Visceral leishmaniasis is common in less developed countries, with an estimated 500,000 new cases each year. Because of the diversity of epidemiological situations, no single diagnosis, treatment, or control will be suitable for all. Control measures through case finding, treatment, and vector control are seldom used, even where they could be useful. There is a place for a vaccine, and new imaginative approaches are needed. HIV co-infection is changing the epidemiology and presents problems for diagnosis and case management. Field diagnosis is difficult; simpler, less invasive tests are needed. Current treatments require long courses and parenteral administration, and most are expensive. Resistance is making the mainstay of treatment, agents based on pentavalent antimony, useless in northeastern India, where disease incidence is highest. Second-line drugs (pentamidine and amphotericin B) are limited by toxicity and availability, and newer formulations of amphotericin B are not affordable. The first effective oral drug, miltefosine, has been licensed in India, but the development of other drugs in clinical phases (paromomycin and sitamaquine) is slow. No novel compound is in the pipeline. Drug combinations must be developed to prevent drug resistance. Despite these urgent needs, research and development has been neglected, because a disease that mainly affects the poor ranks as a low priority in the private sector, and the public sector currently struggles to undertake the development of drugs and diagnostics in the absence of adequate funds and infrastructure. This article reviews the current situation and perspectives for diagnosis, treatment, and control of visceral leishmaniasis, and lists some priorities for research and development.


Visceral leishmaniasis, known as kala azar in India, is the cause of much death and disease in less developed countries. It is one of several diseases caused by over 20 species of *Leishmania*; it is transmitted by sandfly bites.1 Cutaneous and mucosal leishmaniasis cause scarring, destruction of the mouth and nose, and severe disability. Typically, patients with visceral leishmaniasis present with fever, cough, abdominal pain, diarrhoea, epistaxis, splenomegaly, hepatomegaly, cachexia, and pancytopenia. Peripheral lymphadenopathy is common in some foci.

*Leishmania donovani* is the primary cause of visceral leishmaniasis in the Indian subcontinent and East Africa, *L infantum* in the Mediterranean region, and *L chagasi* in the New World. The last two species are identical. Human beings are the only known reservoir of *L donovani*. Canines,
especially domestic and stray dogs (figure 1), provide the reservoir for *L infantum* and *L chagasi*. These differences have major consequences for control and for the emergence and the spread of drug resistance.

Visceral leishmaniasis is endemic in 62 countries, with a total of 200 million people at risk, an estimated 500 000 new cases each year worldwide, and 41 000 recorded deaths in the year 2000. As is the case for other tropical diseases, epidemiological data are incomplete, and official figures are likely to underestimate grossly the real prevalence of the disease. Both the number of recorded cases and the geographical areas affected have grown in the past two decades.

Over 90% of cases of visceral leishmaniasis occur in five countries: India (especially the Ganges and Brahmaputra plains), Bangladesh, Nepal, Sudan, and northeastern Brazil. Population displacement as a result of war, drought, famine, or rural-urban migration underlies the recent epidemic in Sudan, which caused a population mortality of up to 36%, and is contributing to the resurgence of the disease in India and its urban spread in Brazil.

There are 30–100 subclinical infections for every overt case of visceral leishmaniasis. Risk factors for development of clinical disease include malnutrition (figure 2), immunosuppressive drugs, and, especially, HIV co-infection. The number of co-infections will continue to rise, notably in India and Brazil, where the rural visceral leishmaniasis epidemic is increasingly coming into contact. Cases of co-infection are seen as an imported disease in non-endemic areas. Co-infected patients may be difficult to diagnose, respond poorly to treatment, and relapse repeatedly.

Visceral leishmaniasis adversely affects productivity and welfare. In India, the disease attacks older children and young adults. Transmission is mostly domestic, but the male preponderance suggests an occupational association with deforestation, agriculture, cattle grazing, hunting, road construction, or water-resources development projects, and migration of seasonal workers. However, in communities where women’s access to health services is limited, many cases of visceral leishmaniasis in women are likely to go undetected.

The lack of reliable data on the morbidity and mortality associated with visceral leishmaniasis has been both the result of and the reason for the scarce resources allocated to the study and control of this disease. In 2000, the disease burden associated with visceral leishmaniasis, measured in disability-adjusted life years, was estimated to be 1 980 000 (1 067 000 for male and 744 000 for female populations).

**Control strategies**

Different geographical regions have different ecological characteristics, with many species of sandflies as potential vectors and some 100 species of animals as potential reservoir hosts. Control strategies are tailored to the two main epidemiological entities: anthroponotic, when human beings are the sole reservoir, and zoonotic, when dogs are the major source of infection for the vector. In either situation, efficient case management based on early diagnosis and treatment is the key to limit morbidity and prevent mortality.

Effective treatment of patients is also a measure to control reservoir and transmission in anthroponotic foci, particularly for cases of dermal leishmaniasis after kala azar, which are thought to act as a long-term reservoir of the disease. In addition, vector control should be implemented wherever feasible. Used together, the two strategies have been shown to control visceral leishmaniasis in India. Spraying of houses with residual insecticides has been an important measure in the past in India but is not much used now. Insecticides used in malaria-control programmes are effective on leishmania vectors. DDT, being cheap, is the main insecticide used in less developed countries, but the sandfly vector in India, *Phlebotomus argentipes*, is becoming resistant.

In zoonotic foci, the canine reservoir may be important, but dog control represents a major problem because there is no satisfactory strategy currently available. In addition, dogs respond poorly to antileishmanial therapy and require repeated treatment.

To set up an effective control strategy for visceral leishmaniasis is a challenge in endemic areas, which are largely in the poorest countries of the world (India and Bangladesh), in remote places (rural Brazil), or complex settings (civil war in Sudan).

Personal protection may be possible. In foci where sandflies bite at night, impregnated bednets have decreased the incidence of leishmaniasis. One major problem limiting the use and effectiveness of conventional bednets has been the cost of regular reimpregnation. Insecticide incorporated into the polyethylene fibre of bednets might limit the need for reimpregnation. Vaccines are being investigated for both cutaneous and visceral leishmaniasis, but none is yet ready for use.

**Diagnosis**

Diagnosis and treatment follow-up pose a challenge to physicians working in endemic areas. Classically, the...
diagnosis of visceral leishmaniasis is confirmed by demonstration of the parasite. Intracellular leishmania can be identified or cultured from aspirates of spleen, bone marrow, lymph node, or liver. The diagnostic yield is highest, about 98%, for spleen aspirates, which have been used for routine diagnosis in the field, for example in Kenya and Sudan (figure 3). But there are contraindications, precautions are necessary, and complications, though rare, may be serious.

Serological techniques have been adapted for field use. In Kenya, ELISA was 98% sensitive and 100% specific, but there is no commercial kit. Direct agglutination (DAT) is easy to use in the field, and cost-effective, but there is no commercial source of antigen and results are not always reproducible. Testing with a commercially available immunochromatographic strip that uses recombinant leishmanial antigen K39 has proved 100% sensitive and 98% specific in India. Many centres have been evaluating the use of PCR, especially on peripheral-blood samples. PCR is now sensitive to the level of one parasite, and it has been used successfully for diagnosis of visceral leishmaniasis in children in Italy, and for monitoring of relapse in HIV-co-infected patients. But PCR is still not easily usable in the field, where confirmation of clinical diagnosis commonly remains a problem, and patients may not seek medical attention for many months. The mean delay from onset of symptoms to definitive diagnosis was 7.7 months (SD 6.0) in a study in India, and 27.6% of cases were diagnosed longer than 9 months after onset of disease.

Clinical follow-up is generally adequate to detect relapse in immunocompetent patients, but in immunosuppressed patients a non-invasive method of detecting parasite persistence or relapse would be useful. PCR might be suitable.

Table 1. Summary of characteristics of antileishmanial drugs in current use.

<table>
<thead>
<tr>
<th>Drug Regimen</th>
<th>Trade names</th>
<th>Cost (US$)</th>
<th>Total cost (US$)</th>
<th>Issues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pentavalent antimonials</td>
<td>Pentostam (GlaxoSmithKline); Stibanate (Gluconate Ltd, India); Generic Sodium stibogluconate (Albert David Ltd, India); Glucantime (Aventis Pharma)</td>
<td>15–150</td>
<td>629</td>
<td>Resistance, toxicity in HIV co-infection; Higher costs and toxicity when long courses used because of resistance; Quality and price of generic vs proprietary products</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>7–20 mg/kg total dose for up to 20 days, intravenously</td>
<td>Fungizone (Bristol-Myers-Squibb, USA)</td>
<td>60–150</td>
<td>454</td>
</tr>
<tr>
<td>Liposomal amphotericin B</td>
<td>10–20 mg/kg total dose in 5–10 doses over 10 days, depending on geographical area, intravenously</td>
<td>Ambisome (Gilead [NeXstar] and Fujisawa Healthcare USA, Inc)</td>
<td>1000–2500*</td>
<td></td>
</tr>
<tr>
<td>Colloidal dispersion</td>
<td>10–15 mg/kg total dose over 5 days</td>
<td>Amphocil (Liposome technology, Sequus, and Zeneca)</td>
<td>300</td>
<td>458</td>
</tr>
<tr>
<td>Lipid complex</td>
<td>15–30 doses over 3–4 weeks</td>
<td>Abelcet (Liposome Co)</td>
<td>60–150</td>
<td></td>
</tr>
</tbody>
</table>

*Gilead [NeXstar] has an agreement with WHO to provide the drug at a special rate in developing countries: 1 vial free for every 3 vials bought.
Visceral leishmaniasis

Treatment
The therapeutic arsenal against visceral leishmaniasis is limited; the available agents with established efficacy are all injectable. Antimonial drugs, the mainstay of treatment, can no longer be used in northeastern India, where the incidence of visceral leishmaniasis is highest, because of resistance (figure 4). Traditional second-line drugs (pentamidine and amphotericin B) are more toxic and difficult to administer; newer formulations of amphotericin B are not affordable in less developed countries. The first oral medication for visceral leishmaniasis, miltefosine, is now registered in India, but its use elsewhere has not yet been studied. The development of a second oral drug, sitamaquine, is very slow. Trials necessary for the registration of paromomycin (aminosidine) have stalled for lack of funds. Optimum treatment for HIV-co-infected patients has yet to be established.

Current options for treatment
Solustibosan (sodium antimonyl gluconate) was the first pentavalent antimonial agent to be reported as active against kala azar in China and India, in 1937. Pentavalent antimonials (sodium stibogluconate and meglumine antimoniate) are still the mainstay of therapy for leishmaniasis in most of the world (table 1).

Antimonials have several disadvantages: patients have to be admitted to hospital for 3–4 weeks for parenteral therapy; toxic effects may limit the drugs’ use (arthralgia, nausea, abdominal pain, and chemical pancreatitis); HIV co-infected patients are particularly prone to clinical pancreatitis); long-term use at higher doses to combat resistance is restricted by cardio toxicity (ST-segment inversion, QTc prolongation, and, possibly, fatal arrhythmia); brand-name products are expensive; there is a general problem of quality and batch-to-batch variability for both branded and generic drugs; and the poor quality of some generic formulations of the drug in India has led to serious toxicity. The efficacy and safety of generic (Albert David Ltd, Cuttaca) and branded sodium stibogluconate (Pentostam, GlaxoSmithKline) were compared in randomised trials under field conditions in Sudan and Kenya under the auspices of the non-governmental organisation Médecins Sans Frontiéres. No difference was detected, and the investigators concluded that this generic antimonial could be used safely and effectively for the treatment of visceral leishmaniasis. The International Dispensary Association (Amsterdam) has agreed to undertake quality-assurance control and distribution of this product.

The use of antimonials is threatened by the emergence of parasite resistance. Relapse after inadequate treatment with a single drug selects resistant mutants, which are recycled in anthroponotic foci with high rates of transmission. In Bihar, India, up to 65% of new patients with visceral leishmaniasis show primary unresponsiveness, which is associated with in-vitro evidence of antimony resistance. Elsewhere in India, and in Africa and Brazil, primary unresponsiveness is rare. However, the epidemiology of the disease in Sudan suggests that resistance to antimonials should be expected there soon.

Amphotericin B is the current alternative treatment of choice. Its drawbacks are cost, limited availability in some areas, and toxicity—notably infusion-related side-effects (fever, chills, bone pain, thrombophlebitis) and hypokalaemia, renal impairment, and anaemia. These problems are generally tolerable at the doses used in Bihar, where conventional amphotericin B is now the first-line drug in kala azar treatment centres. Though more expensive than the Indian antimonials, amphotericin B has a cure rate of more than 97%, and resistance has not been reported.

Three lipid-associated formulations of amphotericin B are highly effective against visceral leishmaniasis and better tolerated than the conventional preparation. Liposomal amphotericin, Ambisome, was studied through the TDR Programme of WHO in India, Kenya, and Brazil; the minimum doses necessary to provide about 95% cure rates were 6 mg/kg, 14 mg/kg, and 21 mg/kg, respectively. 21 mg/kg is also necessary for Mediterranean visceral leishmaniasis. In an attempt to shorten further the necessary hospital stay, Sundar and colleagues administered a single dose of Ambisome (5 mg/kg) to 46 patients with visceral leishmaniasis; 42 were cured. But even this one dose is prohibitively expensive. The two less expensive preparations of amphotericin B (lipid complex and colloidal dispersion) have been tried in India and Brazil. Efficacy was good, but transfusion-related toxicity was a problem. The high cost at present makes these efficient agents of almost no practical value in less developed countries.

Pentamidine (isethionate or methansulphonate) is progressively being abandoned as second-line treatment for visceral leishmaniasis, because of toxicity and resistance in India, but it might yet find a place for maintenance treatment in HIV co-infection.
HIV-co-infected patients present special problems; they respond slowly to treatment and relapse rates are about 60% in the first year, whichever drug is used. There have been no trials of secondary prophylaxis. The use of highly active antiretroviral therapy may control relapses. India and other less developed countries will not be able to afford these drugs. As a result, relapse and secondary resistance may develop to second-line drugs.

**Perspective in research and development**

One drug is just becoming available in India and two more are in development phases (table 2). Although this progress is encouraging, these drugs are more incidental discoveries than products of a genuine targeted research effort. The substantial knowledge of parasite biology is not yet translating into novel drugs for leishmaniasis.

### Table 2. The research and development pipeline

<table>
<thead>
<tr>
<th>Drug</th>
<th>Institution developing the drug</th>
<th>Status</th>
<th>Expected time to marketing</th>
<th>Issues and problems</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miltefosine</td>
<td>Zentaris (ex Asta Medica)</td>
<td>Registration</td>
<td>Registered in India</td>
<td>Pricing Teratogenicity, restrictions of use in labelling</td>
</tr>
<tr>
<td>Paromomycin (aminosidine)</td>
<td>TDR (discovered by Farmitalia-Carlo Erba)</td>
<td>Phase III with new formulation. Gabromicina (ex Farmitalia-Carlo Erba), no longer produced</td>
<td>1 year?</td>
<td>Regulatory dossier incomplete (lack of funding to complete studies) Potential antimony replacement; can be used in combination with antimonial or other drugs Expected cost of treatment US$45 ($332 including hospital stay)</td>
</tr>
<tr>
<td>Sitamaquine (WR6026)</td>
<td>GlaxoSmithKline (discovered by Walter Reed Army Institute of Research)</td>
<td>Clinical trial phase II</td>
<td>4 to 6 years?</td>
<td>Walter Reed Institute did most of the expensive toxicity and pharmacology work, so the drug could be quite cheap</td>
</tr>
</tbody>
</table>

### Table 3. Summary of the current situation and needs

<table>
<thead>
<tr>
<th>Burden of disease</th>
<th>Current situation, main problems</th>
<th>Research and development needs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imprecise data on incidence</td>
<td>Imprecise data on incidence (estimated at 500 000 new cases each year)</td>
<td>Improve reporting systems to obtain accurate mortality and morbidity data, based on geographic reliable information</td>
</tr>
<tr>
<td>Poverty is a major determinant</td>
<td>and appreciation of disease burden may underestimate needs</td>
<td>Studies on socioeconomic and behavioural risk factors</td>
</tr>
<tr>
<td>Malnutrition is a risk factor</td>
<td>HIV coinfection; the spread of HIV epidemics will increase the number of VL cases and the economic burden of VL in developing countries</td>
<td>Cost-effectiveness analysis of control strategies</td>
</tr>
<tr>
<td>Vector control</td>
<td>Residual-insecticide house spraying has high cost, low sustainability, and logistic constraints that hamper its efficacy</td>
<td>Larger-scale studies to investigate promotional, distribution, and implementation methods of bednets in operational situation in coordination with malaria-control programmes</td>
</tr>
<tr>
<td>Limited information on use of bednets</td>
<td></td>
<td>Studies on the cost-effectiveness of prevention strategies in different epidemiological settings</td>
</tr>
<tr>
<td>Prevention of disease</td>
<td>No vaccine available</td>
<td>Promote research and evaluation of insecticide-impregnated bednets</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>Difficult to diagnose clinically in early phases; treatment is potentially toxic and expensive, so diagnosis should be confirmed by laboratory methods; improved diagnosis techniques may reduce inappropriate drug use because of poor availability of simple diagnostic tools for leishmaniasis</td>
<td>Development of an affordable and effective vaccine as a long-term objective</td>
</tr>
<tr>
<td>Treatment</td>
<td>All drugs in use require parenteral administration except miltefosine; Antimony resistance in the areas with the highest burden (India); Cost of medication and care of patients Amphotericin B in lipid formulations, the most effective drugs available to date, are prohibitively expensive even with shortened regimens Few drugs in the pipeline; Paromomycin is currently not available; completion of studies has been postponed owing to lack of funding.</td>
<td>Improve sensitivity and specificity of rapid tests and adapt them to field situations; multicentre, comparative evaluation of new test, DAT freeze-dried antigen, K39 plus K26, antigen detection in urine; lack of commercial sources of these antigens and tests Assess the validity of rapid tests for leishmaniasis–HIV coinfections Produce affordable diagnosis tests</td>
</tr>
</tbody>
</table>

**VL=visceral leishmaniasis.**
Visceral leishmaniasis

Milletofosine, an alkylphospholipid that was originally developed as an oral antineoplastic agent, is the most advanced drug in development, with substantial contributions from public funds (WHO/TDR). The drug has just received marketing authorisation in India, and Zentaris intend also to register it in Europe. The practical and legal features of its distribution need to be determined by the local authorities, before the drug is released. It is the first highly effective orally administered treatment for visceral leishmaniasis. Dose-finding studies have identified the daily dose of 100 mg/day for 4 weeks for adults and 2·5 mg/kg for children, with cure rates of about 95%. Gastrointestinal side-effects are common but rarely interfere with treatment. Phase III trials comparing the drug with conventional amphotericin B are completed though not yet reported. Nevertheless, a few problems persist that may limit use of miltefosine. It is an abortifacient and a potent teratogen and is toxic to male gonads in dogs. Male fertility data from patients in the phase 3 trial show that the drug is as safe as amphotericin B. The long half-life of miltefosine (2–3 weeks) and its narrow therapeutic index might favour the emergence of resistant mutants. Combination therapy should be considered, to delay the emergence of miltefosine resistance, particularly in anthroponotic foci where resistance could quickly spread. The price of the drug is currently under discussion, but to be useful in endemic countries it should be in the range of, and preferably lower, than that of current first-line treatment options.

Paromomycin (aminosidine) is an old aminoglycoside antibiotic with unique antileishmanial activity. It acts synergistically with antimony in vitro. Clinical trials for visceral leishmaniasis have been carried out in India and Africa (Kenya and Sudan), and in complicated cases imported into the UK. Other studies were done with paromomycin in combination with antimony. The drug is effective, well tolerated, and as cheap as conventional amphotericin B, but it must be administered parenterally. The traditional formulation by Farmitalia Carlo-Erba (now Pharmacia Corporation) is no longer available, and a new formulation produced by the International Dispensary Association must undergo a final phase 3 clinical trial before it can be submitted for registration. These studies, to be undertaken by WHO/TDR in conjunction with a commercial partner, have been postponed for 4 successive years because of lack of funding. We hope that funds may be found urgently to complete the regulatory dossier of this valuable drug.

Sitamaquine (WR6026) is an oral 8-aminoquinoline, the development of which has been slow. It has been under development for over 8 years by SmithKline Beecham (now GlaxoSmithKline) and the Walter Reed Army Institute of Research. To date, little is known about its efficacy and toxicity or prospect of eventual availability. The results of dose-finding trials in Kenya and India are eagerly awaited. Phase 3 studies are about to start in Kenya and India (J Horton, GlaxoSmithKline, personal communication). In Brazil, a small study produced disappointing results.

The azoles and allopurinol are two of several oral drugs that show weak activity against leishmaniasis, but they have not proved useful when used as single-agent therapy. There are no case reports of cures of immunosuppressed patients when the drugs were used in combination, but there have been no formal trials of combinations.

Where do we go from here?

Although visceral leishmaniasis is not ranked among the leading infectious causes of the global burden of disease, it is a life-threatening disease of great medical, social, and economic importance in its endemic areas (table 3). It causes epidemics in non-immune populations, particularly those already suffering the consequences of war, drought, famine, and economic migration. It is an HIV-associated disease, and the impact of the co-infection will be devastating in the Indian subcontinent and Sudan, and possibly in Brazil and the Mediterranean, where it may change the epidemiology of visceral leishmaniasis. With such a variety of epidemiological situations, no single diagnosis, treatment, or control will be suitable for all.

Measures to control transmission vary according to local epidemiology. Where transmission is intense, case treatment alone has little effect, and control of transmission is more important. In general, however, vector-control measures are difficult to apply and become expensive if DDT is banned or resistance develops. The use of impregnated bednets is limited by cost as well as epidemiological patterns. A combination of measures and imaginative studies are needed to define new methods of reducing human–sandfly contact.

Leishmaniasis, like plasmodium, is a cunning parasite, and development of a vaccine may well prove difficult. The place for vaccination is clear, and attempts should be encouraged. Good serodiagnostic tests exist, but many are not suitable for field use, and tissue-invasive tests are necessary to show what is happening to the parasite load. The real needs are for simple tests on urine or saliva, which are known to contain leishmanial antigens. The development of new diagnostics should favour rapid and simple-to-use techniques (panel).

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### Diagnostic tools in development.

- PCR needs more development for visceral leishmaniasis and will probably remain a research tool.
- Conventional serological testing for IgG antibody (immunofluorescence, ELISA) is sensitive and specific but is not adapted to field conditions.
- DAT was found 96.5–100% sensitive and 91–95% specific. Reproducibility problems have, however, been observed under field conditions due to thermal instability of the antigen and reading problems.
- The freeze-dried antigen may solve stability problems.
- A rapid serodiagnostic test with nitrocellulose strips impregnated with recombinant K39 leishmanial antigen (immunochromatographic test) detects antigens of L donovani and L infantum. The estimated sensitivity and specificity were 100% and 98% in India. When used to test clinically suspected visceral leishmaniasis cases in field conditions in Sudan and Nepal, K39 showed a lack of specificity.
- Further field trials should be done in other countries/endoemic areas.
- Antigen detection is useful when antibody production is impaired, as in HIV-infected patients.
- The detection of polypeptide fractions of 72–75 kDa and 123 kDa of leishmanial antigen in urine of patients with visceral leishmaniasis was 96% sensitive and 100% specific; these antigens were not detectable after 3 weeks of treatment, suggesting a good prognostic value.
- At present there is no agreed uniform strategy.
Research will be necessary to test their applicability in field conditions in the less developed countries where the vast majority of visceral-leishmaniasis cases occur. DNA techniques on finger- blood samples need to be standardised and made commercially available for assay of parasite load and follow-up treatment, especially in areas of drug resistance and in immunosuppressed patients. Increased research and funds are needed, with support from both the public and the private sector, for the improvement and deployment of better, affordable, rapid diagnostic tests for use in isolated villages with little infrastructure where most visceral-leishmaniasis cases occur.

The emergence of drug resistance to pentavalent antimonial drugs and the increased toxicity seen in HIV-co-infected patients signal the end of the 60-year run for these drugs. Oral drugs are needed. The TDR/Zentaris partnership is to be congratulated on achieving registration of miltefosine. Efforts is to be congratulated on achieving registration of paromomycin. Experience of several courses of drug treatment. Antimicrob Agents Chemother 1992; 36: 231–7.

The opinion expressed in this paper are those of the authors and may not reflect the position of the employing organisations.

Conflicts of interest
We declare no conflict of interest.

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Visceral leishmaniasis

Personal view