Chagas’ disease: Challenges in Diagnostics

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Aims of KIT Biomedical Research

To contribute to the health of people living in low and middle income countries:

To contribute to infection control by developing and making available simple and robust diagnostic tests and methods

To evaluate and implement new treatment regimes

To contribute to capacity strengthening and policy development

To raise awareness and disseminate information within developed and developing countries
Triatome Bug Stages

1. Metacyclic trypomastigotes in hindgut
2. Trypomastigotes can infect other cells and transform into intracellular amastigotes in new infection sites. Clinical manifestations can result from this infective cycle.
3. Amastigotes multiply by binary fission in cells of infected tissues.
4. Intracellular amastigotes transform into trypomastigotes, then burst out of the cell and enter the bloodstream.
5. Epimastigotes in midgut
6. Trypomastigotes (trypomastigotes ingested)
7. Multiply in midgut
8. Metacyclic trypomastigotes in hindgut

Human Stages

2. Metacyclic trypomastigotes penetrate various cells at bite wound site. Inside cells they transform into amastigotes.

= Infective Stage
= Diagnostic Stage
Triatoma infestans

www.DNDi.org
Estimated global population infected by *Trypanosoma cruzi*, 2009

Sources:
Challenges in Diagnosis

• Currently estimates of Chagas patients are between 8-14 million people. Important to have a better estimate of the number of people infected with Chagas’ disease.

• Crucial for the blood banks and screening programs to have access to accurate diagnostics that can be used in the field, remote situations – simple, quick tools

• Easy read out systems for remote areas

• Diagnostics than can detect infection in chronic asymptomatic stage of the disease and identify all sub-lineages of *T. cruzi* at equal sensitivity – very low parasitaemia

• Diagnostics that can detect active infection – used for treatment outcome and “test of cure”

• Standardisation and quality control for diagnostics
Serological testing:

Rapid diagnostic test: STATPAK: serological test. Detection of antibodies to *T. cruzi*

Used as screening tool by MSF and control programs in the field

<2$ per test, ~93 - 98% sensitive & 97% specific Chippaux et al.(2009)
– Test time 10 minutes

• Conclusive diagnosis may need multiple serological tests (WHO)
• Call for more RDTs with higher sensitivity and specificity (Roddy *et al.* 2008 MSF)

www.chembiodiagnostics.com/products.html
Serological testing:

ELISA (test used in the film)
  Immunosorbent and recombinant
HAI: indirect hemaglutination
IFA: indirect immunofluorescence
Test time 2-3 hours, requires skilled technicians and specialised equipment, not available at the point-of-care.

However, serology can cross react with other disease, *T. rangeli* and Leishmania especially – 2 tests needed (WHO)

Xeno-diagnostics
Cages of nymphs (approx 10 per cage) feed on patient
Intestines dissected out, light microscopy used to detect parasites
Test time up to 90 days
Molecular diagnostics

- PCR
  PCR – exponential amplification of DNA from single strand of DNA
  Sensitive and specific
  Many PCR strategies, “in-house primer sets”
  629 articles
  Deborggraeve et al. 2009 developed easy read out PCR oligo-chromatography dipstick in standardised assay.
Molecular diagnostics

- **PCR**
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- **Quantitative PCR**
  Several rt-PCRs developed, quantify parasitaemia
  Test for treatment outcome
  Duffy *et al.* 2009 recently recommended an RT-PCR strategy for monitoring clinical reactivation and treatment outcome for Chagas’ patients: closed tube, standardised commercial kit. Follow up of patients is possible

All of these methods are expensive, specialised equipment, staff & DNA extraction.
Isothermal reactions

Isothermal reactions operate at one temperature and, therefore, do not require PCR machine – can be used in more remote settings
As sensitive and specific as PCR

NASBA
Nucleic-Acid sequence based amplification
RNA amplification, - 41°C Test of cure

LAMP – Loop-mediated Isothermal amplification
60-65°C for 40-60 minutes.
Complex design of primers

RPA
Re-combinase polymerase activity
~40 °C 60 minutes stable, dipsticks developed

Bohmer et al 2009; Adams et al. 2009 in submission; Piepenburg et al. 2006
Does this affect the Netherlands?

Screening programs

Contamination of blood supplies in Latin America, the US and Spain has lead to testing of blood donors. Many countries in Latin America screen with two serological tests for antigens to Chagas from blood donors. FDA (US) released guidance in March 2009 using ELISA testing on all new blood donors. In the film the public health workers had no clue what Chagas was.

Few reports in the Netherlands (Marcu et al. 2007)
No screening in place, so can not say if there are more people infected.

Some case reports in Suriname (Oostburg et al. 2003)

What happens if we/ USA / Spain/ Latin America screen?
What happens when patients are positive? No effective treatments, drugs may not be licensed
Should pregnant women previously living in endemic areas of Latin America be screened to prevent vertical transmission?

Recent study in Switzerland revealed out of 72 pregnant women from Latin America 9.7% infected with Chagas – now screen all pregnant women from Mexico, Central and South America (Jackson et al. 2009, PloS NTD)

Similar prevalence found in Spain 4.3% (Arandes et al. 2009), however no control program yet initiated.

Drugs are more effective in the acute stage – so early diagnosis best

Should the Netherlands screen pregnant women from endemic areas?
Challenges in Diagnostics

Serological tests available:
Need quality control, comparison tests – very useful at point of care

Molecular tests also available:
Offer sensitive, specific diagnostics, can be standardised
Test of cure can be developed - Specialised staff, expense of equipment

Isothermal reactions:
Not yet available, a good alternative?

Discussion points...

Can we contribute to Chagas’ diagnostics with expertise in this room?
Should the Netherlands screen for Chagas’?
Should pregnant women from endemic areas be screened?