to mutilating scars. The outbreak of CL due to *Leishmania major* in Errachidia province in south-eastern Morocco between 2008 and 2010 left many adolescents with permanent scar tissue in the face or other exposed body parts. We studied the psychosocial impact of CL among these young people.

2 Methods

In 2015 we conducted a cross-sectional survey among high-school students living in boarding schools in two CL endemic areas: Rissani and Tinejdad located in Errachidia Province. A self-administered questionnaire elicited responses about general knowledge of CL and related scars. An open question focused on the possible psychosocial effects associated with these scars. The quantitative data were analysed with Epi Info and the text data with Nvivo software.

3 Results

Almost 20% of 448 respondents reported they had experienced a CL lesion and 87 % said it could possibly or definitely lead to psychological consequences. The text analysis showed that girls more often than boys expanded on the negative psychological effects of CL. The students considered CL as “dangerous”, “serious” and “deathly” leading sometimes to extreme suicidal ideations.

4 Conclusions

The burden of CL in this age group is not negligible. The indelible CL scars lead to self-stigma and social stigma and the emergence of negative psychological effects in this age group. While some students accepted the CL scars and related suffering as their “destiny”, others were eagerly demanding treatment to heal those scars and claiming protective measures against CL.

C0256 VISCERAL LEISHMANIASIS IN SUB-SAHARAN AFRICAN IMMIGRANTS IN A CITY OF SPAIN. WE ARE NOT TALKING ABOUT AN IMPORTED DISEASE.

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1 Background

Visceral Leishmaniasis (VL) is endemic in 65 countries, with an estimated incidence of 500,000 new cases per year. In Spain it´s a hipo-endemic disease. However, an outbreak of Leishmaniasis in 2011 has led to an increase in the number of cases, especially in the south area of the Community of Madrid, where a large percentage of the population are immigrants. The objective is to evaluate the incidence rate of VL in Sub-Saharan African immigrants in the municipality of Fuenlabrada (Madrid, Spain)

2 Methods

A retrospective study of all Sub-Saharan African immigrants with confirmed LV at the Hospital Universitario de Fuenlabrada(HUF), from June, 2004, to March, 2015. The database was completed and designed in a retrospective manner after reviewing the clinical history of cases. The usage of them was approved by the Ethics Commission of the Hospital. The analyzed data were sociodemographic factors, epidemiology and clinical and laboratory results

3 Results

In this period, 132 patients with VL were diagnosed. From these, a 37.9 %(39) were from Sub-Saharan Africa. They were all immunocompetent. In 2012, the annual incidence rate of VL in Sub-Saharan African immigrants was 233.6 cases/100,000 inhabitants, while it was 9.8 cases/100,000 inhabitants in Spanish people (OR 23.76 95% CI 11.2-50.75)($p<0.001$). A 66.7% were males, with an average age of 35.6 years(SD:10.4). A 51.3%(20) were from Equatorial Guinea, a 30.8%(12) from Nigeria, a 7.7%(3) from Cameroon, a 7.7%(3) from D.R. Congo and a 2.6%(1) from Angola. All the cases were VFR-immigrants, with a median length of stay in Spain of 6 years since their last trip to their country of origin(IQR: 1.1-10.3). The median time from symptoms to diagnosis was 15 days(IQR: 12-28). All had fever and some cytopenia. A 66.7%(26) presented pancytopenia. An important ferritin elevation **(**72.2% had levels above 1,000 ng/ml**)** was observed. Diagnosis confirmation was performed by positive serology in a 43.6%(17), by polymerase chain reaction (PCR) in a 28.2% (11), by aspirate marrow in a 25.6 % (10) and by direct observation of the parasites in a 2.6% (1). A 94.8% (37) of the patients received treatment

4 Conclusions

During the outbreak of Leishmaniasis in southern Madrid (Spain), a significantly higher incidence rate of VL was observed in the immigrant Sub-Saharan African population, in comparison to the Spanish population. More studies should be performed to determine if this fact could be explained due to genetic differences or environmental factors.

C0328 INVESTIGATING THE SYLVATIC ORIGIN OF AN OUTBREAK OF CANINE VISCERAL LEISHMANIOSIS IN A MUNICIPALITY OF SÃO PAULO STATE, BRAZIL

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1 Background

The city of Campinas (22°53'20"S 47°04'40"W), São Paulo State, Brazil, has an environmentally protected area (EPA) with Atlantic Forest remnants, numerous water resources and faunal diversity. The EPA has undergone severe anthropogenic environmental changes in recent decades, wherein the wildlife began cohabiting with humans and pets. Within the EPA, an outbreak of human cutaneous leishmaniosis (CL) occurred from 1993 to 1994 and visceral leishmaniosis (VL) has been transmitted among dogs since 2009. We investigated a possible sylvatic cycle of *Leishmania* transmission among the wildlife in the EPA using molecular tools.

2 Methods

We captured free-ranging mammals for four consecutive days per month, from April 2014 to March 2015, using tomahawk traps baited with banana and chicken. The mammals were identified by subcutaneous microchips and blood samples and skin fragments were collected. DNA was extracted using a QIAamp DNA mini kit in a QIAcube (Qiagen®, Netherlands). Polymerase chain reaction was performed with primers for internal transcribed spacer-1 (ITS-1), LITSR and L5.8S, and for the heat shock protein encoding genes, HSP70F and HSP70R. Amplicons were identified by agarose gel electrophoresis and confirmed by Sanger genetic sequencing and basic local alignment with GenBank data.

3 Results

82 wild mammals of six species were sampled: 1/82 (1.2%) *Mazama gouazoubira*, 1/82 (1.2%) *Sciurus* (*Guerlinguetus*) *aestuans*, 8/82 (9.8%) *Callithrix jacchus*, 11/82 (13.4%) *Didelphis aurita*, 18/82 (22.0%) *Callithrix penicillata* and 43/82 (52.4%) *Didelphis albiventris*. Six *D. albiventris* (6/82, 7.3%) were sampled again in a second capture.The molecular identities of ITS-1 amplicons obtained from blood samples of two *Didelphis albiventris* (white-eared opossum; 2/82, 2.4%, 95%CI 0.3–8.5%) were confirmed: one (access KX580706) showed 100% similarity with sequences of *L.* (*L.*) *infantum* (accession no. KR081265.1) and the other (access KX580707) showed 100% similarity with *L.* (*Viannia*) *guyanensis* (accession no. FJ753388.1). This is the first identification of *Leishmania* in wildlife in the region.

4 Conclusions

The occurrence of wild species infected with *Leishmania* spp.in contact with humans and their pets suggests the need for periodic reviews and flexibility in leishmaniosis control and surveillance strategies considering other species may act as reservoirs. The results also highlight the public health risk of human cohabitation with wildlife in sylvatic regions.

C0339 NEW ESTIMATES OF LEISHMANIASIS INCIDENCE IN SELECTED HIGH BURDEN COUNTRIES

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1 Background

In 2007, the World Health Assembly resolution 60.13 on control of leishmaniasis requested the Director-General of the World Health Organization "to monitor progress in the control of leishmaniasis…".
An essential component in assessing the progress in leishmaniasis control is the estimation of the underreported cases.
WHO previously published global estimates of leishmaniasis incidence in 2012. This paper provides new estimates calculated in 2016 for cases reported up to 2014 for selected high burden countries.

2 Methods

The Delphi method was used to approach 53 experts on 18 high burden countries, 14 mainly endemic for visceral leishmaniasis (VL), three for cutaneous (CL) and one for both forms through a structured questionnaire. A second round was arranged for experts representing the same country but with different responses, allowing them to revise their answers after being provided a summary of the previous round. In addition to reported cases, recent literatures and reports were reviewed.

3 Results

A total of 33 responses were received during the first round with at least one response from 17 countries (Algeria, Bangladesh, Brazil, Colombia, Ethiopia, India, Kenya, Morocco, Nepal, Paraguay, Peru, Somalia, South Sudan, Spain, Sudan, The Syrian Arab Republic and Tunisia) and 17 answers during the second round.
Quantitative answers on the underreporting factor were received for 69% (11/16) of the countries, two mainly endemic for CL: Algeria (2.6-4.6) and Tunisia (1.5-4.6) and nine for VL: Bangladesh (1.05-1.8), Ethiopia (1.2-1.8), India (1-1.8), Kenya (2-4), Nepal (1.2-1.8), Somalia (2-4), South Sudan (1.2-1.8), Spain (1-3) and Sudan (1.2-1.8).
For five countries, Brazil, Colombia, Paraguay, Peru and the Syrian Arab Republic, experts proposed that estimates be calculated through surveys to accurately determine underreporting. No feedback was received from Afghanistan.
Upon applying the underreporting factor to the number of cases reported in 2014 from each country, the total estimate for the nine VL countries ranges from 29 969 to 51 008 and from 19 152 to 40 439 for the two CL countries.

4 Conclusions

The new global estimates for leishmaniasis represent a 84%-86% decrease for VL and 83%-87% decrease for CL compared to the last WHO estimates published in 2012, a significant decrease which may be mainly attributed to intensified control measures.
Prevalence surveys would be needed to confirm these estimates.

C0376 EPIDEMIOLOGICAL CHARACTERISTICS OF PEDIATRIC LEISHMANIASIS IN THE OUTBREAK OF SOUTHERN AREA OF MADRID 2009-2016

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1 Background

Since July 2009 an outbreak of leishmaniasis is occurring in the south-west area of the Madrid Autonomous Community. The disease caused by *L. infantum* is usually associated with childhood affectation. The aim of this study is to describe the cases of leishmaniasis in the context of this outbreak in pediatric age and to compare them with adult cases

2 Methods

Descriptive study of the epidemiological variables from cases of leishmaniasis associated to the outbreak, in the period from July 2009 to October 2016 separated according to the age (pediatric age defined as younger than 15)

3 Results

During that period 697 cases of leishmaniasis were reported, 105 of them in pediatric age (15.1%, mean age 4.1 years old). In the outbreak area the mean incidence rate was 19.15 cases per 100,000 inhabitants: 50.83 in children under 2 and 13.03 in those aged between 2 and 14. With respect of the year of symptoms onset: 1 pediatric case was identified during 2009, 11 in 2010, 36 in 2011, 19 in 2012, 15 in 2013, 18 in 2014, 3 in 2015 and 2 during 2016; while adults cases were 5, 86, 161, 141, 76, 75, 40 and 8. The distribution of the variables in the pediatric cases compared to adults was: 52.4% were male vs 60.5% ($p = 0.12$); the country of origin was Spain in 60.0%, sub-Saharan countries 20.0% and 20.0% from others, compared to 80.4%, 10.3% and 9.3% ($p < 0.01$). The clinical presentation in pediatric was 51.4% visceral leishmaniasis (VL), compared to 36.5% in adults ($p < 0.01$) (there are no pediatric cases with lymph node or cutaneous-mucosal presentation). 3.8% of affected children presented intrinsic risk factors but 19.1% in adults ($p < 0.01$) and hospital admission was 51.4% vs 29.1% ($p < 0.01$). Cases in children were classified as confirmed 85.7% and 95.4% in adults ($p < 0.01$). Confirmatory diagnosis was made by biopsy 83.8% vs 92.6% ($p < 0.01$), serology 42.9% vs 21.6% ($p < 0.01$) and culture 3.8% vs 6.3% ($p=0.33$). The mean delay in reporting from the onset of symptoms was 147 days in children in front of 165 in the elderly ($p = 0.23$).

4 Conclusions

The outbreak has affected people of all ages and the highest incidence has occurred in children under two. In pediatric age, compared to adults, a higher proportion of female, foreigners, hospital admissions and VL were observed. We found a lower percentage of cases with intrinsic risk factors, a higher rate of diagnosis only by positive serology and less reporting delay

C0384 MOLECULAR EPIDEMIOLOGICAL SURVEY OF CUTANEOUS EISHMANIASIS IN TWO HIGHLY ENDEMIC METROPOLISES OF IRAN, APPLICATION OF FTA CARDS FOR DNA EXTRACTION FROM GIEMSA STAINED SLIDES

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1 Background

PCR has been used for confirmation of leishmaniasis in epidemiological studies, but complexity of DNA extraction and PCR
approach has confined its routine use in developing countries.

2 Methods

Objectives: In this study, recent epidemiological situation of cutaneous leishmaniasis (CL) in two hyper-endemic metropolises of Shiraz and Isfahan in Iran was studied using DNA extraction by commercial FTA cards and kinetoplastid DNA (kDNA)-PCR amplification for detection/identification of *Leishmania* directly from stained skin scraping imprints.
Patients and Methods: Fifty four and 30 samples were collected from clinically diagnosed CL patients referred to clinical laboratories of leishmaniasis control centers in Isfahan and Shiraz cities, respectively. The samples were examined by direct microscopy and then scrapings of the stained smears were applied to FTA cards and used directly as DNA source in a nested-PCR to amplify kDNA to detect and identify *Leishmania* species.

3 Results

Fifty four of 84 (64.2%) slides obtained from patients had positive results microscopically, while 79/84 (94%) of slides had positive results by FTA card-nested-PCR. PCR and microscopy showed a sensitivity of 96.4% and 64.2% and specificity of 100% and 100%, respectively.Interestingly, *Leishmaniamajor* as causative agent of zoonotic CL was identified in 100% and 90.7% of CL cases from Isfahan and Shiraz cities,respectively, but *L. tropica* was detected from only 9.3% of cases from Shiraz city. All cases from central regions of Shiraz were *L. tropica* and no CL case was found in Isfahan central areas.

4 Conclusions

Filter paper-based DNA extraction can facilitate routine use of PCR for diagnosis of CL in research and diagnostic laboratories in Iran and countries with similar conditions. Epidemiologic changes including dominancy of *L. major* in suburbs of Shiraz and Isfahan metropolises where anthroponotic CL caused by *L. tropica* had been established, showed necessity of precise studies on CL epidemiology in old urban and newly added districts in the suburbs.

C0390 SEROPREVALENCE OF LEISHMANIA SPP. AND TOXOPLASMA GONDII IN DONKEYS FROM PORTUGAL

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1 Background

Leishmaniosis caused by *Leishmania infantum* (syn. *Leishmania chagasi*) is endemic in the Mediterranean basin (including Portugal), the Americas and Asia. In Europe, cases of presumed autochthonous cutaneous leishmaniosis have been sporadically reported in horses from European countries, but not in donkeys. Toxoplasmosis is an important zoonotic disease with worldwide distribution. Cats and wild felids are the only recognized definitive hosts of *Toxoplasma gondii*, while homeothermic animals, including donkeys, act as intermediate hosts.Studies on *T. gondii* infection in donkeys are scarce worldwide. As herbivores, donkeys can serve as sentinels of environmental contamination with *T. gondii* oocysts and the consumption of improperly cooked infected donkey meat can be a health risk to humans. Additionally, the parasite has been found in the milk of naturally infected donkeys, which may pose a risk if consumed raw or unpasteurized. The objectives of this study were to assess seroprevalence to *Leishmania* spp. and *T. gondii* in donkeys from Portugal.

2 Methods

Between February 2015 and February 2016, serum samples were obtained from 186 donkeys from mainland Portugal. The direct agglutination test (DAT, AMC, The Netherlands) was used for titration of IgG antibodies to *Leishmania* spp. Samples were tested for IgG to *T. gondii* with a modified agglutination commercial test (Toxo-Screen DA®, bioMérieux, France).

3 Results

One clinically healthy donkey (0.5%; 95% confidence interval [CI]: 0.0-3.0%) was found seropositive for *Leishmania* spp. with a titer of 800. This animal was a female, with 32 years born in southern Portugal. The seropositive donkey was housed exclusively outdoors and used for work and coaching. The animal had frequent contact with other animals including dogs, cats and other donkeys. Eleven animals were seropositive for *T. gondii*, with an overall seroprevalence of 5.9% (CI: 3.0-10.3%). The seropositive donkeys had titers of 20 (n = 7), 80 (n = 2), 640 (n = 1) and ≥ 2560 (n = 1). The age of seropositive animals ranged from 3 to 17 years. None of the seropositive donkeys were simultaneously seropositive to *Leishmania* spp. and *T. gondi*i, which rules out cross-reactions.Gender, type of housing, ability and clinical status (apparently healthy or sick) were not associated with seropositivity to *T. gondii*.

4 Conclusions

Results of the present study indicate that donkeys in Portugal are exposed to and may be infected with *T. gondii* and *Leishmania* spp.

C0392 THE MOSQUITOES THAT DESTROY YOUR FACE. SOCIAL IMPACT OF CUTANEOUS LEISHMANIASIS IN SOUTHEASTERN MOROCCO, A QUALITATIVE STUDY

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1 Background

Cutaneous Leishmaniasis (CL) is a neglected tropical disease leading to protracted skin lesions and indelible scar tissue. CL is quite common in Morocco and is mainly caused by the anthroponotic *L.tropica* or the zoonotic *L.major*. Most CL lesions develop on the uncovered parts of the body as those are most exposed to thesand fly bites. Often those lesions heal with indelible scars and the disfiguring outcomes can lead to stigma and various psychological problems for patients and their relatives. However, little is known about how the population that is most exposed perceives the problem. Their representations, their level of awareness and attitudes are not documented, which is a major gap for the development of an adequate control strategy. We assessed the social representations of CL in rural communities in Southeastern Morocco.

2 Methods

Between March and April 2015, we conducted 28 focus groups discussions (FGDs) in communities at risk for CL in Errachidia and Tinghir provinces, known endemic for *Leishmania major* and *L. tropica* respectively. We used a non-probability purposive sampling strategy aiming for maximum diversity in age, gender and socio-economic background. To capture the sociocultural diversity, we structured our FGDs around the traditional versus modern contrast by grouping those who usually consult the formal health services and those who prefer traditional healers.Data were analysed using directed content analysis.

3 Results

CL was well known as a vector-born disease. Women were thought to be more exposed to insect bites due to their farming activities. Inadequate waste management and manure kept close to houses increase mosquito nuisance in the villages. CL was not considered dangerous but its psychosocial impact was acknowledged, especially for young single women as facial scars seriously diminish their marriage prospects. Several other elements pointed to social stigma generated by active lesions and scars. A range of traditional herbal and other therapies were cited to treat the CL lesions, sometimes followed by consulting the formal health sector. However, no one knew an effective treatment for CL scars, though these were causing most of the socio-psychological distress.

4 Conclusions

The population most exposed to CL lives in remote rural areas and generally lacks access to health care. CL is a factor of stigmatization especially in young single women. A new treatment for CL with better skin outcomes is urgently needed.

C0396 ECO-EPIDEMIOLOGY OF ATYPICAL CUTANEOUS LEISHMANIASIS IN HONDURAS.

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1 Background

Leishmaniasis is an infectious disease caused by a group of protozoan parasites of the *Leishmania* genus. In Honduras, four clinical forms of the illness have been identified: Cutaneous Leishmaniasis (CL), Mucocutaneous Leishmaniasis (MCL), Visceral Leishmaniasis (VL) and Atypical Cutaneous Leishmaniasis (ACL). This disease represents an important cause of morbidity. The purpose of this study was to characterize the Ecoepidemiology of ACL in Honduras.

2 Methods

Four ACL endemic settings were selected (Amapala, San Francisco de Coray, Reitoca, Alubaren). In order to capture the *Lutzomyia*, CDC miniature light-traps were used according to the Young & Duncan criteria for the taxonomic identification. The patients were enrolled to participate with the collaboration of the Honduran Ministry of Health. The molecular characterization of *Leishmania* species was carried out by restriction fragment length polymorphism (RFLP) analysis. We also determined blood feeding sources of female *Lutzomyia* through internal transcribed spacer (ITS) amplification.

3 Results

156 samples from clinical suspected ACL patients were examined. Of this samples, 130 (83.33%) were positive for *Leishmania spp,* and *Leishmania chagasi/infantum* was the only characterized specie. Using the Young & Duncan criteria, 5,951 *Lutzomyia* were identified, 3,543 (60%) were males and 2,404 (40%) females. Among the captured insects, ten *Lutzomyia* species were identified and the most predominant were *Lu. longipalpis, Lu. evansi* and *Lu. cruciata*. The four endemic settings presented low *Lutzomyia* species diversity (*DMg*=1.04). 450 engorged specimens, of the following species were analyzed: *Lu. longipalpis*, *Lu. cruciata, Lu. evansi, Lu. chiapanensis* and *Lu. sanguinaria.* Most females *Lutzomyia* fed on three sources: *Gallus gallus*, *Sus scrofa* and *Canis familiaris.* Finally, the infection rate found was 0.27%, *Lu. longipalpis* infected with *Leishmania chagasi/infantum.*

4 Conclusions

With the present study, the eco-epidemiology of ACL in endemic settings of Honduras was characterized, and it was confirmed that L*. chagasi/infantum* is the main etiologic agent, *Lu. longipalpis* is the primary vector, and *Canis familiaris* the sand flies major feeding source.

C0400 A NEW FOCUS OF ZOONOTIC CUTANEOUS LEISHMANIASIS IN ISFAHAN PROVINCE, CENTRAL IRAN

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1 Background

To determine the epidemiological features of cutaneous leishmaniasis including human infection, reservoirs and vectors in the city of Aran o Bidgol.

2 Methods

This cross-sectional study was carried out on Leishmania spp. isolated from rodents, sandflies and patients with cutaneous leishmaniasis in Aran o Bidgol. Parasites were identified by random amplified polymorphic DNA-PCR technique and data were reported by using descriptive statistics and frequency percent.

3 Results

Random amplified polymorphic DNA-PCR showed that 71.4% of human isolates were Leishmania major (L. major) and the rest were Leishmania tropica. In addition, 17.8% of Rhombomys opimus and 1.9% of female Phlebotomus papatasi were infected with L. major.

4 Conclusions

The results indicate that L. major parasite is the causative agent of the disease among patients. And Rhombomys opimus and Phlebotomus papatasi are the main reservoir host and vector in the dissemination of L. major in the city. Therefore Aran o Bidgol is introduced as a new focus of zoonotic cutaneous leishmaniasis in Central Iran in order to prevent zoonotic cutaneous leishmaniasis, and control of the rodents and sandflies are suggested.

C0411 MOLECULAR DETECTION OF LEISHMANIA SPP. IN DOGS AND CATS FROM QATAR

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1 Background

Leishmaniosis is increasingly being reported in dogs and cats worldwide and represents a serious threat to both animal and public health. Despite their relevance, no data is currently available regarding *Leishmania* spp. on the northeastern coast of the Arabian Peninsula. Indeed, studies on zoonotic parasitic diseases are extremely limited in the Persian Gulf. Under this circumstance, a research was conducted to assess the occurrence of *Leishmania* spp. in dogs and cats from Qatar, by real time polymerase chain reaction (rtPCR).

2 Methods

From March to July 2016, a survey was conducted at a veterinary medical clinic in Doha, the capital and largest city of Qatar. Ninety-eight owned apparently healthy pets living in Doha and neighboring areas were surveyed: 64 dogs (39 males and 25 females, age range: 4-156 months) and 34 cats (23 males and 11 females, age range: 6-156 months). Blood was collected from the cephalic or jugular vein of each animal. Two drops of whole blood (~80 µl) were spotted onto filter paper, air-dried for 3-4 hours and stored at -20 °C. Genomic DNA was extracted using the QIAamp DNA Micro Kit (Qiagen GmbH, Hilden, Germany), following the manufacturer's instructions and tested by rtPCR targeting a fragment of the kinetoplast DNA (kDNA) of *Leishmania* spp.

3 Results

Overall, six dogs (9.4%; CI: 3.5–19.3%) and two cats (5.9%; CI: 0.7–19.7%) were positive for *Leishmania* spp. and sequencing is currently ongoing. Positive dogs were all male, with age ranging from 6 months to 2 years. Positive cats were male, with 6 months and 2 years old and both had outdoors access.

4 Conclusions

The finding that all the positive animals were born in Qatar and had not travelled outside the country suggests that infection was locally acquired and might be endemic in Doha. These results reveal for the first time the detection and molecular confirmation of *Leishmania* spp. in domestic dogs and cats from Qatar. None of the animals showed any clinical sign, representing a diagnostic challenge to practitioners and a threat to public health, as subclinical infected animals can be reservoirs of *Leishmania* spp. Considering the impact of *Leishmania* spp. on the health of humans and animals, it is crucial to increase scientific knowledge and to plan routine screening and regular prophylaxis against sand fly vectors to reduce the risk of this infection in Qatar.

C0417 MOLECULAR EPIDEMIOLOGY OF A NEW FOCUS OF CUTANEOUS LEISHMANIASIS IN THE JORDANIAN SIDE OF THE JORDAN VALLEY

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1 Background

Cutaneous leishmaniasis (CL) is endemic in many foci of Jordan and the Jordanian Mid Jordan Valley (JMJV) is the most affected with the highest incidence, of more than 313/100 000 cases per year, was reported in 2004/2005. The situation in the northern part of the Jordanian side of the Jordan Valley (NJJV) was different; before 2008, CL has rarely been reported from this area.

2 Methods

From April 2008 to May 2009, passive detection and focused active 'index case cluster'-directed detection were used to trace cases of CL from the NJJV . DNA was extracted from Seven clinical isolates of *Leishmania* promastigotes and lesion scrapings spotted on filter papers obtained from 51 suspected CL patients living in the NJJV. The identity of the causative species of CL in the NJJV was investigated using the polymerase chain reaction (PCR) amplifying the ribosomal internal transcribed spacer 1 (ITS-1) followed by the restriction fragment length polymorphism (RFLP).

3 Results

In 2008/2009, 183 cases were clinically diagnosed of having CL in the NJJV.The parasites in five of the isolates and in 48 PCR-positive scrapings were classified as *Leishmania major*. In two isolates and in one other PCR-positive scraping *Leishmania tropica* was identified. Epidemiological investigations revealed that the *L. tropica* cases were residents of two towns outside the NJJV

4 Conclusions

Herein, we report the clinical and molecular epidemiological features of cases of CL from NJJV with the aim of documenting the new focus of *L. major*.

C0419 COMMUNITY LEISHMANIASIS OUTBREAK IN THE SOUTHERN AREA OF MADRID 2009-2016

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1 Background

Human leishmaniasis is an endemic zoonotic disease in Madrid Autonomous Community which is monitored through the notifiable diseases surveillance. Dogs are the main reservoir and the vector involved is a sandfly of the Phlebotomus genus. Since the beginning of 2011 an outbreak has been detected in the south-west area of the region of Madrid, whose epidemiological data is being analysed.

2 Methods

We present a descriptive analysis of the community outbreak and the main control measures adopted. Also within this study a specific case definition and epidemiological survey were established.

3 Results

From July 2009 to October 2016, 697 cases were reported in the outbreak, the mean incidence rate (IR) was 19.15 per 100,000 inhabitants, from 2009 to 2016 these cases were detected respectively: 6,97,197,160,91,93,43 and 10. The cases lived in the towns of: Fuenlabrada (538; IR 43.02), Leganés (80; IR 6.77), Getafe (67; IR 6,17) and Humanes (12; IR 9,98). All of these towns are geogragraphically close and share large urban parks. The clinical presentation was 38.7% visceral leishmaniasis and 61.3% cutaneous. The mean age of all cases was 48, ranging from 2 months to 95 years old; from all the cases 5.9% were under 2 and 9.2% between 2 and 14; 59.3% were male; the country of origin was Spain for 77.3%, sub-Saharan Africa 11.8% and 10.9% from other countries; 94.0% were confirmed cases by laboratory and *L. infantum* has been identified as the agent. 9.0% of cases had immunosuppressive pathology and 4.0% HIV. Some of them (22.2%) had travel history during incubation period (no case was considered imported). Among the environmental risk factors the proximity of dogs was reported in 27.5% and 3.0% related sick dogs. The surveillance system for canine leishmaniasis had not detected any increase in prevalence. *P. perniciosus* has been identified in high density. The presence of other fauna has been investigated and it has been discovered that hares and rabbits play a role as active secondary reservoirs. Environmental control measures have been intensified: disinsection plan in risk areas and control of Leporidae overpopulation.

4 Conclusions

This is the largest community outbreak described in Spain, with acme in 2011 and subsequent slight downward trend. There are cases in all age groups, most of them immunocompetent. The multifactorial etiology of the outbreak highlights the need of finding a new active secondary reservoir, hares and rabbits. Research and control measures continue.

C0423 PREVALENCE OF CANINE VISCERAL LEISHMANIASIS IN INDIGENOUS LAND XAKRIABÁ, BRAZIL

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1 Background

Canine reservoirs play a central role in the expansion of the visceral leishmaniasis in endemic areas and little information has been obtained in indigenous lands about it. This study was designed to determine the prevalence of canine visceral leishmaniasis (CVL), as well the parasite load of infected animals living in the Xakriabá indigenous land, in southeast Brazil.

2 Methods

Of a sample of 950 dogs of 17 villages were collected blood samples from the ear vein to impregnated in filter paper maintained at -20ºC for PCR assay. The diagnosis of CVL was conducted by different methods: (1) screening by ELISA and confirmation by indirect immunofluorescence (IFI), according to Ministry of Health recommendations at 2011; (2) conventional PCR directed for the *Leishmania* kDNA and (3) real-time PCR targeted to the parasite kDNA. Additionally, the parasite load of positive samples was carried out by qPCR assay. Following the comparative analysis of results among the different diagnostic methods was performed.

3 Results

The prevalence of CVL varied according to the diagnostic method used, 4.3% for ELISA plus IFI, 14.7% for conventional PCR and 8.1% by real-time PCR. Serological groups considered positive for CVL (ELISA + and IFI +, indeterminate ELISA and IFI +) showed positivity differentiated between the PCRs, and 44.9% positive for conventional PCR and 25.6% for the real time PCR ($p < 0.001$). Samples with indeterminate serology (ELISA indeterminate and IFI + or indeterminate ELISA and IFI negative) showed similar positivity for conventional PCR (24.2%) and real-time PCR (25.0%). Between tracks of parasite burden used to classify the samples positive for CVL by real-time PCR (n=77) in 1 cm^3 filter paper impregnated with 15uL of blood, 77.9% of the samples were observed with parasite burden between 0.050 and 0.499 parasites/cm^3, 6.5% with parasite burden between 0.500 and 0.999 parasites/cm^3 and 15.6% with parasite burden ≥ 1 parasite/cm^3 ($p < 0.001$). The 48 samples with positive diagnosis by serology (ELISA and IFI) presented the following parasitic load: 20.8% not quantified, 54.2% between 0.001 and 0.049 parasites/cm^3, 14.6% between 0.050 and 0.499 and 6.25% between 0.500 and 0.999, and 4.2% ≥ 1 parasite/cm^3 ($p < 0.001$).

4 Conclusions

The sensibility of the PCR assays (conventional and real-time) was 3.8 to 10.4 higher than serological test to detect the CVL in the studied area. Supported by CNPq and UFOP.

C0451 INCIDENCE OF POST KALA-AZAR DERMAL LEISHMANIASIS (PKDL) AND VISCERAL LEISHMANIASIS RELAPSE (VLR) AMONGST THE VISCERAL LEISHMANIASIS (VL) PATIENTS TREATED WITH MONOTHERAPY IN BANGLADESH

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1 Background

We aimed to investigate the incidence of Post-kalazar Dermal Leishmaniasis (PKDL) and visceral Leishmaniasis relapse (VLR) among cured VL patients after monotherapy with sodium stibogluconate (SSG), Miltefosine (MF), single dose AmBisome (SDA), multi-dose AmBisome (MDA) and Paromomycin (PMIM).

2 Methods

Study participants are cured VL cases, treated with 4 different monotherapy under various controlled trials in Bangladesh between 2008-2015. Follow up period for every participant was 4 years.Collected information is a mix of retrospective for older arm completed more than 4 years ago (SSG) and mix of retrospective data and from prospective follow-up for other arms. Trained health workers (HWs) visited household of participants and checked for symptoms and sign of PKDL or VLR. Suspected PKDL / VLR cases were referred to the study field clinic where study physician referred probable cases of PKDL / VLR to Surya Kanta Kala-azar Research Centre for further management. The study is on-going and here we report results of an interim analysis.

3 Results

One hundred sixty eight patients for SSG, 150 for MF, 105 for PMIM, 113 for MDA and 126 patients for SDA arm were included. Age for participants (mean ± SD) was lowest in SDA arm (19±12) and highest in SSG arm (28±14), $p<0.001$. Distribution of male and female participants was unremarkable. Fifty-three of 662 particiapnts experienced VLR for a total of 896811 observed days making an average annual incidence of 2%. Incidence of VLR was highest after treatment with MF (4.2%) followed by treatment with PM (4.0%), SDA (2.1%), MDA (0.73%) and SSG (0.45%). Sixty eight of 662 developed PKDL for a total of 915417 observed days making an average annual incidence of 2.71%. Patients after treatment with PM had a highest annual incidence of PKDL (5%) followed by 4%, 2.5%, 2% and 0.8% respectively with SDA, MF, MDA, and SSG. Cumulative number and percentage of patients who had VLR were 22 (15%), 15 (14%), 10 (8%), 3 (3%) and 3 (2%) respectively for MF, PM, SDA, MDA, and SSG. The cumulative number and percentage who had PKDL was 20 (19%), 20 (16%), 14 (9%), 9 (8%) and 5 (3%) respectively for PM, SDA, MF, MDA, and SSG.

4 Conclusions

Incidence of VLR and PKDL among cured VL patients varied depending on therapy for primary VL. The study results will be useful for VL elimination program to choose an appropriate treatment regimen for VL in the maintenance and consolidation phases of the VL elimination program in the Indian sub-continent.

C0457 EPIDEMIOLOGY AND DISTRIBUTION OF LEISHMANIA SPP. IN SOUTH OF MOROCCO

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1 Background

Several species of the genus *Leishmania* are causative agents of cutaneous leishmaniasis in Morocco. Zoonotic cutaneous leishmaniasis (ZCL) due to *Leishmania major* is known to be established in the arid and sub-arid regions of Morocco. Our aims is to study the epidemiology of cutaneous leishmaniasis in Ouarzazate and Zagoura Provinces two regions known since 1914 as ZCL focus, and to update the data concerning distribution of species causing cutaneous leishmaniasis in these regions.

2 Results

Sample collection has been done in 7 different localities in Ouarzazate and Zagoura provinces .Out of 91 samples collected, 66 were found positive for CL after microscopic and molecular analysis by ITS1 typing. After PCR RFLP analysis we have found that the dominant species is *L. major* with 53 samples (80%) followed by *L.tropica* with 12 (18 %) and *L. infantum* with 2 (3%).*L. major* was found in 4 out of 6 localities targeted and *L. tropica* and *L. infantum* was found in Toundount(North of Ouarzazate province) and Agdz(Zagoura province).

3 Conclusions

Our findings suggest a change in the distribution of Cutaneous leishmaniasis due to *L. tropica* and *L. infantum* by its installation in foci further south than what the literature proposes. The vectors of both *L. tropica* and *L. infantum* are present in these regions (*Phlebotomus sergenti* and *Phlebotomus longicuspis*) and the reservoir of *L. infantum*, the dog, is a common animal in the two provinces. This may suggest two hypothesis: (i) Infected reservoirs by *L. infantum* may have migrated from a foci of *L. nfantum* to an area further south where the vector is present (ii) Due to the anthroponotic characteristic of LC due to *L. tropica* the introduction of *L. tropica* in the area may be possible with an external infection from an endemic region. More epidemiological research is needs for a better understanding of the changes in the distribution of Leishmania species in Morocco.

C0465 SEROPREVALENCE OF ASYMPTOMATIC LEISHMANIASIS IN GRANADA

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1 Background

Leishmaniasis refers to a group of vector-bourne diseases caused by an opportunistic intracellular protozoan parasite of the genus Leishmania. According with WHO, Spain is endemic of visceral and cutaneus leishmaniasis.
Although some studies have been conducted in some regions of Spain, is not known the prevalence of asymptomatic leshmaniasis in the population of Eastern Andalusia (Spain).
The objective of this study is to know the seroprevalence of leishmaniasis among the healthy adult population from the province of Granada (Eastern Andalusia) and the distribution in its counties.

2 Methods

From June 2015 to May 2016 we obtained 1260 serum samples from blood donors (606 male and 654 female) in Granada province. The mean age was 42, 89 (range 18-65 years). The sample size was calculated for an estimated prevalence of 5%. Samples distribution by county was: Alhama (n=72) Alpujarra (n=73) Baza (n=89) Costa (n=136) Guadix (n=77) Huéscar (n=31) Loja (n=201) Los Montes (n=80) Valle de Lecrín 132 (10, 48%) and Vega de Granada 369 (29, 29%).
Serum samples we tested by IFI test (Leishmania IFI IgG, Vircell, Granada, Spain) considering positive a titer of 1:80 or higher.
This study has been approved by the Ethical Committee of Research of the University Hospital Virgen de las Nieves (Granada, Spain)

3 Results

Seroposivity rate was 7,9% (100/1260) among them 93 showed a titers of 1:80, 5 showed a titers of 1:160 and finally 2 presents a titers of 1:320.
Seropositivy rate in both gender are 8, 75% (53/606) in male population and 7,18% (47/654) in the female population.
In each counties we obtained the following prevalence value: Alhama 6,94% (5/72) Alpujarra 6,85% (5/73) Baza 12,35% (11/89) Costa 9,55% (13/136) Guadix 9,09% (7/77) Huéscar 1,29% (4/31) Loja 8,45% (17/201) Los Montes 10% (8/80) Valle de Lecrín 8,33% (11/132) and Vega de Granada 5,14% (19/369)

4 Conclusions

This study reveals that the prevalence of Leishmania asymptomatic infection among blood donors in Granada is relatively high, especially in the counties of Baza, Los Montes, Costa and Guadix.

C0478 DETECTING ASYMPTOMATIC INFECTIONS: COMPARISON OF DNA EXTRACTION METHODS AND PCR STRATEGIES, AND IMPLICATIONS FOR FIELD STUDIES.

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1 Background

Asymptomatic infections can impact parasite transmission, can indicate local transmission cycles, and in HIV populations they may be relevant to diagnose before onset of disease. Many studies detect and quantify *Leishmania* in asymptomatic infections. We have compared two DNA extraction methods and PCRs for detecting *Leishmania* in human blood, and we investigated the impact of reaction conditions on sensitivity and quantification.

2 Methods

We tested a manual DNA extraction method (Qiagen) and an automated procedure (Maxwell16, Promega) on parasites spiked in human blood. Two real-time PCRs were compared, targeting the small subunit (SSU) ribosomal DNA, and kinetoplast minicircles (kDNA) respectively. For quantification, the impact of human DNA and simultaneous amplification of normalization genes were assessed. Methods were applied on blood samples from endemic areas in Nepal and Ethiopia.

3 Results

On parasite DNA dilutions, kDNA PCR showed consistent amplification down to 10E-5 parasite genome equivalents (PGE). On the contrary, below 10E-2 PGE, SSU PCR suffered from stochastic effects and often did not amplify. This was also seen in a repeated analysis of asymptomatically infected humans and animals in Nepal: from 10 confirmed infections, only two amplified again. In blood spiked with *Leishmania*, kDNA PCR consistently amplified down to 10E-9 parasites before stochastic effects appeared.
In the presence of human DNA in amounts found in clinical samples, sensitivity of kDNA PCR dropped at least 100-fold, and could only be detected down to 10E-3 PGE. When multiplexed with a human reference gene for normalization, sensitivity dropped further to 1 PGE. Standard curves for quantification calculated from pure parasite DNA (as used in most reports) underestimated parasite load by a factor of 10.
DNA extracted from whole blood of 20 visceral leishmaniasis patients of Ethiopia, showed a more efficient amplification when using Maxwell versus Qiagen procedures: the Ct value of both SSU and kDNA PCRs dropped by 4 units.

4 Conclusions

Our work has significant consequences for interpreting data on parasite load and asymptomatic infection rates. The implications of our findings will be highlighted in relation to studies reporting low parasitaemia. In the coming months, the influence of inhibitors on parasite quantification will be tested. Suggestions for improvements and standardization of molecular detection of asymptomatic infections will be discussed.

C0504 AGE-RELATED TRENDS IN ASYMPTOMATIC AND SYMPTOMATIC LEISHMANIAL INFECTION IN THE INDIAN SUB-CONTINENT

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1 Background

Age-related trends in asymptomatic and symptomatic *Leishmania donovani* infection in the Indian subcontinent (ISC) hold important information about rates of transmission and the duration of immune responses following infection, and yet are poorly understood. In order to target control programmes to achieve and sustain elimination a better understanding of these trends is required. We therefore conducted a systematic review and meta-analysis of age-stratified studies of seroprevalence and clinical visceral leishmaniasis (VL) incidence in the ISC.

2 Methods

A systematic literature search was performed. Eight infection prevalence studies (covering PCR, the Direct Agglutination Test (DAT), rK39 rapid diagnostic test (RDT) and ELISA, and the Leishmanin Skin Test (LST)) and 7 clinical VL incidence studies met the inclusion criteria. Data from these studies was entered into an online database and graphed. Statistical tests were performed to assess agreement between different serological tests performed on the same study population, and results for the same diagnostic from different studies compared to look for trends in seroprevalence with diagnostic cut-off.

3 Results

All DAT studies showed an increase in the prevalence of seropositivity with age up to 30-40 years. Some studies suggested a decrease in DAT seroprevalence with age thereafter, while others suggested a continuing upward trend. Infection prevalence was lower among 0-10 year-olds across nearly all studies and all diagnostics. Clinical VL incidence was also lower amongst children aged 0-5 in most studies, and most studies showed a peak in incidence between the ages of 5 and 20. There were no statistically significant differences in the seroprevalences for different diagnostics in the same study, and no clear trend in seroprevalence with the cut-off for seropositivity.

4 Conclusions

Asymptomatic infection prevalence in the ISC varies with age and according to geographical location and time period. However, there is a lack of standardisation of test protocols and cut-off levels, which makes it difficult to compare different diagnostics and draw firm conclusions about age patterns in seroprevalence. Most studies suggest infection prevalence and clinical disease incidence is lower amongst young children, and that clinical incidence is highest amongst 5-20 year-olds. This may be due to lower exposure to sandfly bites in young children, but the peaked age-incidence pattern may also reflect increasing immunity to the parasite with age.

C0512 SEROLOGICAL EVALUATION OF LEISHMANIA SPP. IN CAPTIVE PRIMATES

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1 Background

Leishmaniasis are zoonoses of worldwide occurrence and of great epidemiological relevance. They affect humans and domestic and wild animals, and may present clinical manifestations. Depending on the relation between the parasite and the host, leishmaniasis may present in the cutaneous or visceral form, having as different agents species of protozoa of the genus *Leishmania*. Although the dog is the main urban reservoir, other species such as non-human primates may participate in the epidemiological cycle of the disease. Thus, the present study aimed to investigate *Leishmania* spp. in captive primates.

2 Methods

The city of Bauru, in the state of Sao Paulo, Brazil, is considered a region endemic for leishmaniasis, with several annual cases of the disease. We investigate the occurrence of *Leishmania infantum*, *Leishmania amazonensis*, *Leishmania braziliensis*, *Leishmania mexicana* and *Leishmania major* in 34 non-human primates belonged to the Municipal Zoological Park of Bauru, SP – Brazil, by the Immunofluorescence Antibody Test (IFAT).

3 Results

Of the 34 serum samples tested for *L.infantum*, *L. amazonensis*, *L. braziliensis*, *L. mexicana* e *L. major*, one sample (2.9%) from primate of the specie *Erythrocebus patas* was reactive to *L. braziliensis* with a titer of 160.

4 Conclusions

The results show that captive primates from zoos inserted to an urban environment can participate in the epidemiological cycle of leishmaniasis, thus contribuiting to the spread of the disease. However, more diagnostic tests are necessary to evaluate the role of these species in the context of the disease.

C0518 MOLECULAR DETECTION OF LEISHMANIA SPP. IN CONJUNCTIVAE OF CATS FROM AN ENDEMIC AREA FOR LEISHMANIASIS

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1 Background

Leishmaniasis are infectious and parasitic diseases occurring worldwide. They are neglected and of great importance in public health. Although the dog is considered the main urban reservoir of the parasite, domestic animals, such as cats, have already been diagnosed with the presence of the protozoan. Several forms of biological samples for leishmaniasis diagnosis are possible, including conjunctival swabs, which is a non-invasive technique. Despite this, there are few studies in the literature that use this technique. Thus, we studied the occurrence of *Leishmania* spp. in conjunctivae of domestic cats from an endemic area for the disease.

2 Methods

The city of Bauru, located in São Paulo, Brazil, is an endemic county for leishmaniasis. We collected samples of conjunctival swabs from the right and left eyes of 36 cats from the Center for Zoonoses Control (ZCC) in Bauru. The samples were submitted to the molecular technique of Polymerase Chain Reaction (PCR) using the primers ITS-1 and HSP70 to detect *Leishmania* spp.

3 Results

Four animals (4/36 - 11.1%) were positive by PCR using primers ITS-1 and HSP70. Of these, one sample was positive in the right eye and three samples were positive in the left eye.

4 Conclusions

We can conclude that the swab of the conjunctivae is a non invasive, reliable and easy diagnostic method for the diagnostic of leishmaniasis in cats.

C0523 SEROPREVALENCE OF LEISHMANIOSIS IN HEALTHY APPEARANCE STREET DOGS IN ESKISEHIR

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1 Background

Visceral leishmaniasis can cause a systemic and chronic disease in the world. Dogs are the main reservoir of the etiologic agent for *Leishmania infantum.* Our study a serological investigation of canine visceral leishmaniasis (CVL) was performed in healthy appearance street dogs in the municipality of Tepebasi located in the central region of Eskisehir, Turkey. Eskisehir located central Anatolia, where the disease is not endemic. We are aimed to determine the seroprevalence of CVL with comparing sex, age and strain in healthy appearance street dogs in Eskisehir province.

2 Methods

Within a two years period, 185 healthy appearance street dogs were couched in Eskisehir city center by officers of the municipality of Tepebasi Veterinary Directorate, Natural Life Center, because of immunization and sterilization. All dogs after general health examination control by a veterinarian and also were taken in appropriate conditions and 5ml blood samples during operation. All sera were separated for Indirect Fluorescent Antibody Test (IFAT) for searching seropositivity against *Leishmania infantum*. The results were evaluated by SPSS version 17.0 program.

3 Results

Total of 35 dogs (19%) were found IFAT as weak positive; 34 dogs in 1/16 and one dog 1/64 titer. There were no clinical symptoms of leishmaniosison these seropositive dogs. Seropositive cases were not significantly differences when evaluated according to dog genders, races and age

4 Conclusions

Leishmaniasis ; importance for public health, and dogs carrying potential reservoir, they often show without symptoms. In healthy appearanced, Street dogs in Eskisehir province may be weak seropositivity of CVL were determined, but it is recommended to take some measures for regular control and prevention of zoonotic infections in owned or street dogs, which could carry important risk for public health. CVL is responsible for the sterilization, embezzlement and different animals of urban dogs. Under control of prevalence with health protection measures it can be taken.

C0524 CUTANEOUS LEISHMANIASIS IN RURAL YEMEN

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1 Background

Yemen, among the Middle East countries, has the highest prevalence of people living in poverty where more than half of the population lives below the poverty line and lacks access to health care and safe drinking water. Out of 17 neglected tropical diseases (NTDs), the country is endemic for at least 11 NTDs, with leishmaniasis (both cutaneous and visceral) being one of the most common; Yemen ranks third in the Middle East region after Syria and Iran in cutaneous leishmaniasis and there has been a notable increase in reported cases during the past five years, particularly among children and adult females. Currently, Yemen is in the throes of a civil war which has caused NTDs to spread to new foci. It has also resulted in a partial-to-complete paralysis of the control surveillance for endemic diseases across the country. However, there is a scarcity of information about the epidemiology of leishmaniasis in Yemen. This study was carried out from June 2015 to April 2016 in the Utmah district of Dhamar governorate, Yemen.

2 Methods

A total of 112 patients with skin lesions were screened for the presence of *Leishmania* parasites. Giemsa-stained skin slit smears were examined by light microscope and then confirmed by nested PCR assay.

3 Results

Microscopic examination detected *Leishmania* amastigotes in 48 smears of the 112 Giemsa-stained smears collected from patients with suspected cutaneous leishmaniasis. Thirty isolates were successfully amplified by nested PCR and sequenced for the ribosomal internal transcribed spacer 1 (ITS1) gene. All isolates were confirmed as *Leishmania tropica* species. Significant associations of infection with age and gender were reported ($P < 0.05$).

4 Conclusions

Cutaneous leishmaniasis remains a public health problem in Yemen with *L. tropica* was the only species identified in the examined isolates. Innovative and integrated preventive measures should be identified and implemented to control this devastating disease in these communities. Further studies on the sand fly vectors and potential reservoir hosts of *Leishmania* spp. in Yemen are required.

C0545 CUTANEOUS LEISHMANIASIS IN TURKEY: COMPARISON OF DIAGNOSTIC METHODS AND GEOGRAPHICAL DISTRIBUTION OF LEISHMANIA SPECIES IN AUTOCHTHONOUS CASES

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1 Background

Cutaneous Leishmaniasis (CL) is a common infection in Turkey, with increasing incidence in recent years. Recent studies confirmed the presence of *Leishmania major*, *L. donovani* and *L. infantum* in autochthonous CL cases, as with *L. tropica* in Turkey. We aimed to present our series of 356 autochthonous CL cases with data on the diagnostic methods and geographic distribution of identified *Leishmania* species.

2 Methods

A total of 360 individuals with one or more skin lesions were initially diagnosed as CL, based on clinical examinations. Their personal data were collected; lesion samples were obtained and examined under the microscope after staining with Giemsa. Samples were also cultivated in NNN Medium and Enriched Medium (EM). Real Time PCR procedure targeting ITS-1 region of *Leishmania spp.* was applied to samples using both amastigotes and promastigotes. To differentiate *L. donovani* from *L. infantum*, single colony genotyping after cultivation in Blood Agar, Heat Shock Protein 70 PCRand RealTime Cysteine Protease b (*cpb*) Gene PCR were done.

3 Results

CL was diagnosed in 356 cases, while all tests were negative in four cases, despite initial clinical signs. Assessment of participants' personal data showed no travel history abroad in the last two years. *L. tropica* was the leading agent (84%), identified not only in southeastern but also in western Anatolia. *L. major*, *L. donovani* and *L. infantum* were all identified in our series. More cases were identified with culture in EM (n=331) than in NNN Medium (n=274).

4 Conclusions

Ourfindings indicated the presence of four *Leishmania* species in CL in Turkey, which may indicate the approaching risk of encountering more visceral leishmaniasis cases tomorrow. Increase in CL incidence in Turkey is due to increase in infection foci within Turkey and recent influx of 3 million Syrians because of the war in Syria. Therefore, urgent, national control programs are necessary to determine the reservoirs, vectors and other contributing factors of CL and develop effective measures. Another interesting finding was the superiority of EM to NNN Medium in the diagnosis, which deserves further analyses.

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C0547 SURVEILLANCE OF VISCERAL LEISHMANIASIS IN MADRID (2009-2016): EPIDEMIOLOGICAL CHARACTERISTIC IN SPORADIC CASES COMPARED TO COMMUNITY OUTBREAK ASOCIATED CASES

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1 Background

Human leishmaniasis is an endemic zoonotic disease in Spain. Since July 2009, there has been a community outbreak of leishmaniasis in the south-west area of the Madrid Autonomous Community. We study the visceral leishmaniasis (VL) cases associated to the outbreak compared with the sporadic cases in the rest of the region during that period.

2 Methods

we carried out a descriptive analysis of VL reported to the surveillance network of Madrid from 1 July 2009 to 31 October 2016. The epidemiological variables studied are compared in cases associated to the outbreak in front of sporadic cases.

3 Results

During the studied period 445 cases of VL were reported in Madrid: 270 (60.7%) were associated to the outbreak and 175 (39.3%) sporadic. According to the year of symptoms onset from 2009 to 2016 patients were detected respectively: 3,32,70,57,28,52,21 and 7 for the outbreak in front of 6,25,19,26, 35,27,19 and 18 for sporadic. The distribution of the clinical variables in the outbreak affected people compared to sporadic cases was: mean age 40.1 years old in front of 41.9 (p=0,46); 66.7% were male vs 70.3% (p=0.42); the country of origin was Spain 60.0%, sub-Saharan Africa 27.8% and 12.2% from other countries compared to 79.4%, 5.1% and 15.4% (p<0.01); the patients from the outbreak that presented intrinsic risk factors were 31.1% in front of 52.6% in sporadic VL (p<0.01); the hospital admission was 83.0% vs 76.6% (p=0.10); and 13.0% of patients from the outbreak had lymph node or cutaneous-mucosal affectation while in sporadic was 2.9% (p<0.01). Most cases were confirmed by laboratory 87.0% for the associated and 90.9% for sporadic (p=0.22), using serology 64.1% vs 44.0% (p<0.01); culture 5.6% vs 34.9% (p<0.01) or biopsy 81.5% vs 73.1% (p=0.04). The mean delay in reporting from the onset of symptoms was 78.9 days for the outbreak in front of 161.7 days for the rest (p<0.01). In the environmental risk factors no significant differences were detected.

4 Conclusions

The findings in the VL associated to the outbreak were a higher percentage of patients of sub-Saharan origin, fewer intrinsic risk factors and more cases with atypical presentation. The percentage of confirmed cases is similar in both groups, but serology and biopsy are used more frequently as a method for diagnosis in outbreak patients. In the area of the outbreak there is a greater awareness of the disease and the delay in reporting to epidemiological surveillance system is less than in the rest of the region.

C0558 SURVEY OF VECTOR-BORNE COMORBIDITIES IN A LEISHMANIA-ENDEMIC CANINE POPULATION FROM PARAGUAY

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1 Background

Canine Visceral Leishmaniasis (CVL) is an endemic parasitic disease in Paraguay with a majority of seropositive dogs observed to be asymptomatic for clinical signs consistent with CVL. Little is known about the full landscape of canine vector-borne diseases (CVBD) infecting dogs in Paraguay and the role concurrent infections may play in the onset of CVL. The objective of this study was to assess the incidence of *Leishmania* and other CVBD agents in a population of pet dogs from *Leishmania*-endemic areas of Paraguay.

2 Methods

A total of 100 serum samples and 400 whole blood samples were collected. The samples originated from 32 unique postal codes distributed across the eastern region of Paraguay. Nucleic acid was extracted from the whole blood samples and real-time PCR was run to detect the following pathogens: *Leishmania*; *Ehrlichia canis*; *Ehrlichia chaffeensis*; *Anaplasma platys*; *Anaplasma phagocytophilum*; *Babesia vogeli*; *Babesia gibsoni*; *Rickettsia* spp.; *Bartonella* spp.; *Mycoplasma haemocanis*; *Candidatus Mycoplasma haematoparvum*; *Hepatozoon canis*; *Hepatozoon americanum*; *Dirofilaria immitis*; and *Trypanosoma cruzi*. ELISAs were performed on serum to detect the presence of antibodies to *Leishmania*, *Ehrlichia* spp., *E. canis*, *E. ewingii*, *E. chaffeensis*, *Anaplasma* spp., *A. platys*, *A. phagocytophilum*, *Babesia* spp., and *Borrelia burgdorferi*, as well as antigen from *D. immitis* and *Angiostrongylus vasorum*.

3 Results

Real-time PCR detected *Leishmania* infections in this population at a rate of 18%. Additional CVBD pathogens detected by PCR included *E. canis* (28%), *A. platys* (11%), *B. vogeli* (9%), *M. haemocanis* (4%), *Cand. M. haematoparvum* (3%), and *H. canis* (2%). All of these pathogens are known to be transmitted by *Rhipicephalus sanguineus* ticks. Overall, 7% of samples were PCR positive for *Leishmania* and at least one other CVBD pathogen with *E. canis* being the most common co-infecting agent. Of the serum samples tested by ELISA, 46% were seropositive for *Leishmania*. High seropositive rates were also observed for *Ehrlichia* spp. (51%), *E. canis* (51%), *Anaplasma* spp. (46%), *A. platys* (23%), and *Babesia* spp. (26%). Overall, 37% of samples were seropositive to *Leishmania* and at least one additional CVBD pathogen.

4 Conclusions

These findings indicate that co-infections with *Leishmania* and other CVBD pathogens, particularly those transmitted by *Rhipicephalus sanguineus* ticks, are relatively common in Paraguayan dogs and should be taken into consideration when diagnosing and treating CVL.

C0575 VISCERAL LEISHMANIASIS IN DOMESTIC CATS (FELIS CATUS) IN BRAZIL

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1 Background

Visceral leishmaniasis (LV) is a disease of worldwide distribution, caused by intracellular protozoa. In Brazil it is caused by the specie *Leishmania infantum* that affects man and a variety of vertebrates, including the domestic cat. Due to the increase in the number of feline cases in Brazil and in other Countries, it was verified the occurrence of visceral leishmaniasis in domestic cats in Teresina, state of Piauí.

2 Methods

In the study were included 18 felines: ten from the University Veterinary Hospital of the Universidade Federal do Piauí and eight from private clinics in Teresina, Piauí State. The animals were submitted to clinical evaluation and diagnosed by direct investigation of the parasite in a sample of bone marrow, popliteal lymph node and skin, using NNN (Novy, MacNeal, Nicolle) biphasic culture medium with 1mL of Schneider's and smear on blade stained with Giemsa. Leishmanias isolated from cultures were subjected to DNA extraction, Polymerase Chain Reaction (PCR), with specific primer to amplify the ITS-1 region of *Leishmania* spp. and Restriction Fragment Length Polymorphism (RFLP) using *HaeIII* enzime, for identification of the *Leishmania* specie. The animals were also tested for Feline Leukemia Virus (FeLV) antigen and for Feline Immunodeficiency Virus (FIV) antibody.

3 Results

In 7/18 (39%) of the cats examined, *Leishmania* was found in popliteal lymph node sample, 4/7 the parasite was also detected in the bone marrow and in 3/7 the parasite was found in the three organs examined (medulla, lymph node and skin). The clinical manifestations observed in the infected cats were: alopecia in the ears and face, nodules in the ears and lips, lesion on the face, on nose and on limbs, uveitis, weight loss and ocular secretion. 29% of cats infected with *Leishmania* were reactive for FIV and none for FeLV. Co-infected animals did not present significant difference in clinical manifestations when compared with those infected only by *Leishmania*. In PCR-RFLP infection by *L. infantum* was confirmed in three cats.

4 Conclusions

With the results of this study we can conclude that domestic cats are affected by visceral leishmaniasis in Teresina, Piauí state, Brazil, an endemic area of the disease for dogs and humans.

C0585 IMPORTED CUTANEOUS LEISHMANIASIS IN HATAY,TURKEY: REPORT OF FIVE CASES

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1 Background

Cutaneous leishmaniasis (CL) is a growing problem because of increasing global travel of workers to endemic areas. In this case series we report five cases of cutaneous Leshmaniasis in international truck drivers, travelling to areas endemic with leshmaniaisis.

2 Methods

The diagnosis of the five cases was established at Mustafa Kemal University teaching hospital in Hatayprovince. Samples from cutaneous lesions were obtained and stained using Giemsa stain. The diagnosis was established by finding the amastigoit form of the Leishmania using 100 x magnifications. All five cases were male patients. The age range was between 38-43 years. There were 5-7 ulcerative or nodular lesions at or near the site of insect exposure in each patient. The lesions duration of existance were variying from 1 month to 1 year.
Turkmenistan, republic of Syria, Islamic republic of Iran, Kingdom of Saudi Arabia and republic of Uzbekistan were among the countries visited by the patients. All patients had their lesion at the travelled countries

3 Results

This case series is the first to be reported as imported cutaneous leshmaniasis in Hatay province. All cases were treated with Glucantime. The result of this case report will help to draw attention to the prophylaxis and awareness of public health of cutaneous leishmaniasisspecially for drivers travelling to the endemic areas.

C0590 SEROLOGICAL AND MOLECULAR BIOMARKERS FOR DETECTION OF ASYMPTOMATIC VISCERAL LEISHMANIASIS INFECTION IN BANGLADESH

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1 Background

Transmission of Visceral Leishmaniasis (VL) is augmented by the presence of asymptomatic individuals in VL endemic region, as they serve as potential reservoir of parasites. Although, majority of the *Leishmania* infected individuals remain asymptomatic, the lack of a precise definition or gold standard for asymptomatic infection complicates the control strategy. An accurate and effective biomarker is crucial designing an efficient strategy to detect, control and monitor the burden of asymptomatic infection. In this study, we evaluated known serological and molecular markers for the detection of asymptomatic infection in endemic region of Bangladesh.

2 Methods

A total of 810 healthy individuals without a history of VL or PKDL from the VL endemic region of Mymensingh, Bangladesh, were screened for asymptomatic *L. donovani* infection. 20 patients with active VL were included as the positive controls and 30 healthy individuals from a non-endemic area were included as negative controls. Sera and peripheral blood buffy coats were collected from all participants. *Leishmania*-specific serum antibody responses were investigated by Direct Agglutination Test (DAT), rK39 RDT and enzyme-linked immunosorbent assays (ELISA) using the rK39, K28 and KRP42 recombinant antigens. A real time PCR assay was performed to detect and quantify the number of Ld genomes in the buffy coat DNA.

3 Results

The prevalence of asymptomatic *L. donovani* infection was assessed at 4.81%(39/810), 3.33%(27/810), 12.96%(105/810), 10.49%(85/810) and 15.06%(122/810) for DAT, rK39 RDT, rK39 ELISA, rKRP42 ELISA and rK28 ELISA respectively. The real time PCR detected *Ld* genomes in 1.48%(12/810) of participants, with a mean parasite load of 125.37 /mL blood. Significant agreement was observed between rK39 and rK28 ELISA(κ=0.831),rK39 and rKRP42 ELISA(κ=0.786), rK28 and rKRP42 ELISA(κ=0.708),and rK39RDT and DAT(κ=0.748). 19.75%(160/810) participants were positive at in least one assay. During a one year follow up period only one participant progressed to active disease, who was positive in all serological tests.

4 Conclusions

Considering sensitivity and accuracy, our data indicate that the rK28 ELISA is the most appropriate method for the detection of asymptomatic *L.donovani* infection. Nevertheless, until stronger prognostic indicator is established, we suggest that individuals positive for asymptomatic *Ld* infection by any of the method should be kept under observation for the risk of progression to active VL disease.

C0604 TIME TRENDS IN VISCERAL LEISHMANIASIS AND L. DONOVANI INFECTION IN NEPAL IN THE CONTEXT OF ELIMINATION

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1 Background

For three consecutive years now, VL incidence in Nepal has reached the elimination target of < 1 case/10,000 population/year at district level. However, this does not necessarily mean that *L. donovani* transmission has been interrupted as well, since the vast majority of infections remain asymptomatic. This study sets out to measure the current age-specific prevalence rate of *L. donovani* infection in communities that were studied 10 years ago in the KALANET trial. This way we aim to assess whether the elimination efforts have indeed resulted in a decreased transmission rate, and as such, enhance our understanding of the impact of the ongoing VL elimination initiative in Nepal.

2 Methods

We organized a repeat cross-sectional epidemiological and serological study, in a population of approximately 10,000 persons for which serological and clinical data exist from 2005. Three additional villages, located in the same endemic area, but with no official records of VL incidence over the last 10 years were also included. We collected data on socio-demographic characteristics and VL-related events, drew venous blood for serological testing (rk39 Rapid Diagnostic Test (RDT) and Direct Agglutination Test (DAT)), and performed Leishmanin skin testing (LST).

3 Results

Results are expected by mid-2017. Main research outcomes are: 1) age-specific prevalence rates *of L. donovani* infection markers in people living in VL-endemic areas, as a proxy of past exposure to *L. donovani*, and this by combining humoral (DAT, rk39 RDT) and cellular (LST) immunity tests ; and 2) trends in age-specific DAT seroprevalence rates over the past decade.

4 Conclusions

We expect preliminary results to be available by March 2017. Lower age-specific prevalence rates would support the success story that is currently being attributed to the elimination initiative. Any decrease in seroprevalence however should still be interpreted against the natural cyclic variations described in this disease. Our data will be used for mathematical modeling of disease transmission.

C0612 VISCERAL AND CUTANEOUS LEISHMANIASIS CASES IN NORTHERN CYPRUSVISCERAL AND CUTANEOUS LEISHMANIASIS CASES IN NORTHERN CYPRUS

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1 Background

The island of Cyprus is located in the eastern part of the Mediterranean Region where leishmaniasis is endemic. In northern Cyprus, data on human leishmaniasis is limited. This report documents visceral and cutaneous leishmaniasis (VL and CL) cases from two consecutive surveys in the northern part of the island.

2 Methods

Survey 1: Between January 2011 and December 2012, three pediatric patients with suspected VL were included in the study. Bone marrow aspirates were used for microscopy and for detection of *Leishmania* species by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). Serological analysis was performed by using immunofluorescence antibody test (IFAT), direct agglutination test (DAT) and rK39 test.
Survey 2: Between September 2014 and December 2014, eight adult patients who were clinically diagnosed with CL were enrolled in this survey. Seven patients were previously diagnosed and treated. One of the eight patients was diagnosed with CL and began to receive the treatment shortly before participating in the study. Blood samples of the patients were tested for *Leishmania* spp. by PCR and DNA sequencing. Also, serum samples were used for DAT and rK39 test.

3 Results

Survey 1: Between January 2011 and December 2012, three pediatric patients with suspected VL were included in the study. Bone marrow aspirates were used for microscopy and for detection of *Leishmania* species by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). Serological analysis was performed by using immunofluorescence antibody test (IFAT), direct agglutination test (DAT) and rK39 test.
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4 Conclusions

The two studies indicate that both VL and CL are present in northern Cyprus. Therefore, preventive measures should be taken for elimination of the disease.

C0629 RISK FACTORS FOR ANTHROPONOTIC CUTANEOUS LEISHMANIASIS IN RESPONSIVE AND UNRESPONSIVE PATIENTS IN A MAJOR FOCUS IN SOUTHEAST OF IRAN

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1 Background

Cutaneous leishmaniasis (CL) due to *Leishmania tropica* is a significant challenge at global range. This study was aimed to evaluate the risk factors which are associated with anthroponotic CL (ACL) in responsive and unresponsive patients in a major focus in southeastern Iran.

2 Methods

Patients were recruited in major ACL foci from responsive and unresponsive cases. The patients were compared for demographic characteristics, clinical status and environmental factors. Twenty risk related factors were analyzed by multiple logistic regression and backward elimination stepwise models. $P< 0.05$ was defined as statistically significant.

3 Results

Overall, 340 ACL patients consisting of 292 (85.9%) responsive and 48 (14.1%) unresponsive cases with active lesions or scars were analyzed by estimating odds ratio (OR). Among 20 variables, 4 major risk factors including lower age group (OR= 0.94, $P< 0.001$), longer duration of lesion (OR= 1.35, $P< 0.001$), history of chronic diseases (OR= 0.048, $P< 0.001$) and poor interior housing conditions (OR=2.32, P=0.016) were significantly associated with incidence of non-healing lesions.

4 Conclusions

Improving interior house construction, protecting children and those with debilitating diseases from biting by sand flies, early detection and effective treatment of the ACL patients are highly important measures for prevention of incidence of chronic lesions in southeastern Iran.

C0636 SPATIAL CLUSTERING OF VISCERAL LEISHMANIASIS CASES OVER THE COURSE OF AN EPIDEMIC IN FULBARIA, BANGLADESH 2002-10: ESTIMATING HOW TRANSMISSION RISK DECREASES WITH DISTANCE.

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1 Background

Visceral leishmaniasis (VL) persists in Bangladesh despite WHO's 2020 elimination target growing ever closer. Since case proximity has been reported as a strong risk factor for infection, there is a need to understand the spatial transmission dynamics of VL. Our analysis of longitudinal VL data from Bangladesh provides valuable insights into spatial dynamics of VL, which can be used to tailor control programmes to eliminate this devastating disease.

2 Methods

We analyse data from a community cohort of 24,781 people (5,118 households over a 12x12 sq km area) during a VL epidemic from 2002-10. We explore how spatial clustering changed with time using semivariograms, which indicate the range and degree of spatial correlation in the occurrence of VL between households.

A spatial kernel transmission model that accounts for variation in risk with case proximity through an inverse square law was developed. Two parameters were estimated: one that determines the rate at which risk decreases with distance from a case; and one representing the monthly rate of infection experienced by each susceptible individual living with an infected person. The incubation period was fixed at 5 months based on a previous analysis of serological data from the same area, and post–kala-azar dermal leishmaniasis and asymptomatic infection were ignored.

3 Results

Weak spatial correlations at a range of approximately 500m were observed in the case presence in households during three biennial periods: 2002-3, 2003-4 & 2004-5. This is in agreement with estimates from the spatial kernel model of 95% infection risk at 110m, 50% risk at 420m and 10% risk at 1050m. The spatial model fitted the data better than a non-spatial model ($p<2E-18$, likelihood ratio test).

4 Conclusions

Over the course of an epidemic, spatial clustering is affected by the dynamics of infection and immunity. By analysing spatial spread at this large scale, we have been able to identify longer range transmission occurring than expected from previous estimates. This forms a proof-of-concept for a more advanced model incorporating a variable incubation period and more disease states.

C0668 SEROLOGICAL AND MOLECULAR SURVEY OF LEISHMANIA INFANTUM IN STRAY CATS IN THE PROVINCE OF BOLOGNA (ITALY).

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1 Background

In May 2013, public health authorities of the Province of Bologna expressed a concern regarding a significative upsurge in human cases of visceral leishmaniosis, pointed out since November 2012. They found indeed 14 cases in six months, which represents an over five-fold increase compared with the annual average of 2.6 cases (range: 0-8) from 2008 to 2012 (Varani et al., 2013). Due to the importance of dogs as primary reservoir of *Leishmania* parasites, a canine leishmaniosis (CanL) surveillance program was launched in 2007, with the purpose of monitoring the presence of sandflies and infected dogs in the public kennels of Emilia-Romagna region (Santi et al., 2014). All data collected demonstrated that the yearly prevalence of CanL in this area dropped from 3.1% in 2013 to 1.1% in 2015. Although no specific information is available about the transmission of *Leishmania spp.* to cats, it is fair to assume that cats are susceptible to infection in the areas where *L. infantum* is present. In 2015-2016 a survey program on stray cats living in the province of Bologna was implemented in order to obtain accurate informations on the prevalence of the disease in this species.

2 Methods

562 sera samples and 566 conjunctival swabs were collected from 566 stray cats captured for the sterilization program in the urban areas of the Province of Bologna. Sera samples were tested using Immunofluorescence technique (IFAT) according to the laboratory procedures described in the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (OIE, 2014). Materials were provided by the National Reference Centre for Leishmaniosis (C.Re.Na.L., Sicily, Italy). Animals with IFAT titre of 1/80 were considered infected, as reported by Pennisi et al. (2012). Conjunctival swabs were analysed using Real Time PCR (Galletti et al., 2011).

3 Results

IFAT was positive (serum titres ranged from 1:80 to 1:320) in 7 of 562 sampled cats (1.24%; IC95% 0.50-2.55). Only 1 of 566 conjunctival swabs (0.17%; IC95% 0.004-0.98) tested using RT-PCR was positive, but IFAT was negative. Each cat was clinically examined prior to sample collection and only one of them has been shown to be affected by ocular conjunctivitis. This cat was IFAT positive (1:320) but RT-PCR negative.

4 Conclusions

Our findings confirm the presence of CanL infection in stray cats living in the Province of Bologna. Despite the seroprevalence in dogs and cats living in the same endemic area seems to be similar, generally cats do not show any clinical symptom.

C0684 MICROPLATE WHOLE BLOOD INTERFERON-GAMMA RELEASE ASSAY FOR MARKER OF LEISHMANIA INFECTION

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1 Background

Whole blood interferon gamma (IFN-γ) release assay (IGRAs) is an *in vitro* immune test that has recently been developed as an alternative to the Leishmanin skin test (LST). An increasing body of evidence supports the use of IGRAs for identification of individuals exposed to leishmania infection but without disease. Requirement of 3 ml of blood preclude this test widely acceptable for its application on larger community studies. This study aimed at evaluating the performance of microplate based IGRA using 200µl blood (direct blood as well as 1:1RPMI diluted blood) with conventional IGRA (3ml blood) for marker of infection.

2 Methods

We employed conventional IGRA and microplate based IGRA with direct as well as diluted venous blood using soluble leishmania antigen (SLA) on patients with active visceral leishmaniasis (VL, n=32), patients with cured VL (n=20), endemic Healthy Controls (EHC, n=21) and healthy control subjects living in non-endemic area (NEHC, n=12). IFNγ levels in culture supernatants were measured by ELISA and kappa statistics was used to access the concordance between test assay formats.

3 Results

The whole blood cells of both active VL and cured VL produced significantly level of IFN-γ in both format of IGRA. Positive correlations were found with active VL blood in IFN-γ production between 3ml vs 600µl, 3 ml vs 300µl, and 600µl vs 300µl, while with cured VL blood it was moderate with 3ml vs 600µl; 3ml vs 300µl. No significant difference in the overall IFN-γ response by both assay formats was detected, and agreement between tests was significant.

4 Conclusions

These findings confirm that 96-well microplate based IGRA is more sensitive as conventional IGRA and promising tool for providing the means to more efficient screening in large scale immunological and epidemiological studies.

C0738 PHLEBOTOMINE SANDFLIES (DIPTERA, PHLEBOTOMIDAE) OF LANZAROTE ISLAND (CANARY ISLANDS, SPAIN): ECOLOGICAL SURVEY AND EVALUATION OF THE RISK OF LEISHMANIA TRANSMISSION

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1 Background

Phlebotomine sandflies are natural vectors of *Leishmania* spp. and their expansion throughout has been evidenced in the last few years due to the global warming and changes in human behaviour, worsening leishmaniasis problem. However, phlebotomine sandflies have been captured in small numbers on the Canary Islands, particularly on the island of Lanzarote, where only one limited survey was carried out almost thirty years ago. The proximity of this island to Morocco, in addition to the high number of tourists, sometimes accompanied by their dogs, from leishmaniasis endemic regions, highlights the importance of studying the sandfly fauna on this island in order to determine the transmission risk of leishmaniasis.

2 Methods

Thirty-eight sampling sites spread across the island were studied with 583 sticky traps, and ecological features were gathered to identify the ecological traits associated to the presence of sandflies.

3 Results

Only 85 sandfly specimens were captured with the following species distribution: *Sergentomyia minuta* (0.15 specimens/m2), which was reported for the first time on this island, and *S. fallax* (1.03/m2). No specimen of the *Phlebotomus* genus was captured.

4 Conclusions

Leishmania transmission risk from a hypothetical infected reservoir introduced in the island, to human or domestic animals, should be discarded, as well as the establishment of an active and stable infectious focus. Therefore, this island of Lanzarote can be considered a tourist place free of leishmaniasis; moreover, the dogs breed in this island could be sent to the European Union, or other countries, without the risk of *Leishmania* introduction.

This study was funded by the Project PI14-01024, Ministry of Economy and Competitiveness, Instituto de Salud Carlos III, Madrid and Feder Funds for Regional Development from the European Union, “One way to make Europe”.

C0748 HUMAN CASES OF VISCERAL LEISHMANIASIS IN A BRAZILIAN ENDEMIC AREA: AN OVERVIEW OF OCCURRENCE, LETHALITY AND HIV CO-INFECTION

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1 Background

Visceral leishmaniasis (VL) represents a challenge in Brazilian public health, mainly due to its high lethality. The death outcome in VL is usually associated with co-infection by the human immunodeficiency virus (HIV). The city of Rondonópolis, located in Mato Grosso State, in Central-Brazilian Region, is an endemic area with intense transmission, which lacks studies. This study described epidemiological and clinical aspects of the occurrence, HIV co-infection and lethality of VL in such area.

2 Methods

All autochthonous cases reported between 2011 and 2015 were included. Data were obtained from the notification forms and death certificates, available at National Disease Notification System and Mortality Information System, respectively. Patients were also contacted to verify the therapeutic itinerary.

3 Results

Rondonópolis reported 83 cases of VL during the period, with highest incidence in 2011 (13.5/100,000 inhabitants). Most of them were males (59.4%), aged 0-4 years (33%), living in urban area (92.6%), especially in peripheral districts. Minors/students (42.2%) and housewives (9.6%) were the most affected occupational group. Most cases (83.1%) were diagnosed at specialized health services. The diagnostic opportunity and therapeutic itinerary were on average 31.9 days and 2.58 health services, respectively. Fever, splenomegaly and weight loss were more common in, respectively, 96.4%, 79.6% and 75.9% of the patients. Treatment was mainly performed with pentavalent antimonial in 71.1% of the cases, with drug failure in 4.8% and cure in 92.8% of the cases. Lethality rate was 6% (n=5), mainly in males (80%), retired (40%), with an average age of 62.4 years. Bleeding (80%), jaundice (40%) and edema (40%) were frequent. The diagnosis was defined only in specialized health services on average 21.6 days after the onset of symptoms. Liposomal amphotericin B (AmBisome) was the predominant used drug. HIV co-infection were reported in 8 cases (9.6%), mainly in males (62.5%), retired (37.5%), with an average age of 48.3 years. Although classical manifestations were frequent, cough and/or diarrhea (37.5%) and edema (25%) were found. The diagnosis of 87.5% of cases was carried in specialized centers on average 42.2 days after the symptoms. Treatment was conducted mainly with AmBisome (62.5%), with one case of drug failure and one death by VL.

4 Conclusions

VL is frequent in the city, with clinical and epidemiological features similar to those observed in other Brazilian areas.

C0753 VISCERAL LEISHMANIASIS IN ARMENIA – FIRST TIME USE OF MOLECULAR DIAGNOSIS

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1 Background

Leishmaniasis historically holds a special place in the infectious pathology of Armenia and associated with a spectrum of clinical manifestations was firstly reported in the country in 1913. The city of Yerevan was considered to be the most active focus of VL. In Yerevan, infected dogs were the main reservoirs. A control program against diseased dogs and sand fly vectors carried out in 1954 and 1955 reducing the morbidity of VL significantly. After a long break a new case emerged in 1999. The number of cases increased continuously. Between 1999 and 2016, more than 81 cases of VL were recorded in the country. Nevertheless, VL is underreported in Armenia because of the lack of trained medical personal and diagnostic facilities.
The aim of the work was a review of the occurrence and epidemiology of VL in Armenia and to apply for the first time molecular diagnosis of VL in Armenian clinical samples by using PCR, with further *Leishmania* species identification by RFLP and sequencing.

2 Methods

25 patients from different regions of Armenia suspected of VL were hospitalized in the Yerevan hospitalbetween 2012 and 2016. Bone marrow aspirates were collected and smear slides were made for further diagnosis. After microscopic examination, DNA was extracted from the slides and ITS1-PCR was performed. RFLP with *Hae*III and sequencing was used for further species identification.

3 Results

We compared the sensitivity of ITS1-PCR with microscopic detection of parasites in clinical samples from bone marrow. From the total 25 samples, 22 were positive by microscopic examination. The causative agent, identified by RFLP, was *L. infantum*.

4 Conclusions

The present work is an important step towards the inclusion of molecular techniques in the current diagnosis of VL in Armenia. Because of the re-emergence of VL in different parts of Armenia and increasing prevalence in neighboring countries, molecular diagnostic laboratories with trained medical personal have to be established. Further typing at intra-species level will focus on epidemiological questions as transmission cycles and mode of spread.
Work supported by funding by the BMBF (FKZ01DK14021) and DAAD (Research Grants for Doctoral Candidates and Young Academics and Scientists).

C0759 MOLECULAR CHARACTERIZATION OF LEISHMANIA INFANTUM CIRCULATING IN HUMANS, DOGS, AND SAND FLIES IN THE EMILIA-ROMAGNA REGION, NORTHERN ITALY

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1 Background

The unusual increase in the number of visceral leishmaniasis (VL) cases observed since 2012 in central areas of the Emilia-Romagna region (E-R), northern Italy, led health authorities to focus the attention on this re-emergent parasitic disease. With the aim to provide a better knowledge of the parasite population circulating in the region, we investigated the genetic characteristics of *Leishmania infantum* strains from different hosts.

2 Methods

Molecular characterization was performed on a panel of 62 *L. infantum* strains obtained from humans, dogs and phlebotomine sand flies between 2013-2016 from different locations abroad the region, including areas involved in recent VL outbreaks. Isolates obtained from other endemic Italian regions were also included in the study for comparison. Species identification was performed by ITS-1 sequencing, and subsequent sub-species characterization was carried out by *k26*-PRC typing. Finally, strains were characterized by multilocus microsatellite typing (MLMT), targeting 15 highly polymorphic markers. Genotype data were analyzed by a Bayesian model-based clustering method.

3 Results

All strains were identified as *L. infantum* by sequencing of ITS1. Capillary electrophoresis revealed different *k26*-PCR product sizes; the amplicon of 626bp, typical of MON-1 zymodeme, was the most representative in both canine and extra-region human isolates. Conversely, all the human isolates from the E-R region shared a distinct, never reported amplicon size.
Overall, the MLMT detected 48 genotypes, with canine isolates showing a higher level of polymorphism. Bayesian analysis inferred two populations; one grouping canine strains together with extra-regional human strains, named Population A, whereas regional human strains and strains from sand flies were grouped in a second population, the Population B.

4 Conclusions

Overall, the study revealed genetic heterogeneity within *L. infantum* population from northern Italy. MLMT proved to be a suitable method for tracking genotypes among different hosts and areas. Despite canine population of the E-R region hosted zoonotic genotypes, recent outbreaks of VL were sustained by a distinct *L. infantum* population, actively circulating in the sand flies and so far undetected in sympatric dogs. Further studies are needed to elucidate the role of wild or domestic animal species in the epidemiology of VL in the region.

C0778 LEISHMANIA INFANTUM ASYMPTOMATIC INFECTION IN PATIENTS UNDER TNF-ALFA INHIBITORS TREATMENT

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1 Background

The clinical manifestation of leishmaniasis varies depending on the parasite virulence and on the host immune response. While immunocompetent individuals have an effective *Leishmania*-specific response that makes the infection asymptomatic or subclinical in most of the cases, immunosupressed subjects are a population at risk of developing the disease. Many immune-mediated diseases are treated with Tumor Necrosis Factor inhibitors (anti-TNFα), a pro-inflammatory cytokine crucial for the control of several opportunistic infections caused by intracellular microorganisms such as *Leishmania*. The aim of this study was to detect asymptomatic infection in patients receiving TNF-α antagonists from the Barcelona area (Spain), where *L. infantum* is endemic and causes cutaneous and visceral disease.

2 Methods

In our study, 192 patients with Crohn disease or ulceratice colitis were recruited at the Vall d'Hebron University Hospital. None of them has a previous history of leishmaniasis. 96 patients were being treated with Infliximab, 89 with Adalimumab and 7 with Golimumab. Anti-*Leishmania* antibodies were tested by ELISA and Western Blotting (WB) and *Leishmania* DNA was detected in peripheral blood mononuclear cells (PBMCs) performing a real time qPCR.

3 Results

Fourteen patients (7.3%, 95%CI 4.3-11.9%) were positive by any technique. By serological techniques, ten patients (5.2%) were positive by ELISA and six positive by WB (3.1%). All patients with positive serology, showed negative results by qPCR. *Leishmania* DNA was detected in two of the 192 patients (1%), who were seronegative. Seven of those positive patients were treated with Infliximab and seven with Adalimumab.

4 Conclusions

Given the prevalence found in the immunocompromised asymptomatic carriers studied and as they are at high risk of developing clinical leishmaniasis, screening for parasite presence of this population is highly recommended. An early diagnosis may help to prevent a possible development of disease.

C0843 VISCERAL LEISHMANIASIS CASES IN NON-ENDEMIC DISTRICTS OF NEPAL: DOES IT SIGNIFY THE EXPANSION OF ENDEMICITY?

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1 Background

Visceral leishmaniasis (VL) is decreasing significantly in Nepal, from over 1200 cases in 2008 to only 200 reported VL cases in the recent year 2015. The majority of these cases represent from the 12 endemic districts in the south-eastern plains of the *Terai* (lowlands). Over the last few years, the number of cases reported from non-endemic districts is increasing and now represents a considerable proportion of cases (65, or 32.5% in 2015). In early 2014 and 2015, field investigation team visited villages in six non-endemic districts, chosen on the basis of reported VL cases in order to collect epidemiological and entomological evidences.

2 Methods

We reviewed VL surveillance data of the Ministry of Health for the period of 2000 to 2015. Six non-endemic districts were selected based on the case load reported. District public health offices were consulted to identify the possible villages for field investigation. House-to-house inventory of VL was done including geo-referencing of houses with (and without) cases. Case-control study (1:4) was conducted, focusing on professional activities and travel history. Sandflies were captured using CDC light traps and mouth aspiration in houses and cattlesheds.

3 Results

A total of 256 VL cases were reported from 22 non-endemic districts during the period of 2000 to 2015. House-to-house survey ascertained 46 confirmed VL cases retrospectively, of which 21 (45.7%) occurred within the last two years. One active VL case was identified during the field work (based on clinical signs and a positive RDT) on the spot. Of the 46 confirmed VL cases, seven were children; most of the cases were at productive age. Age-and sex-matched case-control study showed that exposure to known VL-endemic areas was no risk factor for VL, but having a VL case in neighborhood was. Moreover we were able to capture the vector, *Phlebotomus argentipes* sandfliesin the study villages.

4 Conclusions

The preliminary analysis of the field work suggests the possibility of expansion of VL cases in non-endemic study villages. Further work is required at molecular level to confirm the ongoing local transmission of VL in these areas. These districts are hitherto not considered as endemic for VL transmission in Nepal, hence awareness, surveillance and control activities should therefore be extended in these areas in order to support ongoing VL elimination in Nepal.

C0844 EPIDEMIOLOGICAL CHARACTERISTICS OF LEISHMANIASIS DISEASE IN COCHABAMBA DEPARTMENT, BOLIVIA, DURING THE PERIOD 2014-2015

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1 Background

Bolivia is a country with a high incidence of cutaneous and mucosal leishmaniasis (CL and ML) in South America. *Leishmania braziliensis* transmitted by phlebotomine sand flies is the main etiological agent. The aim of the present study is to describe the epidemiological characteristics of CL, ML and MCL patients attending LABIMED laboratory (Leishmaniasis reference centre of Cochabamba department from the IIBISMED Service, Universidad Mayor de San Simón).

2 Methods

The study was conducted between September 2014 and November 2015 (14 months) in the city of Cochabamba. The study population was comprised by 135 suspected patients with lesions compatibles with CL, ML and MCL referred from different Bolivian Health Centres and coming to the laboratory to be diagnosed. Patient data were collected by a clinical and epidemiological semi-structured questionnaire. Patients were considered to be affected with leishmaniasis when Parasitological Direct Exam (PDE) and/or culture resulted positive for *Leishmania* parasite.

3 Results

86% of the studied cases came from Cochabamba department. Most of the CL cases included in the study came from the tropical region of Chapare province. 59.3% of suspected patients resulted to be positive for one or both diagnoses methods performed (63% by PDE and 83% by culture). Men were the most affected (83.8%) as well as the population range between 18-60 years old (76%). CL was the most frequent clinical manifestation (71.3%), followed by ML and MCL (23.7% and 5%, respectively), the last one with active concomitant cutaneous and mucosal lesions. In the cases of cutaneous lesions, 61.4% presented only one lesion and in 43.9% of cases located in a lower member (eg. legs, feet). In the mucosal cases, nostrils, mouth and larynx were find affected in a similar percentage. Forty-three cases were considered as primo-infection and sixteen patients were considered as treatment failure, considering patients who have finished completely the treatment but did not recover.

4 Conclusions

The present study contributes to the knowledge of leishmaniasis diagnosed in Cochabamba department. Most of the suspected patients could be confirmed by parasitological methods, culture being more sensitive.

This research was funded by ISGlobal, CEADES and UB (ajut institucional per a projectes internacionals i promoció de la Internacionalització).

C0847 MODELING DISEASE OCCURRENCE AND RESPONSE TO ENVIRONMENTAL DRIVERS FOR PREDICTING THE RISK OF CUTANEOUS LEISHMANIASIS IN AMAZONIA

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1 Background

The Cutaneous Leishmaniasis (CL) cycle is a complex inference network between large sets of host and vector species, with large heterogeneities in space and time. To understand the transmission dynamics of the parasite, some studies investigated the spatial distribution of sand fly vectors and co-occurrence with cases. Anyway lack of knowledge about vector and host diversity may weaken the predictive power of the models. In the New World, CL involves sylvatic cycle and is directly influenced by distribution and ecological plasticity of hosts and vectors. A wide set of environmental conditions driving diversity and abundance of species communities, and urbanization of the cycle facilitated by human activities on edge habitats, may contribute to the occurrence and emergence of this disease.

2 Methods

We developed an empirical study based on Ecological Niche Modelling (ENM) to depict the linkages between environmental variables (bioclimatic variables, human footprint, and several forest habitat descriptors: forest cover and forest loss, above-ground biomass, canopy height) and Amazonian cases of CL reported from French Guiana, Colombia, Ecuador, and Amazonas state, Brazil, between 2006 and 2015. Modeling was implemented with Maximal Entropy (MaxEnt) at 2 geographic scales: a small scale, focusing on French Guiana with high resolution environmental data; and a regional scale, encompassing all the Amazonian basin.

3 Results

Both models had highly satisfactory power (AUC=0.897 and 0.919, respectively). In the two models, variables of importance were related to anthropogenic activities (human footprint, distance to forests), to forest habitat structure (above-ground biomass), and climatic factors (pluviometry at local scale, pluviometry and precipitation seasonality at regional scale).

4 Conclusions

The high explanatory power of the ENM applied to clinical cases rather than to putative vectors only allow highlighting the range of environmental variables driving the maintenance of the cycle, without need of deep knowledge of all biotic components. At the two geographic scales, the projection of more favorable conditions for CL occurrence allowed proposing risk maps. In such complex cycles, ENMs can be relevantly used for eco-epidemiological issues as an additional method to identify the risk and locally adapt accurate surveillance efforts. Further, using projections in near future of key explanatory variables, the model could also detect possible new areas of emergence.

C0884 A RECENT OUTBREAK OF AMERICAN TEGUMENTARY LEISHMANIASIS IN THE MAIN TRANSMISSION AREA OF LEISHMANIA (VIANNIA) BRAZILIENSIS IN ARGENTINA

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1 Background

American tegumentary leishmaniasis (ATL), due mainly by *Leishmania (Viannia) braziliensis*, is an emerging - reemerging endemic disease in the northwest of Argentina. This study describes the clinical-epidemiological characteristics of the disease in the last three years.

2 Methods

Five hundred and fifty eight patients with cutaneous or mucocutaneous lesions were recruited in three periods, P1: November 2013 - October 2014, P2: Nov 2014 - Oct 2015, P3: Nov 2015 - Oct 2016. The diagnosis of ATL was based on the finding of *Leishmania* amastigotes on smears of dermal scrapings from the border of their lesions.

3 Results

Three hundred forty-six out of 588 (62%) were diagnosed as ATL cases, 48/91, during P1 (52.7%), 154/230 (67%) in P2 and 144/237 (60.8%) in P3. The ATL cases increased 3.2 and 3-fold in the in periods P2 and P3 comparing to P1, whereas the increases in the no ATL diagnosed cases were 1.8 and 2.2 folds, respectively for the same periods ($p = 0.02$). The 80.6% of the patients with ATL were males. Only 9.5% of them were under 15 years old. The single cutaneous form was the most frequent one, 74% of the cases, while the mucocutaneous one reached 5.2%. The highest LTA cases peak was observed between July and September 2015, in P2. In addition, two smaller peaks were observed: one in January - February and the other in May - June 2016.

4 Conclusions

The increase in ATL cases found could be due to an outbreak in rural areas of northwestern Argentina and in areas of the northern border with the neighbor country Bolivia, mainly affecting farming workers. Further studies of the transmission cycles involving *L. (V.) braziliensis* are needed for a better understanding of the pathology in this area.

C0900 SPATIOTEMPORAL AND MOLECULAR EPIDEMIOLOGY OF CUTANEOUS LEISHMANIASIS IN LIBYA

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1 Background

Cutaneous leishmaniasis (CL) is a major public health problem in Libya. In this study, we continue our survey of eco-epidemiological parameters of CL in Libya that started with a general analysis and molecular identification of the causative agents of cases that occurred between 1995 and 2008. Here we analyze CL cases that occurred during the period of the armed conflict from January 2011 till December 2012 and we investigated demographic characteristics of all cases, the clinical evaluation of patients and molecular characterization of parasites. We explored the spatial distribution of CL cases and projected future distribution into climate change scenarios to establish the expansion potential and risk factors of the disease in Libya.

2 Methods

We investigated 312 CL patients that presented to the Dermatology Department at the Tripoli Central Hospital and came from 81 endemic areas distributed in 10 districts. We compared the results of this study with our previous study done in 1995-2008. Correlation species distribution models were used with the database of occurrence of *L.major* and *L.tropica* for the periods 1995-2008, 2011-2012 and for the whole period (1995-2012).

3 Results

More than half of the patients (56.8%) were males. The average age was 30(range 6 months-85 years). Molecular identification of parasites by a PCR-RFLP approach targeting the ITS1 region was successful for 84 patients with two causative species identified: *L. major* and *L. tropica* comprised 60 (78.9%) and 23 (21.1%) cases, respectively. Seasonal occurrence of CL cases showed that most cases (83.1%) occurred between October and March, *L. major* cases from November–January (53.2%), and *L. tropica* cases mainly in December and January (37%).

Spatiotemporal projection based on climatic and environmental conditions showed that coastal regions have more favourable conditions for *Leishmnia* and are at higher level of risk. Moreover, future projection until 2060 showed an increasing incidence of CL and possible emergence of new endemics in the North-eastern districts of Libya. Conversely, decreased level of risk is expected in the districts Wadi al Shatii, Sabha and Wadi al Hayaa.

4 Conclusions

Future projection of CL until 2060 showed a future trend of increasing incidence of CL and possible emergence of new endemics in the North-eastern districts of Libya. These results should be considered for control programs to prevent emerging of new endemic areas.

C0927 SPATIAL AND TEMPORAL TRENDS OF VISCERAL LEISHMANIASIS BY MESOREGION IN A STATE IN SOUTHEASTERN BRAZIL, 2002-2013

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1 Background

In Brazil, the Visceral Leishmaniasis (VL) urbanization has been documented since the 1980s. This trend represents a challenge for the control of the disease in urban areas. The objective of this study was to analyze the spatial distribution and temporal trends of VL in Minas Gerais State and to identify the greater risk areas of transmission.

2 Methods

An ecological study with spatial and time series analysis was carried out using the new cases confirmed by VL reported in the Brazilian Notifiable Disease Information System (SINAN - Ministry of Health), from 2002 to 2013. Two complementary methodologies were used: thematic incidence maps and Generalized Linear Model (GLM) using Poisson Regression (log-linear). The thematic maps were generated for crude cumulative incidence and smoothed incidence of VL, with 4 time intervals (3 in 3 years) 2002-2004, 2005-2007, 2008 - 2010 e 2001-2013. The Poisson Regression (log-linear) was used to measure the variation in the mean of the number of VL cases from one year to the other by mesoregion of the state of Minas Gerais.

3 Results

The maps showed that during the 12-year time serie, the highest incidences remained in the mesoregions that are located in the north of the state of Minas Gerais (Northwest of Minas, North of Minas and Jequitinhonha), in the East region (Vale do Rio Doce) and in the center of the state (Metropolitan region and Belo Horizonte). Among these mesoregions, it can be concluded that in the mesoregion North of Minas Gerais, the incidence rate decreased sharply. On the other hand, in the Vale do Rio Doce mesoregion, the incidence rate increased over time. The Poisson Regression models showed that the mean number of VL cases increased or remained stable in all mesoregions in the initial periods analyzed and decreased in the last years, except for the Jequitinhonha mesoregion, which in the final period showed a discrete increase (17%) from one year to the next. Among these mesoregions in which the average number of cases increased in the initial periods, the Northwest of Minas Mesoregion stands out between 2002 and 2005, which increased the number of VL cases from one year to the next by approximately 152%.

4 Conclusions

The results of in this study may contribute to a better understanding of the spread of the disease in Minas Gerais, as well as its trends in the chronological series. Also these findings can provide subsidies to assist the actions of the control program of VL.

C0955 GEOPROCESSMENT AND PREDITORS ASSOCIATED WITH TEGUMENTAR LEISHMANIASIS IN SOUTH BRAZIL

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1 Background

Leishmaniasis is a group of infectious and parasitic diseases that remain a public health problem due to their high endemicity and broad geographic distribution. In epidemiology, geoprocessing techniques are tools capable of elucidating the standard behavior of a disease. This study aimed to describe the predictive factors of tegumentary leishmaniasis (TL) in South Brazil using geoprocessing techniques.

2 Methods

The Human Research Ethic Committee of the Universidade Estadual de Maringá (No. 615.628/2014) approved this study. This is a descriptive and transversal study with a quantitative approach. The cases were georeferenced using the elaboration of maps in the Geographic Information System. For the database, 133 cases of TL were selected and reported between 2009 and 2013, in 21 municipalities of Paraná State, Brazil. The variables studied were year of notification, autochthonous cases of municipal notification, age group, gender, and clinical forms. The Microsoft Excel 7.0 software, OpenEpi v.2.2 using a 95% confidence interval (95% CI) and Odds ratio (OR) were used to verify the association between studied variables and TL, and chi-square or Fisher's exact tests were used with a significant level of $p<0.05$.

3 Results

The probability of infection in the rural area was four times higher in comparison to the urban area (OR= 4.12). The prevalent age range for TL was over 20 years (OR= 6. 39). Individuals who declared themselves to be illiterate, even those with incomplete primary education, presented a three fold higher risk (OR= 4.12), compared to higher education levels. In Bandeirantes municipality, the probability of woman acquiring TL was almost twice that of men (OR= 1.79). Bandeirantes presented a risk of almost eight times higher for TL compared to the other municipalities (OR= 8.56). The spread of cases showed a high prevalence in land used to intensive agriculture, soils with great retention capacity at altitudes between 400 and 850 m and areas that belong to drainage basin.

4 Conclusions

The forms of transmission assume variable characteristics even within a geographic area; it is fundamental for building the knowledge of epidemiological profile by health managers. Furthermore, detection and control measures can be adapted and implemented according to each reality.

C0963 TRANSMISSION PATTERNS OF VISCERAL LEISHMANIASIS AND ASSOCIATED SOCIOECONOMIC CONDITIONS IN BRAZIL: A NOVEL APPROACH BASED ON TYPOLOGY CONSTRUCTION.

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1 Background

Ty1ology construction is potentially usefull for better understanding of disease transmission patterns. Associations between Visceral Leishmaniasis (VL) and socieoeconomic conditions suggests that VL ocurrence is strongly related to pooor living conditions, lack of basic urban infrastructure services and low levels of education. In this context, we aimed to explore associations of a typology of VL transmission patterns with socioeconomic conditions in Brazil.

2 Methods

This is an ecological study including 5,560 brazilian municipalities, wich reported information on cases of human VL for at least one year from 2004 to 2012. Statistical analysis were performed in Stata 13 and maps in ArcGIS 10.2.1. We developed a hierarquical scheme for typology construction including selected key feactures of VL ocurrence, as frequency and stability of incidence rate. Demographic and socioeconomic variables were obtained from secondary sources and cluster analysis was applied to identify groups of municipalities with similar socioeconomic conditions. We applied multinomial logistic regression to investigate the associations between the different transmission patterns and the clusters of socioeconomic conditions.

3 Results

A simplified version of the typology with six categories was able to provide relevant summary information on key features of VL patterns. In general, most municipalities were labeled into the "infrequent, low/intermediate", "frequent" and "very frequent, unstable" categories. We found positive associations between the socioeconomic conditions and the typology classification for the "frequent", "very frequent, stable, low/intermediate incidence", and "very frequent, unstable" categories, since the number of municipalities increases with the worsening of the living conditions.

4 Conclusions

This typology contributes for better understanding of VL transmission patterns and its associations with socioconomic conditions at the municipal level. Patterns identified should continue to be evaluated and taken into account for targeting of disease control measures.

C0972 UPDATING CANINE LEISHMANIASIS SEROPREVALENCE IN VALDEORRAS (NORTHWESTERN SPAIN)

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1 Background

Northern Spain had traditionally been considered leishmaniasis-free. Nevertheless, since the early 90's cases of canine leishmaniasis have been reported in northwestern Spain, with Valdeorras being particularly affected. The aim of this work is to update seroprevalence of canine leishmaniosis in Valdeorras for the last 15 years.

2 Methods

This study includes 1890 dogs that were suspected of suffering from canine leishmaniasis (either because they showed clinical signs compatible with the disease or because they coexisted closely with affected animals). All of them were assisted at the veterinary clinic "Servicios Veterinarios del Sil" (located in Valdeorras - Northwestern Spain) from January 2002 to September 2016. These dogs were tested for *L. infantum* using the IFA (immunefluorescent antibody) test (cut-off ≥ 1/100).

3 Results

The seroprevalence rates, with respect to the total population assisted at the Veterinary Clinic (an average of 2220 dogs per year) throughout all these years, did not present great fluctuations. No year the seroprevalence exceeded 2%. Statistically significant fluctuations ($p<0.05$) were observed between some consecutive years: there was a significant decrease in the seroprevalence rates between 2004 and 2005 and between 2007 and 2008, while significant increases occurred between 2005 and 2006 and between 2012 and 2013.
In contrast, when the study was conducted exclusively on the population tested (suspected to have the disease) two periods with highly significant differences in seroprevalence rates were observed ($p<0.01$): the first period from 2002 to 2007 (with a mean seroprevalence of 40.62%) and the second from 2008 onwards (with an average seroprevalence of 18.21%). However, the annual distribution of seropositive cases with respect to antibody titer was statistically similar ($p=0.29$) between these two periods.

4 Conclusions

After 2013, in which the highest seroprevalence was detected (1.9%), an average seroprevalence rate of 1.7 ± 0.2% was estimated in Valdeorras (Northwestern Spain). The significantly higher seroprevalence rates observed in the group of dogs tested between 2002 and 2007 could be attributed to the fact that during this period serological tests were performed mainly on dogs showing clinical signs compatible with canine leishmaniasis. In contrast, since 2008, there has been increasing control over the population living in close proximity to animals affected by the disease, regardless of the presence or absence of symptoms.

C0982 EVOLUTION OF LOW ANTIBODY TITERS IN NATURALLY ACQUIRED CANINE LEISHMANIASIS

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1 Background

It is well known that IFA (immunefluorescent antibody) test is a useful tool for the diagnosis and follow-up of canine leishmaniasis. Nevertheless, the clinical approach of cases with low antibody titers has scarcely been reported, which is particularly relevant in deciding when to perform a new serological test or when to start the specific treatment.
The aim of this work is to assess the evolution of antibody titer in dogs displaying low antibody titers (equal, immediately below or immediately above the cut-off) at the time of diagnosis.

2 Methods

The present work includes 95 dogs assisted at the veterinary clinic "Servicios Veterinarios del Sil" (in Valdeorras - Northwestern Spain). All of them were tested for *L. infantum*, using the IFA test (cut-off ≥ 1/100), and all of them exhibited low antibody titers: equal, immediately below or immediately above the cut-off, at the time of diagnosis. Serological follow-up of these dogs was carried out for at least one year.

3 Results

Of the 95 dogs included in this study, 66 had an antibody titer immediately below the cut-off (1/50) at the time of diagnosis. 24.2% of them evolved to positive titers. The rest of the animals either maintained the antibody titer (30.3%) or became seronegative (45.2%) during the first year of follow-up.
On the other hand, 29 dogs displayed initial titers equal (11 cases) or immediately above (18 cases) the cut-off. Only 8 of these dogs were treated specifically against canine leishmaniasis. Although significant differences were not achieved (p=0.064) with regard to the evolution of the antibody titer, the majority of untreated dogs displaying initial titers of 1/100 maintained the titer or became seronegative (7/10 cases), whereas in most dogs without treatment and initial titers of 1/200 the antibody titer increased (7/11 cases) during the following 12 months.

4 Conclusions

Our results showed that the great majority of the dogs displaying initial titers of 1/50 or 1/100 maintained the antibody titer or became seronegative. However, it was estimated that 25 ± 5.0% of the cases increased the antibody titer during the first year of follow-up. It would be helpful to test these dogs during the first year of follow-up in order to detect seroconversion as soon as possible.
On the contrary, two-thirds of the dogs with initial titers of 1/200 increased antibody titer over the next 12 months. Specific treatment of these dogs should be considered, even in the absence of clinical signs.

C0986 VISCERAL LEISHMANIASIS DURING THE CONFLICT IN SOUTH SUDAN AND THE CONSEQUENCES FOR EAST AFRICAN COUNTRIES

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1 Background

Visceral leishmaniasis (VL), caused predominantly by *Leishmania donovani* and transmitted by both *Phlebotomus orientalis* and *Phlebotomus martini,* is highly endemic in East Africa where approximately 30 thousands VL cases are reported annually. The largest numbers of cases are found in Sudan - where *Phlebotomus orientalis* proliferate in *Acacia* forests especially on Sudan's eastern border with Ethiopia, followed by South Sudan, Ethiopia, Somalia, Kenya and Uganda. Long-standing civil war and unrest is a dominant determinant of VL in East African countries. Here we attempt to identify the correlation between VL epidemics and civil unrest.

2 Methods

In this review, literature published between 1955 and 2016 have been gathered from MSF, UNICEF, OCHA, UNHCR, PubMed and Google Scholar to analyse the correlation between conflict and human suffering from VL, which is especially apparent in South Sudan.

3 Results

Waves of forced migration as a consequence of civil wars between 1983 and 2005 have resulted in massive and lethal epidemics in southern Sudan. Following a comprehensive peace agreement, but especially with increased allocation of resources for disease treatment and prevention in 2011, cases of VL declined reaching the lowest levels after South Sudan declared independence. However, in the latest epidemic that began in 2014 after the onset of a civil war in South Sudan, more than 1.5 million displaced refugees have migrated internally to states highly endemic for VL, while 800,000 have fled to neighboring countries.

4 Conclusions

We find a strong relationship between civil unrest and VL epidemics which tend to occur among immunologically naïve migrants entering VL-endemic areas and when *Leishmania*-infected individuals migrate to new areas and establish additional foci of disease. Further complicating factors in East Africa's VL epidemics include severe lack of access to diagnosis and treatment, HIV/AIDS co-infection, food insecurity and malnutrition. Moreover, cases of post-kala-azar dermal leishmaniasis (PKDL) can serve as important reservoirs of anthroponotic *Leishmania* parasites.

C1000 PREVALENCE OF ASYMPTOMATIC VISCERAL LEISHMANIASIS CASES USING WESTREN BLOT AND REAL-TIME PCR IN AN ENDEMIC AREA IN MOROCCO

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1 Background

Human visceral leishmaniasis (HVL) is a serious parasitic disease, causing high morbidity and mortality in the developing word. The pathogenesis of HVL is complex, and the clinical presentation ranges from asymptomatic infection to severe and fatal disease. There is evidence that in endemic areas of VL only about 20% of the subjects infected by *Leishmania* will develop symptomatic VL. The majority of the infected individuals have a sub-clinical infection that may remain completely asymptomatic. In Morocco, no data about asymptomatic VL are available although they are valuable to complete the epidemiological picture of the disease and help evaluating current control measures.

2 Methods

A total of 130 persons including children and adults were chosen randomly from two rural areas Galaz and Bouadel in Taounate province which is one of the endemic sites for VL. Serum samples were collected and screened using Real-Time PCR (qPCR), Western blot (WB) and Test of the Diangnosis Rapid (TDR) rk30.

3 Results

The prevalence of asymptomatic infection was 74.6%. Thirty six (27, 7%) out of a total of 130 samples come out positive by using Real-Time PCR (qPCR) and western blot analysis with the presence 14 and/or 18 kDa bands,thirty four (26,2%) of these 130 samples were positive only by qPCR and twenty seven (20,8%) samples were positive by WB and negative by PCR, while all samples are found negative by TDR (rk39).

4 Conclusions

Our findings showed that the presence of antibodies against 14 and 18 kDa antigens and presence of the parasite in the peripheral blood are important for the diagnosis of symptomatic and asymptomatic infections. WB and qPCR was found to be effective in the detection of asymptomatic persons in the epidemiological studies in endemic area. It was concluded that asymptomatic VL infections are frequent in Taounate province.

C1020 HUMAN ASYMPTOMATIC LEISHMANIA INFANTUM INFECTION. NEW INSIGHTS IN PATIENTS WITH CHRONIC RHEUMATIC DISEASES TREATED WITH BIOLOGICAL AGENTS AND LIVING IN RURAL AREAS OF NORTHERN ITALY.

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1 Background

Recently, *Leishmania infantum* (Li) began to spread in Italian Northern regions, such as Emilia Romagna (ER). Aim of our study was 1. to assess the level of Li circulant kDNA in patients affected by chronic rheumatic diseases (CRD), treated with biologic drugs (BD) and as a consequence, more susceptible to the parasite; 2. to correlate the positivity to the area of residence; 3. to study the immune response in this subset of asymptomatic carriers.

2 Methods

We retrospectively analyzed PBMC-DNA and sera from both 50 patients affected by CRD, treated with BD for at least 5 years and healthy controls from ER. All the analyzed patients had a CD4/CD8 ratio>2. Each sample was tested with a qPCR for Li kDNA. Cytokines were quantified by a Luminex technology (Bio-Plex, BIORAD Laboratories, Italy). Data analysiswas performed with the Chi Square Test, software Stata (v. 13.1) and Graph Pad Prism (v.5).

3 Results

Among the 50 CRD patients, 18 (36%) resulted positive for Li PBMC-DNA, with high parasite loads (1 to 136 parasite/ml in 4 patients, 1.000 to 40.000 in 11 patients and over 1.000.000 in 3 patients). Positive patients mainly come from rural areas of ER region with a statistically significant difference (SSD) ($p<0.0003$) and displayed higher levels of IL-12p70, IFN-g, IL-2ra, TNF-a, IP-10 cytokines (elevated SSD) and IL-10 and IL-13 (low SSD) compared to healthy controls. All the above cytokines were also up-regulated compared to rheumatic patients negative for Li kDNA, although no SSD was reached owing to the small sample size.

4 Conclusions

qPCR performed on PBMC detected higher DNA rates compared to those found on whole blood in asymptomatic carriers. This may be due to the fact that PBMC are target cells for Li and BD effect may impair the control of the parasitic infection. The statistically significant IL-10 and IL-13 level values are related to a both natural Treg cells or Th-2 response, which supportreactivation of Li in these patients. In contrast, the higher levels of pro-inflammatory cytokines associated to the high CD4+ lymphocyte rate, may strongly amplify the Th-1 response in positive patients with the following disruption of amastigotes and the subsequent protection from the onset of symptoms, despite the detection of high parasite loads. Further studies are needed to increase the sample size and to investigate the kinetics of parasitic infection and immunological response especially in patients under BD in rural areas.

C1029 FACTORS ASSOCIATED WITH TEGUMENTARY LEISHMANIASIS MORTALITY AND LETHALITY IN BRAZIL: TEMPORAL AND SPATIAL ECOLOGICAL MODELING

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1 Background

Tegumentary leishmaniasis (TL) is associated with significant morbidity but as a rule considered a nonlethal condition. Nonetheless, an unexpected high number of deaths related to the disease is reported in Brazil every year. Given the scarcity of studies addressing this issue, the aim of this analysis was to assess temporal and spatial variability in the LT mortality and lethality, besides the association of these rates with several factors.

2 Methods

This is an ecological study based on data retrieved of the Brazilian Diseases Information System between 2007 and 2012. Mortality was standardized by age distribution of the Brazilian population in the middle of the period (2010). Generalized Linear Models (GLM) and Time Series were used to perform temporal analysis. Spatial context factors were explored via GLM and spatial correlation was evaluated by Global and Local Moran indices. Bayesian approach and Integrated Nested Laplace Approximation method were applied.

3 Results

Between 2007 and 2012, 139,254 cases of LT were reported with 108 death, a mortality of 0.078 per 100,000 inhabitants and lethality of 0.078%, both rates remained stable over the period. The population age group presenting the higher incidence was that ranging from 20 to 59 years, most of them are men (63.6%), however, 68% of deaths occurred in patients older than 60 years. Of these fatal cases, 21% were diagnosed without any laboratorial test. The mortality among men was 1.81 times the female rate, while the lethality among women was 1.49 times that observed for men. The median time between the beginning of the treatment and death was 36 days. Fifty-eight per cent of deaths occurred in patients presenting the cutaneous clinical form (CL) and, the other 42%, in patients presenting mucosal leishmaniasis (ML). Interestingly, lethality among ML cases was 11.35 times that one observed with CL cases and lethality for relapsed cases was 2.35 times that observed for "first episode cases". Lethality and mortality were significantly different depending on the Brazilian Federal Unit, allowing the identification of priority areas for control actions aimed at improving the approach and treatment of the disease. In addition, the spatial models highlighted several context factors influencing the variability of LT mortality.

4 Conclusions

Mortality related to LT are a significant public health problem in Brazil. These data represent an alert and may guide future studies aimed at understanding and modifying this reality.

C1053 AN AGENT-BASED MODEL FOR LEISHMANIA SP. TRANSMISSION CYCLE AND INFECTION.

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1 Background

In Algeria, cutaneous (CL) (due to *L. infantum*, *L. major* and *L. killicki*) and visceral leishmaniasis (VL) (due to exclusively *L. infantum*) both co-exist in various areas of the country.
Previous studies identified species, vectors and reservoirs of leishmaniasis, but they remain localized and do not provide a global vision of the epidemiological status of this disease in Algeria. In this study we designed a model of the transmission cycle of leishmaniasis using an "Agent-based Modelling" system in order to better understand the behavior of *Leishmania* species in Algeria.

2 Methods

This study was performed using NetLogo 5.3.1 software (an agent set programming language) to design a model of the transmission cycle of leishmaniasis.
We set up our agents and the total population's number through a literature review according to clinical manifestations (CL and VL forms), parasite species (*L. infantum*, *L. major* and *L. Killicki*), vectors' species (*Phlebotomus (P.) longicuspis*, *P. perfiliewi*, *P. perniciosus*, *P. papatasi* and *P. sergenti*) and reservoirs (Human, Dog, *Canis (C.) aureus*, *Meriones (M.) shawi*, *Psammomys (P.) obesus* and *Ctenodactylus (C.) gundi*) present in Algeria. The code entered allowed Agents to move, carry leishmaniasis, transmit it, to heal, to die…

3 Results

Beside the 3D interface of the program, results obtained are illustrated using "infection counters": graphs that represent the percentage of infection according to the different parameters detailed above.
We found the transmission dynamic of *Leishmania sp* to be rather slow, which we hypothesize is mainly due to the long incubation period and the potentially long persistence of parasites within infected humans.
Taking into account the differences in the number of *Leishmania sp.* / clinic present in the area, *Leishmania sp.* spread in different ways. *L. infantum* (CL) represents a graph with peaks and become the main leishmaniasis infection through time ("ticks"). The treatment of cases had almost no effect on the overall intensity of *L. infantum* (CL) transmission.

4 Conclusions

This artificial model can be a valuable tool to understand role of the agents' cycle and predict the spread of leishmaniasis. Also, this work can be further extended in several ways (simulate other models of *Leishmania sp.* infection especially host-parasite cellular interactions).

C1061 SINGLE LOCUS GENOTYPING FOR MOLECULAR TRACKING OF LEISHMANIA DONOVANI IN THE INDIAN SUBCONTINENT

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1 Background

Visceral Leishmaniasis (VL) in the Indian subcontinent (ISC) is caused by *Leishmania donovani*. The main parasite population in ISC is genetically very homogeneous, because of a recent origin. Therefore, popular molecular tools such as microsatellites are inadequate for distinguishing populations and tracking parasites. Recently, whole genome sequencing (WGS) identified at least ten different genetic groups termed ISC001 to ISC010. We designed a simplified assay, based on single locus genotyping (SLG), to distinguish them. The aim was to develop a tool for following parasite genotypes over time and geographical range, and for studying correlation with clinical outcome.

2 Methods

Prior to this study, 98 clinical *L. donovani* isolates from Nepal collected between 2002 and 2011 were characterised by WGS. For seven of the 10 genotypes found, we identified a unique homozygous single nucleotide polymorphism (SNPs) or indel (insertion/deletion). Subsequently, we developed 7 PCR assays to allow for dideoxynucleotide sequencing of each polymorphic position. Using this technology, named ISC-SLG, we determined the genotype of 106 additional strains collected between 2011 and 2014. The genotypes were analysed in function of geographical origin of the patient, time of diagnosis, and treatment outcome.

3 Results

ISC-SLG identified the genotype of 59% of the strains, but 41% could not be linked to any of the genotypes investigated. Combined with WGS data, the most predominant genotype ISC006, was found throughout the sampling period, while other genotypes were absent from some sample years. Genotype ISC001 was predominantly found in hilly districts. Treatment failed consistently in patients treated with Sodium Stibogluconate and infected with ISC005 parasites. Two out of nine treatment failure patients showed a different genotype before and after relapse.

4 Conclusions

We provide a proof-of-principle that ISC-SLG allows to distinguish *L. donovani* populations, and highlight its potential for epidemiological follow-up of VL transmission in the post-elimination phase in the ISC. Further development of this assay includes the identification of the isolates that could currently not be typed, as well as the translation for direct application to clinical samples.

C1093 EPIDEMIOLOGICAL ASPECTS OF VISCERAL LEISHMANIASIS IN AN ENDEMIC AREA IN NORTHEAST OF BRAZIL.

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1 Background

Visceral Leishmaniasis (VL) is an infectious disease widely endemic in Brazilian territory. Brazil presents the highest VL prevalence among American countries. In the last years, changes have been observed regarding to epidemiological profile of VL, as increasing on the prevalence, especially in urban and peri-urban area. Studies analyzed the environmental factors that can lead to increased risk for *Leishmania* infection; the epidemiological factors include presence of dogs. The Brazilian northeast, as state of Sergipe, is considered a region with elevated risk of VL infection and hence requires priority attention. Thereat, this study aimed to analyze the epidemiological aspects of human VL in state of Sergipe, Brazil.

2 Methods

Data were obtained from SINAN (the Brazilian notification of injury information system) and from information bank about leishmaniasis of the Health Secretary from state of Sergipe and Aracaju (Sergipe capital). We performed descriptive analysis and calculated the incidence coefficients and distribution frequency. For statistical analysis, we used the chi-square test by GraphPad Prism 7.

3 Results

We identified 406 cases of human VL, among 2008 and 2014, in the state of Sergipe. While in Aracaju city were notified 159 cases (39.16% from total of patients), being the city responsible for the majority of VL cases reported in Sergipe. In that same years, there were confirmed 10 deaths due to VL infection in this state, being tree in Aracaju. Moreover, the incidence coefficient (IC) of VL in 2014 was higher in Aracaju (0.5 per 10.000 inhabitants) than in Sergipe (0.3 per 10.000 inhabitants). Correlatively, the frequency of VL in dogs remained stable from 2008 to 2012 and increased significantly in 2013 and 2014 in Aracaju.

4 Conclusions

The results showed an increase of VL in state of Sergipe and Aracaju among 2008 to 2010. These areas remain, thus, endemic for human and dogs VL. Moreover, the exponential increase of VL cases in Aracaju suggests that the disease is in expansion and urbanization process. Our data provide that Aracaju has presented a worrisome scenario regarding to VL epidemiology. It reinforces the requirement of improvements in intervention strategies to VL control in Aracaju.

C1121 IDENTIFICATION OF VULNERABLE MUNICIPALITIES FOR THE TRANSMISSION OF VISCERAL LEISHMANIASIS IN THE STATE OF RIO DE JANEIRO, BRAZIL

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1 Background

The Brazilian Control Program of Visceral Leishmaniasis (VL) classifies municipalities by the average number of human cases in the last three years, with specific control actions recommended for each category. Rio de Janeiro State (RJ) has small numbers of human VL cases; but it should not be neglected due to the high number of infected dogs, mortality and urbanization of the disease. Taking preventive measures in areas without VL is crucial to avoid its spread. This study aims to identify vulnerable municipalities of RJ and guide future entomological surveys, by mapping the spatial distribution of the disease (human and canine) and of its vector, *Lutzomyia (Lutzomyia) longipalpis*.

2 Methods

Occurrence of *L. (L.) longipalpis*, human and canine cases of VL were obtained from the National Information System of Notifiable Diseases, from the Health Department of Rio de Janeiro State and from the literature. The data were integrated in a Geographic Information System and analyzed for distribution of VL and identification of vulnerable municipalities, those were further classified as receptive (with records of *L. (L.) longipalpis*), non-receptive (without records of the vector) or pending entomological investigation.

3 Results

In 2001-2015, human VL occurred in 23 of the 92 municipalities of RJ. In the last three years, only 7 municipalities had records; all classified as sporadic transmission (Barra Mansa, Rio de Janeiro, Paraty, Volta Redonda, Angra dos Reis, Macaé and Teresópolis); 2 municipalities have records of canine VL since 2007, but without human cases (Mangaratiba and Maricá); 21 vulnerable municipalities were identified, with 4 being receptive, 3 non-receptive and 14 pending entomological investigation.

4 Conclusions

Visceral Leishmaniasis transmission in RJ is currently sporadic. Approximately 60% of RJ municipalities have no information on sand flies, which shows a clear need of entomological studies. Among the 54 municipalities pending entomological investigation, 14 are vulnerable and must be prioritized, because they are close to municipalities with recent VL transmission. Upon detection of the vector in vulnerable municipalities, the recommended control actions are: environmental management and canine survey for early detection of VL cases. Financial Support: Papes VI 407707/2012-3, CNPq: 446274/2015-1, FIOCRUZ/ Plano Brasil Sem Miséria/CAPES, IOC/FIOCRUZ.

C1123 TRENDS IN THE PRESENTATION AND CLINICAL COURSE OF TEN CHILDREN WHO SUCCUMBED TO VISCERAL LEISHMANIASIS IN TERESINA, BRAZIL FROM 2013-2015

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1 Background

Over the past decade, urbanization has expanded the endemic distribution of Visceral leishmaniasis (VL) throughout Brazil. While multiple factors have been linked to VL fatality, there is a dearth of information on which risk factors are associated with poor prognosis in the pediatric population. With children under 10 representing 48.9% of annual fatalities in Brazil, it is important to identify pediatric transmission patterns, especially in urban areas.

2 Methods

This observational retrospective study at IDTNP (Teresina, PI, Brazil) looked at pediatric case fatalities from VL diagnoses. Ten patients between the ages of 4 months and 11 years with a positive diagnostic marker for VL who died were included. Paper medical records from June 2013 to November 2015 were analyzed for patient's background, symptom evolution, treatment, timeline, and cause of death.

3 Results

Of the 10 patients studied, 8 were under 2 years of age and 8 were from urban areas. Average symptom duration prior to hospitalization was 30.5 days, with fever being the predominant symptom (20.3 days). Patients were hospitalized for an average of 5.26 days. Once admitted, 70% of patients had fever for an average of 2.3 days. The clinical presentations included: fever (100%), distended abdomen (100%), pallor (80%), hepatosplenomegaly (80%), emesis (60%), irritability (60%), edema (50%), and altered breathing patterns (50%). Lab values included: anemia (100%), thrombocytopenia (100%), leukopenia (100%), and neutropenia (50%). 8 patients received amphotericin B liposomal with an average treatment length of 3 days. Antibiotics were given to 8 patients with an average treatment length of 4.25 days. 9 patients received transfusions of plasma, platelets and/or RBCs. Causes of death were infection (40%), hemorrhage (30%), sepsis and shock (30%), and pancreatitis (10%). Lack of hospital resources, personnel or ICU beds (70%) negatively impacted timely treatment.

4 Conclusions

Fever, distended abdomen, anemia, thrombocytopenia, and leukopenia had the highest predictability of fatality. This data suggests that the time delay from symptom onset to treatment initiation, often limited by resource availability, was a more important contributor to fatality than treatment choice. Furthermore with 80% of patients from urban areas, this study proposes that there may be an association between urbanization and lack of available resources hindering optimal treatment for severe pediatric VL cases.

C1140 GEOGRAPHIC DISTRIBUTION OF LEISHMANIA SPECIES IN COLOMBIA USING PCR-RFLP OF HSP70 GENE AS TYPING TOOL

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1 Background

Leishmaniasis is an endemic disease in most of the Colombian territory. In the last decade, deforestation and human intervention and migration into sylvatic regions has caused changes in the transmission cycle of Leishmania parasite in our country. According with the last, it is necessary to characterize the distribution of Leishmania species in Colombia.

2 Methods

This study included 688 clinical samples from patients with confirmed diagnosis of leishmaniasis: 431 isolates, 76 aspirates, 52 biopsies and 129 smears. Samples came from 28 of 32 departments in Colombia and were collected between 1999 and 2016 by Centro Dermatológico Federico Lleras Acosta, a referral center for leishmaniasis in Bogotá. 93.2% of the samples were from patients with the cutaneous manifestation of the disease whereas 6.8% were from patients with mucosal leishmaniasis. Typing was performed using restriction fragment lengh polymorphisms (RFLP) of mini-exon and *hsp70* genes. Distribution maps of Leishmania species were created using ArcGIS 10.3 software.

3 Results

Typing results showed as predominant species: *L. (V.) panamensis* (52,9%), *L. (V.) braziliensis* (40,7%) *L. (V.) guyanensis (*4,8%) and *L. (L.) amazonensis* (1,6%). *L. (V.) braziliensis* was identified as the main etiological agent of mucosal leishmaniasis (44/47 cases). Distribution maps showed presence of *L. (V.) panamensis* in the western of the country and *L. (V.) braziliensis* at the southeastern of Colombia. In the center valley formed by the mountain chains which crosses the country from southwest to north, we can found presence of two to four species of Leishmania, particularly *L. (V.) panamensis/ L. (V.) braziliensis.* Considering the zone of influence of the referral center, the municipalities with the most number of typified samples are in the central area of the country (Andean Region) and shows a typical ecosystem of tropical moist forest, with an average altitude of 905 m.s.n.m., mean annual temperature of 23,6°C and mean annual precipitation of 2450 mm.

4 Conclusions

Seeing the association between species, clinical manifestation and response to treatment, and the difficulties in typing Leishmania species around Colombia, the knowledge of this geographic distribution could be used as an indirect method to correlate the species which is causing the infection with the epidemiological provenance of the patient.

C1200 SUSCEPTIBILITY OF DOG TO VISCERAL LEISHMANIOSIS AFTER BEEN SUBMITTED TO VACCINATION OR THE USE OF AN INSECTICIDE COLLAR

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1 Background

Visceral leishmaniosis (VL) is a zoonotic disease caused by protozoan from the *Leishmania* genus. In Brazil it is transmitted by the female sand fly's bite *Lutzomyia longipalpis*, and has the dog as its main urban reservoir. To evaluate the effectiveness of using either the Leishmune® subunit vaccine, or the Leish-Tec® recombinant vaccine, or the Scalibor® dog collar, or the combination of these measures, a cohort study was previously performed in dogs from a VL endemic urban area (Panorama, SP, Brazil), from 2012 to 2013. This study showed highest effectiveness of the Scalibor® dog collar to prevent dog infection. The aim of the present study was to evaluate the dynamics and current susceptibility to VL of these dogs that took parte in the previous study group, two years after the last intervention.

2 Methods

The residences where the dogs from the previous study use to live were visited and biological samples were obtained (n=105) to evaluate the presence of antibodies anti-*Leishmania*, by the immunochromatographic test TR-DPP® and confirmed by EIE-ELISA® (Bio-Manguinhos), performed according to the manufacturer guidance. Fisher's exact test was performed by BioEstat 5.0. program.

3 Results

None of the owners reported to have continued applying any individual control measure used in the previous study. Among the dogs from the previous study, 31.6% (88/278) of the dogs were lost due to death or compulsory euthanasia, moved to other places 25.17% (70/278) and only 37.8% (105/278) were found alive and could be evaluated. Thus, one of the limitations of this study was the small number of dogs within each group. The overall positivity of the dogs to VL was 18.1% (28.6% positive to DPP and confirmed by ELISA 21%) and it was higher in dogs that had received the Leishmune® vaccine (42.9%), followed by the dogs that got this vaccine associated to the Scalibor® dog collar (23.5%), although no significant differences between groups was observed by the Fisher's exact test. Surprisingly, dogs of the control group showed the smallest positivity percentage.

4 Conclusions

Measures of individual protection against VL in dogs (vaccination or dog insecticide collar) are efficient if applied properly and continuously.

C1221 EPIDEMIOLOGY AND BURDEN OF CUTANEOUS LEISHMANIASIS IN SUB-SAHARAN AFRICA: EVIDENCE FROM A SYSTEMATIC REVIEW

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1 Background

Cutaneous leishmaniasis (CL) is the most common form of leishmaniasis, with 0.7 to 1.2 million cases per year globally. However, the burden of CL in sub-Saharan Africa is poorly documented. We carried out this review to assess the state of knowledge of CL epidemiology and ascertain its unique features in this region.

2 Methods

We systematically searched PubMed, CABI Global health, Africa Index Medicus databases. There were no restrictions on language/publication date. Case series with <10 patients, species identification studies, reviews, non-human and non-CL focused studies were excluded. Findings were extracted and described. The review was conducted based on PRISMA guidelines, the protocol was registered in PROSPERO (42016036272).

3 Results

From 387 identified records, 53 met eligibility criteria and were included in the qualitative synthesis. CL epidemiology was reported from 13 of the 48 sub-Saharan African countries (3 eastern Africa, 9 western Africa and 1 from southern). More than half (29/53) were from western Africa, notably Senegal (6), Burkina Faso(5) and Mali(3). All studies were observational: 29 were descriptive case series (total 13,257 cases),24 followed a cross-sectional design. 42/53 studies were carried out before the year 2000. The prevalence in hospital settings among suspected cases ranged between 0.1-14.2% and may not reflect the true burden. At community level, based on screening of lesions and/or scars, prevalence varied widely between studies. Outbreaks of thousands of cases occurred in Ethiopia, Ghana and Sudan (n=6). Polymorphism of CL in HIV-infected people is a concern (n=6): a 4.8% co-infection rate reported in Cameroon. Parasitological diagnosis is mentioned in 42/53 studies, but the parasite was either assumed or based on historical account. Regional differences exist in clinical manifestations. We found high variability across methodologies, leading to difficulties to compare data. Key data gaps in CL burden here include population-based CL prevalence/incidence, risk factors, economic cost, and surveillance.

4 Conclusions

The evidence on CL epidemiology in sub-Saharan Africa is scanty. Though considered as a high burden neglected disease, the epidemiology is poorly established. There is a need for population-based studies to better define the CL burden. Endemic countries should consider research and action to improve burden estimation and essential control measures including diagnosis and treatment capacity.

C1234 SEROPREVALENCE OF LEISHMANIASIS AND TRYPANOSOMIASIS IN EQUINE "BAIXADEIRO" FROM AMAZON PANTANAL, MARANHÃO STATE, BRAZIL.

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1 Background

Baixada Maranhense is located in the northern mesoregion of Maranhão state, Brazil. It is formed by low plains that flood in the rainy season, creating huge ponds between the months of January and July, because of this ponds, it is also called Amazon Pantanal. In this region, a breed of native horses called "baixadeiros" stands out. The main species responsible for trypanosomiasis in equines are *Trypanosoma equiperdum* and *Trypanosoma evansi*, the last is endemic in Mato Grosso state Pantanal, a region with similar environmental conditions to Amazon Pantanal. American cutaneous leishmaniasis in horses has been reported in Brazil since the 1950s, but there is no a characteristic clinical pattern. Regarding visceral leishmaniasis, seropositive animals were found in endemic areas, but the role of horses as reservoir was not fully elucidated. This study aimed to determine the seroprevalence of trypanosomiasis and leishmaniasis in "baixadeiros" horses.

2 Methods

A total of 138 blood samples were collected from 2011 to 2013 from "baixadeiro" horses from Amazon Pantanal. For the detection of anti-*Leishmania* spp. and *Trypanosoma* spp. antibodies, the immunofluorescence antibody test (IFAT) and the immunoenzymatic assay (ELISA) were performed. Samples with titers greater than 40 and cutoff greater than 0.3175 and 0.3270 absorbance, respectively, were considered positive.

3 Results

For *Leishmania* spp., 25.4% (35/138) were reagents in the ELISA, 20.3% (28/138) in the IFAT and 5.8% (8/138) were reagent in both. For *Trypanosoma* spp. 45.9% (62/138) were reagents in the ELISA and 10.8% (15/138) in the IFAT. Statistical association ($p = 0.0034$) was observed for *Trypanosoma* spp. and *Leishmania* spp. using the ELISA technique, indicating a cross-reaction between the parasites or a possible co-infection. The cross reaction between the species of the family Trypanosomatidae is quite common, besides being phylogenetically close, there is also the possibility of culturing these agents to leave their promastigotes less mutable and with less antigenic difference.

4 Conclusions

The results suggest that this horse breed is in contact with parasites that cause leishmaniasis and trypanosomiasis, demonstrating the need for a more accurate investigation of the real role of horses in these diseases, in order to assist in disease control measures, especially in places where the environment favors the proliferation of their vectors.

C1240 SURVEY OF PREVALENCE AND INCIDENCE OF AMERICAN CUTANEOUS LEISHMANIASIS IN TWO ENDEMIC REGIONS IN PERNAMBUCO STATE-BRAZIL.

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1 Background

The American Cutaneous Leishmaniasis (ACL) is a parasitic disease and considered a neglected disease in the New World. It is caused mainly by *Leishmania (Viannia) braziliensis* and it affects the skin and mucous membranes. The disease, in their all clinical forms, affect 12 million of people in the World, with an annual incidence of 1,5 to 2 million of new cases. More than 350 million of people are under risk to get the disease. This study verified the prevalence of the disease and the force of infection on human population of two endemic regions from Pernambuco State, Brazil.

2 Methods

The study was carried out in 2 different endemic regions of *Zona da Mata* of Pernambuco (Brazil): Amaraji (Mata Sul) and Vicência (Mata Norte). The cases were recruited by active search. A survey by the Montenegro skin test were applied in all patients. In order to differentiate new cases (incidence) from old cases (to calculate prevalence) and to detect the disease in immunosuppression patients, real time PCR (qPCR) was performed in blood samples from them. The qPCR used the target kDNA *Leishmania (Viannia)* spp.and the set of primers was B1 and B2. The DNA from blood samples were extracted using a commercial kit (Qiagen®). The cases were incidente when only skin test was + with no treatment nor lesion.This study has ethical approve by Centro de Pesquisas Aggeu Magalhães/FIOCRUZ Ethical Committee.

3 Results

The total of population that study investigated to leishmaniais was 70 patients with age between 0-67 years (median=23) in Amaraji and 265 in Vicência with age between 1-71 (median=23). The incidence of ACL in Amaraji in 2014 was 10% and the prevalence was 20%. Vicência had incidence of 9,1% and prevalence of 43%. The majority of patients in Amaraji were male (40%) and in Vicência was the opposite, female was 56,5%. Preliminary results comparing qPCR and skin test are shown on table 1.

Results of qPCR in blood * qualitative Skin test			
		Qualitative skin test	
		+	-
qPCR in blood	+	19	19
	Undetectable	0	1
Total		19	20
Locality - Vicência Sensitivity=100%(83.2-100);Specificity=5%(0.9-23.6)			

4 Conclusions

The results of incidence and prevalence show that the two regions studied remain important rates of infection by *Leishmania* (*Viannia*) *braziliensis* in residents of locality. Therefore, early diagnosis and appropriate treatment is extremely important to prevent transmition and infection. More studies with qPCR are needed.

C1254 EPIDEMIOLOGY OF VISCERAL LEISHMANIASIS IN KARAMOJA SUB-REGION, NORTH EASTERN UGANDA

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1 Background

Visceral leishmaniasis (VL) or kala-azar (KA) is caused by protozoan parasites which are transmitted by bites of infected sand flies. KA is fatal if not treated and is endemic in certain foci where it affects mainly pastoralist communities in north eastern Uganda. Its epidemiology in the region is not well understood yet it is important to know for control. A retrospective study was conducted with emphasis on the epidemiology during 9 years (2008-2016), at Amudat hospital, the only treatment, research and training centre in Uganda located in KA endemic area.

2 Methods

Data of patients treated between 2008-2016 (9 years) at Amudat hospital was used to analyze the following variables; number of patients treated, age, sex, disease distribution and seasonal variation.

3 Results

KA patients (948) were diagnosed and treated between 2008-2016. Average number of cases was 105.3/year. Highest number of patients was in 2015 (148) and lowest in 2009 (72). Majority of patients were below 14 years(62 %) with those below 4 years comprising 10%, the youngest was 6 months old. When those below 28 years were included in the analysis, the percentage increased (88%). More males (74%) compared to females (26%) were affected. One case of PKDL and one VL/HIV co infection were observed over the period. Out of 7 districts in Karamoja sub region, KA was reported from; Amudat (33%), Moroto and Napak (28%), Kotido (13%), Kaabong (10%), Nakapiripirit (8%) except Abim district. Some cross border cases came from Turkana region, Kenya (8%). Generally there was no clear pattern of wide variation between dry and wet seasons with regard to high or low patient numbers (Mean SD: 12). However, the highest total number of patients treated over the 9 years was in July (108/948; mean numbers: 12; SD: 48) while the lowest total number was in September (67/948; mean numbers: 7; SD: 10).

4 Conclusions

Results confirm active transmission of KA in Karamoja sub-region and the disease extending across the border to Kenya. Furthermore it suggests the presence of parasites and vector(s) in the region. The epidemiology follows an infantile pattern with more males being affected than females. There was apparently no clear pattern of variation during the dry and wet seasons with regard to high or low patient numbers. Nevertheless, the highest and lowest numbers of patients treated over the 9 years were in July and September respectively. Kala-azar is more widespread in Karamoja sub-region than originally thought.

C1275 SPECIES COMPOSITION OF PHLEBOTOMINE SAND FLIES AND INFECTION OF MAMMALS WITH LEISHMANIA SPP IN THE VICHADA DEPARTMENT, COLOMBIA.

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1 Background

The Vichada Department is a Colombian area with great landscape anthropization as consequence of several projects related with the agro-industry. Because of this, there is increased migration of people to this department to cope with the high workforce demand required in the agriculture trade. This could promote the occurrence of different parasitic disease foci. Inside Vichada, historically many Leishmaniasis cases have been reported in the municipality of Cumaribo associated with illegal crops. However, in Santa Rosalia and La Primavera, areas with profound ecological change, there is little knowledge on the biological elements involved Leishmaniasis transmission risk. The aim of this study was to assess the presence of phlebotomine fauna and the epidemiological role of mammals in two municipalities Vichada, Colombia

2 Methods

The study was conducted in March of 2016. Phlebotomines were collected during five consecutive nights, using CDC light traps. Each night, a total of 10 traps were placed inside houses and peridomestic area. Phlebotomines were kept in vials containing 70% ethanol until mounting and identification. Transects with 15 Tomahawk® traps were set over five-night period. Mammals were anesthetized for sample collection and inspected for Leishmania ssp by skin and blood cultures and molecular tools. In addition, 55 samples of domestics dogs were tested by Leishmania ssp infection with IFAT.

3 Results

A total of 291 phlebotomine sand flies belonging to 8 species and 4 genera were collected. *Nyssomia antunesi* was the only species with epidemiological importance. Three new species (*Ev. (Ald) serícea*, *Ev. (Eva) georgii*, *Sc. sordelli* are reported for the Vichada Department and *Ps. squamiventris squamiventris* is reported for first time in Colombia. Eight synanthropic mammals were captured (7 Didelphis marsupialis and 1, *Proechomis sp)*; however, none were positive for leishmaniasis. The canine Leishmania spp seroprevalence was of 53.7 % (29/55). However, complementary test as ELISA for Leishmania spp and IFAT and ELISA for *T. cruzi* should be performed in order to discard cross-reaction or co-infection.

4 Conclusions

This study suggests that *C. familiaris* is a potential reservoir in domestic transmission cycle of *Leishmania* spp. Furthermore, *D. marsupialis* does not appearto have epidemiological importance. Finally, the presence of *Ny antunesi* and infected dogs demands the implementation and maintenance of epidemiological surveillance programs for these two municipalities.

C1285 LYMPHATIC FILARIASIS AS A RISK FACTOR FOR KALA-AZAR IN BIHAR, INDIA

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1 Background

The outcome of infection with *L. donovani* depends on the host immune response. The host immune response in turn could be influenced by other factors, one of which could be co-infection with other pathogens. Earlier we have documented a possible association between clinical visceral leishmaniasis (VL) and lymphatic filariasis (LF). As part a household survey conducted in 2013, we asked for presence of elephantiasis or hydrocele among members of households enrolled. Reported prevalence was 5.1% among the population but 10.4% among those who had suffered from VL since January 2007 (RR 2.1, 95% CI 1.5-3.0). In 2015 we initiated a case control study with the aim of further investigating this association.

2 Methods

Between June 2015 and November 2016, we enrolled 289 VL cases registered at the KAMRC treatment center in Muzaffarpur, Bihar, India. For each patient we also enrolled one village matched control. Cases and controls were tested for LF infection with the ICT filariasis test. We also recorded age , gender and infections with soil transmitted helminths. A conditional logistic regression model was fitted to quantify the association between LF and VL

3 Results

On univariate analysis we found associations between ankylostoma and VL and between ascaris and VL but not on multivariate analysis. In the final model we retained age group, gender and the factor of interest, LF infection. The association with age group is in all probability an artifact because controls were selected among those accompanying patients, most of whom were adults whereas many patients were children. LF infection was more common among VL patients (OR 1.4) but the association was not statistically significant (p=0.16).

4 Conclusions

Although we did find a significant association between VL and a proxy indicator of LF, i.e. presence of hydrocele or elephantiasis, we could not confirm this in a case control study involving 289 VL cases. The association appears to be weak, if at all present.

C1286 DISTRIBUTION, ABUNDANCE AND INFECTION OF SANDFLIES WITH LEISHMANIA PARASITES IN THE DEPARTMENT OF CORDOBA, COLOMBIA.

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1 Background

Leishmaniases include a spectrum of diseases caused by the flagellate protozoan parasite *Leishmania*, an obligate intracellular parasite transmitted to humans and other mammals by the bite of a female Phlebotomine sand-fly. In Colombia, nine *Leishmania* parasite species have been reported and 14 species of sandflies have been proven as vectors making it one of the three Latin American countries with the highest number of species. The aim of this study was to determine the presence and distribution of sand-fly species and identify their infection percentage in the Department of Cordoba, Colombia.

2 Methods

The study was developed in 12 different townships of the department of Cordoba, Colombia. In each township 24 houses were selected for sampling, where two mini CDC light traps were placed in the intra and peridomicile from 18:00h to 06:00h. Captured sandflies were preserved in 70% ethanol and taken to the lab for specie identification following taxonomic identification protocol described by Young & Duncan 1994 and Galati 2013. Molecular biology assays were performed in order to identifyinfected species. Conventional PCR using genetic targets HSP-70 and ITS-1 was used to identify the presence of the parasite followed by High Resolution Melting for *Leishmania* species identification.

3 Results

In total 18,664 Phlebotomine sandflies were captured. Fourteen Phlebotomine sand-fly species were recorded, with the most abundant species being *Pintomyia evansi* (72.15%), *Micropygomyia cayennensis* (9.39%), *Lutzomyia gomezi* (7.29%), *Psychodopygus panamensis* (4.62%), *Evandromyia dubitans* (3.15%) and *Pintomyia rangeliana* (1.54%). Molecular biology assays were performed in 8,653 females with Leishmania infection being detected in 5.79% of them. Leishmania species identified were as follows: *Leishmania infantum* present in 11.5% of the processed *Pi. evansi; Leishmania panamensis* present in 7.7% of the *Lu. gomezi*, 3.3% de las P*s. panamensis* y 2.2% de las *Pi. rangeliana* and finally *Leishmania guyanensis* present in *1.72%* of the *Mi. cayennensis.*

4 Conclusions

The township San Andres de Sotavento presented the highest number of captures with 75,5% followed by Sahagun and Moñitos. Furthermore, San Andres de Sotavento was also the township with the highest percentage of infection with 64.3% of the female sandflies analyzed being from this site. *Leishmania panamensis* was detected in sandfly species of the townships of Los Cordobas and Valencia.

C1311 VISCERAL LEISHMANIASIS IN A LARGE URBAN SETTING OF BRAZIL: AN ECOLOGICAL STUDY WHIT SPATIAL ANALYSIS

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1 Background

Visceral Leishmaniasis (VL) is a Neglected Tropical Disease responsible for the morbidity and mortality of thousands of people in several countries, especially in developing nations. Historically, VL was characterized in Brazil as a rural endemy, but the disease suffered an epidemiological transformation by spreading to urban areas of the country. Reports of the occurrence of VL in large urban centers have became increasingly more frequent as shown by studies conducted in the southeastern metropolitan areas of São Paulo, Rio de Janeiro and Belo Horizonte. VL is considered one of the main endemic diseases of the Belo Horizonte, being found widely distributed in all regions of the city. This study proposes to analyze the distribution of human and canine VL in the period of 2007-2012 in the municipality of Belo Horizonte and to verify the possible existence of correlation between the cases.

2 Methods

This is an ecological study carried out with secondary data from the Municipal Health Department of the Belo Horizonte city. For the mapping, the confirmed cases of human LV obtained from the Information System of Notification Diseases (SINAN) and the cases of seroreagent dogs diagnosed in census and sample serological survey in the period 2007-2012 were used. The spatial units used for analysis were the coverage areas of the 149 Basic Health Units of the city of Belo Horizonte. The spatial analysis of human and canine cases was performed with Arcgis® software version 10.0 (Redlands, Ca, USA). Kernel tools were used to determine the density of canine cases and the directional ellipse to identify clusters and direction of human and canine events.

3 Results

A total of 665 human cases of VL were mapped according to the year of occurrence. A total of 37,569 dogswith positive serology for LV were also mapped according to the year of diagnosis for the production of density maps and standard deviation ellipses. LV was widely distributed throughout the municipality and the canine and human ellipses presented similar overlap and direction.

4 Conclusions

The human and canine LV is distributed in all regions of the city of Belo Horizonte. The results presented herein will enable the Municipal Health of Belo Horizonte to devise a more effective management plan for VL in which specific strategies would be applied to areas presenting different levels of risk.

Keywords: Visceral Leishmaniasis, Spatial analysis.
Support: FAPEMIG and CNPq.

C1339 TRENDS OF LEISHMANIA INFECTION BY QUANTITATIVE PCR IN ASYMPTOMATIC SUBJECTS LIVING IN THE AREA ENDEMIC FOR VISCERAL LEISHMANIASIS

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1 Background

Studies employing serological, DTH or conventional PCR techniques suggest a vast proportion of Leishmania infected individuals living in regions endemic for Visceral Leishmaniasis (VL) remain asymptomatic. This study was designed to assess whether quantitative PCR (qPCR) can be used for detection of asymptomatic or early Leishmania donovaniinfection and as a predictor of progression to symptomatic disease.

2 Methods

The study included 1057 healthy individuals living in endemic region (EHC) including both serology-positive and negative subjects who were followed up to 2 years. TaqMan based qPCR assay was done on peripheral blood of each subject using kDNA specific primers and probes.

3 Results

We found that 319/1057 (30.17%) EHC population was positive with qPCR at the base line and among them 39 (3.69% of 1057 subjects) had more than 1 calculated parasite genome/ml of blood. During follow-up in the next year, 905 blood samples were collected, and 346/905 (38.23%) were found positive by qPCR containing 45 (4.97% of 905) EHCs having more than one parasite genome/ml. Importantly, no significant qPCR positivity were detected in seroconverters compared with its matched sero negative controls during 2 years

4 Conclusions

Our findings suggest that qPCR could be used to identify the infections at very low levels of parasitemia that can be used as molecular tools for VL elimination and post elimination surveillance program.

C1397 LEISHMANIASIS: EXPERIENCE OF A TERTIARY HOSPITAL IN ENDEMIC AREA.

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1 Background

Leishmaniasis is an infectious disease caused by Leishmania protozoa, transmitted by the bite of phlebotomine sandflies. It causes a spectrum of clinical syndromes, of which the most common are cutaneous (CL) and visceral leishmaniasis (VL). Clinical presentation is highly variable and is dependent on multiple factors, such as Leishmania species and patient characteristics

2 Methods

This study was conducted in the infectious diseases department of Monastir hospital from January 2006 to November 2016 during which 34 cases of Leishmania were diagnosed and managed.

3 Results

We collected 34 cases of leishmaniasis including 29 cases of CL (85%) and 5 cases of LV (15%). There were 24 women and 10 men (sex ratio = 0.4). The average age was 44 years (19-78 years). For comorbidities, we found three diabetic patients, two patients with corticosteroid therapy and one patient with systemic lupus. The majority of diagnoses were reported during the winter in 16 cases (47%), with an average delay of 14 weeks. The mean duration of hospitalization was 22 days. For LC, the different localizations were: lower limbs (18 cases), superior members and face (9 cases each). Peri-articular location was noted in 7 cases. The mean number of lesions was 3 (1-6).Tthe diagnosis was confirmed by the analysis of different clinical samples depending on the disease form: serum, bone marrow, peripheral blood and skin biopsy. Direct examination with microscopy in 23 cases (68%), PCR in 13 cases including 5 cases of bone marrow PCR , Serology in 3 cases and histology in 2 cases. Glucantime was prescribed in 29 cases (85%) and metronidazole in 3 cases. The mean duration of treatment was 14 days (2-21 days). Adverse effects were observed in 12 cases (35%), including 10 cases of stibio intolerance and 6 cases of stibiotoxicity. Stopping treatment was imposed in 11 cases. The treatment was replaced by metronidazole in 4 cases and Amphotricin B in 3 cases. The evolution was favorable in all cases.

4 Conclusions

Our contry is endemic for leishmaniasis. The cutaneous form is managed in the first and second line structures. However the visceral form and the cutaneous necesiting a systemic treatment are managed in the tertiary hospitals.

C1403 INTRANATIONAL CONFLICT, INTERNATIONAL HEALTH: AN OUTBREAK ANALYSIS OF CUTANEOUS LEISHMANIASIS IN THE LEVANT FOLLOWING REFUGEES FROM THE SYRIAN CIVIL WAR

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1 Background

The Syrian Civil War has had a powerful impact on the healthcare landscape in the Levant. The therapeutic geographies of the region have changed dramatically due to the conflict, with hospitals destroyed, doctors killed or unable to practice, and millions of refugees fleeing the war placing an enormous burden on the healthcare systems in the region. The movement of refugees from Syria into Lebanon has also given rise to an increase in the incidence of disease. Leishmaniasis cases increased from 0-6 cases/year between 2000 and 2012, to 1033 cases in 2013. Despite taking in a similar number of Syrian refugees, Jordan did not see a similar increase in leishmaniasis cases in the same period. A comparison of clustered analyses between these two countries between 2012 and 2015 could indicate why such a disparity exists.

Classified by the WHO as one of the world's most neglected diseases, leishmaniasis is caused through vector contact with the phlebotominae sand fly. The most prevalent form in the region, cutaneous leishmaniasis, is treatable with a course of intralesional pentavalent antimonials. Access to healthcare and the cost of treatment can be prohibitive in circumstances where 50% of refugees cannot afford healthcare and only 37% have any NGO assistance. Understanding leishmaniasis and its relation to conflict and refugee populations is necessary to prevent further outbreaks.

2 Methods

The purpose of this study is to spatio-temporally define the outbreak of leishmaniasis in Lebanon as compared with Jordan to establish any relationship between leishmaniasis cases and the influx of refugees from Syria. Both the Lebanese Classical Reporting System and the Jordanian Public Health Surveillance Program have identified cutaneous leishmaniasis as a mandatory reportable disease and data for cases is publicly available. A discrete Poisson model will be used in SaTScan to analyze spatial and temporal clustering of high rates of cutaneous leishmaniasis cases in Lebanon and Jordan at the district level.Statistical regression analyses will be used to relate clustered analyses to housing, education, poverty, and environmental factors to account for possible associations.

3 Results

We expect to see differences in leishmaniasis cases in the spatio-temporal patterns in Lebanon and Jordan. Final results will be projected in Esri ArcGIS.

4 Conclusions

Programmatic and healthcare landscape differences will be used to indicate a rationale for the incidence disparity.

C1416 SURVEY OF SEROLOGICAL TESTS FOR VISCERAL LEISHMANIASIS IN DOGS EXAMINED AT A VETERINARY DIAGNOSTIC LABORATORY IN NITERÓI, RIO DE JANEIRO, BRAZIL BETWEEN 2013 AND 2016.

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1 Background

Despite the low number of canine visceral leishmaniasis (CVL) notifications in the state of Rio de Janeiro, there has been an increase in the number of reported cases in recent years, although the number of tests made for CVL is still small in the region. The present study aimed at performing a survey of the tests for CVL carried out in a veterinary diagnostic laboratory that examines animals from the oceanic region of Niterói and Maricá, Rio de Janeiro, Brazil.

2 Methods

The survey used data filed between July 2013 and November, 2016 of animals living in the region and that had at least one positive serological test for CVL (Elisa, Indirect Immunofluorescence (IIFT) and immunochromatographic-TR-DPP® test).The results were expressed in absolute and relative values.

3 Results

Diverse hematological examinations were performed in 68,340 dogs during that period. Of these, 287 (0.42%) animals fulfilled the inclusion requirements of this study, being 37 in 2013 (12.9%), 79 in 2014 (27.5%), 101 in 2015 (35.2%) and 70 in 2016 (24.4%).Positivity in at least one serological test was determined in 37/287 (12.9%) animals. Of these, 33 were evaluated only by IIFT and ELISA techniques; 31 obtained positive results by both techniques and 2 only by IIF titration.Only three animals were evaluated by the three techniques, and all of them were positive to TR-DPP and with positive IIF titer, but they had indeterminate results in ELISA and one animal was evaluated only by a positive IIFT result. Regarding positivity distribution among the years studied, 3/37 (8.1%) dogs were found positive in 2013, 12/79 (15.2%) in 2014, 7/101 (6.9%) in 2015 and 14/70 (20%) in 2016.

4 Conclusions

We proved that there was an increase in the number of seropositive animals for CVL in the studied period. However, when we compare the proportion of examinations for CVL in relation to the others, we still observe a lower number than expected, considering the disease expansion in the region. Therefore, serological inquiries and entomological studies are necessary to solve this problem, in addition to continuing education policies for veterinarian physicians.

C1433 INCIDENCE OF LEISHMANIA DONOVANI INFECTION AMONG AGRO-PASTORALISTS TRIBES IN UM-ALKHARE AND BAZURA VILLAGES, GEDARIF STATE IN EASTERN SUDAN

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1 Background

Visceral leishmaniasis (VL) is a life-threatening parasitic disease, transmitted by a sand fly. It is is still one of the world's most neglected diseases, affecting largely the poor developing countries; 350 million people are considered at risk of contracting leishmaniasis, and some 2 million new cases occur yearly. This study aimed to estimate the VL incidence in Um alkhare and Bazura villages located in known endemic southern part of Gedaref State in Eastern Sudan.

2 Methods

In the period of (March 2014 – February 2015), this study was conducted among Agro-pastoralists tribes in the two Villages comparing the epidemiological and clinical spectrum of VL infection. One hundred seventy five (175) clinically suspected as VL cases were enrolled. Bone marrow (BM) and lymph node (LN) smears were aspirated for parasitological examination and serum tested by rk39 for *Leishmania donavani* antibodies. Socio-demographic and other determinants governing VL infections were also studied.

3 Results

The results obtained revealed that the parasitological finding from BM and LN and seroprevalence by rK39 was 64/175 (36.6%) and 74/175 (42.3%) respectively. Sensitivity and specificity for rK39 was determined to be 79%; 70% respectively. Parasitological confirmed cases showed clinical signs and symptoms such as, severe irregular fever, splenomegaly and LN enlargement. In this study we observed that Hausa tribe has a highest VL infection (2o cases), followed by Masaleet (14 cases) and then other tribes from western Sudan. The increased infection rate was reported to occur during October coinciding with high transmission season. It was also observed that prevailing Balantis trees, cracking soil and Leishmania reservoir have direct correlation with VL infection.

4 Conclusions

The use of microscopical BM or LN aspiration proofed to be still the gold standard diagnostic techniques. Socio-demographic, environmental and clinical determinants are important risk factors for VL infections.Further genetic studies based on wide community survey is highly recommended to verify susceptibility of different agro-pastoralists tribes to VL infection.

C1440 EPIDEMIOLOGY OF AMERICAN CUTANEOUS LEISHMANIASIS (ACL) IN AN ENDEMIC AREA OF FOREST EXTRACTIVIST CULTURE IN XAPURI MUNICIPALITY, ACRE STATE, BRAZILIAN AMAZONIA

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1 Background

In the Amazon region, leishmaniasis is one of the main neglected tropical diseases. During the period from 2001 to 2013, the state of Acre attained the highest incidence of all the Brazilian states. The study aimed to analyze clinical and epidemiological aspects of cutaneous leishmaniasis (CL) in Xapuri, Acre, Brazil.

2 Methods

Data from 2008 to 2014 registered in the notification records of the disease of the Information System of the Complaints of Notification (SINAN were used and analyzed with the chi-squared test.

3 Results

A total of 906 cases were registered with 60.2% occurring in men and 39.7% in women. The groups from 0 to 4 years of age (48.0%) and from 5 to 19 years of age (23.3%) were the most affected. Regarding the clinical forms, 77.7% presented CL and 22.3% muco-cutaneous leishmaniasis (MCL). Among the cases with information on the diagnostic methods used (896) Montenegro's skin test predominated (66.4%), with a positive result of 95.8% for CL and 99.3% for MCL. Treatment with N-methylglucamine antimony was performed in 99.4% of the cases, but discontinuously used in the majority of patients.

4 Conclusions

This study presents information which may be used as a tool for the epidemiological surveillance and control of the disease in Xapuri, a region which depends essentially on forest resources and ecological tourism.

C1515 VISCERAL LEISHMANIASIS IN CENTRAL ANATOLIA: A RETROSPECTIVE ANALYSIS OF HOSPITALIZED CASES (2000-2016 YEARS).

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1 Background

Vicceral leishmaniasis (VL) has been seen in most part of Turkey since of many years, especially cutaneous leishmaniasis (CL) getting spreading throught the country of western part. Leishmaniasis is a notificable parasitic disease that increased of incidence last year in Turkey and also causing a public health problem. Eskisehir and surrounding city is placed in central Anatolia and here is non endemic in VL. Aim of the study was to describe the epidemiological and laboratory findings of patients with VL who were admitted to Eskisehir Osmangazi University hospital of Microbiology laboratory between 2000-2016 period.

2 Methods

In this descriptive study was conducted 2000-2016 years period to laboratory records in Eskisehir Osmangazi University hospital of Microbiology laboratory. Laboratory records of 45 VL seropositive children were examined of age, gender, test results, and also regions where they came from. In our laboratory, parasite identification is done by bone marrow microscopy, NNN culture, rK39 dipstick test and Leishmania Ab EIA test. The results that were positive with at least two of these tests were evaluated.

3 Results

A total of 45 positive VL cases in 16 years period. Positive cases belong to children under 16 years of age. 21 cases were detected in the age range of 5-10 years and 8 cases were observed in the range of 1-16 years. In addition to of VL positive cases were defined of 17 of girl and 28 of males. There were no VL positive cases in Eski?ehir city center. VL cases were respectively from Eskisehir neighboring provinces of Afyon, Kütahya, Bilecik and surrounding rural area. Seropositivity was highest who had rural area and owners of domestic dogs and other animals. Serum samples were usually sent with prediagnosis of hepatosplenomegaly, fever, anemia.

4 Conclusions

In Turkey, the majority of study population of VL are pediatric group. Our records emphasized that the need for research to better understand of VL behavier in non endemic areas with developing effective and sustainable control strategies.

C1530 INCIDENCE TREND OF RURAL CUTANEOUS LEISHMANIASIS IN GONBAD-E- QABUS CITY (GOLESTAN, NORTH OF IRAN), DURING 2009-2012

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1 Background

Recently, incidence of cutaneous leishmaniasis (CL) has been increased. The present study aimed to investigate the incidence trend of CL in Gonbad-e-Qabus, (Golestan province, North of Iran).

2 Methods

This descriptive study was carried out for patients referring to health center of Gonbad-e-Qabus, during 2009 to 2012,. Early diagnosis of CL was carried out by direct smear and staining with Giemsa. In addition, to detect species of Leishmania spp, specific polymerase chain reaction (PCR) method was performed.

3 Results

During 2009 to 2012,1398 samples were tested and the results of direct smears in 946 subjects (67.6%) were positive. Among them, 576 cases (60.8%) were male and 370 (39.2%) were female. The majority (39.2%) of the positive cases belonged to 2009 ($P < 0.0001$). The highest frequency was in the age group under 5 years (35.9%) and lowest in age groups > 40 (0.7%). Furthermore, Leishmania spp was isolated from all the patients with Leishmania major by specific PCR.

4 Conclusions

Our results represented high incidence of the CL among less than 5 year-old-subjects which indicated high endemicity in the area. Although, based on interventions of Gonbad's Health Center, new cases of CL were decreased throughout 2012-2016

C1534 VISCERAL LEISHMANIASIS IN SELECTED COMMUNITIES OF HAMER AND BENNA-TSEMAI DISTRICTS IN SOUTH WEST ETHIOPIA; SERO-EPIDEMOLOGICAL AND LEISHMANIN SKIN TEST SURVEY

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1 Background

Visceral leishmaniasis (VL) known as Kala-Azar is a very serious disease caused by *Leishmania donovani* parasites. An estimated 500,000 VL cases occur annually, mostly in India, East Africa and Brazil. In Africa, the worst affected regions are southern Sudan, with estimated 15,000-20,000 cases annually and Ethiopia with 4,000-5,000 diagnosed VL cases a year. The aim of this study was to determine the prevalence of asymptomatic and symptomatic VL and also to determine the level of exposure (infection) to Leishmania parasites in the study areas.

2 Methods

A community based cross-sectional survey was conducted between 25th of July and August14th of 2013 in 15 selected villages of Hamer and Benna-Tsemai districts which are found in southern Ethiopia. Study localities were selected purposefully based on information obtained from patient records in Arbaminch Hospital lesihmaniaisis research and treatment centre. Venous blood was collected for the detection of antibodies using DAT (Direct Agglutination Test) and LST (Leishmanin Skin Test) was also performed to detect the exposure to the parasite. Data was analyzed using SPSS-20 and a P-value of < 0.05 was considered indicative of statistical significance.

3 Results

1760 individuals 975(55.3%) females and 785(44.7%) males were included. 44.1% of the study subjects were less than 10 years of age .statistically significant variation in the rate of exposure to the parasite was observed in different study sites and age groups. Positive LST response has also shown an increasing trend with age. High DAT positivity was observed in lower age groups. The overall LST and DAT positivity were 8.6 and 1.8% respectively

4 Conclusions

asymptomatic VL infection in the area is not negligible and could have a great contribution for anthroponotic transmission of the disease; thus, concerned bodies should take into consideration the implementation of prevention and control strategy for VL

C1546 ASYMTOMATIC INFECTION IN A COHORT OF PATIENTS UNDER ANTITNF TREATMENT. EXPERIENCE OF LEISHMANIASIS OUTBREAK IN FUENLABRADA, SPAIN

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1 Background

The largest leishmaniasis outbreak in Europe was declared since June 2009 in Fuenlabrada (Madrid, Spain). Our aim was to estimate, in this high exposure setting, the prevalence of asymptomatic *Leishmania* infection in the cohort of patients under antiTNF treatment in Fuenlabrada, and to analyze the risk factors for the development of clinical leishmaniasis

2 Methods

Cohort of patients under antiTNF treatment in Fuenlabrada was checked for exposure to Leishmania. A *Leishmania* infected case (LIC) was defined as a positive result in, at least, one of following blood tests: *Leishmania*-specific PCR, serology (ELISA or IFAT) or SLA-cell proliferation test as marker of cellular immunity. LIC could be symptomatic (cutaneous or visceral) or asymptomatic (no previous history of symptoms of active leishmaniasis). LIC vs not infected, and visceral leishmaniasis vs asymptomatic LIC, were analyzed with statistical tests looking for risk factors.

3 Results

562 patients of the cohort treated with etanercept (160), adalimumab (151), infliximab (136), certolizumab (69) and golimumab (46) were followed since 1st June 2009 to 31th December 2015. 95 representative patients were selected for exposure test to *Leishmania*, including 10 symptomatic patients (5 visceral and 5 cutaneous). 23 (27%) of the 85 asymptomatic patients fulfilled the definition for *Leishmania* infection, none of them became symptomatic during the study period. Total prevalence of the cohort was estimated 28.3%.
Multivariate analysis performed between *Leishmania* infected cases (LIC) who developed leishmaniasis vs asymptomatic showed association to illness for concomitant use of methotrexate (OR 7.1, IC 1.4-37.6)(p=0.023) or steroids (OR 14.7, IC 1.3-156.9)(p=0.021) and negative SLA-cell proliferation test (OR 6.3, IC 0.82-47.6)((p=0.093

4 Conclusions

26% of our antiTNF cohort patients has been infected by *Leishmania*, but remain asymptomatic. Only 5 from 562 had visceral leishmaniasis. Concomitant use of methotrexate or steroids seems independent risk factors for leishmaniasis in antiTNF patients. Patients infected by *Leishmania* and negative SLA-cell proliferation test are at high risk for clinical leishmaniasis.
This work has been supported by "Red de Investigación Cooperativa en Enfermedades Tropicales" (RICET + RD12/0018/0008), VI PN de I + D + I 2008–2011, ISCIII- Subdirección General de Redes y Centros de Investigación Cooperativa; and fondos FEDER

C1556 NEW FOCI FOR CUTANEOUS LEISHMANIASIS - ANKESHA DISTRICT, AMHARA REGION, ETHIOPIA

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1 Background

Cutaneous leishmaniasis is found in more than 80 countries in the world. With estimated 29 million people at risk and 20,000 to 30, 000 new cases per year Ethiopia is one of the ten high burden countries. From studies so far in more than 90% of the cases *Leishmania aethiopica* is incriminated as the causative species. The aim was to investigate cutaneous lesion outbreak in Ankesha district, Awi zone, Amhara region.

2 Methods

A preliminary rapid assessment survey was done from November 2013 to January 2014. **37** cases and 74 apparently healthy individuals were interviewed. Skin slit lesion sample was taken from 25 cases. Identification of *Leishmania* parasite was done by smear microscopy and culture. PCR; restriction fragment length polymorphism was done for species identification.

3 Results

Two clinical form of CL were observed; 33 (89%) localized cutaneous leishmaniasis and 4 (11%) diffused cutaneous leishmaniasis. All of CL lesions were observed on the exposed parts of the body such as the face and upper and lower limbs. 16 samples were positive by smear and culture. 6 samples were typed as *L. aethiopica.* The community did not know the cause, transmission and prevention mechanism of CL.

4 Conclusions

We confirmed that the cause of cutaneous lesions among the residents of Ankesha district is CL. The average duration of lesion in the 37 participants was three month, range with one to six month showing the probability that CL appeared in this kebele recentl. 6 samples were typed as *L. aethiopica*. None of the interviewees had knowledge about the cause of CL. The presence of CL in Ankesha was not known before thus underlining the need for further survey to certain the claim of new outbreak, and identify associated risk factors to designed efficient and effective control.

C1562 SPATIO-TEMPORAL EVOLUTION OF CUTANEOUS AND VISCERAL LEISHMANIASIS IN MOROCCO

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1 Background

In Morocco, three Leishmania species: *Leishmania major*, *L. tropica* and *L. infantum* cause cutaneous leishmaniasis (CL), while *L. infantum* causes visceral leishmaniasis (VL). The main reservoirs for *L. major*, *L. infantum* and *L. tropica* are, respectively, rodents, dogs and human. The geographical distribution of different forms of leishmaniasis in Morocco is linked to well described bioclimatic zones. In Morocco this parasitic disease represents a great health problem, not only because of the number of cases recorded each year, but also because of the geographic expansion to previously free areas and the emergence of overlapping foci of CL (due to *L. tropica*) and VL (due to *L. infantum*) in several provinces of Morocco. In addition, Moroccan Leishmania species show high genetic diversity which translates into a diversity of clinical forms and sometimes more rare forms and/or resistant forms.
Diversity, extension and persistence of *Leishmania* species are closely related to socio-economic, climatic and environmental factors.

2 Methods

Objective of our study is to determine and analyze the potential factors can influencing the epidemiological evolution and persistence of leishmaniasis in Morocco during the last twenty four years (1990–2014). During the period 1990-2014, the Moroccan Ministry of Health noted 64,973 cases of leishmaniasis including 62,551 (96.27%) cases of CL (19249 (29.62%) cases of *L. major* and 43302 (66.64%) cases of *L. tropica)* and 2422 (3.73%) cases of VL by *L. infantum*.
Our study was carried out in endemic and non endemic areas from July 2013 to July 2015; and focused on reservoirs, vectors and microenvironment conditions.

3 Results

For microenvironment, our results showed many conditions as risk factors for leishmaniasis in Morocco such as the presence of domestic mammals; type of building materials,, non-ventilated and obscure dwellings, poor housing and domestic sanitary conditions, the presence of the Souk (weekly gathering of the people in a market) and Fandeks (accommodation of the animals until saling in the souk). For macro-environment, in the last decades, the irregularity of the precipitations with the increase of the temperature, are observed in Morocco as consequence of climate change.

4 Conclusions

These climatic conditions influence the local water management, population movements, growth of the human population and urbanization probably allow the persistence and maintenance of the transmission cycle of Leishmania

C1587 LEISHMANIASIS IN IRAN, UPDATE 2015

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1 Background

Leishmaniasis has a long history in Iran. This study aimed to show the trend in Leishmaniasis incidence from 1983 to 2015, and to describe the epidemiological characteristics in 2015

2 Methods

in a retrospective cross-sectional study, data were extracted from the national leishmaniasis surveillance system for visceral Leishmaniasis (VL) and cutaneous Leishmaniasis (CL).

3 Results

The number of cases after conducted new strategy for CL control decreasing from 24200 in 2006 to 19693 in 2015.19 from 30 provinces in Iran are endemic for cl Although in many endemic provinces decreased the number of CL cases but in two provinces (khoozastan, Illam in south west border of Iraq) increased. In 2015, 46% of cases had one lesion and 22% two lesion and 1.8% had more than ten lesions. Bicouse the main type of CL in Iran is zoonotic the transmission increasing from august and pick was in November.
in 2000 the number of VL cases were 346 cases and decrease to 77 cases in 2015, main endemic provinces for VL are Ardebil in nourth and Fars in south.

4 Conclusions

Many factor cause increasing the number of CL cases. one of the main factor is poor knowledge and attitude of people how living in the endemic areas about health protection and also the climate change, poor environment sanitation, no plan for case finding and facilities for rapid treatment.
The main elements of cutaneous and visceral leishmaniasis control program in Iran are as follows:
Strengthen leishmania surveillance system and establishing laboratory network from the national to peripheral level.
Establishment of standard diagnosis and treatment protocol.
Provision of educational programs from different social levels and revision of the national guideline, standardization cases definition and treatment results.
Early diagnosis and rapid treatment of patients, reservoir control program,
Apply direct agglutination test in periphery levels for VL diagnosis.
Collection of infected owner and stray dogs in visceral leishmaniasisi endemic foci.

C1604 STUDY OF BIOMETRIC INDICATORS FOR CUTANEOUS LEISHMANIASIS FROM SOUTHWEST OF IRAN FOR THE PERIOD OF 2001-2011

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1 Background

Background and Objectives: *Leishmania* is a protozoa parasite with extensive clinical signs and symptoms cause leishmaniasis. It is a zoonotic infection transmitted through bite of phlebotomine sandflies and seen in cutaneous (cutaneous leishmaniasis), visceral (kala-azar) and muco-cutaneous forms. Endemic form of infection reported from different parts of Iran and epidemiological information is inadequate and this study was designed to examining prevalence process and epidemiological characteristics of cutaneous leishmaniasis during eleven years from 2001 to 2011.

3 Methods

Methodology: This is a cross-sectional epidemiologic study that examined 2637 patients with cutaneous leishmaniasis in Southwest of Iran during 2001-2011. The data of all patients who referred to the Prevention Unit of Health Center were recorded epidemiologic data summary forms. The data and parameters included age, gender, occupation, season, residence (urban, rural), and lesion location.

4 Results

Findings: Of 2637 cases, 1174 (44.5%) were females and 1463 (55.5%) males. The maximum rate of infection was observed in age group under-10-year-old (45.3%) and minimum rate was seen among those aged over 60 years (0.9%). A number of 1557 patients (59%) were living in cities and 1080 (41%) in rural areas. From occupational point of view maximum and minimum frequency distributions were seen in students (49.9%) and farmers (0.6%), respectively. From seasonal perspective, maximum and minimum frequencies of the disease were documented in winter (52.33%) and summer (7.62%), respectively. The site of lesions on the body were seen in order, in hands (37.5%), face (30%), feet (26.3%), and other organs (6.3%).

5 Conclusions

Conclusion: Biometric indicators including age, gender, occupation, season, residence type (urban, rural), and lesion location in endemic regions have had significant effects on prevalence of cutaneous leishmaniasis and findings can be of help for effective assessing disease to in place preventive actions.

C1614 LEISHMANIA IN SYNANTHROPIC RODENTS (RATTUS RATTUS): NEW EVIDENCE FOR THE URBANIZATION OF LEISHMANIA (LEISHMANIA) AMAZONENSIS

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1 Background

Black rats (*Rattus rattus*) are suspected to serve as a reservoir for the *Leishmania (Viannia) braziliensis* complex in Brazil and are potential sources of infection to peridomestic phlebotomine sand flies. This study aimed to detect parasites from *Leishmania* genus, to determine the prevalence of anti-*Leishmania* spp. antibodies, to identify circulating species of the parasite, and to determine epidemiological variables associated with infection in rats caught in urban area of Londrina, Paraná, Brazil.

2 Methods

Animal capture was carried out from May to December 2006; traps were distributed in 35 points of storage and/or recycling of solid waste, and in junkyards, of the five regions in the municipality. Serological (ELISA and IFAT) and molecular (PCR) methods were performed for the detection of anti-*Leishmania* spp. antibodies and DNA from *Leishmania* spp. The number of samples tested by each method varied according to the availability of the collected material. DNA was extracted from total blood, and nested-PCR, targeting a conserved central part of the SSu rRNA from the *Leishmania* genus, was performed in triplicate. The positive samples were sequenced twice by Sanger method to determine the species of *Leishmania*. A questionnaire about environment, sanitation, and rat characteristics was applied and analyzed with EpiInfo (3.5.4) software.

3 Results

In total, 181 rodents were captured; all were identified as *Rattus rattus*,and none presented clinical alterations. Forty-one of the 176 (23.3 %) animals were positive for *Leishmania* by ELISA and 6/181 (3.3 %) were positive by IFAT. Nine of 127 tested animals (7.1 %) were positive by PCR; through sequencing, seven were identified as *L. (L.) amazonensis*, one as *L. (L.) infantum*, and one could not be sequenced. Four rats were positive using more than one test. The presence of different animal species (other than rats) at collection points was a protection factor against leishmaniosis in rats (p=0.032), when considering just IFAT results. Of the nine positive animals, based on PCR, five originated from the same region of the city (North); among them, three were captured at the same address.

4 Conclusions

This was the first description of synanthropic rodents naturally infected by *L. (L.) amazonensis* (in the world) and by *L. (L.) infantum* (in South Brazil). Regarding *L. (L.) amazonensis*, this finding provides new evidence of the urbanization of this etiological agent.

C1618 CHALLENGES OF VL ELIMINATION PROGRAMME BY THE EXPANSION OF THE ENDEMICITY TO WESTERN REGION OF NEPAL: EVIDENCES FROM ASYMPTOMATIC INFECTION AND TRANSMISSION

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1 Background

The Kala-azar elimination programme is being challenged by the expansion of endemicity, which was evidenced by the documentation of local transmission of visceral leishmaniasis in two eastern hilly districts of Nepal. Additionally, several sporadic cases were recently reported from other non-endemic western Terai and hills, which are far away from classical endemic foci of Nepal. Sporadic cases in non-endemic districts are usually considered as import cases, so in order to collect evidence for local transmission we set out to detect asymptomatic infection in village contacts through indirect (serological) and direct (PCR) methods.

2 Methods

Altogether 320 human blood samples and 23 blood samples from domestic animals were collected from inhabitants of villages that reported VL cases, located in four districts: Doti, Kailali, Surkhet and Palpa, which are non-endemic western plain and hill districts in Nepal. Real time PCR targeting kDNA minicircles and DAT were used to detect *Leishmania* infection. Additionally, sandflies collected from the hills were also analysed by PCR.

3 Results

PCR showed that 15.9% of humans and 4.3% of animals were infected by *Leishmania* parasites. DAT revealed that 11.8% of humans were infected, whereas 3 goats and 1 pig showed elevated antibody titre. Sandflies were also infected.

4 Conclusions

Our study documented an asymptomatic infection rate similar to classical endemic foci of Nepal and India. Expansion of KA in non-endemic district during elimination programme is alarming and needs to be addressed urgently.

C1622 A RETROSPECTIVE STUDY (2011-2015) OF HUMAN VISCERAL LEISHMANIASIS AT A TERTIARY HOSPITAL IN VALENCIA (SPAIN)

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1 Background

The World Health Organization considers Leishmaniasis to be one of the most important tropical diseases. In endemic areas, such as the Mediterranean Basin, a large percentage of the population acquires the parasite but does not develop the disease; the presence of immunedeficiencies may determine if a subclinical infection develops symptomatology.
The objectives of the present study are: i) to determine the geographical distribution of Visceral Leishmaniasis (VL) at a tertiary hospital in the Mediterranean Basin; ii) to identify the risk factors that predispose to infection and iii) to recognize the most characteristic clinical manifestations of VL.

2 Methods

Descriptive study of patients with suspected VL attended at the University and Polytechnic HospitalLa Fe in Valencia (Spain) (July 2011-July2015). The epidemiological, clinical and microbiological characteristics were studied using the medical records management system. The baseline comorbidities analyzed were HIV, hepatitis C virus infection, leukemia, etc. The diagnosis of VL was performed by direct techniques (Giemsa staining of biological samples of patients), amplification techniques of nucleid acids, and serological studies.

3 Results

369 (195men/174 women) Patients with suspicion of VL were included, of whom 26 were diagnosed with VL, 50% of whom were children under the age of 5 and 46.1% of patients presented diseases that compromised their immunological state.The most frequent clinical manifestation was fever (80.8%), followed by asthenia (38.5%) and anorexia (34.6%).Splenomegaly was the most frequent finding in physical examination (76.9%), followed by hepatomegaly (69.2%). Pancytopenia (53.85%) was the most frequent finding in laboratory tests.DNA amplification was the most sensitive technique, being positive in bone marrow in 92.3% of the patients but DNA amplification in blood was less sensitive (58.3%).

4 Conclusions

Children under the age of 5 represented the group of the population with the highest number of VL cases.Coinfection of HIV with LV was presented in 15.38% of patients, although it is a disease related to the rural scope, 48.12% of the patients lived in urban nuclei.
Study supported by Projects: RICET (RD12/0018/0013 and RD16/0027/0023), RETICS-FEDER, ISCIII and PROMETEO (2012/042 and 2016/099), Spain.

C1630 BURDEN OF CUTANEOUS LEISHMANIASIS IN GILGIL - CENTRAL RIFT KENYA

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1 Background

Cutaneous Leishmaniasis (CL) is endemic in over 90 countries and recurrent outbreaks have been described in Kenya, Ethiopia and South Sudan. In Kenya the disease occurs in rural areas and is geographically distributed in Central Rift Kenya where it occurs as localised CL, diffuse and cutaneous leishmaniasis recidivan (CLR). The purpose of this study was to establish the burden of CL in Gilgil sub county -Central Rift Kenya.

2 Methods

A structured questionnaire was administered to collect information on demographics, clinical characteristics, environmental and risk factors. Clinical and laboratory diagnosis was done. Clinically by identification of persons with nodule or nodule-ulcerative plaques with or without crusting. Laboratory diagnosis was by observation of amastigotes in Giemsa-Stained tissue smears. A comprehensive line list for CL positive patients was developed and treatment initiated.

3 Results

A total of 233 cases were positive for CL, with duration of illness ranging from three months to six years. Affected ages were 5 months to 68 years. Males and females were 103(44.2%) and 130 (55.8%) respectively. Children below five years were 92(39.5%). Localised CL cases were 99(48.7%), disseminated CL 57(24.5%) and cutaneous leishmaniasis recidivan 77(37.9%). Number of lesions per patient ranged from 2 to 60 and were mainly located on the face and neck in 202(86.7 %). Mucosal lesions were seen on the mucocutaneous borders of the eyelids, lips, and nostrils in 11(4.7 %) patients. Factors associated with CL included houses located near forest edges, living in forest caves, cracked mud houses and engaging in forest activities like charcoal burning, bee keeping and hunting. Out of 233 patient who had CL, 137(59%) didn't complete primary level education and 201(86.1%) live below the poverty line. Out of those diagnosed, 9 (3.9%) were HIV co-infected. Laboratory diagnosis was done for 120 cases out of which 38(31.6%) tested positive for amastigotes. Treatment by intralesional infiltration of 0.8–5 mL (100 mg/mL) of Sodium Stibogluconate was given to all patients on a weekly bases and 60% completely healed by end of the fifth month. Challenges in treatment included high dropout rate, scarring, resistance, pain and relapse.

4 Conclusions

The study indicates that the disease is of public health importance and needs interventions in sustained case management, improved diagnostic, surveillance and vector control activities.

C1643 DETERMINANTS OF ANTHROPONOTIQUE CUTANEOUS LEISHMANIASIS BY CASE-CONTROL STUDY IN MOROCCO

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1 Background

In Morocco, cutaneous leishmaniasis (CL) is an endemic parasitic disease. It is transmitted by the Sandflies (Diptera: Psychodidae) and caused by three species of the genus *Leishmania* (Kinetoplastida: Trypanosomatidae): *Leishmania major* (*L. major*) and *Leishmania tropica* (*L. tropica*) responsible of endemo-epidemic forms and *Leishmania infantum* (*L. infantum*) responsible of sporadic cases (Rhajaoui et al., 2007).
CL caused by *L. major* is observed in southern foothills of the Anti and Haut-Atlas with 2000 cases per year. CL due to *L. tropica* is mainly present in the center of Morocco with an incidence of about 1000 cases per year (Kahime et al., 2014). While LC caused by *L. infantum* is a sporadic form mainly in the north of the country. Overlapping ranges, of the two last forms of leishmaniasis, is mainly observed in the northern part of the center of the country (Rhajaoui et al., 2007; Rhajoui, 2011).
To characterize the population at high risk for *L. tropica* CL, an epidemiological case-control study was conducted in Al Haouz Province, with a total of 140 cases and 200 controls.

2 Methods

The total population of province of Al Haouz was estimated to 573128 inhabitants in 2014; with 14.40% are urban inhabitants and 85.20% rural inhabitants (Moroccan High Commission for Planning, 2014). Al Haouz lies at altitudes of 600–1700m above sea level and has an arid to semi-arid climate.
We have adopted a case-control study. Thus, for each patient (case) and his witness, anonymous questionnaires have been met, where we recorded all the information concerning the civil status, activity, life style and conditions of immediate environment.

3 Results

Identifying the risk factors of Anthroponotic CL infection in Al Haouz Province offers significant information to design and develop effective control measures. Our results show that the presence of animals, individual houses with cracked walls, age and gender were associated with increased risk of CL infection. On the other hand, healthy living, education, vaccination and use of insecticide were associated with reduced risk of CL infection.

4 Conclusions

Our findings have implications for planning and control of CL in Morocco. Firstly, individual protective measures such as use insecticide and improve hygiene conditions could easily be adopted for the prevention of CL in our study area; and secondly improving housing conditions such as removing cracks from houses and keep breeding away from the house would help to reduce risk of CL in Al Haouz.

C1668 INVESTIGATION ON SANDFLY POPULATION IN A RECENTLY PROVED FOCUS OF VISCERAL LEISHMANIASIS IN TUNISIA. LEISHMANIA INFECTION RATE

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1 Background

An entomological study of phlebotomine fauna was conducted in a region where transmission life cycle of visceral leishmaniasis has been recently confirmed and many sporadic cases of cutaneous leishmaniasis has been observed. A global epidemiological analysis was carried out including i) identification of sand fly species predominant in the studied region, ii) detection and typing of *Leishmania* species in infected sand flies to identify potential vectors and estimate infection rate of *Leishmania* in sand flies, iii) and typing mammalian species in blood meal of fed female sand flies to identify potential reservoir of Leishmaniasis due to *L. infantum.*

2 Methods

Morphological and molecular methods were used for the species identification of 643 sand flies caught by CDC traps and sticky papers. Then, the 199 female sand flies were tested for the presence of *Leishmania* DNA. Also, the blood meal of fed sand flies was analyzed by molecular method (PCR and sequencing).

3 Results

We identified seven species belonging to the genus of *Phlebotomus (P. perniciosus, P. papatasi, P. chabaudi, P. longicuspis, P. riouxi, P. perfilewi,* and *P. sergenti)* and three belonging to the genus of *Sergentomyia* with an abundance of *Phlebotomus perniciosus* (56%), the confirmed vector of *L. infantum,* followed by *P. papatasi (25%),* the unique vector species of *L. major*. Moreover, *Leishmania* DNA has been found in 15 *P. perniciousus*, 6 *P. papatasi*, and 3 *P. longicuspis*. An infection rate of 3,7% is retained in the studied phlebotomine population. Most of infected san flies (86%) were unfed having infective form of *Leishmania* parasite. Moreover, *L. infantum* has been identified in five unfed *P. perniciosus* and two *P. longicuspis*. Also, *L. major* was identified in three *P. papatasi.* This result suggests that this region is endemic for both VL and CL explaining repetitive sporadic cases observed. Furthermore, four species were identified in sand flies blood meal (human, rabbit, horse, and cow) making these species a potential reservoirs of leishmaniasis due to *L. infantum*. The isolation of *Leishmania* from infected sand flies mammalian species are needed to confirm their role in transmission life cycle.

4 Conclusions

We confirm here that the studied region is endemic for both CL and VL and we suggest again the occurrence different transmission life cycle of leishmaniasis of *L. infantum* with *P. perniciosus* and *P. longicuspis* as potential vectors. Also, human, horse and cow could be potential reservoirs.

C1675 THREAT OF FOUR EXOTIC LEISHMANIA SPECIES TO THE EUROPE

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1 Background

Recent political and social events in the Middle East causing an increase in migration to Europe demonstrated the necessity to identify the risk factors originating from infectious diseases. Leishmaniasis as a vector-borne zoonotic disease, with different clinical features (such as cutaneous, visceral, mucocutaneous) affects especially people living in tropical and subtropical regions of the world. Unfortunately dense concentration of refugees, tough environmental conditions, and limited health care access contributes to the higher burden of leishmaniasis in Middle Eastern countries. According to WHO, "Turkey's health system has demonstrated strong capacity and flexibility in adapting to changing needs, outbreaks of leishmaniasis have been prevented". But possible outbreak risk for leishmaniasis is still at the border of Europe with increased number of new cases in Turkey. It has been aimed to evaluate the autochthonous clinical examples of different strains of *Leishmania* affecting both humans and dogs in Manisa province in western Turkey.

2 Methods

Samples from cutaneous, visceral and canine leishmaniasis suspected cases were obtained by lesion, bone marrow and lymph node aspirations, respectively. Smears were stained with giemsa and inoculated to enriched *Leishmania* medium. All clinical materials were tested with real time polymerase chain reaction (q-PCR) with probes that detect the ITS1 region of Leishmania parasites and results were confirmed by hsp70 PCR-N/sequencing and cpb gene PCR/sequencing in order to reveal L. donovani/L. infantum discrimination.

3 Results

Here we present 2 CanL (caused by *L. infantum* and *L. tropica*), 3 VL (caused by *L. infantum, L. tropica* and *L. donovani*) and 3 CL (caused by *L. major, L. tropica* and *L. infantum*), totally 8 leishmaniasis cases from Manisa region. Giemsa stained samples from all cases were amastigote positive. We were also able to cultivate all samples with using enriched medium.

4 Conclusions

Considering the neighbourhood locations of western Turkey with Greek island and main land, risk assessment and awareness should be evaluated in mobile migrants, refugees and travellers. Especially in the presence of competent vectors for leishmaniasis in southern European countries. To be able to respond effectively to possible *Leishmania* threat for Europe in large groups, accurate information and experience are critical.

C1702 THE BURDEN OF VISCERAL LEISHMANIASIS IN BRAZIL AND ITS STATES: FINDINGS FROM THE GLOBAL BURDEN OF DISEASE STUDY 2015

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1 Background

Transmission of visceral leishmaniasis (VL) in Latin America is expanding in Brazil, Paraguay and Argentina. The VL, disease typical of rural areas, has been urbanized and expanded in Brazil since 1980, with high case fatality rate. We used the Global Burden of Disease-2015 (GBD-2015) estimates to examine the burden of VL in Brazil and its states (2000-2015).

2 Methods

: Descriptive study using the GBD-2015 estimates with 95% uncertainty intervals were analyzed: Age-standardized death rates, Years of Life due to premature mortality (YLL), Years of Lost due to Disability (YLD), Disability-Adjusted Life Year (DALY=YLL+YLD).

3 Results

Similar results were found when compared 2000-2015. In 2015, death rate was 0.24/100,000 (0.20-0.30); YLL 11.06/100,000 (8.87-14.06); YLD 0.05/100,000 (0.03-0.08); DALY 11.10/100000 (8.92-14.10). The highest death rates were found in Tocantins (1.64) and Piauí (0.64), northern and northeast of Brazilian states, respectively; all other states had rate <0.5. In the ranking among the 27 Brazilian states, ranging from 1 (best performing) to 27 (worst), Tocantins death rate (higher rate; 27th position) was 2.5 times higher than Piauí (2nd highest rate; 26th). Death rates by age were higher in <1 year old (1.52) and ≥80 years old (0.83); in all other age groups the rates were ≤0.6. The highest DALY/1000.000 were found in the following states and regions: North (Tocantins-27th position: 71.4; Amazonas-21th: 14.1), Northeast (Piauí-26th: 28.8; Ceará-25th: 19.6; Maranhão-24th: 17.1), Midwest (Mato Grosso do Sul-22th: 14.6); Southeast (Minas Gerais-23th: 14.7). DALY/100.000 by age were: <1 year old (128.5); 1-4 (25.3); 40-69 (ranged from 10.1-11.2); ≥ 70 years old (5.6-9.1). DALY/100.000 was higher for male (14.36) than female (8.05). In 2015, age-standardized incidence rates in Brazil, Paraguay, and Argentina were low (<1.0/100,000); however, Brazilian DALY was 262 and 1,049 times higher, respectively. The main component of the Brazilian DALY was YLL (premature death).

4 Conclusions

The death rate from VL is low in Brazil; however, the fatality rate is high. In spite of the availability of transmission control program, diagnosis and treatment provided by the Brazilian public health system, the mortality and DALY have not been reduced from 2000-2015. Therefore, early diagnosis, knowledge of prognostic factors for death, and adequate clinical management should be improved to reduce fatality rate from VL in Brazil.

C1722 HSP20 TARGET IS ABLE TO DETECT ALLELIC POLYMORPHISMS IN AUTOCHTHONOUS LEISHMANIA ISOLATES FROM A BOLIVIAN REGION.

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1 Background

The Plurinational State of Bolivia is one of the Latin American countries with the highest prevalence of leishmaniosis, highlighting the lowlands of the department of La Paz where about 50% of the total cases have been reported.

2 Methods

Nineteen Bolivian *Leishmania* isolates were studied by sequencing the *HSP20* and *cytochrome-b* (*cyt-b*) genes as well as the *internal transcriber spacer 1* (ITS-1) spacer region.

3 Results

The sequencing of the HSP20 gene led to the identification of three major *Leishmania* taxons circulating in Bolivia: *L. braziliensis* (nine isolates), *L. mexicana/L. amazonensis* (8 isolates) and *L. lainsoni* (2 isolates). Additionally, within each taxon, SNPs were observed. Thus, two isolates (INL-181-11 and INL-162-12) belonging to the *L. braziliensis* clade showed an SNP at position 291 of *HSP20* coding region. Moreover, after a visual inspection of the chromatograms, one isolate (INL-018-12) within the *L. lainsoni* clade was characterized by a double peak of T/C at position 279. Interestingly, double peaks of T/C at positions 114, 129 and 147 were also visualized in all isolates grouped within the *L. mexicana/L. amazonensis* clade. Given that HSP20is encoded by a single copy gene, in order to explain the existence of double peaks at some positions of the gene sequence, two hypotheses were considered: i) some isolates may contain a mixture of genetically different parasites; ii) there exist sequence polymorphisms between the allelic copies of the HSP20gene. In order to experimentally test these hypotheses, one of the isolates typed as *L. (L.) mexicana*, 646-10, was cloned by limiting dilution and plating on agar plates. After amplification of the HSP20 target and sequencing, it was observed that the double peaks persistent and no sequence differences regarding the parental isolate were observed. By contrast, analysis of the PCR product, by cloning in the vector pCR2.1 and sequencing with the T7 primer, showed that the double peaks were not technical artefacts as they were not observed after cloning of the PCR product. Moreover, expected sequence polymorphisms were found among the individual clones from the PCR product.

4 Conclusions

In conclusion, allelic polymorphisms within the same strain was demonstrated, and this genotypic features should be taken into account when typing isolates for molecular epidemiology, clinical studies and experimental pharmacotherapy purposes.

C1742 RESULTS OF A COMMUNITY-BASED HOUSE-TO-HOUSE SURVEY: KNOWLEDGE, ATTITUDE, AND PRATICES RELATED TO CUTANEOUS LEISHMANIASIS IN KABUL PROVINCE, AFGHANISTAN

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1 Background

Every year more than 10 million citizens in Kabul are at risk of cutaneous leishmaniasis (CL). Reported incidence is around 17,000 in Kabul each year, with an estimated expansion factor of 3 to 5. CL has a social burden of stigma and marginalization, impacting quality of life, particularly of women and children. The study objectives were (1) Assess knowledge, attitudes, and practices towards transmission, prevention, and treatment, (2) Estimate the prevalence Kabul residents, (3) Identify risk factors.

2 Methods

In 2010, a community-based house-to-house survey was conducted in Kabul City. A sample of 9,614 individuals was included in the study, with estimated 5% non-respondents. Descriptive statistical analysis was conducted for prevalence, and for knowledge, attitudes, and practices. To assess risk factors statistical inference analysis was performed using variance analysis and direct logistic regression on household and respondent level to investigate the likelihood of ACL in households and respondents, respectively. Ethical clearance was obtained from the Internal Review Board at the Ministry of Public Health in Kabul, Afghanistan.

3 Results

Only 52 % of respondents were male in the knowledge, attitude, and practices (KAP) component of the survey and only 30% of the respondents had heard of CL. Yet 91% believed the disease to be curable. Households, which correctly identified sandflies as vectors, were more likely to take the necessary prevention measures. Households with mesh netting (69%) and indoor residual spraying (52%) were more common than bed net use (24%). The overall active lesion prevalence was 2.44%. Active lesions were more common among females than males (3.03 % vs. 1.90%). Household members aged between 0 and 15 had a higher prevalence of lesions (2.82%).In a variance analysis the following risk factors were identified: age (p=0.00), number of residents (p=0.00), construction type of the house (p=0.02) and use of bed nets (p=0.001). A direct logistic regression on the likelihood of lesions on the household level confirmed that the number of household members (odds ratio=1.11) and the use of bed nets (odds ratio=0.5633) are the most important predictors for active ACL ulcers.

4 Conclusions

Effective health communication, education and promotion approaches in CL to mobilise communities on early recognition of symptoms, to seek early treatment and undertake preventive measures against sandfly bites need to be developed.

C1773 LEISHMANIASIS AS NEGLECTED DISEASE IN PARAGUAY: CRITICAL FACTORS DETERMINING THE BURDEN ON HUMAN PATIENTS

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1 Background

Both tegumentary (TL) and visceral leishmaniases (VL) are endemic in Paraguay, and considered as health problems in the country. Overall, these diseases require several techniques to achieve diagnosis, not available in endemic areas (TL), or not easy to fall into the diagnostic schemes of physicians (VL). Although there is a special Programme focused in the disease control at the Ministry of Health (MH), there are several weaknesses and fails which affect patients for the achievement of correct diagnosis and getting proper treatment. These weaknesses range from strict centralization of the Programme at the MH, to the way in which the patients are managed.

2 Methods

In this descriptive study, we cover all the aspects concerning the neglect of both the patients and the leishmaniasis disease, in a critical view. These factors determine the burden of leishmaniasis in Paraguay. Besides of this descriptive analysis, we performed a pilot questionnaire in patients collecting both quantitative and qualitative aspects of the neglect. The data considered in the descriptive approach were those taken from 2013 till beginning of 2017.

3 Results

There are complex aspects concerning the public institutions of Paraguay involved in the management of the control programme of the leishmaniases in Paraguay. These aspects affect, as a main result, the regular availability of drugs. It turns out often in either an inappropriate therapy for singular cases, or a delay of the treatment, sometimes excessively extended in time. The availability of appropriate diagnostic methods, especially regarding TL, relies on circumstantial factors, which are unstable and temporaries.

4 Conclusions

The weak scheme of the MH for prevention, diagnosis, treatment and control of the leishmaniases in Paraguay is typical of neglected diseases. But in the particular case of Paraguay, this situation is not reflected by the large investment made by public funds on the disease. This results in a poor quality of the health services available for both TL and VL patients. This critical condition is in accordance with several aspects revealed by patients in the questionnaire. Only when public institutions, particularly the MH, were cleared up, the service offered to the population regarding leishmaniases control, would be better.

C1779 ELIMINATION OF VISCERAL LEISHMANIASIS IN INDIA THROUGH EXISTING THERAPEUTIC AND VECTOR CONTROL PROGRAMS: A SYSTEMATIC DYNAMICAL MODELING STUDY

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1 Background

In the developing countries of Eastern Africa, Eurasia, South and Central America, Visceral Leishmaniasis (VL) is a neglected disease causing the deaths of millions of people. It is difficult to effectively control this disease, owing to the fact that infection remains asymptomatic for most of the time after being exposed to the parasite. The focus of this study is to understand how treatment of reservoirs and vector control programs impact prevalence of asymptomatic individuals in low and high transmission regions.

2 Methods

A mathematical model is developed and analyzed to effectively capture the transmission dynamics of VL under different control programs and thereby an attempt has been made to deduce an effective solution to distribute limited resources to reach elimination targets. The model captures early detection and active surveillance, reservoirs (such as Post Kala-azar Dermal Leishmaniasis (PKDL) cases), adherence to treatment, and health seeking behaviors of population. The impact of both human and vector control programs are evaluated.

3 Results

The study evaluated the impact of various public health policies as different types of control interventions are implemented. The results indicate PKDL reservoir treatment rates is directly related to reduction of VL prevalence with more than one fifth of the decrease in number of VL cases when combined with any of the three single vector control programs (long-lasting insecticidal nets (LLIN), indoor residual spraying (IRS), or larvae source management (LSM)). We found that insecticide spraying has the largest impact on reduction (about ¾ of sandfly density) with only around 50% reduction due to either by LLIN or LSM in high transmission regions.

4 Conclusions

High levels of reservoir treatment along with existing levels of vector control program can significantly impact the rate of occurrence of new VL cases in high transmission regions. In low transmission regions, treatment of VL cases is a significant factor to reduction in prevalence of VL. Success in diagnosis and treatment of reservoir requires specific behavior from patients and health care providers, which facilitate those practices. Socio-economic status of patients might have also been playing significant association in patient delay to seeking healthcare.

C1801 INTERLEUKIN-2 AS A MARKER FOR DETECTING ASYMPTOMATIC INDIVIDUALS IN AREAS WHERE LEISHMANIA INFANTUM IS ENDEMIC

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1 Background

Asymptomatic individuals must be identified if the true prevalence of *Leishmania infantum* infection in endemic areas is to be known and if the potential of such persons to act as disease reservoirs is to be understood. For this reason, the aim of this study was to establish a simple field method for detecting asymptomatic subjects in areas where *Leishmania infantum* is endemic.

2 Methods

Cell proliferation assay (CPA) with soluble *Leishmania* antigen (SLA) was used as a reference test to detect asymptomatic individuals from the blood bank of the Hospital of Fuenlabrada (Madrid, Spain). From the 330 blood samples tested, 47 asymptomatic individuals (CPA-positive) and 50 negative controls (CPA-negative, NVL) were included in this study. From the medical department were collected samples from active visceral leishmaniasis patients (VL) (n=18), and cured VL patients (CVL) (n=18). Blood from NVL, VL, asymptomatic and NVL were tested using whole blood assay. Briefly, 500 µl of blood was placed on its own in a tube (negative control), and another in a tube containing SLA, and incubated at 37ºC for 24 h. The levels of IFN-γ, TNF-α, IL-10 and IL-2 were determined in SLA-stimulated plasma using the Cytometric Bead Array.

3 Results

Stimulation of whole blood with SLA was associated with significantly higher plasma of IFN-γ, TNF-α and IL-2 concentrations in CVL ($p<0.0001$) and asymptomatic ($p<0.0001$) compared to NVL participants. VL patients returned highest IL-10 levels ($p<0.0001$). Although, IFN-γ and IL-2 were increased in both CVL and asymptomatic individuals, IFN-γ levels were predominately high in CVL, while IL-2 was more patent in asymptomatic subjects. When compared with the reference test CPA, IL-2 appears as a new, 100% sensitive and specific marker for asymptomatic individuals (compared with 100% and 84.78%, respectively for IFN-γ). TNF-α and IL-10 showed a lower sensitivity and specificity than the other cytokines.

4 Conclusions

In conclusion, IL-2 in SLA-stimulated plasma is as a new marker for identifying asymptomatic subjects in *L. infantum* endemic areas with better specificity than IFN-γ. In order to validate the accuracy of this tool in the field, a transversal prospective study (involving a large number of samples and different transmission areas) is currently underway.

C1812 NEW SEROPREVALENCE FOR MAPPING L. INFANTUM INFECTION AND EPIDEMIOLOGICAL DATA AFFECTING DOGS IN SPAIN.

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1 Background

Canine leishmaniosis (CanL) caused by *Leishmania infantum* is a widespread endemic disease in the Mediterranean basin. Prompted by a human leishmaniosis outbreak in Madrid (Spain), since 2009 this zoonotic disease has been paid renewed interest due to public health concerns. The present study updates *L. infantum* infection seroprevalence in dogs in Spain and examines the epidemiological role of host and habitat variables as risk factors for CanL infection.

2 Methods

Over the period 2011 to 2016, several cross-sectional studies were conducted on a total number of 1638 dogs from 70 different locations spanning 25 Spanish provinces including regions never sampled before. The seroprevalence of *L. infantum* infection was determined by the indirect immunofluorescence anti-bodies test (IFAT).Chi-squared and t tests were used to analyse relationships between *L. infantum* seroprevalence and several epidemiological variables including dog sex, age, weight, size, breed, use given, habitat and corresponding bioclimatic zone.

3 Results

Seroprevalences of *L. infantum* infection were mapped by province. Percentage seroprevalence distributions by each bioclimatic belt were: Mountain (5.38), Hillside (3.47), Supramediterranean (7.76), Mesomediterranean (11.90), Thermomediterranean (20.86). Seropositivity for *L. infantum* was related to size (large breed dogs versus small) and age, such that significantly higher seroprevalences were recorded in adults dogs (5-6 years).

4 Conclusions

These data provide an updated prevalence map of CanL in Spain.

C1813 A COMPARISON OF DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF SUBCLINICAL AND CLINICAL FORM IN DOGS WITH LEISHMANIOSIS

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1 Background

Canine leishmaniasis (CL) caused by *Leishmania infantum* is the most common and an important zoonotic disease. Turkey as a natural bridge between Europa and Asia plays important role to disease transmission and effective disease control programs. There is no available data about the CL in Bursa, one of the biggest city and very closed to Istanbul in Marmara Region of Turkey. Researches of CL have been focused mainly on molecular detection methods, but in practice veterinary clinicians needs to more practical approaches to describe CL and then discriminate by using clinical characteristics from sub-clinical (asymptomatic patients) to clinical CL. Thus, in the present study, demographic and clinical characteristics of subclinical and clinical form of CL were compared.

2 Methods

In this study, dogs were divided into two groups; control (healthy dogs, n=20) and test group (dogs with CL). Test group was further divided as a subclinical (n=20) and clinical form (n=30) of CL. Sub-clinical form included dogs appearing healthy and with positive for *L. infantum* antigen by a rapid screening test (Anigen Leish Ag test), whereas clinical form included seropositive dogs to *L. infantum* antigen with 3 or more clinical complaints and abnormal results of physical examinations. Control and test groups were also checked for other vector borne diseases, and dual infections were excluded from the study. Hematological and serum biochemistry panel were performed.

3 Results

There were not statistically differences on demographic data and physical examination results between sub-clinic and clinic form. Dogs with clinical form had lower RBC and platelet counts ($P<0.05$), but higher RBC and platelet distribution widths, than those of dogs with sub-clinical form. Serum albumin and total protein levels in subclinical form were lower than in clinical form, but serum globulin was higher ($P<0.05$) in clinical form, compared to sub-clinical form. When compared to healthy controls, sub-clinical dogs showed lower RBC, WBC and platelet counts, and clinical dogs showed lower RBC, lymphocyte and platelet counts, with a statistical significant level. Lower serum albumin and Na levels but higher liver enzyme activities were observed in sub-clinical form, and the magnitude of abnormal test results was bigger in clinical dogs.

4 Conclusions

Anemia and thrombocytopenia with higher serum globulin might be used to discriminate from clinical to asymtomatic dogs with CL. Demographic data is not useful for clincal form of CL.

C1818 EPIDEMIOLOGICAL ASPECTS OF VISCERAL LEISHMANIOSIS IN DOGS IN THE ADMINISTRATIVE REGIONS OF THE FEDERAL DISTRICT / BRAZIL.

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1 Background

Visceral Canine Leishmaniasis (LVC) is the most emergent zoonosis that affects dogs in South America, especially Brazil. Today it has reached, through its main vector, the *Lutzomya longipalpes*, dogs and humans from various regions of Brazil. The purpose of this study was to determine seroprevalence and to identify risk factors for LVC in the Administrative Regions Lago Norte and Sobradinho II of the Distrito Federal / Brazil, in 2000s years.

2 Methods

The Distrito Federal is located in the Central-West Region of Brazil and has an approximate population of 2,570,160 inhabitants. The region of choice for the study was the peri-urban area of ??Sobradinho II where the communities of Bananal and Engenho Velho are located, with a focus on visceral leishmaniasis transmission and the urban area of ??the Administrative Region of Lago Norte represented by Varjão and Lago Norte. Blood was collected from 6,659 dogs from different regions. The serological test was the Indirect Immunofluorescence Reaction (IFR), used for the detection of antibodies against Leishmania in dog sera.

3 Results

It is observed that the lowest prevalence of 6.66% occurred in Varjão and when compared with the other study areas there is a significant difference with Engenho Velho, Bananal and Lago Norte with a prevalence rate of 13.13% , 15.40% and 22.32% respectively. When analyzing by region, it was observed that the locality of Engenho Velho presents a chance of 1.09 times more of dogs presenting infection, Bananal 2,13 and Lago Norte 3,16 when compared to Varjão. Different from the Lago Norte region, dogs from the community of Engenho Velho that owns a vegetable garden were 12 times more likely to become infected with Leishmania than dogs from homes that did not have vegetable gardens. When evaluating the prevalence, by age it is observed that the young age group presents 10.90% of reagents to the average age one to six years with 22.40% of seroreagent animals and the age group superior to six years of age with. About the animals examined, 15.95% had some type of clinical alteration, and 84.05% had no characteristic of the disease.

4 Conclusions

It can be concluded that Visceral Canine Leishmaniasis is distributed in the different regions of the Distrito Federal studied presenting with greater risk factors the locality, the age. In symptomatic seroreagent animals, the most evident clinical signs are slimming and skin lesion and weight loss, alopecia in the eyes and ocular secretion.

C1821 IP-10, MIG, MCP-1 AND IL-8 AS NEW BIOMARKERS TO DETECT ASYMPTOMATIC LEISHMANIA INFANTUM INFECTION

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1 Background

Asymptomatic *Leishmania* infection represents a major challenge to control the disease. Previous studies have demonstrated that the interleukin-2 and interferon-gamma (IFN-γ) are goods markers to detect asymptomatic subjects. Despite that, there is a need to identify new biomarkers to detect asymptomatic *Leishmania* spp infection.

2 Methods

Whole blood assay with soluble *Leishmania* antigen (SLA) was used as a reference test to detect asymptomatic individuals from the blood bank of the Hospital of Fuenlabrada (Madrid, Spain). From the 550 blood samples tested, 40 asymptomatic individuals and 20 negative controls (NVL) were included in this study.Blood samples were stimulated with soluble *Leishmania* antigen (SLA) for 24h at 37ºC. We have explored the production of IFN-g-induced protein 10 (IP-10), the monokine induced by IFN-γ (MIG), monocyte chemoattractant protein-1 (MCP-1) and interleukin-8 (IL-8) in SLA-stimulated whole blood and compared them with levels of IL-2 and IFN-γ. The levels of analytes in SLA-stimulated plasma were detected by Cytometric Bead Array.

3 Results

We found significant higher plasma levels of IFN-γ, IL-2, IP-10, MIG, MCP-1 and IL-8 concentrations in asymptomatic subjects controls (mean: 747.7; 1088; 19837; 11938; 96022; 59884 pg/ml, respectively) when compared with negatives (1.99; 1.82; 1778, 310.9, 6517, 13736 pg/ml, respectively). Compared with the IL-2 to detect asymptomatic subjects (100% sensivity (Se) and specificity (Sp)) IFN-γ showed 100% Se and 97.5% Se. Likewise, IP-10had 100% Se and 90% Sp;MIG had 100% Se and 85% Sp; MCP-1 had 79% Se and 82% Sp, and IL-8 had 80% Se and 83% Sp. In comparison with the low levels produced by IL-2 and IFN-γ in SLA-stimulated plasma, the patent levels of IP-10, MIG and MCP-1 suggested the combination of these three chemokines to easily detect asymptomatic subjects (100% Se and 95% Sp).

4 Conclusions

IFN-γ, IL-2, IP-10, MIG, MCP-1 and IL-8 in SLA-stimulated whole blood have proven to be goods biomarkers to detect asymptomatic infection. Further studies are needed to explore the potential of IP-10, MIG and MCP-1 for the detection of asymptomatic *Leishmania* infection with the perspective to develop and immunocromatographic test for field assay.

C1838 MULTIKINE (CHEMOKINE/CYTOKINE) DETECTION IN FILTER PAPER TO IDENTIFY ASYMPTOMATIC LEISHMANIASIS CAUSED BY LEISHMANIA INFANTUM AND LEISHMANIA DONOVANI

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1 Background

Whole blood assay is a field tool rapid, stable, robust and sensitive. This assay has been demonstrated to be a great tool to detect asymptomatic subject in the field, but a simple way to store plasma samples in the decentralized settings is needed. Protein Saver cards offer significant logistical and safety alternatives to transporting and storing liquid samples and may aid in detection and validation studies. For that reason, the main objective of this work was to find an easy tool to detect asymptomatic individuals in field. For that, we have compared chemokine and cytokine from SLA-stimulated plasma sample with the results obtained from plasma eluted from dried plasma spots (PE-DPS).

2 Methods

We analyzed samples from asymptomatic individuals (n=40) and negative control (n=20) from a *Leishmania infantum* area in Spain (Fuenlabrada), and asymptomatic individuals (n=12) and negative control (n=13) from *Leishmania donovani* area in Bangladesh (Mymensingh). Blood samples were stimulated with SLA for 24h at 37ºC. From each sample, 40µL of SLA-stimulated plasma was added in Protein Saver Cards. The production of interleukin-2 (IL-2), interferon-γ (IFN-g), IFN-g-induced protein 10 (IP-10), interleukin-8 (IL-8), monocyte chemoattractant protein-1 (MCP-1) and the monokine induced by IFN-g (MIG) in SLA-stimulated plasma and PE-DPS was quantified using a multiparameter-based immunoassay.

3 Results

We have described previously that these analytes can be goods markers of asymptomatic infection (Poster C1821). In both, SLA-plasma stimulated and PE-DPS levels of IFN-g, IL-2, IP-10, IL-8, MCP-1 and MIG were significantly higher for asymptomatic subjects compared with negative controls. Further, the correlation between PE-DPS and SLA-stimulated plasma were excellent in both groups of asymptomatic subject from *L. infantum* and *L. donovani* endemic areas. In addition, we demonstrated that samples in dried plasma spots (DPS) could be stable 10 days at -20ºC and room temperature obtaining same results.

4 Conclusions

In conclusion, this proof of concept has successfully demonstrated that the combination of whole blood assay and PE-DPS samples is a new approach to ease and accurately detect asymptomatic subjects. Further, studies needed to be done to validate it in a larger population that includes other areas of transmission and decentralized settings.

C1842 PALEOPARASITOLOGCAL STUDIES OF LEISHMANIOSIS AROUND THE WORLD AND TURKEY

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1 Background

Parasites of the *genus leishmania* are obligatory intracellular protozoa belonging to the order kinetoplastida. They are causative agents of complex diseases with variable clinical and epidemiological diversity and they cause serious public health issues in many countries.

2 Methods

More than 500 articles of international and national book chapters and papers containing the keyword paleoparasitology and leishmaniasis in the world and Turkey" are researched and studied.

3 Results

Paleoparasitology is the study of the history of parasitism in animal and human host population. The origin and evaluation of leishmaniasis have been under discussion regarding some epidemiological and parasitological aspects. The history of archaeological samples has led the parasitological analysis in time and space. There are species whose presence are described in reptiles. Some experimental studies were conducted in mammals, including one human volunteer. An early report of diseases (1580) in South America was called "Mal de los Andes". In 1939 it was defined as cutaneous leishmaniasis. Between 1950 and 1980 there was a period characterized by CL studies. During 1960's it was recognized mucocutaneous leishmaniasis. First cases in Turkey were encountered in 1916, which was named as "*leishmania splenique*". Lately, histological data are very important in the laboratory tests to detect Leishmania DNA in archaeological remains. Paleoparasitological examinations are promising to clarify the gap that still exists in the relation to leishmaniasis in human.

4 Conclusions

The report from the WHO states that leishmaniasis is present in 98 countries in five continents. Every year about 1.3 million new cases is added to the existing 300.000 cases of visceral leishmaniasis. Due to warfare and high immigration rate in Turkey, the spread parasites are increased. This review is prepared within the idea, that the most successive way to cope with this disease is to follow the traces of its history. Thus, prehistorical documents are essential since they can help to unveil the space and time of leishmaniasis.

C1844 SEROPREVALENCE OF LEISHMANIA INFANTUM IN DOGS FROM GREECE

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1 Background

Canine leishmaniosis (CanL) is a common disease in Greece, as in other Mediterranean countries, remaining endemic with increased numbers of infected dogs and occasionally humans. The primary objective of this study was to investigate the seroprevalence of CanL and the risk factors in clinically healthy dogs from all geographical regions of Greece by detecting the presence of specific antibodies against *Leishmania infantum.*

2 Methods

Blood serum samples were obtained from 740 clinically healthy dogs, during March to December 2016, from 37 different municipalities of Greece. Also, animal data were recorded including information such as gender, age and date of the last antiparasitic treatment. The examination of each sample was performed using the rapid in *vitro* test Speed Leish K™ (Virbac, France).

3 Results

Specific antibodies against *L.infantum* were detected in 88/740 (11.9%) dog blood serum samples. Seropositive dogs to *L.infantum* were detected in 21 from 37 (56.8%) municipalities scattered in the different geographical regions of Greece. In all the regions there were detected positive cases. Increased percentages were detected in companion dogs living outdoors ageing from 3 to 5 years old. There was no significant difference between the gender of the seropositive animals. Finally, mongrel dogs were more commonly challenged than the ones of the various breeds.

4 Conclusions

The results of the present study confirmed that *L. infantum* remains a common parasite in the clinically healthy canine population of Greece and therefore its early diagnosis and prevention are of major importance in the country.

C1848 IMPORTED LEISHMANIASIS IN LONDON: MANY TYPES OF DISEASE PRESENTATIONS

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1 Background

The Hospital for Tropical Diseases (HTD) at University College Hospital London (UCLH) is a national center for treatment of leishmaniasis. We see all types of leishmaniasis and unusual presentations in immunocompromised patients. We report a 2 year period of our cases.

2 Methods

We retrospectively reviewed 42 cases of leishmaniasis treated at HTD between 2014-16. We collected data on demographics, travel exposure, causative species, lesion location and number, treatment regimens and outcomes.

3 Results

27(64%) were male, median age was 33.5 years, (range 18-82 years. We had 34 cases of CL, 3 MCL, 4 of VL. Regarding CL 16 (38%) of cases were Old World leishmania infections and 62% New World Infections. 24(57%) patients had infections with *Leishmania Vianna,* sp *L.Braziliensis,* identified by leishmania DNA PCR. 11 (26%) were acquired in the Peruvian Amazon. Amastigotes were identified in 20 (61%) of PCR positive biopsies.

Three patients (9%) presented with mucocutaneous leishmaniasis (MCL), two of which were *L.Donovani* infections acquired in Spain with vocal cord lesions and without skin involvement. Both cases had significant co-morbidities. They were using inhaled steroids.

Visceral Leishmaniasis: 4 patients (11%) presented with visceral leishmaniasis (VL). Most were immunocompromised and all had delayed diagnosis. Treatment courses were complex and individually managed. One patient had VL caused by *L.siamensis* acquired in Guyana.

All cutaneous leishmaniasis (CL) cases are managed as outpatients. 21(64%) received daily IV Sodium Stibogluconate (SSG), 17(81%) of these were *L.Vianna* infections. Most patients reported side effects with a wide range in severity. 6 (18%) received treatment with oral Miltefosine with no treatment failures.

In patients receiving IV SSG two (8%) failed on first line treatment, one was subsequently treated with oral Miltefosine and one with intra-lesional SSG, both successfully. Side effects included mild reversible transaminitis, reversible QT prolongation, mild anaemia and leucopenia.

Three patients were HIV positive: one patient with CL and two patients with VL.

4 Conclusions

A wide range of leishmaniasis presentations are seen at the HTD. The majority of CL in our patients is in young adults, with *L.Viannia* from the Peruvian Amazon and Central America. Medical immunosuppression causes unusual presentations for MCL and VL. VL cases are associated with immunodeficiency and are difficult to manage.

C1860 EUROLEISH.NET: CONTROL OF LEISHMANIASIS, FROM BENCH TO BEDSIDE AND COMMUNITY

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1 Background

Controlling leishmaniasis at a global level requires the development of new control tools and strategies. This R&D process should be conducted by a new generation of scientists trained in a state-of-the-art inter-disciplinary and international environment.

The aim of "EuroLeish.net: Control of leishmaniasis, from bench to bedside and community" project is to train the next generation of scientists who will contribute to the control of leishmaniasis. Public and private research centres of excellence in Europe and abroad have joined forces to develop innovative tools for the control of leishmaniasis. The multidisciplinary training programme combines basic and translational research to provide integrated solutions for the improvement of diagnosis, treatment and prevention of leishmaniasis worldwide. A cohort of 15 PhD fellows from diverse backgrounds are being trained in the network for 3 years.

The research methodology and approach implemented in this training programme is based on the development of state-of-the-art individual research projects (Excellence) taking advantage of the synergies and complementarities offered by the different partners in the network (Exchange). Controlling a complex disease such as leishmaniasis requires a holistic approach and the interaction of researchers from different disciplines conducting studies in different fields. The structure, designed to integrate the research projects, will ensure (1) the Interdisciplinarity by promoting the exchange of skills and experiences between fellows and research groups working on different disciplines (e.g. immunology and molecular biology or health economics and epidemiology) and (2) the Multidisciplinarity by enhancing the use of different methodologies, approaches and resources to achieve the network objectives.

The training of the EuroLeish.net fellows is articulated around the 15 research projects ranging from drug discovery to health economics. The outcomes of the studies should provide a significant progress in the science of leishmaniasis control, while consolidating training and research network links in this field. EuroLeish.net researchers are part of a singular network of scientists and institutions. The EuroLeish.net network includes 33 academic and non-academic institutions, 9 institutions will host the 15 fellows, and the other 24 Partners will provide training, mainly through secondments.

C1870 LEISHMANIASIS IN UZBEKISTAN - AN EPIDEMIOLOGICAL UPDATE

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1 Background

In Uzbekistan visceral (VL), zoonotic cutaneous (ZCL) and anthroponotic cutaneous leishmaniasis (ACL) are endemic. Historically Uzbekistan was the republic with the highest prevalence in the former USSR. In the first half of the 20th century > 6000 VL cases occurred, however due to control measures leishmaniasis was nearly eradicated by the end of the 1960s. In the last two decades the number of cases is again increasing. The objective of this study is to present an epidemiological update and implementation of modern molecular diagnostics and ecological niche modeling for risk assessment of leishmaniasis in Uzbekistan.

2 Methods

In 2014-2016 103 clinical samples (12 VL and 91 CL cases) from 6 endemic regions (Samarkand, Jizzakh, Navoi, Kashkadarya, Surkhandarya, Fergana) were studied. In addition, reservoir studies were carried out in Kashkardarya and entomological surveys in Jizzakh, Surkhandarya, Samarkand, Kashkardarya. Molecular identification of the causative agents at species level was carried out followed by pilot multilocus microsatellite typing (MLMT) to reveal the diversity of circulating genotypes at strain level. Ecological niche modeling was performed based on registered cases and entomological data and spatiotemporal distribution models were calculated for risk assessment.

3 Results

2013-2015 916 CL cases were registered in 7 regions; the number of VL cases was 52 in 4 regions. ITS1-RFLP of the collected clinical samples identified the causative agents of CL as *L. major* and *L. tropica*, and of VL as *L. infantum*. MLMT placed *L. infantum* from Uzbekistan in a cluster close to *L. infantum* from Tajikistan, Southern Caucasus and Turkey in a combined data set of 520 strains from 19 countries, and *L. tropica* close to a subpopulation from Turkey and the Middle East in a data set of 269 *L. tropica* strains from 12 countries. Spatiotemporal projection based on climatic and environmental conditions identified the main regions at risk in the presence and future.

4 Conclusions

This study provides an update of the prevalence of leishmaniasis in Uzbekistan. Moreover, molecular diagnostics was established for the first time in Uzbekistan. The etiology of leishmaniasis was defined in the studied regions and for the first time causative agents of CL were typed by molecular methods at species and strain level. Important conclusions about spatial and temporal dynamics of the disease could be provided by inferring species distribution models allowing development of appropriate control measures.

C1877 FALSE OR SUB-CLINICAL VISCERAL LEISHMANIASIS

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1 Background

Visceral leishmaniasis (VL) diagnoses, sometimes presents considerable difficulties related to the false negativity of direct parasitologic methods as bone marrow aspiration or lymph node biopsy, from 13% to 22.5% and also to false negativity of serological techniques from 6 to 10%.We encountered cases which imitated VL clinically and biologically, with positive serology , but didn't result VL in the end. We defined them false VL (FVL) or sub-clinical (SCVL). We evidence the nosology and diagnose of FVL and SCVL

2 Methods

We calculated frequency of FVL in all cases with seropositive , but didn't results VL in the end. We identified their real causes using specific protocols. To know persistence of antileishmania antibodies we have analyzed their performance and levels in 6 cases with VL and 4 with FVL in order. We included 34 cases, 16-71 years old, leishmaniasis-like with ELISA antileishmania IgG positive, during 1995- 2015.

3 Results

11 of 34 cases with leishmaniasis-like symptoms and positivity of antileishmania IgG resulted FVL. Mimetic leishmaniasis resulted: Non Hodgkin Lymphoma 2; colagenosis 2; monocytic leukemia 1; splenic lymphoma 2; abdominal typhus 1, ovarial abces 1; chronic myeloid leukemia 1, hair cell leukemia 1 case.
IgG level and it's follow up in 6 cases with VL:
titre at the time of diagnoses was 19.6 to 37.5UI/ml; their normalization was found 7-11.5 months after treatment with glucantime.
IgG level and performance in 4 cases with FVL
Initial titre : 11.4 - 26.0 UI/ml; normalization was observed 5-13 months after treatment of primary diseases

4 Conclusions

In 26.6% of cases with VL –like syndrome and seropositivity of antileishmania IgG weren't true visceral leishmaniasis.Their nozologic structure included 8 different pathologies.
Normalization of antileishmania IgG level in our cases occurred 7 to 11.5 months after specific treatment.

C1881 PROBLEMS ENCOUNTERED IN VISCERAL LEISHMANIASIS IN ALBANIAN ADULTS

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1 Background

Leishmaniasis in Albania are in addition to a number of factors, such as climate change,increased vector resistance to pesticides and to classical antileishmaniasis drugs.We have encountered problems in the identification and treatment of visceral leishmaniasis (VL) in Albanian adults

2 Methods

We analyzed 120 cases of Visceral leishmaniasis, 16-68 years old, during the period 1990-2015, classifing difficulties and problems encountered in particular groups by sections of the antropozoonosis elaboration. On each section listed clinical-biological problems by frequency which they met to our patients.Diagnostic methods studied were, bone marrow aspiration 80, ELISA anti-leishmannia IGM and IGG 15, both methods 33, biopsy of lymphonodes

3 Results

From 120 cases with VL, 76 resulted problematics.Based on atypical clinical aspects:atypical symptoms; lack of fever 5 cases, lack of enlarged spleen 3, beginning of illnes as pneumonia 2 cases, diffuse lymphadenopathy without fever 3, herpes zoster 1,chronic diarrhea1,vasculitis 1 case.Laboratory atypical data: lack of hematological alterations 6 cases,lack of hipergamaglobulinemia 4,lack of parasite in hip bone aspiration 6, in sternal bone aspiration 4 cases,lack of parasite in both forms of bone marrow aspirations 2,negativity in ELISA of specific Ab IgM 3,negativity in the ELISA Ab specific IgM and IgG 1 case. Mimetic Co pathology: Leishmaniasis plus Tuberculosis 3 cases,LED 1, lymphomas 4,hair cell leukemia 2,bone marrow aplasia 1,cirrhosis 1 case.Serious side effects of antileishmaniasis drugs:death 2 cases and severe miocarditis 1case from glucantime, shock and acute renal insufficiency by lomidina 1, vasculitis from Allopurinoli 1. Clinical resistance to treatment 12 cases. The decrease mainly partial and temporary of fever or not improvement of other clinical-biological sings. This phenomenon was observed in persons with co- pathology, similar with VL. Parasitological resistance to etiological treatment: from glucantime 3 cases, allopurinol 3, ambisome 1

4 Conclusions

The incidence of VL problems was 63.3%. Difficulties encountered related, with clinic atypical aspects 13.3%; atypical biologic data 9.1%; bone marrow aspiration false negative12.5% and serious side effects to treatment 9.1%. In 10% of cases the disease was accompanied with co pathologies similar to VL semiotics . Parasitological resistance to etiological treatment was seen in 3.3 of cases.

C1915 FILARIA AND LEISHMANIA CO-INFECTION IN HUMAN ASYMPTOMATIC VISCERAL LEISHMANIASIS

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1 Background

Leishmaniasis and filariasis, coinfection can occur in an endemic area. The immunomodulation triggered by filaria infection might influence the clinical signs and progression of both diseases. The aims of the study were to determine the prevalence of filarial infection in asymptomatic *L. donovani* infected individuals and their association of filarial co-infection with progression to Visceral Leishmaniasis (VL) disease.

2 Methods

TaqMan based qPCR assays were performed to detect filarial species, *Wuchereria banchrofti* and Leishmanial species, *L. donovani* in 833 serologically positive and negative, Leishmania infected healthy individuals (Asymptomatic) living in endemic region Muzaffarpur District Bihar, India.

3 Results

330 (39.6%) Individuals were infected with Leishmania and 69 (8.3%) had filarial infection (23 were co-infected). After fallow up Seven individuals progress to VL in which 2 (8.6%) from co-infected, 3 (0.97%) from *L.donovani* DNA positive individuals and 2 (0.43%) from non-infected individuals.

4 Conclusions

This study shows the increased severity of clinical signs when Leishmania-filarial co-infection is present but this is too early to go for final conclusion because progress to clinical VL numbers is very low.

C2058 PREVALENCE OF INFECTED ASYMPTOMATIC INDIVIDUALS IN FUENLABRADA DESCRIBED A GRADIENT FROM THE PARK (35.4%) TO THE CENTER OF THE TOWN (8%) BY USING THE CELLULAR TEST WBA

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1 Background

The largest outbreak of leishmaniasis declared in Europe has been reported in the southwest of Madrid (Spain). The "Bosque Sur" metropolitan park in Fuenlabrada is an ideal ecosystem to sustain high population density of phlebotomine sand flies where coexist with amount of sylvatic reservoirs as hares and rabbits. From 2009-2012, a total of 446 cases were reported: 35.9% visceral leishmaniasis and 64.1% cutaneous leishmaniasis. Most of the *Leishmania* spp. infections are asymptomatic and its identification is pivotal to estimate the true prevalence of leishmaniasis.

2 Methods

A cross-sectional descriptive comparison of 805 healthy subjects with no history of leishmaniasis was conducted from January to November 2015 in the framework of the epidemiological serovigilance survey "V SEROVI", carried by the Health Promotion and Prevention Subdirectorate, Madrid (Spain). The detection of asymptomatic individuals was performed using 1) WBA with a measurement of IL-2 levels whose cut-off was ≥ 50.37pg/ml, and 2) immunofluorescent antibody titer (IFAT) analyses whose threshold titer for positivity was set at 1:80.

3 Results

Up to 20.4% (169/805) of the individuals living in Fuenlabrada were asymptomatic by the cellular test WBA. However, the humoral prevalence measured by IFAT was 0.17% (1/805). Consequently, agreement between the tests was poor (85.9%; κ=0.020; 95% CI -0.007 to 0.048) and the further described results are based on WBA and IL-2 quantification.

According to the incidence reported by Arce et al (2012), prevalence of asymptomatic infection was higher near to the park (35,4%, 30,3% and 24,8%) and decreased when moved into the town (16,8 and 8%) as a gradient. Other towns close to Fuenlabrada reported lower prevalence of asymptomatic infection (Mostoles 8% and Getafe 4%).

From the factors analyzed in this study, age, sex, or type of house showed statistical differences between groups. Of the total of asymptomatic individuals, 28.0% were men and 12.7% were women. 161 (21.1%) of them had Spanish origin while only 3 were foreigners. The percentage of asymptomatic subjects increased significantly along with the age: <2 (0%), 2-5 (1.2%), 21-30 (15.9%), 41-60 (26.2%) and older than 60 (27.9%). In addition, 19.1% of the asymptomatic subjects lived in apartments and 33% in detached houses.

4 Conclusions

A gradient of asymptomatic infection was foundfrom the park (35.4%) to the center of the town (8%) by using WBA as a field tool. Seroprevalence of asymptomatic infection was 0.17% by IFAT, indicating that this technique underestimated the number of asymptomatic subjects infected by *L. infantum.* In addition to the distance to the main focus, age, sex, or type of house were important to get infected in the area. A study to assess the real prevalence of leishmaniasis in the whole Community of Madrid is underway, including others intrinsic and extrinsic factors.

C2059 VISCERAL LEISHMANIASIS AS A RE-EMERGING PROBLEM IN ARMENIA, SEROEPIDEMIOLOGICAL STUDY, 2015-2016

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Armenia

1 Background

Leishmaniasis is vector-borne disease caused with *Leishmania*, transmitted by *Phlebotomine* sandflies. The first case of visceral leishmaniasis (VL) in Armenia was officially registered in 1913. During 1926-1969, 919 VL cases were reported at different altitudes, the main focus was Yerevan. A 1970 dog-survey showed 7.3% infection. From 1969-1999, no VL cases were reported, however, 115 indigenous human cases of VL with 6 fatal have been reported since 1999, mainly in Tavush, Syunik, Lori regions and Yerevan (the incidence rate is 0.03-0.6 per 100000 population). We conducted seroprevalence study among non-symptomatic children up to 5 y.o. in 2015 and children with clinical manifestation in 2016 to assess the real prevalence of VL in Armenia. The seroprevalence of canine leishmaniasis was investigated in order to understand the zoonotic transmission.

2 Methods

In 2015 from the Medical facilities' register children ≤10 y.o. by systematic random sampling and in 2016 - children in recent VL foci with any of leishmaniasis' symptoms (weight loss, irregular fever, anemia) were tasted using immune-chromatographic rapid tests rK 39. Domestic and stray dogs living within 1.5km radius of a human case residence were sampled and tested as well.

3 Results

In 2015 from 1238 children tested with rK 39 only four were positive (0.3%), two with clinical symptoms. In 2016 373 children with VL clinical symptoms were tasted with 8 positives (2.1%). In 2015-2016 in these regions, 209 dogs were tested with 18 positives (8.6%): 4 in Syunik (9.3%), 10 in Tavush (16.1%), 2 in Lori (6.5%), 2 in Yerevan (3.2%).

4 Conclusions

VL is a re-emerging problem in Armenia, with a high epidemic potential because of its canine reservoir. To strengthen intersectional collaboration is recommended, with vector-control and host-reservoir treatment programs. Medical facilities must be provided with tests to conduct investigations as soon as any of VL symptoms revealed.

Leishmania-HIV co-infection

C0092 EXPLORING PROGNOSIS IN CHRONIC RELAPSING VISCERAL LEISHMANIASIS AMONG HIV-INFECTED PATIENTS: CIRCULATING LEISHMANIA DNA

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1 Background

The approach of visceral leishmaniasis (VL) affecting HIV-infected patients is still surrounded by many uncertainties, especially regarding the criteria to institute and discontinue secondary prophylaxis for VL. The aim of this study was to evaluate the *Leishmania* parasitism kinetic assessed by polymerase chain reaction (PCR) as a possible tool in the prognostic assessment in a context in which patients are receiving highly active antiretroviral therapy and secondary prophylaxis.

2 Methods

From 2011 to 2013, 178 Brazilian patients presenting clinical signs and symptoms compatible with VL were evaluated and 90 patients had their VL diagnosis confirmed, of whom 46 were HIV co-infected (51%). Of the 46 VL-HIV co-infected patients identified in the large cohort, seven patients died between the VL treatment period and the first follow-up visit and seven others were excluded because of a negative (three) or not available (four) *Leishmania*-PCR test before VL treatment. Therefore, 32 *Leishmania*-HIV co-infected patients were prospectively enrolled and followed up during a period of 12 months, of whom 16 relapsed clinically.

3 Results

Approximately two-thirds of all clinical relapses occurred in the first six months after the end of VL treatment (11/16 patients, 69%). Relapsing (R) and non-relapsing (NR) patients had similar antiviral therapy use rates, CD4 lymphocyte count medians and HIV load levels at VL-diagnosis. However, the time between HIV and VL diagnoses was longer in the R than NR-group. At the 12-month follow-up, R-patients presented a significantly lower CD4 lymphocyte count than NR-patients, although no difference in HIV load control. Comparison between Kaplan-Meier relapse-free survival curves (time to clinical relapse from the end of VL treatment) using a log rank test showed that patients presenting circulating *Leishmania* DNA (peripheral blood) had a significantly higher risk of clinical VL relapse within 4 months after a positive test ($p=0.001$) than patients presenting negative *Leishmania*-PCR.

4 Conclusions

Two groups with distinct clinical prognosis unpredicted by their CD4 count at the moment of VL diagnosis and not related to their HIV load control were confirmed. Our results reinforce that a negative PCR could be a useful tool to support prophylaxis interruption among patients with CD4 counts above 200 cells/mm^3 and that a positive PCR suggests imminent VL relapse.

C0142 CASE STUDY: MILTEFOSINE FOR THE TREATMENT OF RECURRENT VISCERAL LEISHMANIASIS IN TWO HIV POSITIVE PATIENTS IN BRAZIL.

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1 Background

Visceral leishmaniasis (VL) became an important opportunistic infection in people immunosuppressed by Human Immunodeficiency Virus (HIV) in endemic countries. VL/HIV co-infection represents, in Brazil, about 1,1 % of VL cases. Miltefosine, an oral drug, was released as an alternative in patients with leishmaniasis and could be an option for cases of VL- HIV co-infected patients. The use of this drug in such patients shows a positive initial response, however the relapse rate is high.

2 Methods

We reviewed the medical records of two HIV positive men native from the northeastern region of Brazil. Both men received many cycles of AMBL before attempting treatment with miltefosine.

3 Results

Case 1: A 56-years- old man diagnosed with VL/HIV co-infection in 2013. He was admitted for the sixth time to our hospital in april 2016 complaining of epistaxis. During the hospitalization was found hepatosplenomegaly, severe pancytopenia and an immunofluorescence for leishmaniasis reporting 1:80 dilutions. It was initiated a combination of AMBL and, for the first time, oral miltefosine for 28 days. By the time of the discharge he was maintaining the hepatosplenomegaly and pancytopenia. Thrombocytopenia improved slightly, and epistaxis was controlled. He was consecutively followed up at the outpatient setting at the Brasília University Hospital and it was made a myelogram with evidence of persistent amastigotes forms of Leishmania.
Case 2: A 39-years- old man diagnosed of VL/HIV co-infection in 2012. After the third treatment for VL (2015), he was investigated for dyspepsia. He performed an upper digestive endoscopy with a biopsy that showed amastigotes of Leishmania. He was hospitalized and physical examination revealed hepatosplenomegaly. Hemogram showed pancytopenia. After treatment with AMBL a PCR of peripheral blood showed persistence of Leishmania. A cycle of 30 day oral miltefosine was administrated, with clinical improvement and mild pancytopenia at discharge, but persistence of positive PCR for Leishmania in peripheral blood.

4 Conclusions

Although both patients in this report reached clinical benefits from control of symptoms and even weight gain, the use of miltefosine in association with AMBL was ineffective in achieving the patient´s parasitological cure.

C0228 KATEX URINE ANTIGEN TESTING FOR DIAGNOSIS OF VISCERAL LEISHMANIASIS AND MONITORING OF TREATMENT RESPONSE IN HIV CO-INFECTED PATIENTS FROM ETHIOPIA

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1 Background

Visceral Leishmaniasis (VL) diagnosis and treatment monitoring in HIV patients is problematic. Since serological tests perform poorly in immunosuppressed patients, invasive parasitological confirmation by spleen aspirate is often done. This procedure is painful, prone to complications, and hampers scale-up of care. Parasite antigen detection in urine is a promising non-invasive alternative. It has not yet been validated in HIV patients.

2 Methods

We conducted a diagnostic accuracy study nested within the pre-screening phase of a cohort study in VL-endemic North-Western Ethiopia between 2011 and 2013. 71 adult male HIV-coinfected VL patients received Katex testing (Kalo Biological, UK) at VL diagnosis, weekly during treatment, and at test of cure (TOC) after 28 days. Both VL diagnosis and treatment failure (positive TOC) was aspirate-confirmed. 59 adult male controls without VL symptoms were Katex tested to calculate sensitivity at VL diagnosis.

3 Results

Among VL patients, median age was 32 years (inter-quartile range [IQR] 28-37), median CD4 count was 54.5 cells/µL (IQR 37-96), 61% were on anti-retroviral therapy, and 44% had VL relapse. 59%, 47%, 44%, and 7% of patients received sodium stibogluconate, ambisome, miltefosine, and paromomycin for VL treatment, respectively. 25% of patients had treatment failures at day 28.
Katex sensitivity and specificity for VL diagnosis were 83% (95% confidence interval [CI] 71-91) and 98% (95% CI 91-100) overall. Sensitivity was 81% (95% CI 61-93) and 84% (95% CI 67-95) in patients with and without previous VL episode; 100% (95% CI 85-100) and 72% (95% CI 55-86) in patients with aspirate parasite grade 6 and below 6; and 91% (95% CI 76-98) and 79% (95% CI 61-91) in patients with CD4 count below and above 50 cells/µL.
Katex was positive at VL diagnosis in 93% of patients with treatment failure and in 74% among those with treatment success, in 93% and 63% at day 7, in 85% and 41% at day 14, in 85% and 36% at day 21, and in 75% and 45% at day 28, respectively.
For treatment failure, Katex sensitivity, specificity, positive predictive value, and negative predictive value was 75% (95% CI 43-95), 55% (95% CI 36-72), 38% (95% CI 19-59), and 86% (95% CI 64-97).

4 Conclusions

Katex sensitivity for VL diagnosis was moderate overall, and good in patients with high parasite loads and low CD4 counts. Diagnostic accuracy for treatment failure was poor. Non-invasive VL diagnostics remain a high priority to improve and scale up VL care for HIV patients.

C0300 MBL2 VARIANTS INFLUENCES THE MBL MANNAN BINDING CAPACITY OF PATIENTS WITH VISCERAL LEISHMANIASIS (VL) AND HIV-VL CO-INFECTED PATIENTS

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1 Background

Co-infection visceral leishmaniasis (VL) and HIV/AIDS emerges as a process whose pathogens can act synergistically. Mannose-binding lectin (MBL) modulates inflammation and has a major role function on innate immunity. MBL expression is influenced by single nucleotide polymorphisms (SNPs) in *MBL2* gene. We evaluated the binding capacity of MBL to mannan regarding the genotypes of exon 1 and promoter region -550 and -221 of *MBL2* and the direct agglutination test (DAT) in patients co-infected with LV and LV /HIV-AIDS.

2 Methods

We used 73 samples, subdivided into 19 patients with LV, 33 patients LV/HIV-AIDS and 21 HIV DAT negative (<1:6400). ELISA evaluated mannan-binding capacity; DNA was extracted by commercial kits according manufacturer's instructions. The promoter region was genotyped using Taqman probes and exon 1 using SybrGreen dye using real time PCR approach. Data analysis was performed using the GraphPad Prism program

3 Results

We found an increased binding capacity of MBL to mannan in subjects with genotypes HH, YY and AA. MBL binding capacity to mannan was higher in patients with LV compared to LV/HIV-AIDS ones (p= 0.0126). LV/HIV-AIDS patients showing intermediate/low genotype production of MBL had higher antibody titers (p=0.0007).

4 Conclusions

Our results demonstrated that *MBL2* variants influence the binding capacity of MBL to mannan and coinfected individuals carrying genotypes associated with intermediate/low serum MBL seemed to be related to disease progression.

C0346 HIV/AIDS AND LEISHMANIASIS IN INDIA: FACTORS THAT CONTAINED THE EPIDEMIC OF CO-INFECTION

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1 Background

After HIV epidemic several countries endemic for leishmaniasis reported co-infections of Leishmania with HIV. The most affected country was Spain. The co-infection of these two pathogens leads to rapid disease progression, more severe manifestations and poor response to treatment with tendency to relapse. In AIDS patients the non-visceralizing species of Leishmania can visceralize and visceralizing species can cause dermal or mucosal manifestations. India has witnessed attaining the dubious distinction of having maximum number of HIV positives and also of visceral leishmaniasis.

2 Methods

A systematic review of literature from India is presented here to understand what were the factors that contributed to keep the epidemic of this co-infection low.

3 Results

On reviewing the literature since first case of visceral leishmaniasis (VL) and HIV was published in 1999 from India till 2014, hundreds of cases of HIV-leishmania co-infections have been reported, but the proportion has been low (0.029-0.4%) as compared to the other countries. So far, more than 89 cases of VL-HIV and 10 cases of cutaneous leishmaniasis (CL) with HIV have been published since 1999. Of these 10 cases, 5 had simple CL and 5 cases manifested with diffuse cutaneous leishmaniasis (DCL). In addition, one case of post-kala-azar mucosal leishmaniasis (PKML) in a full blown AIDS patient has also been reported. In two cases no clear description could be ascertained whether these were cases of DCL or post-kala-azar dermal leishmaniasis (PKDL). Though the first case of VL-HIV co-infection was reported from sub-Himalayan state of Uttarakhand, most cases of co-infection are now reported from VL endemic state of Bihar.

4 Conclusions

Most cases occurred during 1997-2007. After 2007 the number of new cases dropped down; most probably due to low HIV prevalence in VL and CL endemic regions and free supply of highly active anti-retroviral therapy (HAART) for HIV infected patients.

C0365 VISCERAL LEISHMANIASIS IN HIV HOSPITALIZED PATIENTS: COMPARISON BETWEEN HIV/VL CO-INFECTED AND HIV MONO-INFECTED GROUPS IN PERNAMBUCO, BRAZIL

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1 Background

Visceral leishmaniasis (VL) is the clinical form of leishmaniasis more associated with HIV and accelerates the progression to AIDS. The diagnosis of VL may be done in the presence of typical symptoms such as fever, pallor and hepatosplenomegaly, associated with a positive serological or parasitological test. However, this classic presentation is not always observed in people with HIV. This research aims to investigate the prevalence of this coinfection in patients in Pernambuco, Brazil, and to compare HIV/VL individuals with HIV mono-infected regarding epidemiological, clinical and laboratorial aspects.

2 Methods

It was a cross-sectional study with comparison of groups. It was conducted with inpatients from three referral public hospitals for infectious diseases in Pernambuco. The sample consisted of inpatients with HIV, in a period of data collection of six consecutive months in each hospital. It was used four diagnostic tests to VL: rK39-ICT, DAT, KAtex and PCR in peripheral blood. The definition of case of co-infection HIV/VL was the presence of at least one positive test for VL. This study was approved by the ethics committee on research from the Aggeu Magalhães Research Center.

3 Results

The sample was composed by 207 individuals. The prevalence was 16.91%. The prevalence was different according to the diagnostic test: rK39-ICT 1.47% (3/204), DAT 11.27% (23/204), KAtex 1.08% (2/185) and PCR 3.86% (8/207). More than half of the sample was composed of single and male individuals. 140 (67.63%) reported fever and 152 (73.43%) loss of weight. In the univariate analysis, it was found difference between the groups regarding educational level ($p = 0.004$), pallor ($p = 0.009$) and serum albumin level ($p = 0.009$). Educational level was higher in the group of individuals with negative tests for leishmaniasis. Cutaneous pallor was more frequent in coinfected individuals and the mean of serum albumin level was higher in this group.

4 Conclusions

Prevalence of HIV/VL coinfection was high, with many cases without typical symptoms of VL. There was a great variation in the prevalence according to the diagnostic method used, besides a low coincidence of test positivity.The boundary between latent and active infection does not seem well defined in hospitalized patients since their own clinical condition hinders this observation. New laboratory techniques, or improvement of the currents, are needed for faster and more accurate diagnosis, and with a definition of active or latent infection.

C0548 MICRORNA (MIR-122 AND MIR-210) EXPRESSION PROFILES IN VISCERAL LEISHMANIASIS/HIV CO-INFECTED PATIENTS IN NORTHEASTERN BRAZIL

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1 Background

Following the emergence of the human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS), the number of visceral leishmaniasis-HIV (VL/HIV) co-infections has increased worldwide, mainly in Brazil. Epigenetic studies are essential for determining possible markers of susceptibility and pathophysiological mechanisms related to VL/HIV co-infection. MicroRNAs (miRNAs) are untranslated single-stranded RNA molecules of 22-24 nucleotides in length involved in several regulatory processes, modulating gene expression and modifying pathological status of the host. This study aimed to investigate miR-122 and miR-210 expression profiles in VL/HIV co-infected and HIV monoinfected patients.

2 Methods

Serum samples from seventeen VL/HIV co-infected and ten HIV monoinfected patients were enrolled. MiRNAs were extracted from VL/HIV (Group 1-test) and HIV patients serum samples (Group 2-control). Therefore, cDNA was obtained by advanced microRNA cDNA synthesis kit (thermo scientific), whose miR-122 and miR-210 detection was done by specific Taqman Probes (thermo scientific) and Real Time PCR (thermo scientific) was used as apparatus.

3 Results

Regarding epidemiological features to group 1 and group 2, 58.8% vs 50% were males, respectively (p=0.70). Mean age was 36.9 vs 34.6 years (p=0.49). Concerning clinical and laboratory features to group 1 vs group 2, anemia was observed in 64.7% vs 50% (p=0.69), lymphopenia in 41.2% vs 50% (p=0.71), leucopenia in 64.7% vs 40% (p=0.26) and neutropenia in 35.3% vs 40% (p=0.99); irregular fever was detected in 76.5% vs 70% (p=0.99) and splenomegaly was clinically meaningful in VL/HIV group, 58.8% vs 10% [p=0.02 OR 12.9 CI (1.31-125.8)]. The mean CD4+ T-lymphocytes count was 164.2 vs 138.6 cells/mm^3 (p=0.81), group 1 and 2, respectively. Regarding miRNA expression profiles to group 1 vs group 2, miR-122 was not detected in both groups; miR-210 was detected in 82.4% vs 90% (p=0.99), mean ct values were 29.4 vs 29.3.

4 Conclusions

Splenomegaly was significantly associated with VL/HIV co-infected group into agreement with classic features of visceral leishmaniasis. Preliminary miRNA expression results found presence of miR-210 in serum samples from both groups. Thus, it could be a possible biomarker to infections. However, it is necessary to increase the number of samples and functional expression studies of miRNA targets that could modulate the clinical spectrum.

C0827 CHARACTERIZATION OF LEISHMANIA INFANTUM IN VISCERAL LEISHMANIASIS ASSOCIATED WITH HIV CO-INFECTION IN NORTHEASTERN BRAZIL

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1 Background

Visceral leishmaniasis (VL) caused by *Leishmania infantum* is a severe disease that taking lives of thousands of people every year. The human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS) has increased the number of VL-HIV/AIDS co-infection cases reported in Europe, Africa, Asia and Latin America. The aim of the present study was describe clinical, epidemiological and molecular characterization of *L. infantum* isolated from visceral leishmaniasis/HIV co-infected patient.

2 Methods

A 21 years HIV-positive female lived in Middle Scrub Zone in northeastern Brazil, endemic area for visceral leishmaniasis presented clinical features for visceral leishmaniasis was enrolled in this study. She had presented visceral leishmaniasis clinical features and was referred to Hospital Universitário Oswaldo Cruz. Bone marrow cytology, Novy-MacNeal-Nicolle culture, kDNA PCR, and typing methods, multilocus enzyme electrophoresis and ITS1-RFLP PCR were assayed.

3 Results

Bone marrow cytology, Novy-MacNeal-Nicolle culture and kDNA PCR were positive. Typing methods, multilocus enzyme electrophoresis and ITS1-RFLP PCR, have confirmed infection by *L. infantum*.

4 Conclusions

PCR has proved to be safer and more affordable than others characterization methods; highlighting ITS1-RFLP PCR, which can determine *Leishmania* spp. diagnosis in non-endemic and endemic areas, favoring the prognosis and prescribing the appropriate treatment to patients.

C1103 IMMUNOLOGICAL PROFILE OF HIV/LEISHMANIA INFECTED PATIENTS

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1 Background

There are records of HIV/*Leishmania* infected patientsin 35 countries worldwide, but the great number of recorded cases was in south west Europe. These patients can present atypical clinical condition with absence of fever and splenomegaly, longer time for the diagnosis of visceral leishmaniasis (VL), longer hospitalization time and frequency of opportunistic infections, these characteristics contribute to greater morbidity and lethality. Such facts stimulate researchers in the quest for differences between patients with VL and coinfected, so it is important to check the immunological profile of HIV/*Leishmania* infected patients.

2 Methods

The present study involved 10 patients with symptomatic VL (VL group - control) and 12 patients with HIV/*Leishmania*. Patients were evaluated for clinical and laboratorial parameters, membrane surface and intracellular of leucocytes immunophenotyping, besides plasmatic cytokines and BM supernatant dosage.

3 Results

Co-infected patients presented a reduction in the number of T CD4 lymphocytes in PB and BM. As well as co-infected patient's TNFα and IL-12 levels were elevated in peripheral blood and bone marrow. There was an increase in %CD14$^+$TNFα$^+$ and %CD14$^+$IL6$^+$ in PB of co-infected patients when compared with VL patients. Elevation of %CD14$^+$IL-10$^+$, %neutrophils IL-6$^+$ and %TC8$^+$IL-6$^+$ in PB when compared to BM in co-infected patients.

4 Conclusions

The results obtained allow us to suggest that higher IL-12, TNFα levels and the greater %CD14$^+$ TNFα$^+$ by HIV/*Leishmania* patient's monocytes suggests activation of T lymphocytes and monocytes. Thus, these cells can present a differentiated profile in HIV/*Leishmania* when compared with VL patients.

C1114 IMMUNOSENESCENCE AND DECREASED SPECIFIC T CELL PROLIFERATION ARE RELATED TO FREQUENT RELAPSES IN VISCERAL LEISHMANIASIS-HIV COINFECTED PATIENTS

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1 Background

HIV-associated visceral leishmaniasis (VL/HIV) has increased in Brazil in the last years. Both diseases cause immunosuppression and augmented cellular activation, enabling the maintenance of *Leishmania* infection, causing frequent relapses. Recently, we showed that VL/HIV patients experiencing frequent VL relapses (VL/HIV-R) presented lower immune reconstitution and higher levels of cellular activation and microbial translocation compared to VL/HIV-non relapsing (VL/HIV-NR), over a prospective follow-up. Despite this, immunosenescence was equally elevated in both groups, raising the hypothesis that the aforementioned immunological status may be related to the functional impairment of the immune response. Our aim was to evaluate whether VL/HIV-R patients present qualitative differences on T-cell effector response against specific antigens in comparison to VL/HIV-NR.

2 Methods

For this, eight VL/HIV patients under ART and secondary prophylaxis followed-up since active VL to 12 months post treatment were evaluated. PBMC was stimulated in vitro with concanavalin A (ConA), *L. infantum* and p24 antigens. Lymphocyte proliferation ($BrdU^+$ T-cells), replicative senescence ($CD57^+CD27^-$) and senescent memory T cells ($CD57^+CD45RA^-$) were investigated. Plasmatic cytokine (MIP-1B/IL-6/IL-7/IL-12/IL-17/IL-13/TNF) levels were also evaluated.

3 Results

Our results showed that VL/HIV patients presented lower T cell proliferation levels compared to VL patients only and healthy individuals, even in response to ConA. Despite this, VL/HIV-NR presented higher percentages of $BrdU^+$ $CD8^+$ and $CD4^+$ T-cells than VL/HIV-R in response to all the specific stimuli during the phases evaluated. Specific senescent T-cell percentages in VL/HIV patients were significantly elevated compared to control groups, especially on $CD8^+$T cells. Also, VL/HIV-R showed higher levels of $CD57^+CD27^-$ on $CD4^+$T cells and mainly $CD8^+$T cells in comparison to VL/HIV-NR at any time to all the stimuli. Interestingly, the majority of the senescent T cells from the VL/HIV-R match to memory $CD8^+$ T cells. Finally, VL/HIV-R presented a tendency to higher levels of pro-inflammatory cytokines compared to VL/HIV-NR, mostly in the active phase of VL.

4 Conclusions

Our results suggest that the inflammatory status of the VL/HIV-R patients contribute to the elevated senescence degree, which in turn are related with the low levels of specific-T cell proliferation. Such factors may worse the effector immune response, enabling the frequent relapses.

C1147 SPATIAL DISTRIBUTION OF VISCERAL LEISHMANIASIS/HIV CO-INFECTION IN THE STATE OF PERNAMBUCO, BRAZIL

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1 Background

Visceral Leishmaniasis (VL), historically considered a rural disease, is an infection caused by *Leishmania* spp. In the Northeastern Brazil, most of cases occur through the bite of the female sand fly. The increase of the HIV incidence in rural areas and the dissemination of VL in urban and suburban areas have contributed to a higher numbers of VL/HIV co-infection cases. This study aimed to describe the geographical distribution of VL/HIV co-infection individuals in the State of Pernambuco, Brazil.

2 Methods

This is a cross sectional, descriptive and based on retrospective analysis of co-infection registered cases in a secondary database from 2014 to 2015. Epidemiological data (origin, gender, age and educational level) were collected and areas of high occurrence were highlighted.

3 Results

There were identified 35 (16.9%) co-infected individuals, 25 in the Metropolitan Region of Recife, 62.9% male, 74.2% in the age group of 25-49 years, and 68.5% of low educational level (1 to 8 years of study). We observe a growing series of this co-infection in urban areas.

4 Conclusions

Thus, it is important to recognize the most affected areas, so that health services are able to act in a satisfactory way to combat these diseases.

C1225 VISCERAL LEISHMANIASIS AND HIV COINFECTION IN AFRICA

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1 Background

HIV has made visceral leishmaniasis (VL) to re-emerge as an important opportunistic disease. It is seen in over 35 countries. The co-infection is associated with several challenges and is addressed as "double trouble".

2 Methods

This is a review of the challenges faced with VL and HIV co-infection in East-Africa and lessons learned based on literatures and field experiences.

3 Results

The Northwest part of Ethiopia is the most highly affected region with the co-infection. Although the HIV prevalence is showing progressive decline in the general population (1.2% in 2015), it has relatively remained high among VL patients (10-20%). With the co-infection serologic diagnostics are performing poorly; and there is high rate of treatment failure (30-50%), death (10-15%) by end of treatment) and relapse (about 60% in the first year of treatment). Most of these patients are also affected by malnutrition and/or tuberculosis, making the situation "triple trouble". Recent clinical trials in Northwest Ethiopia aimed to improve the treatment outcomes of these patients showed partial success.

4 Conclusions

VL-HIV co-infection is a deadly gridlock that remains with a lot of challenges and high mortality rate. It needs urgent attention in the aspects of reliable diagnostics for treatment follow up, test of cure and patient management.

C1262 LOW LEVELS OF SOLUBLE CD40 LIGAND IN ASYMPTOMATIC LEISHMANIA DONOVANI CO-INFECTED HIV PATIENTS

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1 Background

The production of IL-10 and IFN-y is dependent on costimulatory molecules such as CD40/CD40L. Its serum soluble derivate sCD40L was recently shown in a human *L. infantum* model to improve control of parasite and was associated with clinical resolution of visceral leishmaniasis (VL). They observed that many individuals living in high risk endemic settings in Brazil – with unknown infection status - had high levels of sCD40L, suggesting that it may contribute to protection. Because HIV patients are at increased risk of progression to VL with high mortality and HIV infection conjointly increases sCD40L, we compared – by matching for their HIV status - sCD40L concentrations in asymptomatic *L.donovani* infected and non-infected HIV patients in North-West Ethiopia.

2 Methods

We conducted a case-control study comparing cases of asymptomatic Leishmania infection with non-infected controls, matched on CD4 count, months on ART and ART regime. Asymptomatic infection was defined as positivity for any of the *Leishmania* markers tested: Direct Agglutination Test (DAT), rK39-Rapid diagnostic test (RDT), and urine antigen (KAtex) test. Serum samples were tested for sCD40L on the Luminex 200 (Bio-Rad). Robust mixed-effects logistic regression model was used, corrected for sex, body mass index category and presence of opportunistic infections (OIs).

3 Results

A total of 96 HIV patients were included with a median age of 39.5 years (Min-Max: 21-61y), of which 85.4% male. This included 48 asymptomatically infected patients with *L. donovani* and 48 matched non-infected individuals. The median CD4 count was 386 cells/µL (IQR: 278-490), 92% were on ART. The median sCD40L value was 1370 pg/ml with values ranging from 0 to 5225 in cases and from 372 to 10000 in controls. Every 500 unit increase in serum sCD40L concentrations decreased the odds of being infected with 0.88 times (p=0.014).

4 Conclusions

In general, lower levels of sCD40L were observed in asymptomatic infected individuals, suggesting an immunosuppressive mechanism of the parasite on the CD40 signaling pathway, independent of their HIV infection. These results match with the stated protective role of sCD40L and indicate the importance of the CD40-CD40L pathway in early human immune responses against leishmaniasis in HIV patients. Low sCD40L levels should be further evaluated as early disease/severity marker and merit study as potential prophylactic/therapeutic target in VL-HIV patients.

C1284 DEVELOPMENT AND EXTERNAL VALIDATION OF A CLINICAL PROGNOSTIC SCORE FOR DEATH IN VISCERAL LEISHMANIASIS PATIENTS IN A HIGH HIV CO-INFECTION BURDEN AREA IN ETHIOPIA

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1 Background

In Ethiopia, case fatality rates among subgroups of visceral leishmaniasis (VL) patients are high. A clinical prognostic score could contribute to optimal management and reduction of these case fatality rates. The aim of this study was to identify independent predictors of death from VL, and to develop and externally validate a clinical prognostic score for death in VL patients, in a high HIV co-infection burden area in Ethiopia.

2 Methods

We conducted a retrospective cohort study in north west Ethiopia. Predictors with an adjusted likelihood ratio ≥1.5 or ≤0.67 were retained to calculate the predictor score.

3 Results

The derivation cohort consisted of 1686 VL patients treated at an upgraded health center and the external validation cohort consisted of 404 VL patients treated in hospital. There were 99 deaths in the derivation cohort and 53 deaths in the external validation cohort. The predictors of death were: age >40 years (score +1); HIV seropositive (score +1); HIV seronegative (score -1); hemoglobin <6.5 g/dl (score +1); bleeding (score +1); jaundice (score +1); edema (score +1); ascites (score +2) and tuberculosis (score +1). The total predictor score per patient ranged from -1 to +5. A score of -1, indicated a low risk of death (1.0%), a score of 0 an intermediate risk of death (3.8%) and a score of +1 to +5, a high risk of death (10.4-85.7%).The area under the receiver operating characteristic curve was 0.83 (95% confidence interval: 0.79-0.87) in derivation, and 0.78 (95% confidence interval: 0.72-0.83) in external validation.

4 Conclusions

The overall performance of the score was good. The score can enable the early detection of VL cases at high risk of death, which can inform operational, clinical management guidelines, and VL program management. Implementation of focused strategies could contribute to optimal management and reduction of the case fatality rates.

C1323 DISCONTINUATION OF PENTAMIDINE SECONDARY PROPHYLAXIS FOR VISCERAL LEISHMANIASIS IN HIV PATIENTS

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1 Background

The profound immunosuppression in visceral leishmaniasis (VL) and HIV co-infections leads to recurrent VL disease and subsequent death. The relapse rate is related to the degree of immunosuppression and is high in the first one year after treatment. Secondary prophylaxis prevents relapse of opportunistic conditions in HIV and is important at least until the antiretroviral therapy causes immune reconstitution. While pentamidine as a secondary prophylaxis reduces the relapse rate of VL in HIV patients, when to stop and the outcomes of patients after discontinuation of the secondary prophylaxis is not known.

2 Methods

This was a prospective cohort study of HIV-VL co-infected patients after discontinuation of their monthly pentamidine secondary prophylaxis. Pentamidine was stopped when the CD4 count was more than 200cells/µl after 12 months; or patient had taken pentamidine for 18 months if CD4 count was less than 200cells/µl by 12 month; or patient had relapsed during follow up with pentamidine. The patients were followed every three months for one year and outcomes assessed in terms of disease free survival, relapse, death and drug related adverse events.

3 Results

A total of 74 VL patients with HIV were initiated on pentamidine monthly infusion. By 12 months, 28 had CD4 count greater than 200cells/µl while 17 had CD4 count less than 200cells/µl. The other outcomes were 15 relapsed, 5 died, 7 lost to follow up and 2 interrupted. Those with CD4 count less than 200cells/µl by the 12th month were given additional doses of pentamidine for 6 more months before stopping irrespective of their CD4 count. Of the 45 patients who completed the planned secondary prophylaxis regimen, all the 28 patients who had CD4 count level above 200cells/µl survived without relapse. However, from the 17 patients with CD4 count less than 200cells/µl, 10 (59%) had disease free survival, 3 relapsed and 4 were lost to follow up one year after discontinuation of pentamidine. There was no long term pentamidine related adverse event observed.

4 Conclusions

While pentamidine secondary prophylaxis decreased VL relapse, patients with slow immunological recovery remained at high risk of poor outcomes despite prolonged secondary prophylaxis. Early case detection and intervention before profound immunodeficiency is important. Findings urge for the need to explore other strategies to improve the outcome of VL-HIV co-infected patients with profound immunodeficiency.

C1390 SEROPREVALENCE OF LEISHMANIA INFECTION IN HIV-INFECTED PATIENTS IN A BRAZILIAN URBAN AREA

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1 Background

In Brazil, prevalence of visceral leishmaniasis in HIV-infected patient is about 8%, however these data are related only active disease. Regarding asymptomatic carriers of *Leishmania* is not much investigated. In this work we evaluated the seroprevalence of Leishmania infection in a cohort of HIV-infected patients.

2 Methods

TTwo hundred and forty HIV-infected patients were enrolled in this study. Blood samples were collected and processed to detect antibodies against different Leishmania antigens using ELISA. Soluble antigen of *L. major*-like (ELISA *L.major*-like), antigen of *Leptomonas seymori,* rK39 antigen (ELISA rK39), rK28 antigen (ELISA rK28). Indirect Immunofluorescent antibody test (IFAT) using Leishmania major-like promastigotes was performed too. Demographical and epidemiologic data from subject were obtained through hospital records analysis.

3 Results

Overall, 68.8% were males and 34.6% were from endemic visceral leishmaniasis area. Regarding to ELISA *L.major*-like, ELISA *Leptomonas,* ELISA rK39, ELISA rK28, the number and percentage of positive tests were, respectively: 60 (25%); 9 (3.8%); 3 (1.3%); 6 (2.5%). Eleven patients (4.6%) had IFAT positive test.

4 Conclusions

ELISA *L. major*-like had the highest positivity, probably due to the detection of a wider variety of antibodies directed against soluble antigen components, differently of IFAT, that detects mainly antibodies against surface antigens. *L.major*-like antigen has characteristically worst specificity than other ELISA antigens, presenting corss reactivity with others diseases, such as Chagas disease. As expected because it seems to be associated with active disease, ELISA-rk39 had a lower yield. Some studies have been demonstrating better sensitivity of rk28 antigen compared to rK39 antigen, but no studies evaluate this fact in HIV-infected patients. The highest positivity of ELISA rK28 in relation to ELISA rK39 in this study could indicate that, in this group of individuals, rK28 can present a good performance, as in immunocompetent patients. ELISA *Leptomonas* have performance similar to *L. infantum* antigen for visceral leishmaniasis diagnosis, but this antigen has never been studied in HIV-infected patients. The prevalence of *Leishmania* spp. infection is high among HIV patients in Brazil suggesting that they should be routinely investigated for coinfection, even in area with no autochthonous transmission reported.

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C1519 SEROSURVEY AND ASSOCIATED RISK FACTORS OF ACUTE LESHMANIA DONOVANI INFECTIONS AMONG FEBRILE HIV INFECTED INDIVDUALS IN ABUJA: A HOSPITAL-BASED CROSS-SECTIONAL STUDY

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1 Background

Leishmania donovani (L. donovani) is an obligate intracellular pathogen. L. donovani and HIV coinfection is neglected clinical entity in sub-Saharan African. L. donovani infections have been shown to deplete host cellular immunity and proceed to severe diseases. As part of the ongoing research efforts in Nigeria to improve the healthcare of HIV infected individuals through diagnosis and treatment of coinfections, this study was instigated.

2 Methods

Three hundred and forty blood samples were indivdually collected from HIV infected individuals with fever > 10 days attending University of Abuja Teaching Hospital, Nigeria between 7th April to 10th October 2015. EDTA anticoagulated blood was tested for CD4+ cell counts, while sera were tested for anti-L. donovani IgM antibodies using flow cytometry and Enzyme Linked Immunosorbent Assay (ELISA), respectively. Interviwer-based questionnaires was used to collect participants' sociodemographic variables. Data were analysed for statistical association and relation between seropositivity of L.donovani IgM and risk factors using MedCalc software version 16.8.4.

3 Results

Of the 340 participants studied, the seroprevalence of anti-L.donovani IgM was 10.9%. The mean CD4+ cell count of those with L. donovani IgM seropsitivity (n =32) was 119.4 cells/mm3. There was statistical relation between CD4+ cell counts and L.donovani IgM seropsotivity. There was statistical association between L. donovani IgM seropositivity with age of paticipants (p = 0.014), residential area (p=0.033), living condition (p=0.0001) and occupation of participants (p=0.06). However, there was no statistical association of L. donovani IgM seropositivity with gender, income status and eduactional level of participants (p >0.05).

4 Conclusions

Findings from this study revealed that L. donovani is an etiological agent of acute fever in HIV infected patients and signficantly associated with CD4+ lymphopaenia in coinfected patients. Its recommended that L. donovani screaning be considered during differential diagnosis of HIV patients with prolong fever.

C1545 POSITIVE SLA-CELL PROLIFERATION TEST AND INTERFERON GAMMA PRODUCTION HELPED WITHDRAWAL OF VISCERAL LEISHMANIASIS PROPHYLAXIS ON HIV COINFECTED PATIENTS

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1 Background

Secondary prophylaxis for visceral leishmaniasis (VL) is recommended in Leishmania-HIV coinfected, but it is not clear when to stop it. SLA-cell proliferation test and IFN-γ production have been tested as markers of specific cellular immunity. Largest leishmaniasis outbreak in Europe was declared since June 2009 in Fuenlabrada (Madrid), Spain. In this setting, our aim was to validate the usefulness of this tests for withdrawal of secondary prophylaxis in these patients

2 Methods

From January 2013, HIV infected patients with previous or active VL were recruited to 1st November 2016 (201 weeks). Leishmania-specific PCR, serology (ELISA or IFAT), SLA-cell proliferation test, and analysis of several cytokines production after stimulation with SLA, including IFN-γ, were used besides habitual test for HIV. Relapse was defined as symptomatic VL after the end of the treatment

3 Results

14 VL cases in HIV patients were identified. 1 patient was lost and 3 died of events not related to leishmania. 10 patients completed the follow up (131 to 201 weeks):

- 3 patients were clinical cured and without secondary prophylaxis when were recruited. SLA-cell proliferation tests and IFN-γ production were positive. No relapses in 201 weeks follow up.
- 3 patients were asymptomatic but on prophylaxis when were recruited. SLA-cell proliferation tests were positive as well as IFN-γ production in 2 of them, while the other one produced low levels of the cytokine. CD4 levels were 152, 189 and 359. Prophylaxis was stopped. No relapses in 131 weeks follow up.
- 4 patients had negative SLA-cell proliferation test, and 3 of them had no detectable IFN-γ production after stimulation with SLA. Two patients relapsed, other one is asymptomatic but with PCR positive. The last patient remains asymptomatic with PCR negative and IFN-γ production positive, prophylaxis was recently stopped

4 Conclusions

In this small sample of treated Leishmania-HIV coinfected patients, positive SLA-cell proliferation test and IFN-γ production after stimulation with SLA were markers of specific cellular immunity, and allowed, when positive, the withdrawal of secondary prophylaxis for VL, not matter the CD4 level.

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C1629 VISCERAL LEISHMANIASIS COINFECTION AND AIDS IN BRAZIL, 2001 TO 2010

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1 Background

The urbanization of visceral leishmaniasis (VL) and the concurrent ruralization of HIV infection in Brazil have led to an increased number of *Leishmania*/HIV coinfected persons. The objective of this study was to describe the clinical and epidemiological profile of Brazilian patients with VL coinfected with HIV as compared to those VL patients without coinfection.

2 Methods

A descriptive and exploratory study was carried out using secondary data extracted from the databases of the Ministry of Health of Brazil concerning the cases of VL and AIDS of all 21 states that reported cases of VL from 2001 to 2010. A probabilistic record linkage method was used to obtain cases of VL/AIDS coinfection.

3 Results

We retrieved 760 cases of VL/AIDS coinfection. Coinfection was more prevalent in adult males, with an incidence of 0.01 and 0.07 cases/100.000 inhabitants in 2001 and 2010, respectively. Fatality rate was 25.0% among coinfected patients, being three times higher than in the non-coinfected, where the value was 8.3%. Weakness, weight loss, cough, other associated infections and hemorrhagic phenomena were more commonly found in the coinfected group. The relapse proportion was 6.3% in coinfected patients and 3.1% in the non-coinfected group.

4 Conclusions

The association between VL and AIDS is relatively recent and has been increasing in Brazil. This study contributes to the knowledge of the clinical profile, magnitude and prognosis of VL/AIDS coinfection in the country. Considering that a significant proportion of the coinfection burden in Latin America is concentrated in Brazil, the increasing number of coinfected cases represents a challenge for the health system, especially with regard to the impact on VL lethality. The observed behavior can also be analyzed as a model of what can occur in other countries of the continent where VL has reported as an emergent disease.

C1661 SYNERGISTIC EFFECT OF NELFINAVIR AND AMPHOTERICIN B ON LEISHMANIA INFANTUM AMASTIGOTES

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1 Background

One of the main hazards for control of visceral leishmaniasis (VL) is its interaction with human immunodeficiency virus (HIV) infection. Patients with HIV infection increases the risk of developing active VL by between 100 and 2320 times. Treatment of VL/HIV coinfected patients is less effective than in HIV-negative patients, and relapses are much more frequent. Many experts advocate for combined therapy among VL/HIV coinfected patients in order to increase efficacy, especially in those patients with multiple relapses, as well as to decrease the emergence of resistant parasites. The liposomal amphotericin B has been indicate as the first-line therapeutic option on VL/HIV coinfected patients. Also, highly active antiretroviral therapy for HIV patients reduce the incidence of new VL cases, increases survival rates and diminish relapse rates. Recently conducted field studies point to the use of liposomal amphotericin B in combination with miltefosine, followed by secondary prophylaxis and antiretroviral drugs as a promising therapeutic scheme. The purpose of the present work is to evaluate in vitro the effectiveness of combining the antiretroviral drug nelfinavir (NFV) with amphotericin B (AMB) against *Leishmania infantum* amastigotes in mouse macrophages.

2 Methods

Infected macrophages were treated with different combinations of drugs. After 48 h, supernatants were removed, and the macrophages washed and stained with Giemsa. The percentage of infection was determined by counting 100 cells in three different cultures. The drug interactions were evaluated with a modified fixed ratio isobologram method. The interaction index (FIC) was calculated as [MIC combination A/MIC alone A] [MIC combination B/MIC alone B], where MIC is the minimum inhibitory concentration that produce total death of the culture parasite population.

3 Results

A greater reduction in the percentage of infection and the number of amastigotes per macrophage was observed when drugs are combined compared when used individually. The NFV/AMB combination has a synergistic effect on amastigotes proliferation inside macrophages. The FIC ratio was 0.1, which allow classify the interaction as strong synergism.

4 Conclusions

The NFV/AMB combination could represent a future alternative to monotherapeutic anti-leishmanial agent in VL / HIV coinfected patients.

C1678 TREATMENT RELAPSE AT BASELINE: IMPACT ON UNPLANNED VISITS IN A RANDOMIZED CLINICAL TRIAL OF HIV/VL CO-INFECTION TREATMENT FOR PATIENTS IN ETHIOPIA

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1 Background

In clinical trials, unplanned visits occur in addition to predefined protocol-specific visits. Whilst important safety data can be collected, they impact study budgets and timelines. We compared the frequency and reasons for unplanned visits at two sites enrolling Visceral Leishmaniasis (VL)/Human Immunodeficiency virus (HIV) co-infected patients in Ethiopia.

2 Methods

HIV/VL co-infected patients with primary or recurrent VL infections were enrolled at Abderafi MSF Heath Centre and Gondar University Hospital Leishmaniasis Research and Treatment Centre. Patients came for 3 protocol follow up visits but also returned to the hospital when unwell. We assessed unplanned visits by patients enrolled at the two sites. The frequency and reasons for unplanned visits were compared both between the sites and between those with primary and recurrent infections. Data were cleaned and analyzed using STATA®.

3 Results

59 participants were enrolled in the study (Gondar=31, Abderafi=28): 28 had primary infection (Gondar=15, Abderafi=13) while 31 were recurrent cases. 257 unplanned visits occurred across both sites; 29% (74) by primary and 71% (183) by recurrent cases. 16.4% (42) of the unplanned visits occurred in Gondar and 83.6% (215) in Abderafi. Reasons for these unplanned visits were: 21 (8.2%) suspicion of relapse, 213 (82.9%) concomitant illness not requiring hospitalization, 1 (1%) for assessment prior to initiation of pentamidine prophylaxis, and 22 (8.6%) for other specified reasons. For visits due to suspicion of relapse, in Gondar 4 (36.4%) were primary cases, and 7 (63.6%) were recurrent cases, whereas in Abderafi all were from recurrent cases. For concomitant illnesses, in Gondar 5 (35.7%) were from primary cases, and 9 (64.35%) from recurrent cases; in Abderafi 54 (27%) were from primary cases and 145 (73%) from recurrent cases. In Gondar, 10 (62.5%) visits for other reasons were from primary cases and 6 (37.5%) from recurrent cases; in Abderafi 1 (16.6%) were from primary cases and 5 (83.4%) from recurrent cases

4 Conclusions

Patients enrolled with recurrent VL made more unplanned visits than those with primary infection. There were more unplanned visits in Abderafi than in Gondar. Patient demographics and site characteristics may account for these differences, and could affect study budgets, timelines and outcome. These factors should be taken into consideration when planning future studies involving HIV positive patients with recurrent VL

C1825 CO-INFECTION HIV-LEISHMANIA IN MOROCCO: CASE OF MARRAKECH-SAFI REGION

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1 Background

In Morocco, the incidence of the AIDS marked an increase since 1986. On 2011, there were more than 29.000 people living with HIV infection. As the most of Mediterranean region countries, leishmaniasis in Morocco are a grave health problem. These affections are widespread throughout the country, from the mountains of Rif up to the palm groves of the Anti-Atlas. Co-infections HIV / *Leishmania* became a real health problem, because of the increasing of geographical overlapping of these two affections. The seropositivity in the HIV, causes an immunosuppression favorable for *Leishmania* development. coinfected patients play a major role as reservoirs and sources of infection for the sand fly vector, because of the abundance of the parasite in their peripheral blood.

2 Methods

Our study presents an overview on the incidence of the co-infection HIV/ *Leishmania* in the region of Marrakech-Safi, where both *Leishmania* and VIH were recorded. Through the analysis of epidemiological data on both affections, as well as the estimation of the vector power of leishmaniasis by entomologicals investigations in the studied region.

3 Results

a retrospective study of epidemiological data of both affections (Leishmaniasis and HIV), showed, by mapping, the area with high risk for cutaneous leishmaniasis-HIV and visceral leishmaniasis-HIV co-infections.The entomological investigations showed the co-existence in our study area of all potential vectors of the three Moroccan Leishmania species: Phlebotomuspapatasi proven vector of L. major, P. perniciosus and P.longicuspis potential vectors of L. infantum and P. sergenti proven vector of L. tropica.

4 Conclusions

In conclusion, our study area is at a very high risk of the co-infection HIV/Leishmania because of the presence of VIH-AIDS cases (18% of the total cases in Morocco) and the co-existence of anthroponotic cutaneous leishmaniasis by L. tropica with sporadic cases of visceral leishmaniasis by L. infantum. Epidemiological surveillance of coinfection VIH-Leishmania in Morocco and specially in Marrakesh-Safi region is needed to control both leishmania and VIH infections.

C1841 BENEFITS OF SPLENECTOMY FOR LEISHMANIA-HIV CO-INFECTED PATIENTS.

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1 Background

Visceral Leishmaniasis (VL) or kala-azar has behaved as an opportunistic disease for HIV-infected patients. This association has been described in several regions of the world. Both diseases have a prejudicial synergism over cellular immune response. Around 50% of the co-infected patients evolve to chronic or recurrent kala-azar, with permanent splenomegaly, kala-azar definitive therapy failure, HIV with persistent low cd4+ cell count and hematological pancytopenia profile. This hostile territory probably ows to hypersplenism, which besides being responsible for pancytopenia, is a leishmania and HIV-filled lyphocytes reservoir. Based on this, we described a series of 10 leishmania-HIV co-infected patients who were subjeected to splenectomy and the benefits of the procedure.

2 Methods

This is a series of cases involving patients with recurrent VL, co-infected by the HIV, followed at the natan portela tropical diseases institute, in Teresina, piaui, Brazil, from 2008 to 2016. The ellegibility criteria for surgery were patients who had not diminished or had kala-azar recurrence after at least two months of treatment and six months of observation; patients with confirmed recurrent VL and HIV, who engaged in secondary kala-azar prophylaxis and antiretroviral therapy, in clinical conditions able to undergo surgery and who accepted the procedure. The carried surgery was total laparoscopic splenectomy for the 10 patients.

3 Results

Total splenectomy was carried in 10 patients, from which eight patients remain in ambulatory follow-up and two deceased lately (8th and 10th months, after surgery), after apparent sepse and pneumocystis pneumonia. Only one patient developed immediate postoperative complications. The anesthesiologist and/or cardiologist did not have to make ICU reservations for any patients during postoperative phase. Average hospitalization period after splenectomy was 5.58 days. All patients increased cd4 count, reduced new hospitalizations and enhanced pancytopenia. One HIV patient died immeadiately before splenectomy from herpes encephalitis.

4 Conclusions

Total splenectomy can bring benefits for the group of leishania-HIV co-infected patients who failed treatment for both infections and developed hypersplenism. However, the risks and benefits must be carefully reflected and discussed with the patients.

C1899 RELAPSE OF VISCERAL AND CUTANEOUS LEISHMANIASIS IN A PATIENT WITH ADVANCED HIV INFECTION DESPITE LIPOSOMAL AMPHOTERICIN B THERAPY AND PROPHYLAXIS. DIAGNOSTIC AND THERAPEUTIC ISSUES.

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1 Background

Coinfection with HIV and Leishmania is a major health problem worldwide. Recent climate changes favours the spread of Leishmania infection even in some Italian Northern regions, traditionally considered non-endemic areas.

2 Methods

We report a case of a multi-drug-resistant HIV positive patient on antiretroviral (ARV) therapy with multiple failures, suffering from fever, diarrhea and several skin ulcerations on the limbs and trunk. Initial physical examination revealed several large, ovoid skin ulcerations, ranging from 5 to 15 mm in diameter, with raised erythematic borders and a clear exudate. The patient lived in the Italian Northern region Emilia-Romagna and never traveled abroad. CD4+ cells count was 127/ml (CD4+/CD8+ ratio 0.11) and HIV-RNAq 540,000 cp/ml, as the patient did not assume ARV correctly. Histopathological examination of colonic mucosa by biopsy revealed a colic leishmaniasis, while a qualitative PCR performed on a sample aspirate of the skin lesions disclosed the presence of *L. infantum* DNA.

3 Results

An intravenous liposomal amphotericin B (LAmB) regimen was promptly started, at 3 mg/kg per day for 7 days, and, subsequently, 3 mg/kg once a week for 5 weeks with a rapid improvement of fever and diarrhea, while the cutaneous lesions of the limbs and trunk began to heal 2 weeks after starting treatment. Six months following therapy, the patient developed a visceral leishmaniasis (VL) and cutaneous leishmaniasis (CL) relapse despite a secondary prophylactic regimen with LAmB was maintained. The patient was then treated with miltefosine x OS 30 mg/kg for 15 days, with a rapid clinical improvement but a progressive deterioration of the renal function which required hemodialysis treatment. During hospitalization the patient died due to refractory pancytopenia and acute renal failure.

4 Conclusions

In HIV-coinfected patients with Leishmania, the impaired immune function may: (i) favour the reactivation of latent Leishmania infection and induce, as in this case, a simultaneous presentation of VL and CL with a poorer therapeutic response; (ii) increase the risk of relapse after treatment, although miltefosine could represent in certain cases, an interesting option alone or in combination therapy. Continental Italian Northern regions are now considered at risk for Leishmanial disease, especially for HIV patients. Molecular methods, not yet well validated for cutaneous leishmaniasis, could be a reliable diagnosis tool.

Operational research

C0074 IRANIAN MEDICINAL PLANTS WITH PROMISING ANTILEISHMANIAL ACTIVITY: A SYSTEMATIC REVIEW AND META-ANALYSIS

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1 Background

Leishmaniasis is a major public health problem worldwide. The aim of the present study was to investigate medicinal plants with anti-Leishmania activity which used in Iran

2 Methods

Data were systematically gathered from five English databases including Ebsco, Science Direct, PubMed, Google Scholar and Scopus, four Persian databases including Magiran, Irandoc, Iran medex and the Scientific Information Database (SID) from1999 to April 2015. Information obtained included plant family, extraction method, concentrations of extracts, animal models and parasite strains.

3 Results

A total of 68 articles including 188 experiments (140 in vitro and 48 in vivo) between 1999 and 2015, met our eligibility criteria. Thoroughly, 98 types of plants were examined against three genera of Leishmania spp. For the heterogeneity study conducted, it was showed that there was a great deal of variation among studies. Based on random effect, meta-analysis pooled mean of IC50 was obtained 456.64 (95% CI: 396.15, 517.12). In conclusion, most Iranian plants used as anti-leishmanial activity were Artemisia species, Allium sativum, Achilleamille folium, Peganum harmala and Thymus vulgaris.

4 Conclusions

The present systematic and meta-analysis review provide valuable information about natural products with anti-Leishmania activity, which would be examined in future experimental and clinical trials and herbal combination therapy

C0206 OPEN SOURCE ELECTRONIC MEDICAL RECORD DISTRIBUTION SYSTEM FOR NEGLECTED TROPICAL DISEASES: USE CASE WITH CUTANEOUS LEISHMANIASIS

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1 Background

The use of technology resources to support processes in health systems has generated robust, interoperable and dynamic platforms. Hospitals and institutes handling patient information require tools that facilitate decision making for the medical staff, either for diagnosis or treatments. In the case of institutions working with neglected tropical diseases (NTD), there is a need of customized systems. The establishment of a personalized virtual medical records platform would facilitate the analysis of treatment evolution in patients as well as integrate more accurate data about the epidemiology and clinical aspects. In this project, a personalized open electronic medical record system (EMR) based on OpenMRS was developed, implemented and tested with the local outpatient service of cutaneous leishmaniasis (CL).

2 Methods

Requirements and specific needs for the management of patients with CL and other neglected diseases were identified through interviews to the healthcare staff. With the collected information, a set of forms and functionalities were developed under the OpenMRS guidelines, using community shared modules. All the personalized information was packaged in a public distributable module called NTD Health. The whole process was controlled by testing protocols with the healthcare personnel. In the case of leishmaniasis, a detailed follow up of the implementation was carried out, and further data analysis was proposed. This process was fostered through a local workshop on Health Informatics Tools and an open online course available for free.

3 Results

The project has passed a first phase of local implementation in Medellín, Colombia, thanks to the local services provided by the Program for the Study and Control of Tropical Diseases - PECET. The platform is cloud-based accessed and has been tested in different environments. The system can be upgraded and improved by the user without technological barriers, prompting the personalization of health care systems able to interoperate with other platforms. In addition, the EMR provides data transfer protocols between servers and application for further mobile integration.

4 Conclusions

The NTD Health implementation has demonstrated improvements in local health services of CL using an open and personalized software tool. Based on the results, the platform could become a useful tool for other institutions to improve their health practices and potentially improve the quality of life of NTD patients.

C0280 VISCERAL LEISHMANIASIS IN BENISHANGUL-GUMUZ REGIONAL STATE, WESTERN ETHIOPIA: REEMERGING OR EMERGING?

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1 Background

Kala-azar is a growing public health problem in Ethiopia. Benishangul-Gumuz regional state was previously not known to be endemic for the disease. In response to a case report from the region, we conducted a rapid assessment survey.

2 Methods

A pretested questionnaire was used to capture sociodemographic and clinical histories pertinent to kala-azar. Study participants with complaints of fever and headache for 2 weeks or more were tested for kala-azar and malaria. All participants were screened with the leishmanin skin test and the direct agglutination test for exposure to Leishmania, defined as a positive result with either or both tests.

3 Results

Of 275 participants, 20 were exposed giving an overall leishmaniasis seroprevalence rate of 7.3%. Among the 20 positive individuals, 19 were farmers and nine of them reported no travel history outside their district. It appears that kala-azar is emerging in Dangur and Guba districts of Benishangul-Gumuz regional state, probably in connection with human encroachment into one or several previously out-of-reach zoonotic foci.

4 Conclusions

We recommend integrated epidemiological surveys for confirmation and early containment of disease transmission in the area.

C0340 STIGMA CAUSED BY CUTANEOUS LEISHMANIASIS IN SURINAME AND PATIENT HEALTH CARE SEEKING BEHAVIOR

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1 Background

Health-related stigma and its dramatic consequences for those stigmatized have long been a crucial concern for public health authorities globally. Cutaneous leishmaniasis has been identified as a major cause of stigma in several countries (Ecuador, Afghanistan) and affected people (often women) are often excluded from social life. CL is also a major health concern in Suriname and anecdotal evidence exists that severely mutilated people may experience stigmatization. However, before concluding that stigma spoils the lives of people with a particular disease or disability and is a major obstacle to obtaining/providing adequate health care, it is necessary to first determine whether there is actual stigmatization related to the condition concerned. Furthermore, people tend to seek traditional ways of treatment before entering into the regular health care system. This delays treatment and may exacerbate disease. It is therefore important to assess patient's perceptions on health care in relation to CL.

2 Methods

The purpose of our study is to nuance the concept of stigma through a detailed ethnographic exploration of the experiences and views of patients and others affected by the parasitic skin disease cutaneous leishmaniasis (CL) in Suriname. Furthermore, health care seeking behavior was studied. Qualitative data on the perceptions, treatment (including health care seeking behavior) and illness experiences of CL in Suriname was collected in 2009 and 2010 among 205 CL patients at the Dermatology Service in the capital city Paramaribo, and among 321 people in different rural hinterland villages.

3 Results

The exploration reveals the complex and sometimes confusing statements of patients and observers of social reactions to the disease. CL is not generally a stigmatized disease in Suriname (though this is not to deny that stigmatization may occur occasionally). Access to the regular health care is hampered due to costs, long travel distances, fear and traditional believes. Traditional medicine is focused on the use of natural products, but is also extended to the use of harsh chemicals such as battery acid and herbicides.

4 Conclusions

Although CL is not generally a stigmatized disease in Suriname, patients can feel embarresed by the illness and avoid contact with community members. In particular in the hinterland, people tend to opt for traditional treatment. There is a need for better inform the general public through education and awareness campaigns.

C0438 DELAY IN DIAGNOSIS, TREATMENT AND REPORTING OF KALA-AZAR PATIENTS IN BANGLADESH

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1 Background

Early diagnosis and complete treatment, and effective disease surveillance are essential elements of the national kala-azar elimination program (NKEP) in Bangladesh. There is limited information in Bangladesh about VL patient's diagnostic delays at upazilla health complex (UHC), as well as VL reporting delays from UHC to central level. This study aimed to fill these gaps by investigating and documenting patient's health seeking behaviour, diagnosis and treatment delays, associated risk factors, and delays of VL patients reporting from UHC to central level.

2 Methods

We conducted this study in 10 UHCs in the VL endemic areas of Bangladesh. UHC Program Managers were interviewed through a structured questionnaire to collect the information on current reporting system from UHC to central level. Four UHCs were selected randomly for patient interviews to understand the delay of VL diagnosis and treatment; 60 VL patients treated from August 2014 to December 2015 were recruited in the study. Patients were interviewed at home using a structured questionnaire.

3 Results

The median of the patients' age was 23.5 years (IQR 10.2-35.0); 53.3% were female. The overall median time from first feeling ill to treatment being started was 78 days (IQR 38-128), composed of 7 days (IQR 5-14) for first seeking health care (patient's delay), 70 days (IQR 32-120) for establishing a VL diagnosis (diagnostic delay) and 1 day (IQR 1-2) for providing treatment (treatment delay). The main reason for the diagnostic delay was healer shopping and informal health providers failing to referring suspected cases to the UHC. Sixty percent of patients first sought healthcare from an informal health provider, but only 3% were referred .Patients' healer shopping was highly correlated ($r = 0.683$, $p=0.01$) with the diagnostic delay. Seventy percent of the UHC reported VL cases to central level monthly and 30% weekly. Reporting media was 100% by e-mail, 80% by surface mail and 60% by web-based system.

4 Conclusions

The VL patients' healer shopping practices, including informal health providers' failure to identify and refer VL suspects are the main cause of the persisting delays for treating cases and interrupting transmission. Proper IEC/BCC activities can improve current healer shopping practice in the community; hence reduce delay in diagnosis of VL. Web-based VL surveillance is feasible and should be scale up.

C0449 INSECTICIDAL WALL PAINTING FOR SAND FLY CONTROL – A CLUSTER RANDOMIZED CONTROLLED TRIAL

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1 Background

We aimed to investigate the efficacy of insecticide paint containing Alphacypermethrin 0.7%, D-Allethin 1.0%, and Pyriproxyphen 0.063% (5A IGR NG interior PaintTM) for indoor sand fly control and compared its efficacy with existing sand fly control methods in support of the VL elimination initiative of the Indian Sub-continent.

2 Methods

The study was conducted in seven randomly-selected villages with similar VL burden in the Mymesnsingh district, Bangladesh. A cluster was defined by 50 randomly-selected households in each village. Before the intervention we measured indoor female *P. argentipes* sand fly density in each cluster for two consecutive nights by CDC light trap. From these, we then selected five clusters with similar sand fly density which were randomly assigned to one of four intervention within each cluster and one control cluster. Interventions were: households wall painting with5A IGR NG interior Paint (IP), installation of Durable Wall Lining (DWL), impregnation of bed-nets with KO Tab 1-2-3 (ITN), and indoor residual spraying with deltamethrin (IRS). One cluster served as control without intervention (control). Follow-up entomological activities included sand fly density measurement using the CDC light trap and the WHO Cone Bioassay test on treated and untreated walls respectively in intervention and control households at 1, 3, 6, 9 and 12 months after intervention. We also collected socio-demographic information and household heads' knowledge and practice about VL and VL vector control. The study is going on and here we report the 6-month follow-up results. Efficacy was measured by Difference-in-Difference analysis and was expressed in percentage.

3 Results

Baseline sand fly density (mean ± standard deviation) per household per night in IP, DWL, ITN, IRS and Control cluster was respectively 0.82 (0.93), 0.28 (0.54), 0.29 (0.54), 0.51 (0.81) and 0.28 (0.57). Differences between clusters were insignificant. Percent sand fly density reduction by IP, DWL, ITN and IRS compared to the control cluster was at 3 months 83%, 11%, 14% and 31% respectively and at 6 months 70%, 29%, 17% and 59%. Acceptability of all interventions was very high and no serious or clinically-significant adverse event was observed in intervention clusters.

4 Conclusions

IP was the most effective tool to control indoor sand fly density. The long term evaluation of IP efficacy and operational research for assessing the suitability of this tool for community based work is highly desirable.

C0466 SEMINAL PARAMETERS IN DOGS TREATED FOR CANINE LEISHMANIASIS: A COMPARATIVE RANDOMIZED FIELD STUDY BETWEEN MILTEFOSINE AND MEGLUMINE ANTIMONIATE PROTOCOLS.

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1 Background

Miltefosine (MIL) is routinely used to treat canine leishmaniasis (CanL) for its good efficacy and tolerance despite occasional transient gastrointestinal side effects (Dorlo et al., 2012). Studies conducted on male rats showed a reversible testicular atrophy and impaired fertility induced by supra-therapeutic doses of MIL (Sindermann&Engel, 2006). The effects of two dosing regimens of MIL were evaluated and compared to the standard Meglumine antimoniate (Meg) protocol (Oliva et al., 2010) on serum Testosterone concentration (T) and seminal parameters of samples from dogs suffering from CanL.

2 Methods

17 males (age 1-8 years, various breeds) living in South Italy were enrolled. All dogs were classed as stages II and III CanL using the Leishvet staging system (Solano-Gallego et al., 2009). They were randomly divided in 3 groups: A (5 dogs), B and C (6 dogs each). In group A, Meg was administered subcutaneously (100 mg/kg/day for 28 days); in group B MIL was orally administered at the standard dosage (2 mg/kg/day for 28 days); in group C a novel MIL protocol was tested (1.2 mg/kg/day for 5 days then 2.5 mg/kg/day for 25 days). All dogs received allopurinol (10mg/kg BID) throughout the entire study. Dogs were observed for 180 days. At D: 0, 30, 60, 90, 180, blood and semen samples were collected and analyzed for T and seminal parameters (concentration, % of cells showing total (MOT) and progressive (PMOT) motility, % of rapid cells, VAP, VCL, VSL, ALH, STR, LIN, BCF) by Computer Assisted Sperm Analyzer. Differences were analyzed statistically using Kruskal-Wallis and Mann-Whitney tests.

3 Results

Differences in T were not statistically different at any time. At D30 the sperm cells concentration was statistically higher in group C vs B ($P<0.05$). At D90 the % of MOT and PMOT were statistically better in group C vs A and B ($P<0.05$); in group C the % of rapid cells was also highest compared to groups A and B ($P<0.05$). VCL was better in group C vs B at D30 ($P<0.01$) and better vs A and B at D90 ($P<0.05$). BCF was also better in group C vs B at D30 ($P<0.05$). Differences in the other kinetic parameters were not statistically different.

4 Conclusions

This study demonstrated that MIL treatment compared to Meg induces less morphological sperm cells changes, with better kinetic parameters (MOT, PMOT, % of rapid cells, VCL, BCF) and that the novel tested MIL dosage maximizes the positive effects.

C0473 ACHIEVING THE VISCERAL LEISHMANIASIS ELIMINATION TARGET IN INDIA WITH EFFECTIVE VECTOR MONITORING.

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1 Background

Visceral leishmaniasis (VL) is a vector-borne neglected tropical disease of public health importance in Bihar and is transmitted by the bite of an infected *Phlebotomus argentipes* sand fly. Since the inception of the VL elimination programme in 1934, indoor residual spraying (IRS) has been conducted with dichlorodiphenyltrichloroethane (DDT). In 2015, in response to evidence showing a rapid decline in *Ph. argentipes* susceptibility, the elimination programme switched to the pyrethroid insecticide, alpha-cypermethrin (alpha). Entomological monitoring is crucial to ensure insecticide efficacy during the transmission season.

2 Methods

Susceptibility tests were conducted to assess the current resistance status of *Ph. argentipes* across Bihar using WHO diagnostic tests. To assess the intensity of DDT and potential pyrethroid resistance, dose mortality curves were established using CDC bottle bioassays and F1 *Ph. argentipes*. The efficacy of DDT and alpha IRS was assessed by a testing process treating tiles made of three of the most common surfaces in Bihar (mud, brick and limewash).

3 Results

Susceptibility tests of F1 and field caught *Ph. argentipes* from Bihar showed 100% mortality rates when exposed to 0.1% alpha and 0.05% deltamethrin. Reduced mortality rates were observed when *Ph. argentipes* were exposed to 4% DDT (24.6-37.5%). The DDT CDC bottle bioassays showed that after 45 minutes of exposure at the diagnostic dose (100µg/bottle) 30% of sand flies were killed and 69% at 10x the diagnostic dose. Mortality breaching the 98% WHO limit for susceptibility was only found at 10x the diagnostic dose after 75 minutes and 5x the diagnostic dose after 150 minutes, demonstrating the intensity of resistance. However, with alpha, after 30 minutes of exposure at the diagnostic dose (12.5 µg/bottle), 89% of sand flies were killed, whilst mortality ranged between 96-98% for 2x, 5x and 10x the diagnostic dose. Efficacy of DDT on F1 *Ph.argentipes* sand flies exposed for 30 minutes, was 13.45-21.97% on tiles treated at 1g/m^2, conversely 0.025g/m2 treated alpha-cypermethrin tiles was 33.33-83.33%

4 Conclusions

High levels of DDT resistance has been detected in field and laboratory settings, whilst alpha is shown to be effective. Monitoring entomological indicators across Bihar promotes informed decision-making, provides early indications of sub-optimal impact and could support the programme in achieving its 2017 elimination target.

C0479 ACHIEVING AND SUSTAINING VISCERAL LEISHMANIASIS ELIMINATION – A POLICY BRIEF

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1 Background

Current efforts to eliminate visceral leishmaniasis (VL) as a public health problem in the Indian Subcontinent are proving successful. The challenge now is long-term durability. Policy briefs can promote ongoing investment; elimination is as much a political as a scientific question, requiring the mobilisation of human resources and financial capital to ensure success. Political will is key to ensure that the aspiration of eliminating VL is achieved by 2020 and sustained.

2 Methods

The case of Bangladesh was examined. The national control programme has implemented the "No Kala-azar Transmission Activity (NKTA)" and the elimination target is being achieved. The current situation was evaluated through reviewing relevant literature and consulting with the country research and control communities. A stakeholders analysis and policy landscape were conducted.

3 Results

The policy landscape identified stakeholders and policy priorities. The interests and roles of national and international stakeholders involved in agenda setting/policy making, funding/supporting, and executing were analysed. Three areas for policy priorities were identified: 1. Ensure the target of lowering visceral leishmaniasis incidence rates to below 1/10,000 is reached in every upazila; 2. Successful transition of visceral leishmaniasis operations in Bangladesh from attack phase to sustainable maintenance phase; 3. Focused research and development by relevant stakeholders with national and international commitments to the success of the programme. The role of policy briefs was explained.

4 Conclusions

Both scientific evidence and policy briefs are important and should mutually support each other. They both point to further investment being required to ensure elimination as a public health problem and to prevent resurgence. A policy memo can assist in reaffirming the importance of successful elimination, to avoid wastage of the existing investments and progress made. Policy memos may therefore serve as a powerful tool to sustain interest and motivation in completing VL elimination.

C0563 ACTIVE CASE DETECTION OF VISCERAL LEISHMANIASIS CASES ALONG WITH LEPROSY, TUBERCULOSIS AND MALARIA THROUGH CHRONIC FEVER CAMP APPROACH IN NEPAL

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1 Background

Nepal has made significant progress towards eliminating visceral leishmaniasis (VL) with about 90% reduction in VL incidence in 2015 compared to2005, when the VL elimination programme started. Having reached the maintenance phasein most endemic districts, methods of identifying cases are required which are more efficient and adapted to low VL incidence in the country. The objective of this studywas to test the effectiveness of active case detection (ACD) of VL and post kala-azar dermal leishmaniasis (PKDL) and other febrile diseases through chronic fever camp approach at the community level.

2 Methods

Chronic fever camps were organized in 7 villages of Saptari district reporting VL cases (total population 44,323). Communities were informed of the camp date, time and place through female community health volunteers and health workers of the local health facilities. A campteam from the District Public Health Office examined for VL, PKDL, tuberculosis, malaria and leprosy cases among the febrile cases. Immediately after the camps, house-to-house VL screening was conducted.

3 Results

A total of 398 chronic febrile cases attended the camps (0.9% of the total population) yielding one VL, three tuberculosis and one malaria cases. Post-camp house-to-house screening involving 7,211 households identified 679 chronic fever and 461 skin lesion cases, but none of them were positive for VL, PKDL, leprosy, tuberculosis and malaria. The overall risk of chronic fever in these villages (between self-reported and actively-detected cases) was 0.024 per 10,000. The camp approach was found cost-effective for conducting ACD of febrile illnesses.

4 Conclusions

ACD of VL and PKDL along with leprosy, tuberculosis and malaria through camp approach is feasible and sensitive. Using a common platform of ACD of all febrile illnesses increases cost-effectiveness and sustainability.

C0625 EFFICACY OF INDEX BASED INTERVENTION PACKAGES FOR EARLY CASE DETECTION AND SANDFLY CONTROL IN BANGLADESH

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1 Background

We investigated the efficacy of three different index case (IC) based intervention packages for early detection of visceral leishmaniasis (VL) and post-kala-azar dermal leishmaniasis (PKDL) as well as sand fly control in support of the VL elimination program on the Indian sub-continent.

2 Methods

A randomized clustered trial was conducted in six unions of a kala-azar endemic sub-district of Bangladesh. The interventions were i) indoor residual spraying with insecticide (IRS) and case search through house-to-house visits in 60 households (HHs) around recently reported VL/PKDL cases (IC), known as no kala-azar transmission activity (NKTA); ii) a combined fever camp at the village of the IC for detecting VL/PKDL and other febrile diseases plus installation of insecticide impregnated durable wall lining in 60 HHs around the IC HH (DWL), and iii) the combined fever camp at IC village plus impregnation of existing bed-nets with slow release insecticide tablet, KO Tab 123 (ITN). Efficacy was measured through yield of new cases, vector density reduction and corrected sand fly mortality (WHO Cone Bioassay) at six months.

3 Results

A total of 49 IC based interventions were implemented in 28 villages from September 2015 to March, 2016: 18 (11 villages) for NKTA, 12 (10 villages) for DWL and 19 (7 villages) for ITN. Newly detected VL/PKDL cases per IC based camp search were 0.5 and 0.43 respectively for DWL and ITN. In addition, 3 cases with enteric fever and 7 cases with other febrile illness were detected in DWL areas. These numbers were 5 and 11 for the ITN arm but none for the NKTA arm. However NKTA was the most effective intervention for sand fly reduction up to six months followed by DWL. The mean (95%CI) sand fly reduction by NKTA and DWL were respectively -2.73 (-1.00, -4.44) and -1.23 (-1.00, -1.45). Sand fly reduction by ITN up to six months was not consistent 0.77 (-0.31, 1.85). DWL had the best WHO Cone bioassay results 95% (95%CI, 93%-97%) followed by NKTA 75% (95% CI, 72%-78%) and ITN 74% (95%CI, 70% - 79%) at six months.

4 Conclusions

IC based combined fever camps are useful for early detection of cases of VL, PKDL and other febrile illness. IRS of NKTA is most effective for sand fly control for the last push towards elimination. Study findings will help designing the strategy for achieving and maintaining the elimination goal.

C0645 SUSCEPTIBILITY STATUS OF DIFFERENT INSECTICIDES ON VISCERAL LEISHMANIASIS VECTOR, PHLEBOTOMUS ARGENTIPES (DIPTERA: PSYCHODIDAE) IN BANGLADESH AND NEPAL

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1 Background

Phlebotomus argentipes is the vector for visceral leishmaniasis (VL) in the Indian sub-continent. In Bangladesh, indoor residual spraying was applied since 2012 in VL endemic areas using deltamethrin. In Nepal, IRS was started in 1992 for VL vector control with the insecticide lambda-cyhalothrin (ICON). Irrational use of insecticides may develop vector resistance but information on insecticide resistance is limited for Bangladesh, but few studies showed DDT is resistance levels in India. The objective of this study was to generate information on insecticide susceptibility of *P. argentipes* in support of the VL elimination initiative on the Indian Subcontinent.

2 Methods

Susceptibility tests were performed using WHO test kits following the standard procedures regarding alpha cypermethrin (0.05%), deltamethrin (0.05%), lambda cyhalothrin (0.05%), permethrin (0.75%), malathion (5%) and bendiocarb (0.1%) in different parts of these countries. Adult *P. argentipes* were collected in Bangladesh from six upazilas (sub-districts) namely Fulbaria, Madhupur, Pirganj, Bera, Terokhada and Godagari and in Nepal from three districts namely Morang, Sunsari and Saptari.

3 Results

The results show that the VL vector in the study areas was highly susceptible to all insecticides at 60 minutes of exposure. In Bangladesh, mortality was 100% at 15 minutes as well as 30 minutes of exposure, while study sites in Nepal showed some diverse results: the mortality rate was less than 90% for 15 minutes of exposure to alpha cypermethrin and deltamethrin in Morang and Sunsari districts respectively. After 30 minutes of exposure the mortality was above 95%.

4 Conclusions

Rational use of insecticides is highly recommended to national control programs as well as insecticide rotation. Resistance monitoring should be part of the programme.

C0669 PREFERRED TREATMENT OUTCOMES OF CUTANEOUS LEISHMANIASIS PATIENTS IN BRAZIL, BURKINA FASO, COLOMBIA, IRAN, MOROCCO, PERU AND TUNISIA

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1 Background

Many treatments have been tried on Cutaneous Leishmaniasis (CL), but few if any can be confidently recommended as effective, safe and acceptable. CL is a visible disease and the patient's requirements in terms of treatment and treatment effects are all the more important, but the patient's needs are rarely considered. We sought patients' opinions about desired treatment outcomes *via* individual interviews.

2 Methods

Participants were selected based on a purposive, maximum variation sampling approach along a defined patient spectrum covering e.g. *Leishmania* species, age and gender, and clinical presentation. Individual semi-structured interviews were conducted with patients with confirmed CL at 8 sites in 7 countries (Brazil, Burkina Faso, Colombia (two sites), Iran, Morocco, Peru and Tunisia) in their mother tongue, after obtaining their consent.

In-depth interviews, following a collaboratively developed topic guide, were covering patients' overall disease experiences, symptoms and treatments, as well as the cultural and social context, expectations, preferences, and concerns. Thematic analysis of translated transcripts was based on a coding framework, developed based on initial interviews, using the OSOP method and NVivo® software. Analysis was focussing on elements with reference to treatment outcomes, but also touching upon related topics such as treatment preferences and inclusion/exclusion criteria for treatment.

3 Results

80 adult patients (around 10 per site), aged 18–73 years, 61% male, were enrolled in the study. Their experience with treatments varied from traditional to medical (topical, local, systemic) treatment. Across all countries, treatment outcomes identified as important from a patient's perspectives did not necessarily correspond to outcomes considered in clinical trials to-date, and complement them. Some of these themes are region- or gender-specific, whereas others are general. For example, CL patients expect treatment to get rid of the parasite ('parasitological cure'), to achieve permanent healing ('no relapse') and not to leave a scar, to reduce pain, to improve general fitness.

4 Conclusions

The study provides a rich insight into preferred outcomes for CL treatments, as well as related topics, from the perspective of a broad and diverse patient population representing different forms of the disease. They will hopefully be of use for the research community to inform trial design and target product profiles.

C0687 FOLOWING THE COURSE OF AN OUTBREAK OF CUTANEOUS LEISHMANIASIS IN PARAGUAY

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1 Background

Cutaneous leishmaniasis is one of the neglected diseases in America. Patients who suffer the disease have less access to appropriate diagnostic and treatment. Majority of the cases occurs in forest areas far from health services with roads in poor conditions. More over drug stock for timely treatment of the cases is not the common situation. Fluctuations of the supply due to irregular buying of the medicines, threaten a proper cure of the patients. It is known that a delay in the treatment of the cutaneous form of leismaniasis increase the probability to develop the mucosal form. The aim of this study was to describe the actual epidemiological and clinical situation of an outbreak occurred one year ago.

2 Methods

After a year of an outbreak of cutaneous leishmaniasis without regular treatment of the cases, in order to follow the course of the patients, we went in November 2016, to the burst, in a forest area in Caaguazú, Paraguay, to describe the occurrence of new cases, to observe what happened with the old cases and to capture sandflies. Blood samples, smears from the ulcers and skin biopsies were taken from the patients. In addition, CDC traps were put to capture sandflies. Indirect immunofluorescent assay was performed with blood samples of the patients and skin samples were taken to make smears, histopathology, PCR and culture, to detect *Leishmania*.

3 Results

From 11 patients, we found five new cutaneous leishmanias patients with active lesion, four with healed ulcers without treatment and two cured with treatment. Of these five new patients, three were positive for skin smear o culture. Three of the five new and three of the six old patients had a positive serological test. PCR from the biopsies provided *Leishmania braziliensis* complex. Phlebotomine captured were identify as *Nyssomyia neivai* y *N. whitmani.*

4 Conclusions

To have appropriate diagnostic and treatment for the cutaneous leishmaniosis cases must be the main goal, because a delay increase the likelihood to develop mucosal form, as sandflies vectors are present and *L. braziliensis* is the agent.

C0694 INCREASING THE AWARENESS OF LEISHMANIASIS IN A LOW-ENDEMIC SETTING

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1 Background

Leishmaniasis is a rare imported disease in Norway and is not included in the mandatory Norwegian Surveillance system for Communicable Diseases (MSIS). Thus, reliable epidemiological data is lacking. Due to increased international travel, a growing part-time community in southern-Europe of Norwegian retirees, and increased immigration from endemic areas, the prevalence of leishmaniasis could be increasing. Therefore, a Norwegian multicenter study was initiated in order to gain more reliable data on the occurrence of the disease in the country, evaluate molecular diagnostic methods in a Norwegian setting, and raise awareness of the disease in the medical community.

2 Methods

A national multi-center observational study involving all the university hospitals and health-regions in the country was conducted. Patients with suspected leishmaniasis were included prospectively. The study was approved by the Regional Committee for Medical and Health Research Ethics (REK).
Skin biopsy, blood, bone-marrow and spleen samples were collected according to the clinical manifestation. Clinical data included suspected time and place of onset of infection, clinical manifestation, number and location of lesions, fever, weight-loss, skin pigmentation, hepatosplenomegaly, any differential diagnosis considered, treatment and outcome. For the visceral patients, biochemical data included Hgb, leukocytes, C-reactive protein, ESR, ALAT, albumin, INR, bilirubin. For all patients results from any diagnostic test for leishmaniasis was included.

3 Results

21 patients were included over a 2-year period. 4 of the 5 participating hospitals had patients with suspected leishmaniasis in the study-period. The patients came from all the endemic regions, 16 with suspected cutaneous and 5 with suspected visceral disease. Initial analysis found that 7 of the patients have confirmed cutaneous- and 2 visceral leishmaniasis. Strains detected included *L. mexicana*, *L. braziliensis*, *L. naiffi*, *L. donovani* and *L. infantum*.

4 Conclusions

During the study-period 21 patients were included, of which initial analysis have confirmed 7 patients with cutaneous and 2 with visceral leishmaniasis. Leishmaniasis, although rare in Norway, remains a disease that medical doctors need to be aware of due to its potential lethal outcome. Even in a low-endemic setting better epidemiological data, diagnostic possibilities and increased awareness is important.

C0713 TOLERANCE AND CLINICAL EFFICACY OF TWO DOSING MILTEFOSINE REGIMENS ASSOCIATED WITH ALLOPURINOL, IN THE TREATMENT OF CANINE LEISHMANIASIS: A RANDOMIZED FIELD STUDY.

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1 Background

The conventional treatment protocol for canine leishmaniasis using miltefosine (MIL) (Milteforan®, Virbac) combined with allopurinol has published evidence of a good safety/benefit ratio. However MIL may occasionally induce gastrointestinal disorders reducing treatment compliance. This study investigated a modified MIL dosing schedule in order to assess possible improved digestive tolerance and increased efficacy.

2 Methods

Dogs naturally infected by *Leishmania infantum,* andshowing clinical signs of the disease, were included in the trial, randomly allocated to 2 treatment groups, and monitored over 180 days.MILwas administered orally at the conventional dosage of 2 mg/kg BW SID for 28 days in group X and at the novel dosing regimen of 1.2 mg/kg BW SID for 5 days followed by2.5 mg/kg BW SID for 25 days in group Y. All dogs received allopurinol at 10 mg/kg BID throughout the entire study. Treatment tolerance was assessed by the incidence of observed adverse events. Efficacy was assessed by the percentage of reduction of the Clinical Score (CS) and the improvement of clinicopathological data during the follow-up; data were analysed using a mixed model ANCOVA Test. The number and timing of relapses were evaluated in each group.

3 Results

34 dogs were enrolled, 18 in group X and 16 in group Y. Adverse events (mild self-limiting diarrhea or vomiting at D2, D3 and D5) were registered in 16.7% and 12.5% respectively. The percentage of reduction of the CS from D0 to D180 was 68.3% in group X and 81.3% in group Y but with no statistical difference. Statistical differences were identified between groups at D90 and D180 for improvement of HCT%, RBC, Hgb, Alb%, Glob%, and Alb/Glob ratio. There was no difference regarding renal and hepatic parameters. Relapses were observed in 3 dogs from group X (one at D90 and two at D180).

4 Conclusions

While the standard protocol demonstrates a good safety/benefit ratio, the modified MIL schedule showed slightly better results in terms of digestive tolerance and for some clinical parameters, suggesting that it may be useful in cases of poor tolerance and/or clinical relapses to previous treatments.

C0736 STUDY OF CUTANEOUS LEISHMANIASIS OUTBREAK IN VAQUERIA, A RURAL REGION OF CAAGUAZÚ DEPARTMENT, IN PARAGUAY

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1 Background

As in many parts of the world, in Paraguay, cutaneous leishmaniasis is a tropical forest endemic zoonotic disease. The first report of an outbreak in Paraguay occurred in 1934, during Paraguay-Bolivia Chaco's' war. Formerly, since 1970 decade, began a continuous increase of the disease report, mainly because of the agriculture border progress into the forest. The cases arose from 45/year to 569/year by the end of 1990 decade. During that time, 80% of the cases were from *San Pedro, Caaguazú, Alto Paraná* and *Canindeyú* departments. After that rise, less cutaneous leishmaniasis outbreaks were detected and, the number of yearly reported cases decreased to 91 in 2015. This work had the aim to describe a cutaneous leishmaniasis outbreak, occurred in *Vaquería*, *Caaguazú* department, Paraguay, in September of 2015.

2 Methods

The work was a descriptive study of the cutaneous leishmaniasis outbreak. Clinical, epidemiological and laboratory aspects of the cases were studied and phlebotomine fauna were identified.

3 Results

Twenty cutaneous leishmaniasis cases were detected, 90% of them had between 16 and 60 years old, with a 34 years mean, mainly man (65%), all the lesions were typical cutaneous open ulcers, between 15 day to 3 month of evolution and 14 cases (80%) present only 1 to 2 sores, located mainly (14/20) in lower limbs. Only one of the patients was negative for serological studies by indirect immunofluorescent assay. Phlebotomines specimens captured in the area were identify as *Nyssomyia whitmani, Migonemyia migonei, Bruntomyia brumpti* and *Pintomyia kuschell*.

4 Conclusions

In this outbreak, several factors were combined, human activities, weather and the presence of phlebotomines vectors, to define the exposure to the agent. Women and less than 12 years old cases indicates intra domiciliary transmission pattern as the dwellings were very close to the forest.

C0740 CLINICAL, HAEMATOLOGICAL AND BIOCHEMICAL PROFILE OF VISCERAL LEISHMANIASIS PATIENTS: A DESCRIPTIVE ANALYSIS AND COMPARISON BETWEEN PRIMARY AND RELAPSE VISCERAL LEISHMANIASIS

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1 Background

We investigated clinic-laboratory profile of visceral leishmanaisis patients and compared those of patients with primary VL and VL relapse (VLR).

2 Methods

Study participants are VL cases under a controlled trial which was conducted between February, 2012 and August, 2013 in Muktagacha Upazila Hospital of district Mymensingh. Hematological and biochemical analysis of collected blood samples were feasible from last 70 of 300 patients, enrolled in that trial. Analysis was carried out in the ISO certified laboratory of icddr,b, Dhaka, Bangladesh.

3 Results

Fever with shivering was present with 41 (58.57%) patients which is significantly higher (p-value =0.000011) among primary VL cases (75.51%) comparing relapse (19.05%). Other common symptoms are weakness (75.7%), anorexia (67.1%), abdominal pain (57.1%), headache (38.6%), vertigo (37.1%), cough (35.7%), vomiting (18.6%), and burning micturition (11.4%). While considering haematological report, 42 (60%) patients had haemoglobin less than 10gm/dl and this condition is significantly higher (p-value = 0.003) among primary VL group 35 (83.3%) comparing with relapse VL 7 (16.7). Total count of WBC, percent neutrophil and monocyte count was less than normal among 27 (38.6%), 58 (82.9%) and 55 (78.6%) cases respectively whereas percent lymphocyte and eosinophil count was more than normal among 54 (77.1%) and 10 (14.3%) cases. Again, 44 (62.9%), 13 (18.6%), 22 (31.4%) and 22 (31.4%) patients had low sodium, potassium, chloride and calcium level respectively whereas 12 (17.1%) and 13 (18.6%) had high phosphate and magnesium level. On the other hand, 65 (92.9%) and 37 (52.9%) patients had low iron and iron binding capacity level. All the patients had low HDL and 68 (97.1%) had both low LDL and cholesterol level. In terms of checking hepatic and renal markers only 20 (28.6%) and 34 (48.6%) patients had high aspartate transaminase and alkaline phosphatase respectively.

4 Conclusions

Primary cases of VL and VLR differed regarding some clinical and biochemical features. In general electrolyte imbalance, sub-clinical hepatic and renal acute injury in significant proportion of VL patients should alert physicians while treating VL cases with anti-leishmanial drugs.

C0755 CO-VARIATES AND OUTCOME OF VISCERAL LEISHMANIASIS TREATMENT WITH AMPHOTERICIN B DEOXYCHOLATE

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1 Background

Background: Amphotericin B Deoxycholate (ABD) is an effective treatment option for visceral leishmaniasis (VL). It has now been using as a backup drug for treatment of VL in Bangladesh in situations when all other recommended drugs are not available or effective. But information about treatment of VL with ABD mostly relies on studies in India. So, sufficient data yet to be available for this treatment option in Bangladesh. So, we aimed to find out the treatment outcome of ABD to treat VL in Bangladesh. Moreover, clinical co-variates help the physicians and policy makers in recognizing early and late recovery group and that is why we also aimed to find out the co-variates of VL treatment outcome with ABD.

2 Methods

Methods: The records of Surya Kanta Kala-azar Research Centre (SKKRC) were evaluated and finally analyzed 34 patient records who were treated with ABD (1mg/kg body weight for 15 days). All of them were confirmed through microscopic examination of splenic smear. Treatment outcome was measured on the basis of resolution of fever and enlarged spleen at two time points: after completing 15 day treatment and 6 months after completing treatment.

3 Results

The median age of the patients was 18 (12-42) years and the median baseline duration of fever and enlarged spleen size were 10.5 (4-30) weeks and 6.5 (2-17) cm respectively. All of them attained complete cure during six-month follow-up and among them 10 (29.41%) achieved complete cure just after completion of 15 days treatment. During treatment fever with shivering 16 (47.1%), hypokalemia 16 (47.1%), vomiting 12 (35.3%) and acidity 5 (14.7%) were the most common side effects. The median value of the duration of fever and baseline spleen size was significantly lower in the group that attained complete regression of enlarged spleen within 15 days treatment. We ran linear regression with log of percent spleen size regression just after finishing 15 days treatment as a dependent variable and found baseline log of duration of fever and log of enlarged spleen size predicted early recovery ($R2=0.70$, $F (1, 32) = 74.49$, $B=5.33$, $p=0.00$ and $R2=0.76$, $F (1, 32) = 101.86$, $B=5.01$, $p=0.00$).

4 Conclusions

The study shows ABD as a safe and effective option for VL treatment in Bangladesh. On the other hand, large scale evaluation including the other drugs in regards to co-variates should be evaluated. The study results are expected to be useful for the policymakers to combat situations with drug scarcity or treatment failure.

C0807 ENHANCING SURVEILLANCE OF THE VISCERAL LEISHMANIASIS ELIMINATION PROGRAMME IN INDIA.

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1 Background

Research clearly demonstrated that indoor residual spraying (IRS) in India with DDT and stirrup pumps was no longer effective at controlling *Phlebotomus argentipes* the vector of *Leishmania donovani* which causes visceral leishmaniasis (VL). A policy change was made to introduce compression pumps and pyrethroid insecticide alpha-cypermethrin to improve the quality of vector control and the chances of reaching the elimination target of 2020. Alongside this a series of sentinel sites was established to continuously monitor the impact of these changes on the programme.

2 Methods

Eight sentinel localities were randomly selected, six in Bihar, one both Uttar Pradesh and West Bengal. A sentinel locality consists of four villages receiving IRS and two villages with no IRS (Non-IRS villages are VL free for 3 or more years). Indicators include clinic records with follow up active case detection, *P. argentipes* abundance using CDC light traps, infectivity by PCR and insecticide resistance status. IRS quality is assessed by insecticide quantification kits and HPLC with coverage being determined by spray squad record cards and household survey.

3 Results

Initial results show that all *P. argentipes* are fully susceptible to the pyrethroid alpha-cypermethrin in all sites but highly resistant (less than 60% mortality) to DDT. There is a low level *kdr* mutation within the populations (11-23%). The abundance data shows lower numbers of sand flies being collected in the IRS villages compared to non IRS villages.

4 Conclusions

DDT resistance remains high in the *P. argentipes* population and is no longer suitable for control. This resistance in part is due to the *kdr* gene, however, the level of resistance is such that there is likely a second mechanism, e.g., glutathione-*s*-transferase involved in this resistance. Little cross resistance was observed to the pyrethroids and studies in *Anopheles* have shown that *kdr* alone may not cause operational failure of an insecticide. Initial base line *P. argentipes* abundance data follows the same seasonal patterns observed previously, the impact of IRS will be assessed after the next round in February 2017.
All data is being collated in a central disease data management system that will allow all stakeholders access to analysed data to improve the decision making process on the elimination programme.

C0820 KNOWLEDGE, ATTITUDE, AND PRACTICES RELATED TO LEISHMANIASIS - EVIDENCE FROM SOUTH INDIA

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1 Background

Cutaneous leishmaniasis (CL, also called ''kala-azar"), is a life threatening neglected tropical infectious disease which mainly affects the poorest of the poor. VL is prevalent in India particularly in the north and south of the country. The objective of the study was to assess knowledge, attitudes, and practices (KAP) of inhabitants of an endemic area in Kerala, South India toward cutaneous leishmaniasis (CL).

2 Methods

The Sample size (237 residents) was calculated based on population. House-to-house survey was done to collect the data regarding knowledge, attitudes, and practices of the inhabitants. The head of each household was interviewed by a trained staff to assess his/her KAP related to CL. A semi-structured KAP questionnaire was used for data collection.

3 Results

Mean age of participants was 39 and more than half of the respondents were in the age group of 31-40. Males constituted 172 (72.5%) of subjects. Most of the respondents (84.3%) were literate. The majority of the study population (83%) had heard about CL and most of these respondents (91%) were aware that CL is presented with a cutaneous lesion. Nearly two-third of the participants (63.5%) stated the bite of mosquito (not specifically sandflies) for CL transmission. The respondents' attitude regarding the treatment of CL was not satisfactory since only 48% believed that CL can be treated by medicine. A noticeable proportion of respondents (21%) believed in indigenous medicine for the treatment of CL. A small proportion of respondents (14%) stated that traditional healers are good at treating this disease. More than two-third (69%) of respondents believed that the disease is preventable although most of interviewees did not know about preventive measures.

4 Conclusions

In this study, insufficient knowledge of community about infection nature, vector, transmission mode and preventive measures of CL, highlights the needs for a health education initiative to enhance the awareness of people about CL. This would improve inhabitants' contribution in control program of CL in this area.

C0836 ASSESSMENT OF KNOWLEDGE, ATTITUDE AND PRACTICE TOWARDS VECTOR CONTROL PROGRAMS AMONG SCHOOL TEACHERS IN INDIA

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1 Background

Vector-borne diseases such as Malaria and Leishmaniasis are still a public health problem in India. One of the effective ways to prevent of these diseases is to improve the vector control program. We carried out this study for school teachers knowledge, attitude and practice regarding vector control programs.

2 Methods

A cross-sectional study was conducted during October to September 2015 in mangalore, Karnataka, India. The participants were 38 school teachers that were selected BY random sampling. The data were collected through a questionnaire that was comprised four sections. All statistical analyses were performed using SPSS software.

3 Results

The findings showed that only 34% of participants had a positive attitude toward the vector control program. Furthermore, 55.6% of participants were agreed with chemical vector control method.

4 Conclusions

The knowledge of participants was rather good about vector control. Since, Community education is a key factor to improve vector control program, we suggest strengthening the vector control education program at different levels of the community.

C0837 ENHANCING VISCERAL LEISHMANIASIS ELIMINATION IN INDIA – RESULTS OF A CLUSTER RANDOMIZED TRIAL.

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1 Background

In India, visceral leishmaniasis (VL) is caused by *Leishmania donovani* and the only vector is *Phlebotomus argentipes*. Historically indoor residual spraying (IRS) with DDT has been used to control malaria with a beneficial side effect of controlling VL. This early success led to the adoption of DDT to eliminate VL in India. However, a systematic analysis and study demonstrated that the current control method was not effective. This led to a policy change from the use of DDT to pyrethroids for IRS and a change in the delivery of insecticides from stirrup pumps to compression pumps. Here we present the first analysis of a cluster randomised trial of different insecticides and delivery mechanisms for IRS in VL elimination.

2 Methods

A cluster randomised trial with 4 arms, combining DDT or pyrethroid with either stirrup pump or compression pump was established. Each arm consisted of 4 IRS and 2 non-IRS villages. Quality of IRS was measured using insecticide quantification kits and HPLC, as was the residual decay rate, entomological indicators included *P. argentipes* abundance and resistance status. IRS coverage and acceptability was monitored via questionnaire as was pump acceptability by spray operators.

3 Results

IRS coverage across the study ranged from 81-97% however the quality of IRS was still below expected ave. 75% of target dose for all arms. *Ph. argentipes* was 100% susceptible to the pyrethroid but resistant to DDT (Ave. 60% mortality). Villages that received IRS with pyrethroid had an impact on *P. argentipes* abundance however, no difference was observed in villages that received IRS with DDT compared to non-IRS villages.

4 Conclusions

Due to the high level of DDT resistance in the *P. argentipes* population the pyrethroid insecticide was able to better control *P. argentipes* abundance, however, numbers of *P. argentipes* caught were lower than expected even in non IRS sites. Initial analysis show no significant difference was found in sand fly abundance between compression and stirrup pump, however, IRS quality data is still being analysed.
Based on this and previous work, IRS with compression pumps and pyrethroid have now been scaled up in India. In order to monitor this a series of 8 sentinel sites compromising 64 villages have been established.

C0856 KALA AZAR MANAGEMENT INFORMATION SYSTEM (KA-MIS): A COMPREHENSIVE MANAGEMENT INFORMATION SYSTEM TO SUPPORT AND MONITOR SUSTAINABLE VL ELIMINATION IN INDIA

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1 Background

India aims to achieve VL elimination, currently defined as annual incidence of less than 1 case per 10000at the block (sub-district) level, by 2017. The case recording and reporting systems of the elimination program, constituting the management information system, were weak at the outset but have been considerably strengthened through the efforts of the national and state program teams with technical assistance from CARE India.

2 Methods

WHO/TDR recommended common reporting formats across affected countries in South Asia. The existing VL disease recording and reporting systems in the National Vector Borne Disease Control Program (NVBDCP) in India were reviewed to identify inconsistencies and gaps. Manual recording formats were refined and standardized to reflect the prescribed diagnosis and treatment protocols. Line list formats were designed to segregate the resident and non-resident cases at the block level and a robust unique identification system developed. An online reporting system was evolved that enabled near real-time reporting of line lists of cases, the tracking of cases as they migrated from blocks of residence and between facilities for diagnosis or treatment and thus automated the reporting of incidence by block of residence. The online database was populated with VL and PKDL case information from January 2013 onwards. Separate modules record and report financial incentives and inventory of supplies. The system is GIS enabled. Extensive cross-checks were implemented for data validation and cleaning, and field experience informed refinement of the system in phases. A varied set of automated reports includes inter alia the generation of IRS micro-plans.

3 Results

The KA-MIS has been implemented in phases across all VL-affected districts of Bihar, Jharkhand and West Bengal. Program staff use the system in all facilities. The geographical localization of cases has acquired high fidelity, and the misclassification of the endemicity status of geographical units is no longer a concern.

4 Conclusions

The KA-MIS has matured to a point where it can reliably support core surveillance and related epidemiological studies, besides providing **precise** case monitoring information for VL elimination. It can be easily adapted for use in any geography globally that reports VL cases.

C0908 "DIFFERENTIAL DIAGNOSIS OF CUTANEOUS LEISHMANIASIS IN A TROPICAL DISEASE CLINIC IN PANAMA "

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1 Background

Panama is one of 18 countries in America where Cutaneous Leishmaniasis (CL) is endemic. The incidence in the country in 2014 was 50.53 cases per 100,000 inhabitants. The average amount of cases per year has been 3250 in last 5 years.
The Gorgas Memorial Institute for Health Studies (ICGES) is the national reference center for the diagnosis of tropical diseases (including Leishmaniasis). Patients with CL and other dermatologic infections are referred to our Unit for diagnostic work up.
CL usually presents as a clean, chronic, non-healing ulcer with elevated edges. Other causes of cutaneous ulcers can simulate leishmaniasis and a specific diagnosis is required. We present a description of the patients referred to our Unit with the presumptive diagnosis of CL.

2 Methods

This is a retrospective chart review involving all the patients seen in our Unit and we selected those with a presumptive diagnosis of CL referred between October 2013 and November 2016. We gathered information on demographic data, reasons for referral and final diagnosis from paper and the electronic medical record. The diagnosis of CL was confirmed either with microscopy, culture or PCR (detecting kinetoplastid DNA). The information is part of our routine epidemiological and clinical surveillance. Measures to protect the confidentiality of the subjects were undertaken.

3 Results

There were a total of 1524 patients evaluated in the clinic during this period. Of those patients, 617 (40.5%) came with a presumptive diagnosis of leishmaniasis. In 557 (90.3%) of the patients CL was confirmed and 60 (9.7%) were diagnosed with other diseases that are considered in the differential diagnosis.
Of these 60 patients, the diagnosis included: 37 (61.7%) ulcerated bacterial infections, 5 (8.3%) ulcers secondary to peripheral vascular disease (venous and arterial), 4 (6.7%) ulcers secondary to spider bites (Loxocelism), 4 (6.7%) skin abscesses, 3 (5%) diabetic ulcers, 2 (3.3%) impetigo,,and 5 (8.3%) had other miscellaneous diagnosis such as sporotrichosis, ecthyma , basal cell carcinoma, cutaneous tuberculosis and chromomycosis.

4 Conclusions

In over 90% of the referrals, the initial clinical impression of CL was confirmed parasitologically.
The most common alternative diagnosis were bacterial causes of skin ulceration but many other etiologies were also detected remarking the importance of a specific etiologic diagnosis even in the setting of a high incidence of CL.

C0961 STUDY OF PROLIFERATION EFFECTS OF HUMAN URINE ON LEISHMANIA MAJOR PROMASTIGOTES

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1 Background

Synthetic culture media are often essential for the laboratory diagnosis of leishmaniasis and laboratory studies concerning metabolic, infectious and antigenic properties of leishmanial promastigotes. Synthetic *Leishmania* culture media are divided into monophasic and biphasic (blood containing agar) types and are used in association with fetal calf serum (FCS) and rabbit blood respectively in mass culture and isolation of various *Leishmania* spieces. In this study, the growth-stimulating effects of human urine on *Leishmania major* (MRHO/IR/75/ER) promastigotes as well as the possibility of substituting strile human urine for FCS and rabbit blood in synthetic *Leishmania* culture media was examined.

2 Methods

Monophasic RPMI 1640 and biphasic agar supplemented media were made using different concentrations of human urine. *Leishmania major* promastigotes were cultured in these as well as in control standard media (RPMI 1640 containing 10% FCS and agar containing 10% rabbit blood). The number of proliferating promastigotes was determined at different times following culture and the related growth curves were plotted. The average number of promastigotes in every medium was ascertained and a comparsion was made among the trials and control media.

3 Results

The maximum cell density of *L.major* promastigotes in the presence of human urine (10%) in RPMI 1640 medium was about 65×10^{6} cell/ml. The average number of promastigotes produced in RPMI 1640 medium containing human urine with concentrations of 2, 4, 6, 12, 14 and 16 percent did not differ from those in the control media ($P>0.05$). the maximum density of *Leishmania major* promastigotes in the biphasic agar supplemented medium in the presence of human urine (20%) was about 21×10^{4} cell/ml which did not differ significantly from the control media (N.N.N) ($P>0.05$).

4 Conclusions

Results indicated the human urine has a promoting effect on the proliferation of *L.major* promastigotes which is independent of urine concentration, hence human urine can be an appropriate substitute for FCS and rabbit blood in *Leishmania* culture media. Human urine is a readily available and inexpensive enhancer of the growth of *L.major* promastigotes and probably all other *Leishmania* spieces.

C1008 UNDERSTANDING LONG-TERM CYCLES OF VISCERAL LEISHMANIASIS IN BIHAR

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1 Background

Visceral leishmaniasis (VL) is a vector-borne disease of public health importance in India, with the highest burden of disease in the states of Bihar, Jharkhand, West Bengal and Uttar Pradesh. VL is currently targeted for elimination by 2017; the primary interventions used for elimination are indoor residual spraying, active case detection and treatment. Historically the disease trend in India has been regarded as cyclical with case resurgence characteristically occurring every 15 years. However, the cause of this pattern remains unclear and as the Bihar VL programme nears the elimination target, explaining previous trends to use within predictive tools to avoid future epidemics has become essential.

2 Methods

To interpret observed cyclical trends, annual climatic indicators including rainfall, temperature and humidity over time periods known to influence disease and vector trends over the year were compared with annual VL case incidence data.

3 Results

Rainfall was found to have a strong association with annual VL case patterns during the monsoon season (June to September) ($p=0.0383$) and prior to sand fly peaks (February to May, September and October) ($p=0.0253$). Whilst annual rainfall was also found to have a close association with annual case incidence ($p=0.0398$), rainfall during the sand fly peaks (March to June, October and November) was not significant ($p=0.0911$). No association between humidity or temperature and VL incidence was detected (all values $p=>0.05$).

4 Conclusions

The VL programme in Bihar has made significant progress in recent years, adopting improved practices for vector control, diagnostics and treatment strategies: changing from DDT and stirrup pumps to alpha-cypermethrin and hand compression pumps, introduction of the rk39 test and wide-scale availability of amphotericin B. Such concerted efforts may lead to short-term success, however to achieve and sustain elimination, a better understanding of external causative factors is required.

C1025 EFFICACY OF INSECTICIDAL WALL PAINT, INSECTICIDE TREATED BED NETS AND INDOOR RESIDUAL SPRAYING FOR VISCERAL LEISHMANIASIS VECTOR CONTROL IN NEPAL MURARI LAL DAS1, LALITA ROY1, CHITRA KUMAR GURUNG2, ANAND B. JOSHI2, PIERO L OLLIARO3, AXEL KROEGER3,6, GREG MATLASHEWSKI4, MEGHA RAJ BANJARA5 1B.P. KOIRALA INSTITUTE OF HEALTH SCIENCES, DHARAN, NEPAL 2PUBL

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1 Background

Visceral leishmaniasis is on the verge of elimination in Nepal. Besides other control strategies, thanks also to Indoor Residual Spraying (IRS) which has high acceptance at community level but is costly and operationally challenging. To explore alternatives to IRS particularly in the post-elimination phase, we investigated the efficacy of insecticidal wall paint (IWP), bed net impregnated with slow release insecticides (ITN) and indoor residual spraying (IRS).

2 Methods

This cluster-randomized controlled trial was started in May 2016 in the VL endemic district of Saptari. Four clusters (with about 100 households each) were selected and randomly assigned to untreated (Control) and three interventions: IWP with Inesfly ™(Alphacypermethrin 0.7%, D-alethrin 1.0% and Pyriproxyphen 0.063%); ITN impregnated with K-OTAB1-2-3 (Deltamethrin, 25 mg/m^2); and IRS with Alphacypermethrin 5% WP. Six households from each cluster were randomly selected for entomological measurements before, one and six months after interventions. Bioassay tests were conducted to evaluate the bioavailability of insecticides in each of the vector control tools.

3 Results

The intervention effect was measured as the difference in differences in female *Phlebotomus argentipes* sand fly count from different times of post intervention to baseline in intervention clusters compared to the control cluster. The sand fly reduction [mean and 95% CI] ranged for IWP from -15.71 (-22.51, -8.91) at one month to -2.88 (-7.15, 1.40) at six months, for IRS from -13.25 (-20.07, -6.43) at one month to 0.88 (-2.37, 4.12) at six months and for ITN from -7.33 (-13.75, -0.91) at one month to 1.63 (-3.64, 6.89) at six months. The reductions were statistically significant at the 5% level for IWP only after six months. Vector sand fly mortality rates in bioassays ranged from 93.97% (76.92%-100%) for IWP, 65.37% (12.77%-86.00%) for ITN and 54% (15.38%-92.31%) for IRS at 3 minutes of exposure on treated surface after six months of intervention.

4 Conclusions

IWP was found effective in reducing vector sand fly density up to at least six months post-intervention. Vector mortality rates were also high. IWP can be used as normal wall paint at community level that may reduce the operational challenge experienced in IRS. IRS and ITN had satisfactory results only at one month of post-intervention. Hence, IWP can be the alternative or complementary vector control tool to IRS particularly for the VL post-elimination program in Nepal.

C1144 USING FIELD EPIDEMIOLOGY TO UNDERSTAND VISCERAL LEISHMANIASIS BETTER: SUMMARY OF FINDINGS FROM ONGOING OUTBREAK INVESTIGATIONS IN INDIA

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1 Background

Visceral leishmaniasis (VL) cases has been on the decline in India since 2011, for reasons that are not fully understood. However, outbreaks of cases in long unaffected villages and regions are being observed. Likewise, there are villages reporting cases despite *Indoor Residual Spraying* (IRS). Understanding the factors causing these outbreaks will help design interventions targeted at achieving VL elimination goals.

2 Methods

A range of field epidemiological investigations are being conducted to explore possible factors underlying the outbreaks in different geographic and programmatic contexts in Bihar, the highest endemicity state in India. The base case incidence data is taken from a carefully aggregated and constantly updated universe of reported VL and PKDL cases. Descriptive analyses are being used to generate hypotheses to explain peculiar patterns of seasonality and clustering, and developing insights into previously unsuspected or unexplained factors that apparently enhance the risk of either infection or activation of disease among the infected individuals. Simultaneously collected data from entomological surveillance and IRS coverage studies, as well as precise location-based information on cases and associated parameters complement detailed case surveillance.

3 Results

Potentially useful hypotheses are emerging from observed higher risk associated with migration for work, nature of work, hookworm infestation and nature of vegetation in these ongoing studies. Combining information from a large number of outbreaks enables testing of potential hypotheses and description of homogeneity (or lack of it) of particular characteristics across geographical contexts having varying past experience of the disease.

4 Conclusions

One potential threat to VL elimination is the emergence of new foci while elimination efforts are sought to be concentrated in the worst affected areas. Application of field epidemiological methods for understanding how such foci emerge and how the emerging cases stand out from the cases reported from endemic areas – in presence or absence of relevant control measures - should help unravel more mysteries about this long neglected disease.

C1199 TREND IN THE HEALTH SEEKING BEHAVIOR OF KALA-AZAR PATIENTS IN MUZAFFARPUR-TMRC HEATH AND DEMOGRAPHIC SURVEILLANCE SYSTEM IN BHAR, INDIA

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1 Background

Muzaffarpur-TMRC Health and Demographic Surveillance System (HDSS) is a dedicated platform for visceral leishmaniasis (VL) research established in 2007 in Muzaffarpur district of Bihar state in India. In the 50 VL endemic villages of the HDSS, annual household surveys are conducted and all VL patients are registered and interviewed. We used the HDSS dataset to get more insight into the healthcare seeking behavior of VL patients.

2 Methods

From the available HDSS data we extracted information on number and types of health providers consulted by VL patients, diagnosis and treatment prescribed; and individual and health system delay.

3 Results

Between March 2007 and July 2015, 332 VL cases were registered (185 men (56%) and 147 women (44%)). We observed declining trend in annual incidence (from 15 per 10000 in 2007 to 0.82 in 2014). Most patients (73%) first consulted unqualified providers; only 3% chose to go to government health facilities. This trend does not improve much over the study period. Mean patient delay was 7.3 days and the average patient visited more than three health facilities before being diagnosed. During the course of health seeking, only 17% of the total visits were given to government hospitals. Mean total health system delay (constituted as diagnosis delay: 37.7 days + treatment delay 6.3 days) was 44 days. Daignosis was based on rK39 rapid test for 93.7%, Amphotericin-B (45.5%) and Miltefosine (23.5%) were the most common treatments while 19.3% received some combination therapy, 11.8% received sodium antimony gluconate. Of 332 patients, though 35.5% were diagnosed at public hospitals, only 22.3% of them took final treatment there. Remaining patients were treated by NGOs (47%) and private qualified doctors (30.72%).

4 Conclusions

Incidence of VL in the HDSS area sharply declined over the past 8 years. Patients usually present early but most of the time they present to unqualified providers despite free diagnosis and treatment available and recent incentives introduced to make patients come to public hospitals. They still incur substantial health system's delays. The VL elimination programme needs to improve to provide timely treament.

C1211 IMPROVING SURVEILLANCE IN INDIA'S VISCERAL LEISHMANIASIS ELIMINATION PROGRAMME.

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1 Background

Data is at the core of any successful vector-borne disease control or elimination programme. At the early stages of control, monitoring data can help prioritize limited funding and resources to maximize impact.During the pre-elimination and elimination phases, surveillance data itself becomes the primary intervention by quickly identifying persistent transmission. It is crucial to integrate data-driven, spatial decision-support tools with health information systems (HIS) as countries strive for elimination.
The Disease Data Management System (DDMS) is an integrated data management system, developed to address the data collection, management and analysis needs of malaria and dengue control programmes. The system is able to collect performance and impact data and provide real-time feedback to decision-makers.
In 2005, India, Bangladesh and Nepal signed an agreement to achieve Visceral Leishmaniasis (VL) elimination by 2015. In order to support this elimination goal, the DDMS was adapted for VL specific data and monitoring within India.

2 Methods

The DDMS was tailored to India's VL monitoring needs using country specific data. To improve granularity of analysis, details regarding VL transmission seasons, insecticides used for vector control and census data were added. The geography within the DDMS was updated to include local geographical divisions to enable spatial mapping of entomological data and quality assurance.

3 Results

The DDMS was initially implemented in 8 districts in Bihar. The captured data collected highlighted several issues with the current DDT-based IRS vector control programme, particularly poor impact of IRS on vector abundance (1 month vs the stated 6), low quality of IRS and high resistance of the vector to DDT. These results prompted a national policy change in insecticide and pump use.

4 Conclusions

The DDMS is a novel data management system adapted for VL. As an integrated data management platform, it empowered the VL elimination programme to transition from DDT IRS with stirrup pumps to pyrethroid IRS with compression sprayers. The DDMS has been further expanded, covering 8 sentinel sites to support the VL programme in India as it moves towards reaching its elimination target.

C1281 RISK FACTORS ASSOCIATED WITH DEFAULTING FROM VISCERAL LEISHMANIASIS TREATMENT; AN ANALYSIS UNDER ROUTINE PROGRAM CONDITIONS IN BIHAR, INDIA

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1 Background

Introduction: The Visceral Leishmaniasis(VL)Elimination Program has a target to achieve a treatment completion rates of above 90%. Default during treatment can be a major hindrance to the elimination effort. This case control study was conducted between Dec 2009 and June 2012 in three Primary Health Centres (PHC) of Muzaffarpur district to find out the magnitude of the problem of default from treatment and identify risk factors and underlying causes.

2 Methods

Patients who defaulted from treatment from the PHCs were considered as "cases", and those who completed their treatment as "controls". Two controls were included in the study for each case. Respondent's opinion and satisfaction with the services provided at the PHCs was also conducted. Logistic regression was performed to assess the contribution of socio-demographic variables on patient status. Further, a discriminant analysis was done (after decomposing the original data) to identify factors that can predict the patient status as defaulter or not, based on factor scores of the components as predictor variables.

3 Results

During the study period 16.3% (89/544) patients defaulted. 87 cases and 188 controls were interviewed through a semi structured questionnaire. Women had a higher risk for default (OR 1.8, 95% CI 1.1-3.0).Treatment received was Miltefosine in 58.6% and Sodium stibogluconate (SSG) in41.4%. Most (86 %) of the defaulters from PHCs completed their treatment at other health care facilities, with 70% of them preferring non-governmental institutions. Most cited reasons for default were to seek second opinion for VL treatment and preference to be treated in specialized VL centres. Discriminant analysis showed only one significant predictor namely dissatisfaction about the medical care received in PHCs.

4 Conclusions

Efforts are needed to enhance the quality of VL care at PHC level, which will be beneficial in increasing treatment completion rates.

C1550 ECONOMIC IMPACT OF VISCERAL LEISHMANIASIS IN BARINGO, KENYA

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1 Background

Visceral Leishmaniasis (VL) also known as Kala-azar is a deadly parasitic disease transmitted by the bite of an infected female sandfly. According to the World Health Organization (WHO), an estimated 200,000 to 400,000 new cases of VL occur worldwide and 20,000 to 30,000 deaths occur annually. A study was undertaken to examine the economic impact of VL on households (HHs) in Baringo County, Kenya.

2 Methods

The study population comprised 108 VL patients in Baringo who had been diagnosed and treated for VL in 2011/2012 at the two District Hospitals. A sample size of 84 was determined to be necessary using the Creative Research Online sample calculator. Questionnaires were used to collect data from 30 HHs. Data was analyzed using descriptive methods.

3 Results

We found that a single VL episode costs Kshs.31 200 (equivalent to USD 390 at 2012 exchange rates), which is triple the average household monthly income or 1.6 times their annual per capita income. These costs are beyond the reach of the majority of poor households and a combination of approaches were undertaken in order to be able to purchase treatments, including asset sales, reliance on community support, spending family savings or borrowing. Despite this, patients still could not meet the cost of VL treatments. Short-term direct costs outweigh short-term indirect costs. However, in the long-run, there are chances that the indirect costs may be much higher than direct costs especially where the patient is incapacitated and is not able to work again.

4 Conclusions

The economic burden of caring for VL patients and the subsequent stripping of household assets together with coping mechanisms can institute a vicious cycle of poverty in a household and may undermine sustainable development of endemic communities. It is recommended that VL disease control programs adopt novel mechanisms to fast track VL patients so that they may reach diagnostic facilities rapidly and receive medical attention. The support systems are necessary if the country is to sustain long-term health, welfare and development of the endemic community and indeed achieve the economic and health aspirations described in Kenya's economic blueprint, Vision 2030.

C1673 STRENGTHENING WEB BASED SURVEILLANCE FOR VL ELIMINATION PROGRAMME IN BANGLADESH

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1 Background

Effective Disease surveillance for kala-azar is one of the important strategies of the national Kala-azar elimination program (NKEP) in Bangladesh. To strengthen the disease surveillance in the country an expert project team from icddr,b assisted NKEP to build up a national capacity with setting up a web-based VL surveillance system in Bangladesh. Here we described the milestone of the project team for strengthening the web-based VL surveillance system in the country.

2 Methods

Methods included series of decision making workshops and meetings with the management of NKEP, Government of Bangladesh (GoB), National Management and Information System (MIS) and Institute of Epidemiology Disease Control and Research (IEDCR) for hosting of the web-based system and finalized the indicator for VL surveillance. Project team conducted the training of trainers (ToT) from the District Civil Surgeon Office (CSO) of VL endemic districts. Trained statistical assistant from the CSO also have been trained statisticians from the upazila health complexes (UHC). Medical officer(s) disease control (MODC) and senior nurses from the VL endemic upazilas also were trained in web based surveillance system especially in collection information and fill up forms.

3 Results

Project team conducted 5 decision making preparatory workshops and meetings with NKEP. The central VL surveillance team in NKEP and the project team jointly finalized the indicators. After that Project team and experts from MIS transferred the indicators in to the District Health Information System2 (DHIS) of the system of MIS. VL surveillance has been successfully piloted in 10 upazilas of 4 districts after training of MODC, nurses and statisticians from the respective upazilla. Another ongoing PV surveillance on VL that was running under NKEP and Directorate General of Drug Administration (DGDA) and icddr,b is considered to come together in to the VL web based surveillance system and it has been made a one platform that is now in DHIS2 system. Programme personnel at central level and 100 endemic UHC staffs are trained. TOT for VL web based system is now in place in 26 VL districts of Bangladesh.

4 Conclusions

In the surveillance system there is kala-azar cases are being entered, which is given by UHC's statistician. For the web based surveillance system Program people are getting the report quickly, analysis the case trend and other analysis.

C1685 TRADITIONAL TREATMENTS OF CUTANEOUS LEISHMANIASIS: PATIENTS' PERSPECTIVES FROM BOBO-DIOULASSO, BURKINA FASO.

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1 Background

The first cases of Cutaneous Leishmaniasis (CL) were reported in Ouagadougou, the capital city of Burkina Faso. This city is now known as an endemic focus for CL but in 2013 there was also an outbreak in Larama (southern Bobo-Dioulasso, the second largest city of the country). Despite the health problems caused by CL, the experiences of CL patients have not been explored in Burkina Faso. As part of a larger international multisite study examining patients' perceptions of treatments, we sought to understand the use of traditional treatments by CL patients in Burkina Faso.

2 Methods

A qualitative study using semi-structured in-depth interviews was conducted with patients with confirmed CL at Larama, Southern Bobo-Dioulasso in Burkina Faso. Participants were selected based on a purposive, maximum variation sampling approach along a defined patient spectrum covering e.g. *Leishmania* species, age and gender, and clinical presentation. All patients provided informed consent.
The interviews, conducted in the patients' mother tongue, followed a topic guide, developed as part of the international collaboration, and explored patients' disease experiences. NVivo® software was used to organise the data and we used thematic analysis to analyse the data.

3 Results

A total of 10 patients with age ranging from 24 and 73 years were enrolled into the study. The majority of the patients was men (60%) and had no formal education (80%). Overall 70% (7/10) had used traditional treatments, mostly at the onset of the disease before seeking medical treatment. Most of the patients thought that CL was not a medical disease. Traditional treatments used were made of local tree's bark or leaves, horse's bones, or wet soil used in the water storage and cooling process. Most of the patients' lesions worsened after having used the traditional treatment.

4 Conclusions

These findings highlight the common use of traditional treatment among CL patients from Larama. There is a need for further education on CL particularly in rural areas of Burkina Faso. These results will inform the design of appropriate interventions for this context, in combination with diagnosis and medical treatment.

C1700 USING PASSIVE SURVEILLANCE DATA TO INFORM CONTROL OF VISCERAL LEISHMANIASIS: 5-10 YEARS OF HOSPITAL-BASED RETROSPECTIVE DATA IN ENDEMIC AREAS F ETHIOPIA.

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1 Background

The Ethiopian visceral leishmaniasis (VL) control programme was established in 2006, following an unexpected VL outbreak in the highlands. Effective surveillance is key to understand VL disease dynamics, trends, early detection of outbreaks and design of prevention and control strategies. VL data are collected monthly through the health management information system (total number of cases disaggregated by sex and age is reported). However, in sites where World Health Organization (WHO) reporting formats were adopted, detailed VL data are recorded but so far these have remained at the health facility level. WHO, the KalaCORE project and the Ministry of Health, Neglected Tropical Diseases unit conducted retrospective collection of these data from the last 5-10 years.

2 Methods

Patient data were collected from individual VL cards in treatment sites in the 4 most endemic regions (Amhara,Tigray,SNNP and Oromia). A standardized format was used, data were entered into Epi-Info (v7.2). The data collected included 23 indicators into 5 blocks, 1) socio-demographic (origin, age group, gender, anthropometrics, migrant status),2) diagnosis, 3) treatment,4) treatment outcome and 5) co-morbidities. Descriptive analyses were conducted and are ongoing.

3 Results

Close to 10,000 VL patient records were collected and entered in database. The Ethiopian VL population is older (15-44 year) compared to neighbouring Sudan (mostly children affected) and 94% were males. Amongst females, over one-quarter were children under 15 years compared to 5% of males. Preliminary analysis suggests a correlation between time from symptoms to treatment and increased mortality due to VL. Data on nutritional status showed in high endemic areas, approximately 50% of VL patients were severely or moderately malnourished.

4 Conclusions

Retrospective data collection based on VL patient data provided valuable information that can contribute to shaping the Leishmaniasis Control Program. The predominance of VL in male migrants in Ethiopia warrants implementing of specific control measures. Reducing the time between symptoms and treatment is key to reducing mortality and improving treatment outcome. Patient data provide evidence for the need of nutritional therapy as part of the control program and female predominance in age group below 15 years deserves further investigation. Prospective collection of passive surveillance data has the potential to implement evidence-based decision making for VL control.

C1705 NEW TOOL FOR ASSESSMENT OF HEALTH FACILITIES READINESS TO PROVIDE VISCERAL LEISHMANIASIS TREATMENT IN AFRICA

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1 Background

The KalaCORE project is a Department for International Development (DFID)-funded initiative to eliminate and control visceral leishmaniasis (VL) in Bangladesh, India, Nepal, Ethiopia, South Sudan and Sudan. VL is a neglected disease which in Africa puts high demands on the health system due to the complex diagnosis and clinical management of patients. Readiness of health facilities to diagnose and treat VL is essential for the good prognosis of patients and a well-functioning control program.

2 Methods

A cross-sectional Excel-based tool (the Health Facility Assessment tool or HFA tool) was developed to collect information on the readiness of health facilities for the provision of VL services and to inform the National VL Control Programs where specific support was needed. The tool was based on previous recommendations issued by WHO. Health facility readiness was defined as *"a health facility where at least one member of medical and laboratory staff was trained in VL in the past year, appropriate diagnostic tests and first and second line anti-leishmania drugs were in place without stock ruptures in the last three months, VL guidelines and standard operating procedures were present, wards and laboratories meeting basic standards were in place and there was a functional cold chain (facilities providing AmBisome)."* The health facility assessment was conducted in 15 and 36 VL treatment facilities across Ethiopia and Sudan, respectively, during 2015.

3 Results

In Ethiopia and Sudan, the HFA tool identified that most health facilities were close to meeting the defined standards. However, health facility readiness could be further improved by on-site training of medical staff, strengthening of supply chains to ensure availability of diagnostics and drugs for treatment of VL without delays caused by stock rupture, minor refurbishment in the clinical wards of some of the hospitals, strengthening the cold chain, and ensuring all supplies and equipment is in place in laboratories for performing the full diagnostic tree for VL diagnosis (including quality control).

4 Conclusions

The KalaCORE project will provide targeted support to VL control programmes in Sudan and Ethiopia to improve health facilities' readiness for effective treatment of VL patients. A standard Excel-based assessment tool was helpful to determine the needs of the program and could be context-adapted for the use in other endemic countries.

C1708 BARRIERS TO ACCESS TO VISCERAL LEISHMANIASIS DIAGNOSIS AND CARE AMONG MIGRANT SEASONAL WORKERS/FARMERS IN WESTERN TIGRAY, NORTHERN ETHIOPIA

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1 Background

Ethiopia bears a high burden of visceral leishmaniasis (VL). While early access to VL diagnosis and care improves clinical prognosis and reduces transmission via human reservoirs, significant obstacles exist. The hundreds of thousands of mobile seasonal workers/farmers (MSW/F) visiting Amhara and Tigray region for the agricultural season may be particularly at risk of VL acquisition and death.

2 Methods

In early 2017, 50 in-depth interviews (IDI) and 11 focus group discussions (FGD) were conducted with current/previous visceral leishmaniasis patients, caretakers, healthy MSW/F, health staff and community leaders, recruited using purposive sampling, in Kafta Humera district, Tigray Region. A preliminary thematic content analysis explored barriers to access to diagnosis and care.

3 Results

While VL transmission was largely attributed to sand flies, participants also implicated mosquitos, termites, contaminated food, unclean water, dirt/lack of sanitation, increased temperatures, person-to-person transmission, evil, fatigue, hunger and disease evolution (malaria evolving into VL). Support of peers, family members, caretakers and farm owners strongly influenced care-seeking; MSW/F unable to receive salary advances, compensation for partial work, or help from peers for completion of contract work were particularly disadvantaged. Some participants used traditional medicine; most preferred care at modern health facilities, though multiple visits were consistently needed to access VL diagnosis. Lack of awareness/money, prioritization of farming activities (on the part of MSW/F or farm owners), inadequate training of health staff and unavailability of diagnostic tests were significant barriers to diagnosis and care. Participants suggested the government, in collaboration with other stakeholders, intervene to ensure MSW/F access to bed nets (especially), food, shelter, water, and health care at farms or sick leave. Additional recommendations included: community health education; training of health staff; availability of diagnostic materials at primary health facilities; surplus medications and health staff for the peak season; improved referral, feedback, and reporting within the health system; and free health care for all VL-related services.

4 Conclusions

Numerous opportunities to overcome barriers to access to diagnosis and care exist. Interventions tailored to the needs of MSW/F may help reduce health disparities and the burden of VL disease

C1712 DEVELOPING OUTBREAK MANAGEMENT GUIDELINES FOR THE VL POST-ELIMINATION AGENDA IN THE INDIAN SUBCONTINENT

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1 Background

Nepal has already achieved the target of elimination of VL as a public health problem and it is probable that Bangladesh will follow by 2017. However, since KA is a re-emerging disease with history of cyclic trends, it is important that the national programmes of these countries are ready to identify and respond to early warning signs of any re-emergence or outbreaks, so that further transmission of disease can be prevented. KalaCORE has supported local governments to develop KA outbreak management guidelines (OMG) that aim to identify and control the outbreaks with the intention of prevent additional cases of the disease to sustain the elimination goals.

2 Methods

OMG outlines the process based on logical series of steps to identify risk factors and establish reactive control measures in case of an outbreak. OMG provides an operational definition to identify outbreaks, and describes how to identify early warning signs using passive surveillance data. The process identifies two multi-disciplinary Kala-azar outbreak management teams (OMT) to be constituted at the time of outbreak - one at the central level, and another at the local (district) level. OMG defines roles and responsibilities of members of OMT, and provides formats and tools, which are to be used by OMT during a response. Finally, OMG outlines the response measures that are to be taken both on a short and long term basis, and how to evaluate the outbreak response for future corrective action.

3 Results

The guideline defined KA outbreak as an episode in which two or more people, thought to have a common exposure, experience a similar illness or proven infection. Outbreak management teams were formed at the central and at the district levels, comprising representation from the government, kala-azar response programme, development partners, microbiologists, public health specialist, epidemiologists, and other stakeholders. The interface between the team and existing the Kala-azar surveillance mechanisms to suspect/detect Kala-azar outbreak was designed, with specific roles and responsibilities allocated for planning and coordination of the outbreak investigation and response.

4 Conclusions

By the time of WL6, we expect to have a number of operational examples of outbreak detection and response to present, with an evaluation of how well the guideline performed.

C1715 IMPACT OF INFORMATION, EDUCATION, COMMUNICATION AND BEHAVIOURAL CHANGE COMMUNICATION ON KNOWLEDGE, ATTITUDES AND PRACTICES FOR VISCERAL LEISHMANIASIS IN INDIA

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1 Background

Visceral Leishmaniasis (VL) is endemic to 54 districts in four states of India, and poor awareness of the disease with inappropriate health seeking behavior is considered to be one of the major challenges facing its elimination as a public health problem. Over the course of 12 months, the KalaCORE implemented an intensive behavioral change communication (BCC) programme for preventative health education and social mobilization focusing on knowledge, attitudes and practices (KAP) related to VL and the importance of Indoor Residual Spraying (IRS). About half of all 11000 endemic villages covering 10 million (directly 2 million) population were covered, and an impact assessment was conducted to quantify the success of the strategy.

2 Methods

Immediately after the 2nd final IRS rounds, VL endemic villages from 14 highly VL endemic districts in Bihar (n=10) and Jharkhand (n=4) were randomly assigned into: villages with BCC activities (intervention villages, N=700 houses) and villages without BCC activities (control villages, N=350 houses). Quantitative analysis was carried out to estimate rate of IRS refusal and qualitative analysis to measure changes in KAPs.

3 Results

IRS refusal rates were lower at 8% in the interventional villages compared to 24% in the control group (OR=0.27, $p<0.0001$). Stratified by occupation, caste, religion and socio-economic status, refusal rates were higher in families involved with agriculture (OR 1.9, $p<0.01$) and labour ($p<0.01$), those with below poverty line status (OR 1.1, $p<0.01$), and lower in those following the Christian religion (OR 0.35, $p<0.01$), and households identifying with scheduled caste (OR 0.92, $p<0.05$). On 19 qualitative outcome indicators related to KAP, intervention village communities were found to be more aware of spread of VL by sandflies (68% vs 7 % $p<0.01$), informed of IRS as effective control measures (82% vs 42% $p<0.01$) and go to Primary Health Centre (PHC) for treatment (84% vs 35% $p<0.01$), motivated to help others for IRS (79% vs 44%%, $p<0.01$), and sought treatment at PHC (77% vs 39 %, $p<0.01$) for prevention of VL and adopting positive treatment seeking behavior compared to control village communities.

4 Conclusions

The findings suggest that community-based health communication resulted in reducing IRS refusal, and in improving knowledge attitudes and practices in relation to VL. Community participation in the elimination effort should a focus as an important component of the elimination strategy.

C1718 FIELD EVALUATION OF RAPID DIAGNOSTIC TESTS FOR VISCERAL LEISHMANIASIS DIAGNOSIS IN SOUTH SUDAN

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1 Background

South Sudan, where visceral leishmaniasis (VL) has a characteristic epidemic pattern, presents the highest burden of VL in East Africa, and 30% of its population is considered to be at risk. The situation in last years has been characterized by civil unrest and population displacement, with difficulties in access to appropriate diagnosis. There was no proper studies done to inform policy decision on the performance of the various RDTs for VL diagnosis. This situation prompted South Sudan Ministry of Health and WHO to conduct an evaluation at the health centre level, with the aim to provide implementing organizations involved in the management and control of VL in the country with supporting information to choose the best field-adapted tool.

2 Methods

A field evaluation of two rK39-based (Kalazar Detect, Inbios; IT-Leish, Bio-Rad) and one rK28-based (*Leishmania* Ab Rapid Test, CTK Biotech) VL RDTs, in comparison with the direct agglutination test (DAT, Institute of Tropical Medicine, Antwerp) was conducted in two health centres in Old Fangak (Jonglei State) and Koch (Unity State) between November 2012 and March 2013, using peripheral blood samples. An additional analysis was conducted with plasma samples from the same participants at the WHO Collaborating Centre for Leishmaniasis, Instituto de Salud Carlos III, Spain, where IFAT was also employed.

3 Results

One hundred and thirty five VL suspects (fever for two weeks or more plus splenomegaly and/or weight loss, and malaria ruled out), 7 febrile and 7 malaria patients were included in the study, together with 117 healthy endemic controls. The RDTs returned the following sensitivity/specificity (%) in peripheral blood: Kalazar Detect 66/96, IT-Leish 71/95, Leishmania Ab Rapid Test 66/94; and in plasma: 67/93, 78/87 and 78/82 respectively for the RDTs, and 76/90 for IFAT.

4 Conclusions

All RDTs presented higher sensitivity in plasma than in peripheral blood, though this might not be the best sample for testing in peripheral health facilities. Among them, IT-Leish showed higher sensitivity using either peripheral blood or plasma samples, so this should be used as a first diagnostic approach in South Sudan, coupled with a strict VL clinical case definition. It is well known that rK39-based RDTs have a limited performance in East Africa, thus rK28-based RDTs have been suggested as an alternative. However, we did not observe that the rK28-based test used in this study could circumvent the limitations that rK39-based test have in this region.

C1720 DECENTRALISING AMBISOME TREATMENT FOR VL ACROSS ENDEMIC AREAS IN INDIA, A NOVEL NODAL APPROACH

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1 Background

The Government of India aims to eliminate Kala Azar (KA) as a public health problem by the end of 2017. A key component of this has been the introduction of single dose liposomal amphotericin B (SDA) as first line treatment. This presented a number of logistical and programmatic challenges for what is a relatively complex treatment requiring storage <25°C to ensure adequate, effective and safe coverage. KalaCORE (KC) supported the implementation of this approach through planning, design, upgrading and training across the four endemic states of India over an 18-month period.

2 Methods

KC initiated a comprehensive assessment and planning exercise with local programme officers and district health officials. This exercise considered the pre-existing burden of disease, decreasing incidence and existing health seeking behaviour patterns to identify the most appropriate primary and secondary level facilities to introduce SDA treatment. The list of sites were selected considering: 1) distance between adjoining facilities, 2) patient load at each facility, and 3) feasibility to accommodate Ice Lined Refrigerators. KC then installed ILRs and provided training to health care providers within selected centres.

3 Results

A total of 85 ILRs were installed at the public health centres, 56 at the primary and 29 at the secondary and tertiary health care level. A strategic decision to move away from the previous model of 'treatment at every centre' towards a system of 'nodal' referral sites was made. This was based primarily on a needs-based safe and sustainable decentralised approach to treatment, where with many primary health care facilities seeing one or less cases monthly, consolidation of care at nodal facilities within reasonable travel distance. Diagnostic capacity was ensured at all facilities, including non-SDA enabled PHCs. As of October 2016, 65% of all patients have treated at district hospital, whereas 35% patients treated at primary health care centres.

4 Conclusions

With the continuous reduction in KA cases across the endemic states in India, a rational need based decentralised system of treatment is key to sustainable health system strengthening. India has achieve good coverage through SDA however there remains a continued need to address issues such as delay in treatment seeking behaviour and supply chain weaknesses.

C1758 STUDY PROTOCOL ON HAND HYGIENE, CUTANEOUS LEISHMANIASIS AND LATENT TUBERCOLOSIS CO-INFECTIONS IN HEALTH CARE FACILITIES IN AFGHANISTAN

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1 Background

Afghanistan is one of 22 countries with one of the highest tubercolosis (TB) disease burden. Chronic wounds, as cutaneous leishmaniasis (CL), ulcers have a high susceptibility for bacterial infections, including latent TB. Prescription of antibiotics against latent TB is common during CL treatment, although drug quality is not guaranteed, patients' are often non-compliant, and bacterial resistances against available antibiotics are of growing concern. Proper hand washing and basic hygiene have proven efficient for infection control in health care facilities worldwide. The present abstract summarizes a study protocol to evaluate the prevalence of latent TB infections, the pharmacoepidemiology, and the hygiene standards and activities in CL patients treated in primary health care facilities affiliated to the National Malaria and Control Program (NMLCP) of the Ministry of Public Health (MoPH) in Kabul.

2 Methods

CL health facilities will be randomly selected out of a total of 850 health care facilities in 10 provinces. Data will be collected with electronic case report forms on smartphones.

3 Results

--to be finalized --- run out of time.

4 Conclusions

--to be finalized--- run out of time.

C1800 WHY DO PEOPLE DELAY IN SEEKING CARE FOR VISCERAL LEISHMANIASIS IN SOUTHERN GEDAREF, SUDAN? A QUALITATIVE STUDY

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1 Background

Gedaref state in eastern Sudan is where 80% of the visceral leishmaniasis (VL) burden in Sudan is reported from. Control of VL here mainly relies on provision of diagnosis and treatment services. However, many people may fail or delay in accessing VL care, as reflected in a relatively long duration between the onset of symptoms and the moment patient seeks treatment. VL is potentially fatal, thus reducing these delays is critical to save lives and the success of the control program. This study sets out to look into community and their health worker(s) perspective of VL and access to care, taking into account the various facets of health seeking behavior and its influencing factors (cultural, socio-economical, etc). This way we aim to voice the community perception on VL access barriers, eventually help to improve the timely use of available care through useful recommendations.

2 Methods

A qualitative study design is chosen to answer the research questions. The setting will be selected villages located along Rahad and Atbara river basin, including those with known access problems. Data is collected through focus group discussions (FGDs) and in-depth interviews (IDI), in the villages and health facilities. Participants will include adults (>18 year old) and adolescents (14-17), selected using non-probability purposive sampling. Key informants include former VL patients, caretakers/families, community members, leaders and health staff. Different categories (age, gender, ethnicity) will be included in the study to ensure maximum variation. The field teams will receive trainings on qualitative research methods and tools are piloted. Data collection will continue until a saturation point is reached and is guided by content analysis.

3 Results

Results are expected by mid-2017. Text data will be analyzed using NVivo. Main research outcomes, in the forms of codes/sub-codes, are expected to include themes of 1) perception of illness and knowledge of VL; 2) Beliefs and preferences for VL care; and 3) Accessibility and barriers perceived and/or experienced to access VL.

4 Conclusions

We expect preliminary results to be available by March 2017. Our data may provide useful insights and better understanding of VL access barriers in this context, therefore further intervention(s) could be adjusted wherever feasible. The qualitative nature of the study provide a rare view from the community lens on this deadly disease.

C2056 NUTRITIONAL SUPPLEMENTS FOR PATIENTS BEING TREATED FOR ACTIVE VISCERAL LEISHMANIASIS: A COCHRANE SYSTEMATIC REVIEW.

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1 Background

Visceral leishmaniasis (VL) increases morbidity, mortality, and healthcare costs. Malnutrition may hasten the progression of the leishmaniasis infection and VL may worsen malnutrition. There is no evidence-based guidance on oral nutritional supplements for patients being treated for VL. We assessed the effects of oral nutritional supplements in people being treated with anti-leishmanial drug therapy for VL.

2 Methods

Cochrane systematic review performed with *Covidence* and *RevMan 5* software. Two review authors independently decided on eligibility of studies. We planned to assess risk of bias in the eligible studies identified (Cochrane EPOC Group criteria). Selection criteria: randomized controlled trials (RCTs), quasi-randomized controlled trials or non-randomized controlled trials including patients of any age being treated for visceral leishmaniasis (VL). Intervention: any oral nutritional supplement given in addition to any anti-leishmanial drug treatment. Tube feeding or parenteral nutrition were excluded. Control: no nutritional intervention, placebo, or dietary advice alone. Primary outcome: primary cure (clinical and parasitological improvement after treatment). Search methods: we searched electronic databases regardless of language or publication status (up to 20 July 2016): Cochrane Infectious Diseases Group Specialized Register; Cochrane Central Register of Controlled Trials; MEDLINE; EMBASE; LILACS; WHO International Clinical Trials Registry Platform; and ClinicalTrials.gov. We also searched conference proceedings, WHO consultative meetings reports, reference lists and contacted researchers in the field.

3 Results

The search strategy generated 181 unique records. None of them was eligible for this review.

4 Conclusions

No study met the review criteria. This absence of evidence does not imply the evidence of no effect for oral nutritional supplements. RCTs are needed to determine the effects of oral nutritional supplements in patients with VL.

Reservoirs

C0257 THE INVOLVEMENT OF THE TWO SPECIES OF PSAMMOMYS SPP (OBESUS AND VEXILLARIS) IN THE LEISHMANIASIS EPIDEMIOLOGY IN TUNISIA: FIRST DETECTION OF LEISHMANIA DNA IN PSAMMOMYS VEXILLARIS RODENT

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1 Background

In Tunisia, three clinico-epidemiological forms of cutaneous leishmaniasis (CL) are present: the zoonotic form CL (ZCL) found in the center, whose only causative agent described to date is *Leishmania* (*L.*) *major*, the chronic CL (CCL) caused by *L. killicki* in the south-east, and the sporadic CL (SCL) due to *L. infantum* which prevails in the north, with an extension to the center of the country. While the reservoirs for the ZCL disease are rodents of the *Psammomy (P.)* and *Meriones* genera, those for CCL and SCL remain unknown. *Psammomys* spp. has long been represented by only *P. obesus* specie, until we established the existence of a second specie *P. vexillaris*. The question then arises of the role of each specie in leishmaniasis epidemiology. The aim of this research was to explore the potential role of *P. vexillaris* as host, and to compare it to that of *P. obesus*.

2 Methods

In the present work, clinical manifestations of *Leishmania* infection were assessed by skin examination on 43 *P. obesus* captured in the center and among the 29 *P. vexillaris* caught in the south-west of Tunisia. Total genomic DNA was extracted from liver biopsies obtained from every animal and used in molecular analysis. To determine the presence of the parasite, real time polymerase chain reaction (RT-PCR) targeting kinetoplast (k) minicircles DNA was performed, and the ribosomal internal transcribed spacer 1 (ITS1) region was next amplified and followed by restriction fragment length polymorphism (RFLP) to identify the *Leishmania* species involved. Furthermore, direct sequencing of ITS1 amplified products was performed.

3 Results

The RT-PCR allows the detection of *Leishmania* spp. DNA in 41,86% of the *P.obesus* specimens and in 27,58% of the *P. vexillaris* individuals. Species identification showed that the parasitized *P. obesus* were infected by *L. infantum*, whereas *L. tropica* and *L. infantum* were identified among infected *P. vexillaris*.

4 Conclusions

This is the first study that demonstrates the presence of *L. infantum* in the deep organs of *P. obesus* captured in the center; our results suggest that this mammal could play a role in the epidemiology of SCL and its expansion in this region. Likewise, this work establish for the first time in Tunisia, the natural infection of *P. vexillaris* by *Leishmania* parasite suggesting a potential epidemiological role as reservoir host for this rodent. Establishing the place and role of each of these twin species is mandatory to create an effective control strategy.

C0377 MOLECULAR DETECTION OF LEISHMANIA INFANTUM AND LEISHMANIA MAJOR IN TURKISH WILD RODENTS

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1 Background

In Turkey, *Leishmania (L.) donovani, L. infantum, L. tropica* and *L. major* play role on visceral leishmaniasis (VL), zoonotic and cutaneous leishmaniasis (CL) and canine leishmaniasis (CanL). *Leishmania major* were recently identified as causative agent for ZCL by molecular methods using clinical samples of patients. So far, no study was performed to screen the *Leishmania* sp. infection in wild rodents in Turkey. In the present study, the role of rodents in the *Leishmania* infection cycle as reservoir animals was investigated

2 Methods

Tissue samples (spleen, liver, and lung) obtained from 384 rodents in 29 provinces were screened for the presence of *Leishmania* spp. by molecular methods. Tissue samples had been preserved in formalin and before the DNA extraction, all tissues were first dried using Eppendorf Concentrator, then they were rehydrated using Qiagen Tissue Lysis buffer and Proteinase K. Tissue samples were later transferred to Zymo Bashing bead and homogenated using Magna Lyzer. DNA was extracted from the homogenate using QIAamp DNA Mini Kit according to the manufacturer's instructions. *Leishmania* detection was carried out by kDNA real time PCR using the primers JW11/JW12, which targeting the fragments of about 166 bp. Species identification was done for positive samples using ITS-1 RT-PCR protocol with primers LITSR/ITS1R-TR1. Melting curve analysis was conducted to determine the species of *Leishmania* positive samples.

3 Results

Of the studied 384 tissue samples, six of them were found to be positive for *Leishmania* sp. DNA by kDNA PCR. The species typing revealed five *L. infantum* and one *L. major* among the positive samples. *Leishmania major* DNA was detected in *Apodemus* sp. and *Leishmania infantum* DNA was detected in three different animals, *Apodemus* sp., *Rhinolophus Euryale* and *Apodemus mystacinus*. All positive samples were collected from Zonguldak province located in northwestern part of Black Sea Region. *Leishmania infantum* positive rodents were all male while *L. major* positive was female.

4 Conclusions

The presence of *Leishmania* spp. DNA in Turkish wild rodents was firstly reported in the present study. Findings of the present study highlight the possible role of wild rodents for ZCL in Turkey. Further studies needed for investigating the role of wild rodents in the transmission of *Leishmania* sp.

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C0426 VISCERAL LEISHMANIASIS IN THE ARGENTINIAN NORTHWEST

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1 Background

In Argentina, as well as in the rest of the American continent, the causal agent for Visceral Leishmaniasis (VL) is *Leishmania infantum*, the main vector species involved in its transmission is *Lutzomyia longipalpis* and its main reservoir is the domestic dog (*Canis familiaris*). The first confirmed autochthonous human case of VL in Argentina was reported in 2006 in the city of Posadas (Misiones) in the northeast of the country, with the presence of *Lu. longipalpis* and canine VL cases. In the northwest of Argentina, *Lu. longipalpis* was registered for the first time in 2013, in the locality of Tartagal (Salta). The aim of this study was to complement previous studies performed in Tartagal with respect to the situation of VL in the argentinian northwest.

2 Methods

A random sampling of canines was performed in June 2016 in different neighborhoods of Tartagal in order to analyze the presence of the parasite in the main reservoir for this disease. Serum samples from 94 dogs, either with or without compatible symptomatology, were collected together with samples from popliteal lymph nodes were possible. Serum was analyzed using rK39 immunochromatography (InBios Kalazar Detect rapid test) and immunofluorescence (IFI) while lymph nodes were analyzed through PCR.

3 Results

With the rK39 technique 2 positive samples for VL where detected, one of the samples was also positive using IFI with a titer of 1/1280, the lymph nodes of this sample were also positive through PCR. This sample was molecularly characterized as belonging to the species *Leishmania infantum*. The remaining samples analyzed through IFI and PCR were negative for *Leishmania*.

4 Conclusions

These results confirm the presence of the parasite that causes VL in canine reservoirs from Tartagal, added to the previous presence of the vector *Lu. longipalpis* in 2013, these results raise an epidemiological alert for this region of the country. It is important and necessary to continue realizing complementary studies to determine the prevalencia of the visceral leishmaniasis in the canine population of this locality and to analyze environmental variables that might be playing an important role in the epidemiology of this disease in Argentina.

C0428 DETECTION OF LEISHMANIA INFECTION IN STRAY DOGS FROM A VISCERAL LEISHMANIASIS-ENDEMIC AREA OF BANGLADESH

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1 Background

Visceral leishmaniasis (VL) or kala-azar is mainly caused by two closely related *Leishmania* species, *Leishmania infantum* and *Leishmania donovani*. *L. infantum* is responsible for zoonotic VL, with dogs as the main reservoir hosts in the Mediterranean, the Middle East, Asia, and South America. In the Indian subcontinent, VL is caused by *L. donovani* and is considered anthroponotic, although the only known vector sand fly species is zoophilic in nature. The role of domestic and stray dogs in VL transmission is still unclear in this area. In this study, we investigated the stray dogs from a VL-endemic area of Bangladesh to support the hypothesis that dogs are an animal reservoir for *Leishmania* parasites in this area.

2 Methods

We screened 50 stray dogs from Mymensingh district, one of the most VL endemic areas of Bangladesh. Dog sera were used to perform rK39 immunochromatographic test (ICT). Internal transcribed spacer 1-PCR (ITS1-PCR) and real-time PCR were performed with the DNA extracted from the buffy coat. Sequencing analysis of the PCR products was conducted to identify the *Leishmania* species.

3 Results

Anti-*Leishmania* antibodies were detected in six (12%) dog serum samples using rK39 ICT. *Leishmania* kinetoplast DNA was detected in ten (20%) buffy coat DNA samples by real-time PCR, five of which were also positive by ITS1-PCR. A sequencing analysis of the amplified products confirmed that the parasitic DNA was derived from *L. donovani*.

4 Conclusions

Our results showed serological and molecular evidence of *Leishmania* infection in stray dogs, strengthening the assumption that dogs are an animal reservoir of *Leishmania* parasites in Bangladesh. However, further analyses on the transmission potential of the parasites between dogs and vector sand flies in nature are needed to reveal the role of dogs in VL epidemiology in Bangladesh.

C0442 SCREENING AND MOLECULAR IDENTIFICATION OF LEISHMANIA SPP. IN VERTEBRATES COLLECTED FROM DINDER NATIONAL PARK (SUDAN)

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1 Background

Sudan is one of the endemic areas of visceral leishmaniasis (VL) in the world. The disease represents a major health problem with outbreaks occurring periodically claiming the life of high number of victims. The disease is caused by many strains/species of Leishmania parasite which is harbored by many species of vertebrate reservoir hosts. The objectives of this study were to investigate the reservoir hosts of Leishamania spp among different vertebrates from two areas: a VL endemic area (DNP) and a non-endemic area (Khartoum State); and to identify and characterize Leishmania spp if present.

2 Methods

Thin blood films from 119 samples from five possible vertebrate hosts (bats, birds, Egyptian mongoose, reed buck and rodents) were microscopically examined and screened for the presence of*Leishmania* parasites. Genomic DNA was extracted from different tissue parts from the same sample. For the molecular detection of the parasite; three genes were successfully amplified: *Leishmania*minicircle kDNA, small subunit - ribosomal DNA (SSU-rDNA), and the internal transcribed spacer (ITS1).

3 Results

Leishmania amastigotes were microscopically observed in 14.3% (3 of 21) of the bats collected from DNP. *Leishmania* specific genomic targets DNA was amplified in 17.6% of the sample (21 of 119) belonging to five vertebrate species: the Egyptian mongoose*Herpestes ichneumon*, the Spiny mice *Acomys albegina*, the Nile rat*Arvicanthis nilotucs*, the Reed Buck *Redunca arundinum*, birds including (the African jacana *Actophiloms africanus*, Pied kingfisher*Cerylr rudis*, greater painted snipe *Rostratula benghalensis)* and two bats: *Glauconycteris variegata* and *Scotophilus nigrita*.
Z study finding of bats, birds and reed buck being infected with*Leishmania spp*. is the first confirmed report in Sudan. More work is needed for further identification of *Leishmania* detectedAll the positive samples were collected from DNP.

4 Conclusions

The study finding of bats, birds and reed buck being infected with*Leishmania spp*. is the first confirmed report in Sudan. More work is needed for further identification of *Leishmania* detected

C0697 IDENTIFICATION OF POTENTIAL VECTORS AND RESERVOIRS OF CUTANEOUS LEISHMANIASIS IN ENDEMIC AREAS OF PARAGUAY

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1 Background

Cutaneous leishmaniasis is endemic in several areas of Paraguay and present a wide variety of possible vectors and reservoirs that have not been identified yet. The main goal of the current study was to identify potential vectors and reservoirs of cutaneous leishmaniasis in Paraguay.

2 Methods

Sandflies and small mammals were captured in six districts of *San Pedro* and *Canindeyú* departments, where cutaneous leishmaniasis had occurred recently. Specific primers for the detection of *Leishmania* genus and complexes were used in a PCR technique. In addition, phlebotomine pool samples were tested to detect human blood.

3 Results

The following species of sandflies were identified: *Nyssomyia whitmani*, *Migonemyia migonei, Evandromyia walker*i, *Pintomyia pessoai, Brumptomyia brumpti, Nyssomyia neivai* and *Pintomyia monticola*. The small wild mammals captured were classified as belongin to the genus: *Calomys, Oligoryzomys, Oryzomys, Akodon, Rattus, Mus, Didelphis, Nectomys, Holochilus, Myocastor* and *Cavia*. Ten pools of *Lu. whitmani* and ten pools of *Lu. migonei*, nine specimens of *Oryzomys buccinatus*, seven of *Calomys callosus*, three of *Olygorizomys flavescens*, three of *Akodon montensis*, one of *Didelphis albiventris* and one of *Nectomys squamipes* were found naturally infected by *Leishmania braziliensis* complex. Human blood was identified in three pools of *Lu. whitmani*, captured in *Canindeyú* department.

4 Conclusions

These results, based in molecular tools, have shown the usefulness of the PCR technique for epidemiological studies and suggest that *Lu. whitmani* and *Lu. migonei* are potential vectors for *Leishmania braziliensis* complex. Additionally, *Calomys callosus*, *Olygorizomys flavescens, Oryzomys buccinatus, Akkodon montensis, Didelphis albiventris* and *Nectomys squamipes* are suggested as potential *Leishmania braziliensis* complex reservoirs in the areas of study in Paraguay.

C0701 CANINE VISCERAL LEISHMANIASIS IN WILD CANINES (FOX, JACKAL, AND WOLF) IN NORTHEASTERN IRAN USING PARASITOLOGICAL, SEROLOGICAL, AND MOLECULAR METHODS

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1 Background

Although many studies had been conducted on various aspects of canine visceral leishmaniasis (CVL) in domestic dogs in the endemic areas of Iran, investigations on CVL in wild canines are rare.

2 Methods

This is a cross-sectional study was conducted from December 2012 to 2013 in northeast of Iran where human VL is endemic. Wild canines were trapped around the areas where human VL cases had been previously identified. Wild canines were collected and examined both clinically and serologically using direct agglutination test (DAT). Microscopically examinations were performed in all the seropositive wild canines for the presence of the amastigote form of Leishmania spp. Some *Leishmania* sp. which had been isolated from the spleens of wild canines, were examined analyzed by conventional PCR and sequencing techniques using α-tubulin and GAPDH genes.

3 Results

Altogether, 84 wild canines including foxes (Vulpes vulpes, n=21), Jackals (Canis aureus, n=60) and wolves (Canis lupus, n=3) were collected. Four foxes and seven jackals showed anti-Leishmania infantum antibodies with titers of 1:320–1:20480 in DAT. Furthermore, one fox and one jackal were parasitologically (microscopy and culture) positive and L. infantum was confirmed by sequence analysis

4 Conclusions

The present study showed that sylvatic cycle of L. infantum had been established in the studied endemic areas of VL in northeastern Iran.

C0721 ECOLOGICAL NICHE MODELING OF MAIN RESERVOIR HOSTS OF ZOONOTIC CUTANEOUS LEISHMANIASIS IN IRAN

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1 Background

Zoonotic cutaneous leishmaniasis (ZCL) caused by *Leishmania major* is a common zoonotic vector-borne disease in Iran. It is transmitted to humans when they are in close contact with infected animal reservoir hosts, because of agricultural activities or building and residence near active colonies of rodents. Gerbils (Rodentia: Gerbillidae) are the main reservoir hosts for ZCL and four species of the desert rodents are proved as the reservoir hosts of the disease in different endemic foci of Iran , i.e. *Rhombomys opimus*, *Meriones libycus*, *Meriones hurrianae* and *Tatera indica*. The purpose of this study is to introduce distribution model of the fourth rodent species as the main reservoir hosts of ZCL in Iran to find at risk areas for the disease.
The ecological niche model of gerbils could be a useful tool for understanding of the ecoepidemiological aspects of leishmaniases.

2 Methods

A data bank was developed including all published thesis, papers, documents and abstracts on reservoirs of ZCL from 1970 to 2015. Maximum entropy method (MaxEnt) was used to find the most appropriate ecological niches for each rodent species.
The maximum possible test AUC was 0.931, 0.846, 0.878, 0.986 and 0.841 rather than 1 for *R. opimus*, *M. libycus*, *T. indica*, *M. hurrianae*, respectively.

3 Results

A total of 59 published papers and 60 MSc/PhD theses were used in the first national database for reservoirs of cutaneous leishmaniasis (CL) in Iran. Based on the results of those studies we found five species are repeatedly reported to be infected with *Leishmania major* in different endemic foci of CL, including *Rhombomys opimus*, *Meriones libycus*, *Tatera indica*, *Meriones hurrianae* and *Nesokia indica*.
Summation of presence probabilities for three main species, i.e. *R. opimus*, *M. libycus* and *T. indica* showed wide areas from 16 provinces of Iran have the favorite ecological niches for them, while 3 provinces have supportive niches for these species.

4 Conclusions

This is the first prediction on distribution of ZCL reservoir hosts in Iran. We found that climatology and topography variables such as seasonal precipitation, annual mean temperature, and slope had high contributions toward the prediction of potential distribution of three main reservoir species . As climate change will affect on climatology variables, the models should be updated periodically with new data and the results should be used in disease monitoring program.

C0747 ACOMYS DIMIDIATUS (RODENTIA: MURIDAE): PROBABLE RESERVOIR HOST OF LEISHMANIA MAJOR, SOUTHERN IRAN

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1 Background

Rodents are the main reservoir hosts of Zoonotic Cutaneous Leishmaniases (ZCL) in different parts of Iran. Nonetheless, no evidence of natural infestation of *Leishmania* parasite has been reported from spiny mice *Acomys dimidiatus* (Rodentia: Muridae).

2 Methods

Between March 2011 and March 2012, 62 wild rodents were captured and checked for leishmanial infestation of their livers and spleens using Polymerase Chain Reaction (PCR) in fourteen regions of Darab district in Fars province, southern Iran.

3 Results

Overall, 62 rodents consisting of 42 *Tatera indica*, 6 *Meriones libycus*, 5 *Me. persicus*, 5 *Mus musculus*, and 4 *Acomys dimidiatus*were identified. Infestation to *Leishmania major* was confirmed in all the captured species of rodents: *Ta. indica* (23.8%), *Me. libycus* (50%), *Ac. dimidiatus*(50%), *Me. persicus* (20%), and *Mu. musculus* (20%).

4 Conclusions

Ta. indica was the reservoir host of *L. major* in this focus of ZCL. Moreover, *Me. libycus and Me. persicus* had a remarkable role in the maintenance of the parasite. *Ac. dimidiatus* was also introduced as a new probable reservoir host of *L. major* for the first time. Yet, the role of this species in the maintenance, transmission, and epidemiological cycle of ZCL needs more specific researches.

C0764 LEISHMANIA INFANTUM INFECTION IN DOGS FROM AN AREA WITHOUT HUMAN CASES OF A BRAZILIAN ENDEMIC LOCALITY

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1 Background

Visceral leishmaniasis (VL) in Brazil is a severe disease caused by the intracellular protozoon *Leishmania (Leishmania) infantum*. Due to the frank process of expansion and urbanization, VL has great relevance in local public health, especially in the municipality of Rondonópolis, located in the state of Mato Grosso, which represents an endemic area with intense transmission. In this region, VL is a zoonosis and the domestic dog is the main animal reservoir, being responsible for the maintenance of the disease among humans. Thus, the determination of the prevalence of canine visceral leishmaniasis (CVL) and the associated risk factors are fundamental for guiding the adopted control measures. However, most of these efforts are focused on areas with large numbers of human cases. In this way, current study aimed to determine the seroprevalence of CVL and associated risk factors in an urban area with no human cases of the municipality of Rondonópolis.

2 Methods

A cross-sectional study was conducted in 2016 in domestic dogs from four contiguous urban neighborhoods (Jardim Altântico, Jardim Europa, Residencial Três Américas and Jardim Paraíso). Serology was carried out by using a rapid immunochromatographic test (Dual-Path Platform - DPP) for screening and enzyme-linked immunosorbent assay for the confirmation of positivity. The owners of the dogs were also interviewed based on a semi-structured questionnaire addressing socioeconomic aspects, environmental characteristics of the home, knowledge about leishmaniasis, behavior of the animal and care given by the owner.

3 Results

Blood samples from 77 domestic dogs were collected randomly and the prevalence of CVL was 14.29% (95% confidence interval, 7.35-24.13) (n=11). With respect to the associated risk factors, 50 dog's owners were interviewed, but no evaluated variable showed statistical significance.

4 Conclusions

These results indicated a high prevalence of CVL in an area without human cases of VL, which reinforces the permanent need for control measures, since canine cases usually precede human cases.

C0798 INVESTIGATION OF THE PRESENCE OF CATS INFECTED BY LEISHMANIA INFANTUM AND ITS IMPORTANCE IN CYCLE TRANSMISSION OF VISCERAL LEISHMANIASIS IN MINAS GERAIS

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1 Background

Leishmaniasis are diseases caused by flagellate protozoa of the genus *Leishmania* and presents great diversity in clinical manifestations. Canine visceral leishmaniasis (CVL) is caused by *Leishmania infantum* in Brazil and it is characterized as severe and chronic infectious zoonotic disease. Dogs have been considered the main reservoir, however, the role of the cat in the transmission cycle has been discussed. This work aimed to evaluate the presence of infected cats and sand flies in an enzootic area for CVL, and the ability of felines to infect their biological vector, *Lutzomyia longipalpis*.

2 Methods

Nine cats residents in São Joaquim de Bicas, Minas Gerais, were studied.

3 Results

The parasitological evaluation demonstrated the presence of amastigote forms in 5/9 (56%) of the bone marrow samples by cytological examination. Besides, *q*PCR increased sensitivity by identifying one more cat infected with *L. infantum* (66%). However, none of the samples were positive in culture with NNN medium. Eight animals were submitted to xenodiagnosis and no infection of any *L. longipalpis* that realized the bloodmeal in these animals was detected. Animals were tested for infection by Feline Immunodeficiency virus (FIV) and Feline Leukemia virus (FeLV) and one animal was positive for FeLV being also infected by *L. infantum*.

4 Conclusions

This work is pioneer in performing xenodiagnosis in cats resident in an endemic area, as well in evaluating aspects of the transmission in the environment in which these animals are inserted. Further studies to identify the real role of cats in VL epidemiology in Brazil are required.

C0929 THE HEDGEHOG ERINACEUS EUROPAEUS AS A POSSIBLE RESERVOIR OF LEISHMANIA INFANTUM IN A MEDITERRANEAN AREA

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2 Background

In the European countries of the Mediterranean basin, leishmaniasis is mainly caused by *Leishmania infantum*, with dogs as the principal reservoir hosts. Although several studies have indicated that other mammals may also act as reservoirs, little information is available on insectivores, although hedgehog have been incriminate as *L. major* natural reservoirs. The aim of this work was to study the possible role of hedgehogs as reservoir hosts of *L. infantum* parasites in endemic European Mediterranean areas.

3 Methods

A total of 64 *Erinaceus europaeus* were collected from different endemic areas of Catalonia, Spain. Samples of spleen, skin (ear) and blood extracted from the heart were collected. Molecular (real time qPCR) and serological (Western blot and in-house ELISA) methods were used to determine the presence of *Leishmania* DNA and specific anti-*Leishmania* antibodies.

4 Results

Of the 64 *E. europaeus* studied, *Leishmania* DNA was detected in 22 specimens (34.4%, 95%CI 23.9-46.6%), in 19/51 spleens (37.3%) and in 5/50 skins (12.5%). In the 28 individuals in which the two tissues (spleen and skin) were studied, 2 specimens gave positive results in both samples. The serology was positive in 12 specimens (40%, 95%CI 24.6-57.7%), 11/30 (36.7%) by Western blot and 6/30 (20%) by ELISA. Four of 12 seropositive individuals were positive by qPCR.

5 Conclusions

The number of seropositive individuals, together with the detection of *Leishmania* DNA in the skin and the spleen suggest that the hedgehog *E. europeus* may play an epidemiological role as a natural reservoir in the studied leishmaniasis endemic area. Nevertheless, the implication of the hedgehogs in the life cycle of *L. infantum* must be deeply studied.

C0942 CANINE LEISHMANIOSIS DUE TO LEISHMANIA (LEISHMANIA) INFANTUM: CLINICAL ASPECTS AND DISTRIBUTION OF THE DISEASE IN NORTHEASTERN ARGENTINA

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1 Background

Canine leishmaniosis (CanL) is endemic in northwestern Argentina. In 2006, the first autochtonous case was diagnosed in Posadas, Misiones. Here, we describe clinical and epidemiological aspects of this ailment.

2 Methods

Two hundred and eleven dogs suspected of having CanL, were surveyed between 2006 and 2016 in several northeastern Argentina cities, particularly in the province of Misiones. Diagnosis was reached through a combination of several methods, the search for amastigotes on smears of bone marrow or lymph node aspirates, PCR, and ELISA using crude extracts or recombinant *Leishmania* antigens. Criteria recommended by Leishvet were applied for clinical staging. *Leishmania* species identification was done by applying Low Stringency Single Specific Primer-PCR method on DNA templates obtained from the aspirates.

3 Results

One hundred and fifty-three out of 211 dogs (72.5%), were diagnosed as CanL. Seventy-nine of them (51.5%) were females. Average age was 6.5 ± 3.6 years. The most frequent clinical stage was IIa, with 64 cases (41.8%). Patients at this stage displayed moderate disease (abundant clinical signs, presence of anti-*Leishmania* antibodies and no detectable alterations of kidney function at biochemical level). Stages I, IIb and III were represented by 29 (19%), 25 (16.3%), and 18 (11.7%) dogs, respectively. Only four patients reached stage IV (2.6%). On the other hand, 13 subjects (8.5%) were asymptomatic. *Leishmania (L.) infantum* is incriminated as the causal agent of CanL in the area, highlighting four cases from Puerto Rico city, and two from Jardín América, in addition to those found in Posadas previously.

4 Conclusions

The predominance of IIa stage indicates that animals are being detected early in the evolution of the disease, thus speeding their inclusion in treatment protocols and therefore their chances of recovery or cure. ELISA serological reactions helped the detection of asymptomatic CanL. The application of a protocol for handling these cases is discussed. Two cities have been added to the geographic distribution area of *L (L.) infantum*, suggesting the expansion of this species in the region of Misiones.

C0990 SEROLOGICAL AND MOLECULAR STUDY OF LEISHMANIA INFECTION IN HEALTHY DOGS FROM VENEZUELA

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1 Background

Venezuela is an endemic country for human and canine leishmaniosis due to *Leishmania infantum* and *Leishmania braziliensis* among other species. However, limited data is available regarding *Leishmania* infection in canines in Venezuela. The aim of this cross-sectional study was to evaluate the rate of *Leishmania* infection in apparently healthydogs from the states of Lara and Yaracuy (Venezuela) by means of serological and molecular methods

2 Methods

Physical examination was performed and blood samples were collected from dogs in urban areas in the states of Yaracuy (n=90) and Lara (n=60)s. *Leishmania infantum* and *L. braziliensis* specific antibodies were assessed by the enzyme-linked immunosorbent assay (ELISA) and *Leishmania* DNA from blood samples was evaluated byreal-time polymerase chain reaction (RT-PCR). A leishmanial genus and *L. infantum* RT-PCRs were perfomed

3 Results

The total rate of *Leishmania* infection in blood samples was 5,3% (8/150) by PCR. In Yaracuy state, the molecular rate of infection was 4,4% (4/90). Only one mixed-breed two years old dog from the state of Yaracuy was positive for *L. infantum* by RT-PCRs. Similar findings were observed in Lara state with a molecular rate of infection of 6,6% (4/60).The PCR-positive dogs presented negatives ELISA results to both *Leishmania* antigens.

The total percentages of *L. infantum* and *L. braziliensis* seropositive dogs were 2,0% (3/150) and 8,6% (13/150), respectively. All dogs from the Yaracuy state were serologically negative to *L. infantum* while four out of 90 (4,4%) dogs were positive to *L. braziliensis* antigen. Three out of 60 dogs (5%) from Lara state were positive for both *Leishmania* antigens. However, higher antibody levels were found for *L. infantum* antigen when compared with *L. braziliensis* antigen. Moreover, six out of 60 dogs (10%) from Lara state were only *L. brazililiensis* positive ELISA.

4 Conclusions

We conclude that the serological and molecular rates of *L. infantum and L. braziliensis* infection observed indicates that dogs are frequently infected by these species in endemic areas for these infections.

C1005 LEISHMANIA INFANTUM INFECTION IN THE EUROPEAN HARE (LEPUS EUROPAEUS) AND WILD RABBIT (ORYCTOLAGUS CUNICULUS), NORTH-EASTERN SPAIN

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1 Background

Leishmania infantum infection has been reported in hares and rabbits and the Iberian hare was proposed as a potential and important reservoir of this protozoan parasite in Spain after the human outbreak of leishmaniosis reported in Central Spain in 2009. However, there is limited information on infection of hares in some geographic areas in Spain including Northeastern (NE) Spain. The main objective of this study was to investigate the presence of *L. infantum* infection in the European hare (*Lepus europaeus*) in NE Spain.

2 Methods

Sera from 125 European hares and 41 rabbits were analyzed for the presence of specific *L. infantum* antibodies by an in house ELISA. The hares and the rabbits were from Girona, Lleida and Barcelona provinces. In addition, whole blood (n=125) and spleen (n=20) of hares, and blood (n=41) and spleen (n=31) from rabbits were tested by real time-PCR (RT-PCR) for the presence of *Leishmania* DNA. In order to compare the prevalence of *L. infantum* infection of hares to that of dogs, sera and whole blood from 174 dogs from same provinces were analyzed by ELISA and RT-PCR, respectively.

3 Results

Sera of 3 out of 125 hares (2.4%; 5.53 ± 0.86 ELISA units (EU)) were confirmed as positive for the presence of antibodies against *L. infantum* and five (4%; 3.19 ± 0.44 EU) were considered doubtful. All the blood and spleen samples from hares resulted negative for the detection of *Leishmania* DNA by RT-PCR. Three out of 41 rabbits (7.32%; 8.92 ± 6.23 EU) were confirmed as positive for the presence of antibodies against *L. infantum* and two were considered doubtful (4.88%; 3.21±0.59 EU). All the blood and spleens from rabbits analysed by RT-PCR resulted negative. Twelve dogs out of 174 (6.90%; 145.92±143.31 EU) were positive for antibodies against *L. infantum*. Moreover, two out of 71 (2.8%) blood samples from dogs were confirmed as positive for *Leishmania* DNA by RT-PCR.

4 Conclusions

These results suggest that *L. infantum* infects hares and rabbits in NE-Spain. However, the observed prevalences of antibodies in both species were lower than found in other areas from Spain. The rabbit and hare have been proposed by some authors to be reservoirs for *Leishmania* sp., and confirmed by xenodiagnoses. However, our results show a very low circulation of the parasite in these species indicating that do not play an important role in the epidemiology of *L. infantum* in NE Spain.

C1033 IMPLICATION OF HEDGEHOGS AS POTENTIAL RESERVOIR HOSTS IN THE TRANSMISSION CYCLES OF LEISHMANIASIS IN TUNISIA

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1 Background

In Tunisia, 4 clinical forms of human leishmaniasis (leish) co-exist caused by 3 *Leishmania* (*L.*) parasites species. Their reservoir hosts include *Psammomys* and *Meriones* rodents or dogs, which are confirmed reservoirs of zoonotic cutaneous and visceral leish, respectively. However, other mammals could be potential reservoir hosts of leish. in Tunisia and worldwide. Our goal was to investigate the potential infection of hedgehogs (H.) often encountered in transmission foci with *L.* parasites in Tunisia.

2 Methods

18 H. specimens were trapped from 3 endemic foci in Tunisia: El Kef, Kairouan and Kebili. From euthanized animals, 129 samples or swabs were taken from liver, spleen, heart, kidney, blood, urine, bone marrow, fat, eye and gum. After DNA extraction, PCR tests targeting: kinetoplast minicercles (13A13B) and a conserved sequence in the small subunit ribosomal RNA gene (Lei70)were applied to detect *L.* infection. *L.* species identification was conducted on positive samples by a nested PCR targeting a repetitive *L.* specific sequence (T2/B4; L1/L4) coupled to an RFLP using 2 restriction enzymes, and direct sequencing of the different PCR products. Results were also confirmed upon cloning and sequencing of some PCR products.

3 Results

Analysis of morphological characters identified the animals as belonging to the species, *Atelerix algirus* (N=14) and *Paraechinusa ethiopicus* (N=4). 13A13B and Lei70 PCR results, showed *L.* infection of the 18 H. specimens. 69 samples and 33 samples, were positive respectively, taken mainly from kidney, liver, spleen, heart and blood. *L.* species were identified in case of 51 samples with *L. infantum* (*L. i*) (N=25), *L. major* (*L. m*) (N=21) and *L. tropica* (*L. t*) (N=1). Interestingly, co-infection with *L. m* and *L. i* was found in 6 different specimens, 3 of them had it in the same organs. An *L. m* and *L. t* co-infection was also found in an eye swab from a 7th specimen. Co-infection results were confirmed with cloning and sequencing. H. infection showed a correlation with the endemic profiles of leish. in the studied foci. Indeed, *L. t* infection was only found in H. samples from kairouan focus. While, *L. m* and *L. i* infection were found in the 3 studied foci.

4 Conclusions

This study shows, for the first time in Tunisia, natural infection of H. animals by *L.* parasite species, inferring this animals could be a potential reservoir host, knowing that there is a correlative relationship between the geographic distribution of *L.* parasites and the parasite infecting studied H.

C1042 THE CLINICALLY NORMAL SKIN OF DOGS WITH DIFFERENT CLINICAL STAGES OF LEISHMANIOSIS

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1 Background

Intact skin of dogs with leishmaniosis, with or without dermatological manifestation, frequently shows microscopic lesions along with the presence of *Leishmania* amastigotes. However, comparative studies between lesional and non-lesional are lacking. They are necessary in order to evaluate and compare the expression of cytokines and toll like receptors transcription in skin lesions and in clinically normal skin of dogs with this disease. The objective of this study was to compare histological and parasitological changes of lesional and macroscopic non-lesional skin from dogs with different clinical stages of leishmaniosis.

2 Methods

Twenty-five dogs, 10 with Leishvet stage I (papular dermatitis) and 15 with at least stage II (ulcerative or exfoliative dermatitis) were included. Two biopsies (lesional and non-lesional skin) were taken from each dog and processed for routine hematoxilin/eosin staining, *Leishmania* immunohistochemistry (IHQ) and *Leishmania* real-time PCR.

3 Results

The study demonstrated microscopic lesions in 14/25 (56%) clinically normal skins. The inflammatory pattern observed ranged from perivascular to interstitial mainly in the superficial and mid dermis. The intensity of the dermatitis was mild to moderate and always more prominent in the lesional skin. Macrophages with lymphocytes and plasma cells were the predominant cells. In macroscopically non-lesional samples, the presence of parasites was demonstrated in 5/25 (20%), 9/25 (36%) and 18/25 (72%) by routine hematoxilin/eosin, IHQ and RT-PCR, respectively. On the other hand, amastigotes were noted in 11/25 (44%), 23/25 (92%) and 25/25 (100%) lesional skin samples by routine hematoxilin/eosin, IHQ and RT-PCR, respectively.
Eleven out of 25 (44%) macroscopically non-lesional samples were histologically normal and did not show *Leishmania* by means of histological stains, however, 5 were RT-PCR positive. Percentage of positive RT-PCR on macroscopically non-lesional samples from dogs with stage I disease was lower than that detected in non-lesional skin from more diseased dogs (Fisher's exact test, $p = 0.0068$).

4 Conclusions

The non-lesional skin of dogs with leishmaniosis frequently shows microscopic lesions and harbours the parasite, demonstrated by routine hematoxilin/eosin staining, IHQ and, more often, by RT-PCR. Moreover, non-lesional skin from dogs with Leishvet stage I is less probable to harbour the parasite than more severe affected dogs.

C1060 SEROLOGICAL STUDY OF LEISHMANIA INFECTION IN HEALTHY AND RETROVIRUS-INFECTED CATS

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1 Background

Leishmaniasis is caused by protozoan parasites of the genus *Leishmania*. More than 20 species can infect dogs and/or humans, and some of them are also infective to cats. Although dogs are considered the main reservoir of leishmaniosis caused by *L. infantum*, cats may also be implicated in the transmission cycle of the parasite, as primary reservoirs, secondary reservoirs, or just accidental hosts.

Serologically positive cats to *L. infantum* infection in endemic areas are less likely to develop clinical signs when compared to dogs. However, feline immunosuppressive viruses such as feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) may impair the normal response to infection and expose cats to other pathogens. Co-infection of retroviruses and *Leishmania* in cats has been sporadically reported; but its importance as risk factor for feline leishmaniasis is still controversial.

The aim of this work was to study the seroprevalence of *Leishmania infantum* in healthy cats and cats infected by FeLV, FIV and feline coronavirus (FCV) and determine if viral infections modulate *Leishmania* seroprevalence.

2 Methods

Antibodies against *Leishmania infantum* were determined by direct agglutination test (ADT) in 153 cats from endemic areas. In all cats, seroprevalence of FeLV, FIV, and FCV were also investigated. Cats were divided into two groups according to the presence of antibodies to either of the retroviruses. Group A corresponded with the seronegative cats (n=111) and group B with the seropositive cats (n=42).

3 Results

Between seronegative cats to retrovirus, 5 (4.5%) had antibodies to *Leishamania infantum* and 106 (95.49%) were negative, while between seropositive cats, 6 (14.28%) had antibodies against the parasite and 36 (85.7%) did not have. The six leishmania seropositive cats in group B were also seropositive to FeLV while only one had antibodies to both FeLV and FIV. This cat presented the higher antibody titres to *Leishmania*.

4 Conclusions

This study shows that seroprevalence of *Leishamnia infantum* in cats can be high in endemic areas and that infected cats rarely present clinical signs. Additionally, antibodies against *Leishmania infantum* were more often detected in cats infected by retroviruses, especially FeLV, than in healthy cats, suggesting that these can modulate feline leishmaniasis.

However, further research is needed to estimate the importance of the cat in the epidemiology of leishmaniasis, especially in regions where feline retroviruses are prevalent.

C1063 ANTIBODY PREVALENCE OF LEISHMANIA INFANTUM INFECTION IN DOGS AND CATS OF MESSINA (SICILY, ITALY)

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1 Background

Dogs are the main reservoir of *Leishmania infantum* (Li) but many studies suggest the cat as possible long term host. The aims of this study are: to provide Li infection prevalence rates in stray cats and in kenneled dogs from Messina (ME) (Sicily, Italy); to evaluate seasonal Li infection prevalence in cats and dogs along one year period; to assess associations between clinical data and Li infection.

2 Methods

Li infection prevalence was investigated in 124 stray cats and 227 kenneled dogs sampled in ME between November 2014 and May 2016 by means of immunofluorescence antibody test (IFAT) (cut off 1:40). Data about signalment, physical examination and cell blood count (CBC) were collected. Positivity to FIV and FeLV in 95 cats was also investigated using SNAP Combo Plus (IDEXX Laboratories, Maine, USA). Results of 47 cats and 61 dogs were randomly selected in order to compare a balanced number of dogs and cats in each season: 15 cats and 16 dogs in autumn, 12 cats and 12 dogs in winter, 4 cats and 11 dogs in spring and 16 cats and 22 dogs in summer. Statistical analysis was performed by using the Fisher's exact test. P values $\leq$ 0.05 were considered significant.

3 Results

Seroreactivity to Li was significantly higher in dogs (58%; ≥1:160 in 24%) than in cats (36%; titer ≥160 in 5%). Twenty cats were FIV antibody positive. No cat was FeLV antigen-positive. No difference in antibody prevalence was found according to: gender, age, Body Condition Score and CBC values both in dogs and cats; health status, occurrence of clinical signs compatible with leishmaniosis and FIV infection in cats. Prevalence of clinical signs or skin lesions was higher in antibody positive dogs. Seasonal antibody prevalence was 27% in cats and 94% in dogs in autumn, 17% in cats no diffenand 25% in dogs in winter, nihil in cats and 45% in dogs in spring, and 37% in cats and 41% in dogs in summer. Autumnal antibody prevalence in dogs was significantly higher than in other seasons and also in this same season in cats. No significant differences were found between seasonal antibody prevalences in cats.

4 Conclusions

Dogs are more frequently found infected by Li, namely in autumn, and have higher antibody titers than cats in endemic areas. Cats however show not negligible anti-Li antibody positivity, not influenced by FIV co-infection. Compared to dogs, infected cats more frequently have a subclinical infection suitable for playing the role of reservoir.

C1107 CONSOLIDATED ANTI-FEEDING EFFICACY OF A DINOTEFURAN-PERMETHRIN-PYRIPROXYFEN COMBINATION AGAINST SANDFLIES IN DOGS EXPOSED THROUGH DIFFERENT STUDIES

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1 Background

Leishmaniosis is transmitted during the blood-meal of female sandflies. Repellent parasiticides can reduce the risk of infection in dogs. Four studies assessed the onset and duration of efficacy of a topical product (4.95 % w/w dinotefuran, 36.08 % w/w permethrin and 0.44 % w/w pyriproxyfen, DPP, Vectra®3D) against sandflies from different origins. This analysis was designed to assess the inter-study efficacy.

2 Methods

54 dogs were involved in 4 studies. In each study, the dogs were allocated to a control or to a treated group based on pre-treatment feeding rate of female sandflies. Three studies were conducted against *Phlebotomus perniciosus* and one study was performed against *Lutzomyia longipalpis*. Administration of DPP was performed on day 0 according to the label. The dogs were exposed under sedation to unfed adult sandflies for 1 h starting from 1 day and at weekly intervals for 4 to 6 weeks after treatment. The insects were collected and classified as fed/unfed and live/dead before calculation of anti-feeding and insecticidal efficacies. The protocols were approved by an ethics committee.

3 Results

The overall feeding rate in the control dogs ranged from 33.0 to 96.4% between studies. The immediate (1-2 days after treatment) anti-feeding efficacy of DPP was 88% against *L. longipalpis* (n=12 dogs) and 96.7% against *P. perniciosus* (n=42 dogs). The efficacy was maintained for 4 weeks above 75% against *L. longipalpis* and above 88% against *P. perniciosus*. The efficacy persists for 5 weeks after treatment at 49% (n=12) against *L longipalpis* and above 87% (n=16) against *P. perniciosus*.

4 Conclusions

The prevention of sandflies blood feeding can be achieved by the administration of advanced topical repellent (DPP). The product performed at a high level from day 1 and for 1 month after administration. A significant residual efficacy is observed for 6 weeks but optimal prevention is expected to be achieved by monthly administration.

C1162 INFECTION SCORE OF LEISHMANIA INFANTUM AS A THERAPEUTIC INDICATOR IN THE TREATMENT OF NATURALLY INFECTED DOGS.

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1 Background

In a retrospective study realized between January 2015 and January 2016, were included 46 dogs diagnosed with canine visceral leishmaniasis (LVC) that were treated with immunotherapy Leish-Tec® associated with Alopurinol® and Infervac® (RIBEIRO et al., 2013).

2 Methods

The infection in these animals was confirmed by serological tests (Indirect immunofluorescence -IFI and Elisa rapid test -IDEXX®), parasitological tests (bone marrow cytology and immunohistochemistry - IHQ) and Real-time polymerase chain reaction - qPCR. Clinical evaluation and laboratory examinations such as hematology, serum biochemistry, urinary biochemistry, urinalysis and abdominal ultrasound were performed every six mouths. All 46 dogs were considered symptomatic and were classified in a disease score of 1 to 4 (SOLANO GALLEGO el al 2009).

3 Results

In a previous assay of the data obtained at the beginning of the therapeutic protocol, it was observed that six of the animals were positive for IHC (13%) and all of them (100%) presented a score ≥ 2. In the analysis of renal function through urine protein-creatinine (UPC) nine of all the animals(19%) presented values ??above normal and from the nine animals, seven (78%) presented a score ≥ 2. In relation to the fractionAlbumin / Globulin (A / G), thirteen of the 46 dogs (28%) presented values ??below 0.6% dL and of these thirteen, nine (69%) had a score ≥ 2.

4 Conclusions

Considering the observed data, it can be inferred that the score ≥ 2 is compatible with the higher chance of high parasitic load, according to the correlation of the IHQ with Xenogiagnosis (TAFURI et al., 2004), and renal disease, either by reduction in value Of the A / G fraction or the UPC value below 0.5mg / dL, which may be a good indicator for the initiation of the therapeutic protocol. Further more, it can auxiliate the the staging of the disease.

C1216 ANTIBODIES OF LEISHMANIA ON WILD AND DOMESTIC CANIDS FROM CENTRAL MÉXICO

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1 Background

Leishmania spp. have been reported in a wide variety of mammals, including canids, which play an important role in the transmission of these parasites between urban and natural environments.There are currently 21 species recorded of *Leishmania* in humans, and in domestic animals and wildlife, Mexico reported three species, including *L. mexicana*, *L. braziliensis*, and *L. infantum*. Prevalence of *Leishmania* in canids in México has been less studied, but studies have been conducted in the southern region of the country. However, the current situation of *Leishmania* infections regarding these canids in the central region of the country is still unknown.

2 Methods

The present study was performed in seven localities from Querétaro state. This study was carried out from January 2014 to July 2015. The canids were captured using coil spring traps for medium-sized canids, which were placed on animal trails at the selected capture sites. To get samples from domestic and feral dogs, we decided to use those captured in the traps, as well as those found on the outskirts of the communities. A 10 ml blood sample was obtained and was placed in test tubes to obtain blood sera and use in serological assays. For the ELISA and Western Blot assays, a partially-purified fraction of FeSODe was used as antigen for L. mexicana and L. infantum.

3 Results

We obtained seven samples from gray fox and 14 from domestic dogs. For FeSODe Indirect ELISA assay, five sera (23.8%) were positive for *L. mexicana*, and eight sera (38%) were positive for *L. infantum* in this test. FeSODe Western Blot. For *L. mexicana,* of the five ELISA-positive sera, four sera (19%) were confirmed positive and one was confirmed negative, while for *L. infantum*, of the eight ELISA-positive sera, six samples (28.5%) were confirmed positive and two were confirmed negative in this test.

4 Conclusions

This is the first record of the exposure from this parasites in wild and domestic canids in central México. The data from this study present a scenario in which the reservoirs of two of the "forgotten" diseases with the greatest impact in Latin America may be closer to highly urban populated areas than we suspected. Nowadays, the extent of these canids' participation in the cycle of transmission of *Leishmania* is unknown. Further studies in anthropic areas are planned in order to identify the principal reservoirs of these parasites in the center of México. Species studied will include mammals that have synanthropic habits.

C1264 NATURAL HISTORY OF CANINE VISCERAL LEISHMANIASIS IN NATURALLY INFECTED DOGS FROM AN ENDEMIC AREA IN BRAZIL

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1 Background

Canine visceral leishmaniasis (CVL) is a systemic and potentially fatal disease caused by *Leishmania infantum*, that can lead to different clinical signs. Natural history, from infection to development of disease, is not clearly unravelled in dogs naturally exposed to *L. infantum*. Understanding natural history of a disease enables predict prognosis and identify opportunities for prevention and control of the disease. The aim of the present study is to describe the disease course, evaluating clinical, serological and parasitological changes during the evolution of natural infection by *L. infantum* in dogs.

2 Methods

A random sample of dogs from an endemic area in Brazil was recruited to the study. After performing a baseline selection, dogs considered positive by diagnostic tests were excluded, while CVL negative dogs and dogs with positive results only in qPCR were followed during one year. In the baseline and every six months' evaluations, dogs were clinically examined, and a clinical score, as well as CVL diagnosis by EIE-LVC, splenic culture and qPCR were performed.

3 Results

In the baseline, 285 dogs were examined, 54%were male, 88%mixed breed and 60%domiciled. Amongst the 285, 55% were identified as negative and 45% as positive. The overall incidence after one-year of follow up was 41%. During the cohort study, 40% were lost to follow up. Considering serological evaluation, 49% of the 138 seronegative dogs remained seronegative throughout the follow-up. Among dogs that became positive, 35% seroconverted after 6 months, being that 57% of them without clinical signs. The other 43% that became seropositive after six months displayed CVL clinical signs, and after one year 52% continued as seropositive and clinically ill, while 3 of them presented recovery of the clinical picture. Twenty-seven dogs became seropositive only after one year, being that 56% subclinically and 44% displaying signs of CVL. To evaluate disease course amongst only qPCR positive dogs, 42% remained as subclinical after one year, while 13% displayed clinical signs after six months and 7% after one year.

4 Conclusions

CVL incidence is high, and although results of diagnostic tests vary widely during follow-up, most dogs manifest disease after one year, except for those who presented only positive qPCR at baseline, who showed a tendency to remain subclinical or turned to negative over time. Longer prospective studies are needed to better characterize disease course and factors that might influence disease dynamic.

C1295 SEROEPIDEMIOLOGY OF DOMESTIC DOGS NATURALLY INFECTED WITH LEISHMANIA SPP. AND TRYPANOSOMA CRUZI IN URBAN AREA OF OVEJAS, SUCRE, COLOMBIA

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1 Background

Leishmania spp. and *Trypanosoma cruzi* are related parasites that belong to Order Trypanosomatida and Family Trypanosomatidae; they have complex transmission cycles, which include many mammalian species. Dogs (*Canis familiaris*) are reservoirs of *Leishmania infantum* and it can play an important role for other species of this genus; in case of *T. cruzi*, dogs can be primary or secondary reservoirs in some regions of America. Ovejas, is an important focus for leishmniasis in Colombia, where it has started urbanization of this parasite; and on other hand, studies about *T. cruzi* have not been conducted. The objective was to assess prevalence of anti-*Leishmania* and anti-*T. cruzi* antibodies in a group of domestic dogs in urban area of the municipality of Ovejas, Sucre, Colombia, as first approach to know the role of domestic dogs (*C. familiaris*) in these complex transmission cycles.

2 Methods

A total of 120 samples of domestic dogs were tested, the serological diagnoses of *T. cruzi* and *Leishmania* spp. was performed by IFAT, ELISA and DPP.

3 Results

10/120 dogs (8,33%) were positives for canine visceral leishmaniasis for DPP test. We found seroprevalence of 45,83% (55/120) for *Leishmania* spp. and 67,5% (81/120) for *T. cruzi* in studied canines. Among the 120 sera samples tested, 46/120 (38,3%) showed positive serological results for both of the parasites (co-infection). Our evaluation indicates that only two samples (1,66%) had cross-reaction for *T. cruzi* and *Leishmania* spp. There was association between dogs seropositives for *T. cruzi* and age (≤ 36 meses), with X^2= 6,064 and P value= 0,0138. In addition, this association was found for dogs seropositives for *Leishmania* spp. and *T. cruzi* (coinfection) with X^2=8,268 and PValue= 0,004 (very significant). The agreement between test IFAT and ELISA was "good" for diagnoses of both seroprevalence *Leishmania* spp. and *T. cruzi*.

4 Conclusions

This study suggest that *C. familiaris* is a potential reservoir in domestic transmission cycle of *Leishmania* spp. in the municipality of Ovejas. It is reported the presence of antibodies for *T. cruzi* for first time in dogs from Ovejas, Sucre, Colombian Caribean Coast. In case of *T. cruzi*, further studies are necessary in order to evaluate the role of domestic dogs in transmission cycle and epidemiology of this parasite in the area. These findings are important in epidemiology of leishmaniasis and chagas disease.

C1346 CHARACTERIZATION OF LEISHMANIA INFANTUM STRAINS BY PCR-RFLP IN WILD LEPORIDAE FROM THE COMMUNITY OF MADRID (SPAIN)

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1 Background

Leishmania infantum is the main species implicated in Leishmaniosis in the Mediterranean basin, where the disease is endemic. Canines are the main domestic reservoirs. Recently, *Leporidae* have been revealed as competent reservoirs of *L. infantum*, being the source of infection during an important outbreak in Madrid (Spain) that involved 697 human cases from 2009 to 2016. *L. infantun* DNA has been detected by quantitative real-time PCR in wild rabbits and hares in two Madrid natural areas outside the outbreak area. Comparison of the strains isolated from different areas could add more knowledge about the sylvatic cycle of *Leishmania* spp. and PCR-RFLP can be used as a cost-effective and reliable method to achieve it.
The aims of this study are a) to characterize the *L. infantum* strains detected in wild *Leporidae* from different areas of the Community of Madrid (outbreak and non-outbreak areas); and b), to know if the *L. infantum* strains maintained by wild *Leporidae* in the outbreak area are the same as those ones found in the non-outbreak areas.

2 Methods

A total of 4 rabbits and 6 hares were studied from the outbreak area; whereas 14 rabbits and no hares and 1 rabbit and 5 hares were analysed from the non-outbreak areas 1 and 2, respectively. Spleen, skin and hair samples were used to characterize *L. infantum* strains by PCR-RFLP using two different restriction enzymes (BslI and MscI-NEBiolabs) to digest the 145 bp fragment present on the high coy of kDNA minicircles.

3 Results

There is a main RFLP profile with both restriction enzymes, corresponding to four bands with BslI and three with MscI. This pattern is shared by rabbits and hares in the outbreak and non-outbreak areas. There is not any difference between tissues.

4 Conclusions

Some *L. infantum* strains circulating outside the outbreak area are the same as those ones inside it, therefore there can be a sylvatic cycle of *Leishmania infantum* maintained by wild rabbits and hares. Special attention should be given to the control of wild *Leporidae* to avoid new outbreaks in different areas of Madrid, depending on the epidemiological circumstances. Further analyses on wild *Leporidae* populations from other non-outbreak areas should be carried out.

C1355 COINFECTION OF LEISHMANIA INFANTUM AND BABESIA CANIS VOGELIIN DOGS FROM URBAN AND RURAL AREA FROM NORTHEAST BRAZIL

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1 Background

Leishmanasis and babesiosis and are vector-borne parasitic diseases endemic in most tropical area. In both diseases, canine are competent reservoir they can serve as available source of nutrition for many blood-feeding arthropods. Canine Visceral Leishmaniasisis caused by *Leshmania infantum (sin Leishmania chagasi)* and it affects millions of dog in Asia, North Africa, Europe and South American. In Brazil it was initially reported in rural areas, however, since eighty decade this disease has been described in urban area, including in the capital of several Brazilian states. *Babesia spp* is other parasite that has been cause great preoccupation among veterinary practice. Depending the *Babesia* species the clinical picture may range since sublinical condition to fatal disease. In Brazil, *Babesia canis vogeli*, transmitted by *Rhipicephalus sanguineus*, has been incriminated as the causative agent of canine babesiosis.

2 Methods

This study was performed in São Luís Island, Maranhão state, Brazil. Two hundred and thirty-four dogs from the São Luis, municipality (134 from urban area and 100 from rural area) were included in the study. The dogs were clinically examined and an epidemiological questionnaire was applied in order to obtain information about breed, age, sex, hair, absence or occurrence of ectoparasites. DNA was extracted from 300µL of whole blood employing the Wizard® Genomic DNA Purification kit (Promega), following the manufacturer's recommendations. Primers were used Lsh1 e Lsh2, for *L. infantum* and Piro-A e Piro-B, derived from 18S rRNA gene for all subspecies of *B. canis*. The PCR products were then subjected to agarose gel electrophoresis in 1.3% for *B. canis* and 1% for *L. infantum.*

3 Results

Independent the studied area the results showed that 16% were found positive for *Babesia spp* and 23.93% for *L. infantum* and 3.8% presented DNA for both parasite. Age, sex and hair type did not constitute as risk factors for both diseases. However, animals with age ranging 6 months and two years old seemed to be more susceptible than animals older. In regard the presence of ectoparasites it was observed that 56% (n=132) of animals were parasitized by ticks. Comparing the PCR survey for *Babesia* and *Leishmania* was observed a statistical difference between urban and rural area.

4 Conclusions

The young animals are more susceptible to coinfection between *Leishmania* and *Babesia*, and urban area presented more coinfection than rural area.

C1356 NATURAL HISTORY OF CANINE LEISHMANIA (L.) INFANTUM CHAGASI-INFECTION IN THE BRAZILIAN AMAZON, WITH EMPHASIS ON ITS PREVALENCE, INCIDENCE AND CLINICAL-IMMUNOLOGICAL EVOLUTION

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1 Background

American canine visceral leishmaniasis (CVL) is a parasitic zoonosis widely spread throughout Latin America and caused by *Leishmania (L.) infantum chagasi*. With respect to the natural history of canine infection in the Brazilian Amazon, it is well known that its prevalence, incidence and clinical-immunological evolution are till now poorly understood. This work sought to amplify our understanding on the clinical canine infection, by focusing not only on the prevalence and incidence, but principally on the clinical-immunological evolution of the infection, aiming to address specific questions on the canine immune responses and their evolution towards death.

2 Methods

This was a cohort study that analyzed for two years the prevalence, incidence, and clinical-immunological evolution of canine *L. (L.) i. chagasi*-infection in 316 mongrel dogs from endemic area of American visceral leishmaniasis in the Brazilian Amazon. The indirect fluorescence antibody test (IFAT-IgG), leishmanin skin test (LST), and parasite search using popliteal lymph node aspiration were combined to diagnose the infection. Data analysis used simple percentages for the rates of prevalence and incidence (at 6, 12, and 24 months), the chi-square test, and the Relative Risk (RR) with 95% confidence interval.

3 Results

IFAT and LST recognized three immune response profiles: (I) IFAT$^{(+)}$/LST$^{(-)}$ (81 dogs), (II) IFAT$^{(-)}$/LST$^{(+)}$ (17 dogs), and (III) IFAT$^{(+)}$/LST$^{(+)}$ (13 dogs), providing specific prevalence of 25,6%, 5,4% and 4,1%; overall rate of 35,1%; accumulated incidence of 5,7% dogs/month, and specific rates of 5,4%, 0,3% and 0,0%, respectively. Parasitological diagnosis was positive in 19% of dogs from the prevalence (85,7%-profile I), and in 11% from the incidence (100%-profile I). The clinical status of 179 dogs revealed 145 (81%) from the profile I (82% subclinical); 21 (11,7%) from the profile II (100% subclinical); and 13 (7,3%) from the profile III (84,6% subclinical). The clinical conversion from the subclinical to sick was higher (40,2%) in the profile I than in the profiles II (5,8%) and III (9%). With regards to immunological conversion, only 3,2% of dogs from the profile I converted to LST$^{(+)}$, while 80% of dogs from the profile II converted to IFAT$^{(+)}$. A 100% death rate due to *L. (L.) i. chagasi*-infection was observed in dogs from the profile I.

4 Conclusions

These results strongly suggested that canine immune response is mainly directed by the profile I, which becomes dogs highly susceptible to infection.

C1360 LATE AND ACCIDENTAL PARASITOLOGICAL DIAGNOSIS OF LEISHMANIA SP THROUGH ASCITIC FLUID IN SYMPTOMATIC DOG LIVING IN A REGION OF LOW DISEASE TRANSMISSION.

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1 Background

In the state of Rio de Janeiro the suspicion and diagnosis of canine visceral leishmaniasis (CVL) is still a challenge, because it is a region of low disease transmission, with underreporting and consequently with failures in diagnosis orientation. Besides being a place with high incidence of other parasitic diseases, there is a mistake when trying to diagnose CVL based on few tools and without previous knowledge, often associated to a pre-established erroneous treatment. The objective of this paper was to report a CVL case, diagnosed late by an accidental finding through ascitic fluid cytology, indicating the difficulty of clinical suspicion and diagnosis in those regions.

2 Methods

A female dog, of the French Bulldog breed, from the Brazilian Northeast, living in Niterói, RJ, Brazil, two years old presenting loss of appetite, prostration, fetal reabsorption, splenomegaly and ascitis was subjected to hematological and biochemical exams, screen for microfilariae and haemocytozoa, histopathological examination of spleen, cytology of ascitic fluid and indirect immunofluorescence and Elisa for CVL.

3 Results

The hematology indicated anemia, leukopenia, thrombocytopenia, hyperproteinemia with increased total proteins and globulin, biochemistry with no abnormalities and negative reactions for haemocytozoa and microfilariae. In view of this, the dog was treated for ehrlichiosis. At the end of treatment there was worsening of the clinical manifestations and splenomegaly and presence of ascites. The organ was removed and submitted to histopathology that showed splenic infarction with hemorrhage and absence of malignancy or parasitic forms. However, the cytology analysis of the ascitic fluid showed the presence of free *Leishmania* sp. amastigote forms in the preparation and inside the macrophages, characterizing CVL, which was later confirmed by serological result.

4 Conclusions

This late and accidental finding proved the difficulty of CVL clinical suspicion and diagnosis in regions of low transmission. Afterwards, it was possible to correlate epidemiological data with the place of origin of the animal, a region of high transmission, and the place of residence, of low transmission, but where an increase in the number of cases has been observed in recent years. It was verified the fragility in identifying the disease circulation, the origin of the infection and the time that animal remained in the environment as a source of infection.

C1441 FIRST REPORT OF NATURAL INFECTION OF THE LESSER EGYPTIAN JERBOA WITH LEISHMANIA MAJOR IN TUNISIA.

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1 Background

Many rodent species and small mammals are implicated in the transmission cycle of *Leishmania* parasites around the world. In the present study, we aimed to investigate the presence of potential rodent reservoir hosts in an endemic area of cutaneous leishmaniasis in Tunisia.

2 Methods

Ten *Jaculus* (*J.*) *jaculus* (lesser Egyptian jerboa, Linnaeus, 1758) were collected in Southeast Tunisia in June 2015. PCR-ITS1-RFLP was used for *Leishmania* parasites detection and identification.

3 Results

Leishmania (*L.*) *major* was detected and identified among four *J. jaculus*.

4 Conclusions

This is the first report of *L. major* identified from *J. jaculus* in Tunisia. This novel finding strengthens our understanding of the transmission cycle of *L. major* parasites, the major responsible of cutaneous leishmaniasis in Tunisia.

C1445 THE GONDI AS POTENTIAL RESERVOIR HOST OF LEISHMANIA PARASITES IN TUNISIA.

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1 Background

Incriminating new rodent species, as reservoir hosts of *Leishmania* parasites is crucial for understanding the transmission cycle of this disease. The aim of this study was to survey natural *Leishmania* infection in collected *Ctenodactylus* (*C.*) *gundi* from two endemic regions in Tunisia: Sidi Bouzid and Tataouine.

2 Methods

Leishmaniasis diagnosis was realized using clinical, parasitological (direct exam and parasites culture) and molecular (PCR-ITS1-RFLP) methods. Total DNA was isolated from the spleens and the livers of 92 *C. gundi*.

3 Results

Leishmaniasis clinical manifestations were detected among 11 rodents (12%). *Leishmania* parasites were detected in 30 (32.6%) rodents using direct exam method. *Leishmania* DNA was detected in 40 (43.5%) *C. gundi* by combining results among spleens and livers. *Leishmania* isolates were con?rmed to be *Leishmania* (*L.*) *major* except for only one specimen which was *L. tropica* /*L. killicki*.

4 Conclusions

These results demonstrated, for the first time, the high natural infection rate of *C. gundi* with *L. major* parasites in Tunisia. Hence, *C. gundi* should be considered as reservoir host of *Leishmania* parasites causing CL in Tunisia.

C1486 LEISHMANIA INFANTUM RFLP ANALYSIS: A SHARED INFECTION BETWEEN WILD CARNIVORES AND LAGOMORPHS, DOGS AND HUMANS IN ITALY.

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1 Background

In Northern Italy the epidemiology of zoonotic visceral leishmaniasis (ZVL) has experienced profound changes in the last decades as far as territorial expansion and increased prevalence of infection in dogs and humans, but also in free-ranging species, like red foxes *Vulpes vulpes*, European Brown Hare *Lepus europaeus* and the invasive Eastern Cottontail *Sylvilagus floridanus*.

2 Methods

A retrospective comparison was made between 79 Restriction Fragment Length Polymorphisms (RFLP) patters obtained from humans (n=73), dogs (n=151), foxes (n=104), hares (n=20), rabbits (n=3) and cottontails (n=23) naturally infected with *L. infantum*. All human (blood serum) and animal (whole blood or spleen) samples were collected from the same area in Northwestern Italy (Piedmont region) from 2007 to 2015. A *L. infantum* specific fragment of kDNA, was analyzed by Restriction Fragment Length Polymorphism (RFLP).

3 Results

Among the 79 RFLP patterns identified in the study area, 18 were shared among different species. Eight patterns identified in infected humans were also isolated in dogs. *L.infantum* in wild foxes seems to be circulating independently from the domestic cycle involving humans and dogs. One RFLP pattern was shared directly between human and cottontail, a second pattern is common to humans, dogs, hares and cottontails. Most notably 4 RFLP patterns are shared between fox and lagomorphs, and 3 between dogs and the studied lagomorph species.

4 Conclusions

Dogs are confirmed as primary reservoir of human infection. in the area. Only one RFLP pattern is indeed shared between humans and foxes. Such a clear separation between the domestic and sylvatic cycles of *L. infantum* is not, however, maintained thanks to the "bridge" role that lagomorphs seem to play. The role of hares and cottontails as reservoir of infection for ZVL should be further investigated to better understand and control how the domestic and wild cycles of *L.infantum* interact.

C1554 PRESENCE OF LUTZOMYIA LONGIPALPIS (DIPTERA: PSYCHODIDAE) AND NATURAL LEISHMANIA (L.) INFANTUM CHAGASI-INFECTION IN THE WILD RODENT, PROECHIMYS SP. (RODENTIA: ECHIMYIDAE), IN THE "SERRA DOS CARAJÁS", SOUTHERN OF PARÁ STATE, BRAZIL

Thiago Vasconcelos dos Santos, Patrícia Karla Ramos, Fábio Márcio Medeiros da Silva, Ana Camila Alves, Luciana Vieira do Rego Lima, Marliane Batista Campos, Rodrigo Ribeiro Furtado, Fernando Tobias Silveira

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1 Background

In Brazil, geographic expansion of American visceral leishmaniasis (AVL) due to *Leishmania (Leishmania) infantum chagasi* is a worrisome issue. In the Brazilian Amazon, some emerging *foci* are worth of epidemiological interest and potentially vulnerable/receptive areas, such as the 'Serra dos Carajás', southern of Pará State, where human disease is so far known to be absent or underreported needs further investigation.

2 Methods

During 2012-2016, ecological studies were conducted in three municipalities of the 'Serra dos Carajás': Canaã dos Carajás (forested area, metalophic savanna 'Canga' vegetation, rural settlements and urban area), Curionópolis ('Serra Pelada' rural settlements) and Marabá (Tapirapé-Aquiri National Forest). Phlebotomine captures were undertaken with ten CDC light traps disposed from 6:00 p.m. to 6:00 a.m., in forested areas and peri/intradomiciliary environments. Mammals were captured in forested areas with 'Tomahawk' and 'Sherman' traps, and subjected to biopsy of the skin, liver, spleen and blood collection. Material was twofold inoculated in Difco B[45] and intraperitoneally/intradermally in *hamsters* for attempting to parasite isolation. Specific DNA diagnosis was performed with RV1-RV2 primers for qPCR from directly biopsies, and also from experimentally inoculated *hamsters*.

3 Results

A total of 307 *Lutzomyia longipalpis* were captured, associated mainly with 'Canga' vegetation (283), but also with domiciliary environments in Canaã dos Carajás, urban (20) and rural areas (3); and Curionópolis, only rural area (1), however not found in the Tapirapé-Aquiri National Forest. Amongst mammals, 70 wild rodents were examined and the spleen and liver from a single specimen of *Proechymis* sp. (from Tapirapé-Aquiri National Forest) was found with *L. (L.) i. chagasi*-DNA. Interestingly, the spleen and liver from the *hamsters* experimentally inoculated with the spleen and liver homogenate of that same *Proechymis* sp. were also qPCR positive for *L. (L.) i. chagasi* following 2 months P.I.

4 Conclusions

Lutzomyia longipalpis findings confirm receptiveness for AVL transmission in the 'Serra dos Carajás'. Positive *L. (L.) i. chagasi*-qPCR result for experimentally inoculated *hamsters* confirms the circulation of the etiological agent and its viability from *Proechymis* sp. which can be, for the first time, regarded as a potential reservoir host in that region.

C1563 FIRST DETECTION OF LEISHMANIA TROPICA DNA IN MUS MUSCULUS (RODENTIA, MURIDAE) IN THE FOCUS OF CUTANEOUS LEISHMANIASIS IN SOUTH MOROCCO.

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1 Background

Cutaneous leishmaniasis is an infectious disease caused by flagellate protozoa of the genus*Leishmania* (Kinetoplastida: Trypanosomatidae). In Morocco, cutaneous leishmaniasis due to *Leishmania tropica* is usually considered anthroponotic and as a public health problem. Anthroponotic cutaneous leishmaniasis (ACL) infection is transmitted to a human host by the bite of an infected female sand fly (Diptera: Psychodidae) of the genus *Phlebotomus. P.* (*Paraphlebotomus*) *sergenti* has identified as the only proven vector (Ajaoud et al., 2013).
L. tropica it has been isolated from several mammalian species, which might serve as reservoir hosts, e.g. dogs in Morocco (Dereure et al., 1991), hyraxes (*Procavia johnstoni*) in Keny (Sang et al., 1992), rodents (Svobodova et al., 2003; 2006; Talmi-Frank,, 2010), rock hyrax in Egypt (Shehata et al., 2009) and gerbil in Israel (Jacobson et al., 2003).
The main objective of our study was to determine the potential animal reservoir of *L. tropica* in Morocco.

2 Methods

A total of 50 rodents were captured, using Sharman traps, from many localities of two L. tropica foci : 19 in Immintanoute (Rhajaoui et al. 2005) and 31 in Tlat Hanchan (Ajaoud et al. 2013) species identification was carried out morphologically according to Aulagnier and Thevenot (1986).
Samples of skin (ear lobe, skin lesion) were harvested for parasite detection by PCR assays. To identify the Leishmania species, sequence of the internal transcribed spacer (ITS-1) products was analyzed.

3 Results

For rodent species, 12 *Rattus rattus* and 7 *Mus musculus* were collected in Immintanoute (38%), while 17 *R. rattus*, 11 *M. musculus* and 3 *R. norvegicus* were collected in Tlat Hanchan (62%). For parasite isolation, one *M. musculus* from each locality was affected by *L. tropica*.

4 Conclusions

This is the first molecular detection and identification *L. tropica* in *M. musculus* in Morocco, suggesting the possible implication of rodent species, in the transmission cycle of *L. tropica* in Morocco.

C1567 FIRST DETECTION OF LEISHMANIA INFANTUM IN DOMESTIC DOGS FROM BURKINA FASO (WEST AFRICA)

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1 Background

Human cutaneous leishmaniasis (HCL) is endemic in Burkina Faso and broadly in West Africa, due to infection with the zoonotic species *L. major.* Visceral leishmaniasis is rarely described in West Africa and no cases have been reported to date in Burkina Faso. The purpose of the current study was to investigate on the presence of *L. infantum* in populations of domestic dogs in Bobo-Dioulasso city in Burkina Faso.

2 Methods

The study was carried out in five districts of the city of Bobo-Dioulasso, Burkina Faso. We conducted an active survey in households with dogs in five districts of the city for canine leishmaniasis screening. Epidemiological data clinical sign were collected for each dog. Venous blood collection and skin lesions biopsies were performed in dogs after informed consent of the owners. The plasma was used for the immunological diagnosis (DiaMed -IT LEISH) and positive samples were confirmed by nested PCR.

3 Results

A total of 85 dogs were included in the study. For the first time, the occurrence of *L. infantum* in Burkina Faso was confirmed in five domestic dogs presenting symptomatic signs by immunochromatographic tests. Biopsy samples were positive for *L. infantum* in PCR analysis for two dogs.

4 Conclusions

Our findings indicate that *L. infantum* is circulating within canine populations in Bobo-Dioulasso. Infected dogs would be the reservoir hosts of this visceralising species. Several priorities for public and veterinary health research including highlighted by this study.

C1576 POSSIBLE RELATIONSHIPS BETWEEN CHANGES IN THE ENVIRONMENT AND THE DISTRIBUTION OF CANINE LEISHMANIASIS IN MOROCCO

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1 Background

The Mediterranean coastal regions of Morocco are known, for a long time, to be endemic for human and canine visceral leishmaniasis (CanL) caused by *Leishmania infantum*. However, both CanL and human visceral leishmaniasis cases are increasingly detected from areas where the disease was unknown is observed.

2 Methods

Because the geographic mobility of CanL could be associated with the environmental changes, we conducted a survey of the natural and human-made changes that occurred during the last 40 years.

3 Results

The survey revealed that a significant natural and human-made changes occured. The most important natural changes are those of rainfall resulting in more frequent floods and longer periods of dryness. Human-made changes are numerous and diversified : level and variations of water resources, rural management (new vegetation, development of irrigation network, new agro-industrial activities, deforestation, road construction, biodiversity losses). In these new areas seroprevalence of CanL (10-41%) and virulence of the isolated *L. infantum* strain are high. On the other hand, in these foci, a *L. tropica* anthroponotic specie has been found to be capable of causing canine visceral infection associated with several of the classical signs and pathology including visceralisation.

4 Conclusions

The natural and human-made changes that occured during the last 40 years could have contributed to the appearance of new populations of Phlebotomine vectors, and/or to increase the pre-existing populations of these insects. These changes have encouraged the emergence of new economic opportunities which are attracting for new human populations that are more sceptible to leishmaniasis. On the other hand, these populations develop cattle, sheep and goats husbandry, built new villages and new inadequate slaughterhouses that are attractive for semi-domestic and/or stray dogs; a situation that is likely to favour an increase in the population of these animals and, consequently, the sources of *L. infantum* either for dogs and human infection.

C1595 CANINE EUTHANASIA: AN EFFECTIVE MEASURE FOR THE CONTROL OF HUMAN VISCERAL LEISHMANIASIS IN AN ENDEMIC TERRITORY IN BRAZIL?

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1 Background

Brazil is currently responsible for about 90% of cases in Latin America, with reports of human visceral leishmaniasis (HVL). In 2012, around 3,038 cases of the disease were reported in humans, with an incidence of 1.57 cases/100,000 inhabitants and a lethality rate of 7.1%. However, it faces difficulties in combating the disease, since it adopts the euthanasia of symptomatic and seropositive dogs as a control measure per current brazilian decree 51,838, of March 14 of 1963 year, unlike some countries such as Italy and Spain, which condemn this practice, since there is no yet scientific evidence that this contributes to disease control. On the contrary, it has generated conflicts between owners, who create resistance in delivering their dogs with high affective value, and public power. We discuss euthanasia as a measure of HVL control, in Brazilian public policies

2 Methods

This is a descriptive, study from the secondary database of the Epidemiological Surveillance, Zoonoses Service and the General Attorney Service of the municipality of Governador Valadares/MG/Brazil, from 2008 to 2015 years

3 Results

It was verified that Governador Valadares is an endemic area after the outbreak of 2008, with some declines, in the years of 2013 and 2014, and intensified in 2015. Was observed a high prevalence of seropositive dogs, despite canine sacrifice together with significant lawsuits against dog owners, deliver the reservoir to make the control measure

4 Conclusions

For an effective public policy several elements must be taken into account, especially interdisciplinarity, emphasizing legal reflections and health, involving issues that permeate human relations in the context of ethics and legislation. It is necessary to advance the understanding of the biology of *Leishmania* and shed new light on the complex interactions occurring within the parasite-host-vector triangle, to discover effective measures in programs of treatment and control of the disease. In addition, exploring how the disease in dogs relates to that of humans and how an improved understanding, in line with the concept of "One Health" may open new avenues for the control of these devastating diseases.

C1664 MOLECULAR DETECTION OF LEISHMANIA VIANNIA SPP. AND OTHERS TRYPANOSOMATID INFECTIONS IN SLOTHS (CHOLOEPUS HOFFMANI AND BRADYPUS VARIEGATUS) FROM A TEGUMENTARY LEISHMANIASIS ENDEMIC REGION IN PANAMA.

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1 Background

American Cutaneous Leishmaniasis (ACL) is a zoonosis very frequent in rural areas of Panama. The development of particular control and surveillance activities for this disease needs a precise knowledge about those species of reservoirs that maintain the sylvatic cycle. In this sense, it is essential to confirm the infection rates, as well as the precise identification of *Leishmania* species found in sloths living in forest areas near Panamanian rural communities where ACL is a health problem. The main objective of this study was to evaluate the frequency of infection with *Leishmania* spp. and other trypanosomatids in sloths captured in forested areas near rural communities.

2 Methods

The main objective of this study was to evaluate the frequency of infection with *Leishmania* spp. and other trypanosomatids found in sloths captured in forested areas near rural communities. For this, a total of 106 sloths (79 *Choloepus hoffmani* and 27 *Bradypus variegatus*), from three LT endemic communities located in the province of Panama Oeste, were investigated. A venous blood sample was taken from each anesthetized animal. It was used to perform blood cultures and DNA extraction for PCR analysis using kDNA, HSP-70 and ITS-1 specific sequences as molecular makers. Additionally, a duplex PCR technique, based on telomeric sequences, was used for the identification of *Trypanosoma rangeli* and *Trypanosoma cruzi* infections. Reference strains of *Leishmania Viannia panamensis*, *T. rangeli*, *T. cruzi* and *Endotrypanum* spp. were used.

3 Results

It was possible to isolate trypanosomatids in 31.1% (33/106) of blood cultures. In addition, DNA from trypanosomatids was detected in 79.2% (84/106) of the analyzed blood samples, *C. hoffmani* (65%) and *B. variegatus* (14.1%). In general terms, 13.2% (14/106) of the sloths were infected with *Leishmania Viannia* spp., 38.7% (41/106) with *T. rangeli*, 2.8% (3/106) with *T. cruzi*, and 53.7% (57/106) with *Endotrypanum* spp. These results confirm the important role played by sloths as reservoirs of *Leishmania* parasites and other trypanosomatids in this country region.

4 Conclusions

Infections with *Leishmania Viannia* spp. were frequent in the evaluated sloths, this results should be taken into account during the surveillance and prevention of LT. The most common trypanosomatid found was *Endotrypanum* spp., a phylogenetically close parasite to *Leishmania* that merits consideration in studies about sloths as reservoirs of LT.

C1689 INCIDENCE OF LEISHMANIASIS IN CATS IN SOUTHERN SPAIN AND COMPARATIVE BETWEEN SEROLOGICAL TEST AGAINST DOGS

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1 Background

Leishmania infantum has settled down in Southern Spain Coast like endemic and its prevalence is about 60-80% in dogs. In this region, Leishmaniasis is an EDO (Notifiable Disease) and epidemiological data come from clinical veterinary practitioners.
The problem is that all these positive dates come from registered pets, with microchip identification System. Outdoor dogs out of system are not tested and epidemiological dates of these dogs are unknown and it is expected to be more incidence than indoor dogs.
The same goes with cats. The latest studies have showed that domestic cats can be a chief reservoirs also, mainly these animals injured by a retroviral diseases like Feline Leukemia (FeLV) or Felines Immunodeficiency (FIV) and Toxoplasmosis.
Based on these studies, it can be expected that outdoor cats which come from settled colonies will show high incidence of *leishmanial infantum*. At least, it has been hope find animals with antibodies against *LI*.
The FeLV and FIV proliferate out between cat who lives nearby, in territorial fights, using same drink or feeding points.
By last, *Toxoplasma gondii* can get down immunologic system and allow cats exposed to the infection.

2 Methods

Its has been selected cats from 40 towns from Axarquía area. Semitropical climatology with dry environment, also the lowest levels are exploited based on irrigation system (avocado, mango, kiwi and custard apple), which allows to maintain a high humidity at ground level, establish an ideal environment for *phlebotomus* proliferation.
Previously, cats tranquillized with xylazine and ketamine. The samples are from blood, bone marrow, spleen, skin and lymph nodes.
Laboratorial techniques are ICT, IFAT, ELISA and PCR

3 Results

Remains to be determined
At the moment it has been detected 3 positive cats by PCR (2 bone marrow and one skin) and one female old cat positive by ICT.
First test was made with 20 µl of plasma and 5 µl of reactive. The positive marker showed a weak line so it was considered positive.
To rule out errors, it was repeat again with the same proportion and in ultimately is was tested with 40 µl of plasma where the positive marker was more clear.
By now, this sample has been considerate positive until bone marrow PCR and plasma IFI and ELISA test will be done.

4 Conclusions

(PROVISIONAL)

Prevalence of LI in cats are more important than it was showed at now. Cats can show less clinical symptoms and negative serological test but show LI DNA in different tissues.

C1697 COMPARATION OF LEISHMANIA INFANTUM POSITIVE RESERVORIES WITH HUMAN TRANSPLANTED PATIENTS IN A HIGH INCIDENCE AREA IN SOUTH OF SPAIN

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1 Background

Our aims were determinate the risk level for transplanted patients in an area with high incidence of L.I. (more than 70% displayed dogs).
Spain has been the first transplant country in the world along 25 years with 100,7 transplanted patients by 1million of population. Mainly organ affected by leishmaniasis is kidney and Andalucía region has more than 400 kidney transplant each year.
Aside from, transplanted population are medicated with immunosuppressant drugs (ISD), which let them exposed to infectious diseases like leishmaniasis. The problem is the clinical risk if they are retired the ISD, because their immunologic system can to reject the organ also leishmaniasis can kill them if it continues ISD treatment.

2 Methods

Spain National Health System files review to localize transplanted population in the 40 towns from Axarquía area by means of Postal districts numbers. Draw a map.
Draw other map with prevalence of leishmaniasis cases in dogs declared in same Postal districts and positives cases founded in the same area of cats. Also dogs and cats from mainly adoption and rescues centers were tested because it is expected show
Collate dates from both maps to stablish risk zones in a colored map which Sanitary Health officials can use to advise all these patients.

3 Results

- Kidney transplanted population: Remains to be determined
- Other organs transplanted population: Remains to be determined
- Incidence of reservoir Leishmaniasis: Remains to be determined
- Chiefly risk areas: Remains to be determined in a colored map.

4 Conclusions

- Transplanted population (TP) from red district (60-70% prevalence) must be advised about how to prevent LI infection by their Physicians and be tested once a year in late autumn.
- TP from orange district (40-60% prevalence) must be advised, tested if they report fly bites and monitored.

- TP from yellow (20-40% prevalence) and green (0-20% prevalence) district must be advised and monitored.

C1699 AN ELISA TO DISCRIMINATE BETWEEN SPECIFIC L. INFANTUM AND L. BRAZILIENSIS CANINE ANTIBODIES

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1 Background

Leishmania species coexist in South America such as *Leishmania infantum* and *Leishmania braziliensis*. Both species can infect dogs. However, there are limited studies regarding serological tests that will distinguish between *L. infantum* and *L. braziliensis* specific canine antibodies. The aim of this study was to set up an in-house quantitative enzyme-linked immunobsorbent assay (ELISA) with both *Leishmania* antigens to evaluate specific canine antibodies.

2 Methods

a previously described endpoint *L. infantum* in house ELISA was slightly modified and *L. infantum* and *L. braziliensis* antigens were used in the same ELISA plate. Thirty-five sick dogs in different clinical stages of leishmaniosis living in Spain were studied. Eighty serum samples from dogs living in an non endemic area (United Kingdom) were used to establish the cut-off for both *Leishmania* antigens. A non parametric test (Wilcoxon signed-rank test) was used to evaluate statistical significance.

3 Results

All sick dogs studied were considered seropositive for both *Leishmania* antigens. However, *L. infantum* specific antibody levels were significantly ($p<0.0001$) higher [mean±standard deviation (SD) of 2738 ± 6134 ELISA units (EU)] when compared with *L. braziliensis* specific antibody levels [mean±SD of 622±1184 EU].

4 Conclusions

This endpoint quantitative in house ELISA appears to help establishing what *Leishmania* species are most likely infecting dogs in endemic areas. These findings are extremely important in areas where these two parasites might co-exist. Therefore, this type of ELISAs should be used to determine the most likely *Leishmania* parasite infecting dogs in South America.

C1714 GOAT (CAPRA HIRCUS) AS A POTENTIAL ANIMAL RESERVOIR FOR KALA-AZAR IN INDIA

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1 Background

Visceral leishmaniasis (VL) is a disease that has both zoonotic and anthroponotic routes of transmission. In India, VL is considered to be anthroponotic, and is caused by *Leishmania donovani.* However, our group had earlier reported anti-*Leishmania donovani* antibodies in some animals. The study was aimed to find the potential role of goats in the maintenance and transmission of VL in India.

2 Methods

Total 932 blood samples of goats consisting 726 from VL endemic states (Bihar, Jharkhand) and 206 from non-endemic areas (Kerala, Tamil Nadu and Delhi) were collected and tested for the presence of *Leishmania* species specific antibodies using whole cell lysate-latex agglutination test (WCL-LAT). *Leishmania* specific PCR was also done to amplify whole ribosomal ITS region sequence to confirm samples positive by serology.

3 Results

Leishmanial antibodies were detected in 31(4.26%) of 726 goat samples collected from endemic states. None of the sample from non-endemic states showed antibody presence. The parasite DNA was detected in 13(41.93%) of 31 serologically positive blood samples.

4 Conclusions

The present finding indicates that goats are potential animal reservoirs of human VL in India.

C1717 ROLE OF WILDLIFE MAMMALS IN THE EPYDEMIOLOGICAL CYCLE OF LEISHMANIA INFANTUM IN SOUTHEAST SPAIN

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1 Background

Although dogs have been historically considered to be the main reservoir of *Leishmania infantum* in endemic areas, the role of wildlife is recently gaining attention and importance. The classic epidemiological cycle dog-sandfly-human is turning into a network of animal species that collaborate with the dog in the maintenance of the parasite under natural conditions. The collection of wild animals for detection of a possible parasitic infection seems to be the first step for reservoir host identification.

2 Methods

Our present study investigates *L. infantum* infection in lymphoid tissue from 266 dead lagomorphs, rodents and wild carnivores in Murcia using a kinetoplast real-time PCR. The area is located in southeast Spain and is endemic for canine and human Leishmaniosis. However, infection in wild animals has not been previously reported. Our study included 231 skin, 69 liver, 255 spleen and 4 lymph node samples from 80 rabbits (*Oryctolagus cuniculus)*, one genet (*Genetta genetta),* 6 badger (*Meles meles*), 48 rats (*Rattus rattus*), 9 martens (*Martes foina*), one ferret (*Mustela putorius furo*), 64 red foxes (*Vulpes vulpes*), 2 Iberian wolves (*Canis lupus signatus*), 2 raccoons (*Procyon* spp.), 4 wildcats (*Felis silvestris*) and 49 wood mice (*Apodemus sylvaticus*).

3 Results

Leishmania infantum kDNA was found in 100% genets, 42% red foxes, 50% wolves, 25% wildcats, 22% martens, 21.2% rabbits, 10% wood mice and 2% rats. The overall detection rate of *L. infantum* by tissues was: 11.8 % spleen, 4.4 % liver and <1% skin.

4 Conclusions

These results confirm previous findings indicating that these wild species can harbor *L. infantum* infection. However, their role as primary reservoirs of this zoonotic protozoan has only been confirmed for lagomorphs using a xenodiagnostic approach. Further similar efforts combined with parasite strain characterization need to be carried to ascertain the role of most of these wild species in the epidemiology of *L. infantum* infection in Spain.

C1730 COMPARATIVE STUDY BETWEEN SOROLOGICAL IMMUNOCROMATOGRAPHIC METHODS, ELISA AND INDIRECT IMMUNOFLUORESCENCE FOR THE DIAGNOSIS OF CANINE VISCERAL LEISHMANIOSIS

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1 Background

The diagnosis of canine visceral leishmaniasis (CVL) is a great challenge for veterinary practice. The detection of antibodies in the serum of dogs has been the main diagnostic tool used throughout the world. The emergence of rapid immunochromatographic tests (RIT) offered the possibility of short-term diagnoses, providing collective and individual interventions that are more economical, practical, agile and, therefore show greater perspectives of efficiency. However, some issues are relevant when it comes to the reliability of the negative or positive results.

2 Methods

Among the existing RITs, this study evaluated the correlation between Kalazar Detected® InBios International rapid test, ELISA and Indirect Immunofluorescence technique (IIF). While the last two were carried out at the Biological Sciences Institute (ICB-UFMG), for the diagnosis of LVC. 120 dogs of different races, sex and ages, from which samples of blood, bone marrow and skin were collected for anti-*Leishmania* antibodies and *Leishmania sp* testing. The dogs were divided into four groups, according to the IIF titer: Group I - 34 were non-reactive (28%); Group II - 37 were reactive at titers of 1:40-1:80; Group III - 23 reactive at titers of 1: 160-1: 320 and group IV - 26 reactive at titers equal to or greater than 1:640.

3 Results

In group I, out of the 34 non-reactive in the IIF, 08/34 (24%) were positive ELISA and 06/24 (18%) in the RIT. From this group, seven animals were parasitologically positive, and among them, 03/07 (43%) were positive in the ELISA and 01/07 (14%) in the RIT. In group II, out of the 37 animals 1: 40-1: 80, all (100%) were positive in the ELISA and 17/37 (46%) in the RIT. In this group, 11 animals were parasitologically positive and, ou of these, 07/11 (64%) in RIT. In group III, out of the 23 dogs 1: 160-1: 320, all were positive in ELISA and 16/23 (70%) in RIT. Among these animals, 13 were parasitologically positive and 12/13 (92%) in RIT. Group IV, among the animals with titers equal to or greater than 1:640, resulted positive in ELISA and 24/26 (92%) in RIT. In this group, 20 had positive parasitological tests and all were also positive in RIT.

4 Conclusions

The intercorrelation between both tests is directly proportional to the titers of anti-*Leishmania* antibodies and none of them was able to identify all animals that tested positive for parasites. Out of titers 1:40-1:80 there was greater correlation between IIF and ELISA than between RIT and IIF or RIT and ELISA.

C1764 OCCURRENCE OF ARTERIAL HYPERTENSION IN DOGS NATURALLY INFECTED BY LEISHMANIA INFANTUM IN THE CLINICAL STAGING OF INFECTION.

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1 Background

Systemic arterial hypertension (SAH) is a disease that affects dogs and has been gaining importance in veterinary practice, but it is still underdiagnosed. SAH is characterized by a sustained increase in systolic blood pressure (SBP) and diastolic blood pressure (DBP) and may be classified as primary or idiopathic, associated with stress (white coat hypertension), and secondary. The last one is the most prevalent in dogs and cats.The most frequent causes of secondary SAH are endocrine, cardiac, vascular, renal disease and also obesity, traumatic brain injury, electrolytic changes and in blood viscosity and iatrogenic disorders. The SAH causes damage to the organic functioning, generating cardiac, renal, ocular and cerebral damages, in those that are considered the main "target organs". Despite these related causes, no infectious contagious disease has been routinely related to SAH.

2 Methods

In order to evaluate the relationship of dogs with canine visceral leishmaniasis (CVL) with the occurrence of SAH, a retrospective study was carried out involving 265 animals in which 60/265 (23%) were positive for CVL.

3 Results

Among the positive dogs, 36/60 (60%) had SAH. These preliminary results indicate a high occurrence of SAH in dogs with LVC. Among the clinical alterations associated with the origin of SAH in dogs with CVL we can mention nephropathies, hyperviscosity syndrome and anemia as the most frequent alterations.

4 Conclusions

Therefore, studies that aim to correlate the causes of SAH in dogs with CVL should be further investigated and veterinary clinicians should be aware of the occurrence of SAH in dogs with CVL.

C1766 PRELIMINARY EVALUATION OF DIFFERENT SOROLOGICAL TESTS IN THE DIAGNOSIS OF NATURAL INFECTION BY LEISHMANIA INFANTUM IN DOGS IN BELO HORIZONTE, MINAS GERAIS.

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1 Background

The correct diagnosis of Canine Visceral Leishmaniasis (CVL) have extremely significance both in the veterinary medical routine and in the public health control programs, for the management of the infected or sick dog or for the withdrawal of the dog from the community. Thus, the evaluation of tests used in the veterinary medical routine and in the public health laboratories is of great use to improve the best diagnosis and management of the canine population inserted in the areas of LVC occurrence.

2 Methods

Thus, the objective of this study was to compare the serological results between serum ELISA tests (IDEXX-Snap® CLATK), DPP® CVL rapid test (Bio-Manguinhos®), Biomanguinhos ELISA and Kalazar Detect (Inbios International®) in the serum Of infected and not infected dogs by *Leishmania*. Serum from 25 negative dogs, from non-enzootic areas for LVC and 74 serum of parasitological positive dogs were used. For the negative animals, 25/25 (100%) were confirmed as negatives in the fast ELISA (IDEXX®) and DPP (Bio-Manguinhos®).

3 Results

In the ELISA test (Biomanguinhos®) 23/25 confirmed negative, 1/25 (4%) was undetermined and 1/25 positive (4%) and in Kalazar Detect® 23/25 (92%) confirmed negative and 2/25 (8%) were positive. Among the 74 serum of dogs with positive parasitological tests, the rapid ELISA serological tests (IDEXX®) showed 59/74 (80%) positive and 15/74 (20%) negative, the DPP (Bio-Manguinhos®) had 72 / 74 (97%) were positive and 2/74 (3%) negative, the ELISA (Biomanguinhos®), 59/74 (80%) positive, 13/74 (17%) negative and 2/74 (3%) were indeterminate and Kalazar Detect®, which demonstrated 60/74 (81%) positive and 14/74 (19%) negative results.

4 Conclusions

There was similarity between the rapid ELISA (IDEXX®) and DPP (Bio-Manguinhos®) tests in relation to negative serum, with 100% assertiveness. Biomanguinhos® and Kalazar Detect® ELISA tests showed similar results with 92% of assertiveness. Concerning to positive parasitological tests, none of the tests reached 100% detection, being the DPP test (Bio-Manguinhos®) the most effective, followed by ELISA (Biomanguinhos®), Kalazar Detect® and Rapid ELISA (IDEXX®) respectively, with discrete differences between them. In this way, we can conclude by the proximity between the tests, being necessary the combination between them for greater assertiveness in identifying true positives and negatives.

C1792 THE ROLE OF THE DOG AS A RESERVOIR FOR VISCERAL LEISHMANIASIS

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1 Background

Zoonotic visceral leishmaniasis (ZVL) is an important public health problem in Albania. An effort to control this disease involves understanding the epidemiology of this infection in both humans and reservoir dogs.

2 Methods

Method During the first half of 2015, 16 human cases of ZVL were reported to the Institute of Public Health in Tirana. Blood collection from household dogs of these human patients and from the dogs living in the same area, but not belonging to patients was conducted in June and July of 2015. Nineteen areas were surveyed in total. The sera of dogs were examined by ELISA to detect anti-*Leishmania* antibodies and blood was used for detection of kinetoplast DNA by real time quantitative PCR. For each case, a detailed questionnaire was completed. Bio-statistical and geo-spatial methods were used for statistical analysis.

3 Results

Out of 305 dog sera, 10 resulted seropositive (3.3%) (95%CI 1.54 – 5.80) for *Leishmania* antibodies and 7.2% (22/305) had borderline results. Most of the cases, (8/305; 2.6%) were from rural areas and only 0.65% (2/305) from urban settings. We found 1.3% of the cases in mountainous areas followed by hilly and plain areas with ~1% each, respectively. Clinical signs compatible with canine leishmaniosis such as dermatitis, alopecia, ulcers, onychogryphosis, pale mucosal membranes, ocular lesions, or weight loss were found in 3 dogs in two localities.

4 Conclusions

In conclusion, this study indicates that *Leishmania* infection is present in dogs in several areas in Albania. Canine leishmaniosis represents a threat to human public health. It is therefore necessary to implement strategies for the surveillance and control of canine leishmaniosis which could reduce the incidence of leishmaniasis in humans.

C1803 RESERVOIRS OF LEISHMANIASIS IN THE SOUTH-WEST OF MADRID AUTONOMOUS COMMUNITY, SPAIN. OVERLAPPING OF BIOLOGICAL CYCLES.

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1 Background

Traditionally, in the Autonomous Community of Madrid (CAM), leishmaniasis was observed in rural or peri-urban areas. However in an urban area of the south-west of Madrid, the number of new human cases has been increasing since 2010. The incidence escalated from 3/100 000 inhabitants in 2009 to 55.7 in 2011. Interestingly, the infected individuals resided near a park where there was a high population of hares and rabbits.

2 Methods

During the period 2012-2016, we have analysed 4404 samples (spleen and skin) from 1750 wild animals (rabbits n=1147 and hares n=610) by *Leishmania* nested-PCR. On the other hand, the *Leishmania* seroprevalence in dogs was studied at the outbreak start (2012) and in the later years (2014-2016) by IFAT. In addition, the molecular characterization of Leishmania isolates obtained from samples of rabbits, hares and dogs was carried out. These results were compared with the data previously obtained from human samples.

3 Results

L. infantum was detected in 21,66 % of the animal tested: 17,8 % rabbits and 28,69 % hares. Canine seroprevalence increased from 1,6% in 2012 to 7,1% in 2016. In the our study area, the *Leishmania* molecular characterization shows that genotype ITS-type-Lombardi are circulating among the human, hares and rabbits, however the ITS-type-A is isolated only from dogs.

4 Conclusions

Our results show that rabbits and hares from the south-west of Madrid can be considered as reservoirs of *L. infantum*. Data show there are a traditional domestic/urban cycle and other wild/peri-urban of leishmaniasis transmission and they are overlapping by proximity. The increase in the population of wild animals contributes to the *Leishmania* spread and its re-emergence; and therefore, leishmaniasis is becoming a serious public health problem despite the traditional control measures.

C1815 RETROSPECTIVE STUDY ON FELINE LEISHMANIOSIS: CASE REPORTS AND PREVALENCE STUDIES CONDUCTED IN SPAIN (1999-2016).

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1 Background

Leishmaniosis is a zoonotic disease caused by intracellular protozoa of the genus *Leishmania* and transmitted by sandflies. Despite dogs are considered its main reservoir, feline leishmaniosis has been reported in South America, USA and Europe, and it has been suggested that cats can play a role in the transmission of the infection. In Spain, a few clinical cases have been reported, and a few studies have been conducted measuring prevalence and evaluating molecular diagnostic methods in cats. The aim of this study was to review case reports, and prevalence studies conducted in Spain from 1999 to 2016, and to describe non-reported clinical cases of feline leishmaniosis attended at the veterinary teaching hospital of the Veterinary School (Universidad Complutense de Madrid).

2 Methods

Scientific databases such as PubMed, Web of Science, Elsevier, and Google Scholar were used. Clinical records of cats attended at the UCM veterinary teaching hospital, and records of IFA and PCR performed at our lab along the same period were also searched.

3 Results

Eight publications describing clinical cases were found, with a total of 24 clinical cases reported of feline leishmaniosis. Three cats were diagnosed with leishmaniosis at the UCM veterinary teaching hospital (data unpublished). Skin lesions were the most common clinical picture observed. Ocular, hepatic and renal involvement was also reported. Prevalence studies showed values ranging from 3.2% to 13.2% even serological diagnostic methods performed and cut-off values established were different. All case reports and seroprevalence studies were from the Mediterranean basin and Madrid, endemic areas for canine leishmaniosis.

4 Conclusions

Up to date, feline leishmaniosis maybe considered uncommon in Spain but reporting new feline leishmaniosis cases can be challenging, as diagnostic methods commonly used for dogs can be inconclusive in cats and prevalence of *L. infantum* infection in cats remains unknown in many regions of Spain.

C1820 MOLECULAR IDENTIFICATION OF LEISHMANIA PARASITE AND MORPHOLOGICAL CHARACTERIZATION OF RODENTS AS RESERVOIR HOSTS OF ZOONOTIC CUTANEOUS LEISHMANIASIS (ZCL) IN ILAM PROVINCE, EAST OF IRAN

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1 Background

Zoonotic Cutaneous Leishmaniasis (ZCL), a neglected tropical disease is a public problem with an increasing infected in some areas of Ilam province. Rodents belonging to the subfamily Gerbillinea are the main reservoir host of ZCL in endemic foci of Iran and other countries. Because of rodent burrows are long standing and have special features in the landscape many other animal species such as *Phlebotomie* sand flies use them as shelter.

2 Methods

Wild rodents were collected using live traps, from rural areas of Mehran and Dehloran in Ilam province. External characters including Head-Body length (HBL), Tail Length (TL), Ear Length (EL), and Hind Foot Length (HFL), were measured by using rural. For cranial measurement rodent skull was boiled in distilled water. Blood samples were taken from the heart under anesthetic conditions.Laboratory diagnosis of Leishmaniasis accomplished by demonstration of parasites relevant tissues by light microscopic and examination of stained specimens, or detection of parasite DNA in tissue samples. DNA was extracted from tissue and blood of each rodent using phenol chloroform and ISH-Horovise method. *cyt* B, ITS and cytochrome oxidase II (COII) genes, were amplified. PCR product was sequenced and analyzed using molecular software.

3 Results

At first we detected *Leishmania* parasite Based on mitochondrial DNA cytochrome B (*cyt* B) gene, at 3 subspecies *Tatera indica, Nesokia indica and Meriones* lybicus in Ilam. Each species of rodents had specific sequences of *cyt* B gene. New specific primers of ITS1F/ ITS2R4 and COIIF/COIIR were designed for detection of *Leishmania* species.

4 Conclusions

All three rodents sampled in Ilam were known as principal and potential reservoir hosts of ZCL in Iran. DNA of all collected samples from different regions were extracted and amplified from the mentioned genes (*cyt B, ITS and COII*). Sequenced samples were shown that this rodent have a unique sequence (haplotype).for detection of *Leishmania* parasite with mitochondrial and nuclear genes, COII is more specific than other genes for identification of *Leishmania* species.

C1861 POPULATION DYNAMICS AND PREVALENCES OF VISCERAL LEISHMANIASIS IN FREE-ROAMING DOGS: A CAPTURE AND RECAPTURE STUDY

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1 Background

The existence of free-roaming dogs is regarded as a public health and animal welfare issue. The proper understanding of these animals' ecology and of canine visceral leishmaniasis (CVL) occurrence in them is useful to build mathematical models aiming to improve the interventions to control these populations and the disease. The present study analyzed the population dynamics and a panel of prevalences of CVL in unrestricted dogs followed in a municipality in Brazil.

2 Methods

We used capture and recapture procedures, with analytical methods based in open populations models. During 14 months, for each 2 months, captures, tags, releases and recaptures of dogs in two regions were conducted. In one of these, the animals were sterilized. Captured dogs were conducted to the Reference Center of Health Surveillance of the city, where they underwent clinical tests and were screened for Canine Visceral leishmaniasis (CVL) by mean of serological tests RIFI and Elisa. Seropositive dogs were euthanized, according to the recommendations of the Brazilian Ministry of Health. Dogs with seronegative diagnosis were microchipped. After the results of LVC diagnosis, and total rehabilitation of the surgical procedure (Area B dogs), animals were placed back on the same place where they had been captured. Recaptured animals were re-examined.

3 Results

The presence of 148 females and 227 males was estimated during the period of study. The average dog/man ratio estimated was 1 dog for each 42 and 51 human beings, in the areas without and with sterilization, respectively. There was a tendency of increase in the population size in both regions. The survival rates were low, however, the mortality decreased as the study was conducted. There was a higher entry of males in the population and a remarkable population turnover, i.e., a large number of substitutions of dogs that died or emigrated, by dogs which were born or immigrated. Twenty four dog were euthanized due to the positive serology for LVC. The initial prevalence of CVL was 11% and decreased as the study progressed. The last prevalence was 3%.

4 Conclusions

Results presented point out that sterilization might not be a viable and effective strategy in regions with low availability of resources and high abandon rates. The large number of entries and deaths made the canine free-roaming population younger and more susceptible to infections as CVL. On the other hand, the decrease in CVL prevalences is an indication of the efficiency of the control strategy implemented.

C1884 INTEGRAL APPROXIMATION TO STUDY DIDELPHIS MARSUPIALIS AND CANIS FAMILIARIS AS POSSIBLE RESERVOIRS OF L. PANAMENSIS IN SYLVATIC AND SUBURBAN CYCLE OF COLOMBIA

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1 Background

The knowledge of leishmanial reservoirs is key to the control of leishmaniasis. Many mammals have been proposed as *Leishmania* reservoirs. Dogs and opossums are animals highly adaptable to human disturbance and many studies indicate high infection rates of these animals in endemic foci of American Cutaneous Leishmaniasis (ACL). Our aim is to determine the role of *Didelphis marsupialis* and *Canis familiaris* as reservoirs of *L. panamensis* in Colombian sylvatic and suburban endemic areas.

2 Methods

Two areas in Colombia were selected: (i) the biogeographical region that borders Panamá, where sylvatic transmission has been identified and (ii) the Andean region, with suburban transmission. Samples of blood and skin were collected. The process for identification and genetic analysis included isolation in culture, IFI, PCR with Hsp70, Multilocus enzyme electrophoresis (MLEE), multilocus sequence typing (MLST), single locus typing with ITS. For parasite load determination, qPCR was employed and finally xenodiagnoses was carried out.

3 Results

There were not statistics difference on animals seroprevalence between study areas, otherwise the infection frequency analyzed by PCR was very low (3%) on the sylvatic area and high (40%) on the suburban area. The parasite load measured with qPCR behaved similarly. *L. panamensis* was the unique identified species and it has very high genetic homogeneity. Only two out of five opossum were xenodiagnostic positive by microscopy on the sylvatic area and five out of eight canines were positive by PCR of the insects used on this test in the suburban area. In the suburban area, the infection frequency changed along the year, getting close to zero in some months.

4 Conclusions

the role of animal changes according to the environment, although some results show low capacity of the animals as reservoirs, their role in the dynamics of transmission must be considered and further studied. The present holistic approach must be considered for future reservoir study.

C1906 IN SEARCH OF SYLVATIC RESERVOIRS OF LEISHMANIA IN PARAGUAY: RODENTS AND MARSUPIALS

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1 Background

In the Americas, protozoa of the *Leishmania* genus are responsible for causing pathological conditions in humans, known as leishmanioses. These diseases have diverse clinical manifestations, ranging from painless skin ulcers to invasion of internal organs, typical of the visceral syndrome. In Paraguay, *Leishmania* species are transmitted by hematophagous insects of the *Lutzomyia* genus, and require mammalian reservoirs to complete the biological cycle. The Departments of Caaguazú, San Pedro, Canendiyú and Alto Paraná, mainly, are endemic areas of transmission of *Leishmania (V.) braziliensis*. The mammalian reservoir of this species, responsible for causing mucocutaneous leishmaniasis in humans, is unknown until now. The only documented research on reservoirs, made in the 1950s by González and Arce Queirolo, could not find *Leishmania* in all the specimens studied.

2 Methods

In the present study, 497 small rodents and marsupials were captured in endemic areas for leishmaniasis in Paraguay, between 2006 and 2009. The captures were performed in rural areas of Departments of San Pedro, Canendiyú, Alto Paraná y Caazapá, and managed according to CIOMS/WHO normative. In search of *Leishmania*, samples were taken from both skin and spleen, and destined to: NNN culture, inoculation in gold hamsters (*Mesocriscetus auratus*) and PCR of kDNA. In order to determinate the species of mammals captured, biometric data of each animal were taken. Also, skulls were prepared to access the craniometrics data and the dental formula.

3 Results

Five small rodents showed suspicious lesions in skin. Twenty seven rodents (belonging to genus *Orizomys, Callomys, Akodon, Oligoryzomys* and *Nectomys*) and 2 marsupials (*Didelphis*) were found positive for PCR of *Leishmania* genus. Most of them were positive for *L. braziliensis* complex. Parasitological techniques were unsuccessful to isolate *Leishmania*.

4 Conclusions

These results obtained give useful evidence about the possible role of these small mammals as *Leishmania* reservoirs. More epidemiological research will be necessary in order to demonstrate the real role of these mammals in the biological cycle of *L. braziliensis* in Paraguay.

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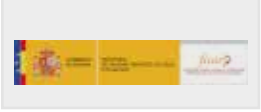

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