TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (2000) 94, 465–471

Review Article

Visceral leishmaniasis control: a public health perspective

M. Boelaert[1], B. Criel[1], J. Leeuwenburg[2], W. Van Damme[1], D. Le Ray[3] and P. Van der Stuyft[1] *[1]Department of Public Health, and [3]Department of Parasitology, Institute of Tropical Medicine, Antwerp, Belgium; [2]Regional Public Health Service, Dordrecht, the Netherlands*

Abstract

Visceral leishmaniasis (VL), also known as kala-azar, is a vector-borne disease caused by a protozoan of the *Leishmania donovani* complex. A phlebotomine sandfly transmits the parasite from person to person or via an animal reservoir. VL is a severe, debilitating disease, characterized by prolonged fever, splenomegaly, hypergammaglobulinaemia and pancytopenia. Patients become gradually ill over a period of a few months, and nearly always die if untreated. Case–fatality ratios are high even in treated patients. Worldwide an estimated 500 000 VL cases occur each year. This study reviews clinical, epidemiological and public health aspects of the disease and shows how critical adequate case detection is for the success of VL control. Examination of the issue of VL diagnosis with respect to the global challenges in VL control leads to the observation that a sound diagnostic–therapeutic algorithm for the health services in endemic areas is badly needed. Serological tests could be an alternative to parasitological diagnosis and the direct agglutination test (DAT) was found to fulfil many criteria for a 'field test', including cost effectiveness. Although research needs on vaccine and better drugs continue to be high on the agenda, a VL test–treatment strategy based on currently available highly sensitive serological tests, such as the DAT, should be introduced in the health services in endemic areas.

Keywords: visceral leishmaniasis, disease control, epidemiology, transmission, clinical aspects, diagnosis, chemotherapy

Visceral leishmaniasis, the disease

Aetiological agent and pathogenesis

The leishmaniases can be divided into 3 major clinical syndromes: cutaneous (CL), mucocutaneous (MCL), and visceral leishmaniasis (VL) or kala-azar. VL is caused by *Leishmania donovani* in India and eastern Africa and by *L. infantum/chagasi* in the Mediterranean basin, western Africa and Latin America (WHO, 1990). In contrast to CL and MCL, in which the protozoan parasite is histologically localized, visceral disease is caused by parasite growth within reticuloendothelial cells throughout the body. BERMAN (1997) qualified this as 'an immunologically important experiment of nature in which micro-organisms survive solely within phagolysosomes'. During the active stage of the disease, patients have anergy to leishmanial antigens as indicated by a negative delayed-hypersensitivity skin test. Yet at the same time, they have intense polyclonal B-cell activation, including high levels of anti-leishmanial antibodies (NEVA, 1990). Established VL represents the failure of specific cell-mediated immunity to control the infection (SUNDAR *et al.*, 1997a). Malnutrition lowers cell-mediated immune responses and has been shown to be a risk factor for VL (CERF *et al.*, 1987). CORKILL (1948) suggested very early that immunodepression of previously infected healthy individuals can result in the development of symptomatic VL. VL has been sporadically described in patients on immune-suppressive therapy (FERNANDEZ *et al.*, 1987; PROCÈS *et al.*, 1997). Since DE LA LOMA *et al.* (1985) published the first casuistic report, the importance of VL has been widely recognized as a co-infection in AIDS (DE GORGOLAS & MILES, 1994; WHO, 1997). The opportunistic behaviour of the parasite has been compared to that of *Mycobacterium tuberculosis* (MONTALBAN *et al.*, 1990). Like tuberculosis, leishmaniasis is now thought to be due to primary infection as well as to re-activation of latent infection.

Transmission cycle

VL is a vector-borne disease and, based on transmission characteristics, 2 types are distinguished: the zoonotic and the anthroponotic form of VL. In the former the parasite is maintained in an animal reservoir (domestic dogs, with foxes, rodents, gerbils as secondary reservoirs) and humans are an occasional host. In the latter form, no animal reservoir exists, and the parasite is exclusively maintained in a human–vector–human cycle. The life-cycle of the parasite has 2 distinct phases: a promastigote phase developing in the gut of the arthropod vector, and an amastigote form which develops intracellularly in the mammalian host. The vector is a phlebotomine sandfly which breeds in warm humid microclimates and is typically found in rodent burrows, termite hills, organic remnants (e.g., cow dung) and rotting vegetation (BRYCESON, 1996). The disease is spread when female sandflies of the genus *Phlebotomus* (Old World) or *Lutzomyia* (New World) ingest amastigotes while taking a blood meal from an infected mammal. These transform to promastigotes within the insect's gut, migrate to the proboscis, and are deposited in the dermis of the new host during the next bloodmeal. Direct person-to-person transmission of VL has occasionally been described (blood transfusion, sexual and congenital transmission, and increasingly, syringe exchange amongst intravenous-drug addicts) (SYMMERS, 1960; NYAKUNDI *et al.*, 1988). The incubation is usually 2–6 months, ranging between 10 days to 2 years (BRYCESON, 1996), but not every infected individual progresses to overt VL disease. There is a spectrum from asymptomatic infection to subclinical disease to the full-blown VL syndrome (HO *et al.*, 1982). Several prospective studies have documented the ratio of incident asymptomatic infections to clinical cases: it varied from 1:2·4 in Sudan (ZIJLSTRA *et al.*, 1994) to 4:1 in Kenya (SCHAEFER *et al.*, 1995), 5·6:1 in Ethiopia (ALI & ASHFORD, 1994), 8:1 in Brazil (EVANS *et al.*, 1992) and 18:1 in Brazil (BADARO *et al.*, 1986). Subclinical or mild forms of visceral disease were described in a Brazilian study (BADARO *et al.*, 1986) as well as in veterans of Operation Desert Storm (MAGILL *et al.*, 1993).

The mammalian host is infectious to the sandfly as long as parasites persist in the circulating blood or in the resident macrophages of the dermis. If humans act as the reservoir host, infectivity for phlebotomines may persist even after clinical recovery, especially when patients develop post-kala-azar dermatitis (PKDL). PKDL cases

Address for correspondence: Dr M. Boelaert, Epidemiology Unit, Institute of Tropical Medicine, Nationalestraat 155, 2000 Antwerpen, Belgium; fax +32 3 247 62 58,
e-mail boelaert @itg.be

are important for the transmission cycle as the nodular lesions contain abundant parasites (ADDY & NANDY, 1992).

Clinical features

Classic kala-azar presents as a prolonged but irregular fever with weight loss, hepatosplenomegaly, lymphadenopathy, pancytopenia and hypergammaglobulinaemia. Thrombocytopenia may cause uncontrollable epistaxis or bleeding from other sites. Differential diagnosis for VL must consider malaria, relapsing fever, typhoid fever, tuberculosis, AIDS, brucellosis, chronic hepatitis, cirrhosis, lymphomas and leukaemia (WHO & ODA, 1996). Disease progression is gradual over a period of 1–4 months. A more acute course of the disease is described in immunologically naive populations (SATI, 1958; HASHIM *et al.*, 1994). Untreated, the disease is fatal, mainly owing to profound cachexia, secondary infections and/or haemorrhage (BERMAN, 1988). In treated VL, reported case–fatality ratios (CFR) vary between 5% and 15% under controlled conditions, and documented causes of death in clinical studies include severe intractable diarrhoea, along with antimonial toxicity (ZIJLSTRA, 1995). Recovery is associated with apparently lasting homologous immunity, whereas cured cutaneous leishmaniasis does not confer protection against kala-azar (BRYCESON, 1996). Skin lesions accompany VL in up to 50% of cases in the Sudan. The initial lesion of VL can be a cutaneous non-itching ulcer, called leishmanioma (MANSON BAHR, 1955; CAHILL, 1964; MANSON BAHR & SOUTHGATE, 1964).

After correct treatment of *L. donovani* VL, 3% of African and up to 10% of Indian cases develop PKDL, a disfiguring skin disease that has been called 'the unhappy sequel of life-saving drug treatment' (DYE & WOLPERT, 1988). Whereas in East Africa PKDL appears shortly after VL symptoms subside (0–6 months), in India the interval is 1–2 years with up to 30 years being reported in exceptional cases. PKDL was first described by BRAMACHARI in India in 1922. It is characterized by a spectrum of lesions ranging from depigmented macules to wart-like nodules over the trunk and face and mimicking the skin lesions of leprosy (ZIJLSTRA *et al.*, 1995; RAMESH & MUKHERJEE, 1995). As PKDL patients are very infectious to sandflies, the syndrome is considered to play a role in disease transmission between outbreaks (DYE & WOLPERT, 1988; ADDY & NANDY, 1992; DYE, 1992). Poor treatment adherence is thought to increase its occurrence, as shown in a prospective community study in Sudan where 56% (48/85) of kala-azar cases developed PKDL after irregular and incomplete treatment (ZIJLSTRA *et al.*, 1995). Mild forms of PKDL self-cure (ZIJLSTRA *et al.*, 1995), but the more severe forms need 4 months or longer treatment with the standard antimonial regimen (RAMESH, 1994). Recurrence of VL after PKDL is uncommon (NANDY *et al.*, 1998).

Public health importance of VL and epidemiological aspects

Zoonotic VL

The zoonotic form of VL (ZVL) is caused by *L. infantum* and occurs in the Mediterranean VL foci, western Africa, tropical and subtropical America, in northern China, western Asia (Iran) and Asia (Pakistan). Domestic dogs are the main animal reservoir for transmission. Whereas in some areas of Portugal, for example, more than 50% of the dogs can be infected, human cases are rare and were mainly described in young children (ABRANCHES *et al.*, 1983). More recently, ZVL has gained attention as an opportunistic infection in AIDS (MONTALBAN *et al.*, 1990; DE GORGOLAS & MILES, 1994). The HIV–VL co-infection has been called 'a deadly gridlock' as both infections tend to mutually reinforce their impact on the immune system (WHO, 1998b). The HIV epidemic induced a major change in the epidemiology of VL in the Mediterranean: from a traditionally zoonotic disease, VL epidemiology involves now person-to-person transmission, either vector borne or through exchange of infected needles. Currently, up to 73% of HIV–VL co-infections are in intravenous-drug users. The latter route raises fear of increased virulence of parasites selected through frequent mechanical transmission. The specific problems posed by the HIV–VL co-infection are not limited to the Mediterranean, and WHO has recently set up a worldwide surveillance system (WHO, 1997). *Leishmania*–HIV co-infections have been reported from 33 countries so far and the WHO worldwide surveillance network includes now 28 member institutions.

Anthroponotic VL

L. donovani has no known animal reservoir, and gives rise to the so-called anthroponotic form of VL (AVL), causing sporadic cases and periodical epidemic waves mostly in rural populations in the Indian subcontinent (India, Bangladesh and Nepal) and in East Africa (Sudan, Kenya, Ethiopia and Eritrea). In India, kala-azar raged across the eastern part of the country in 3 major epidemics between 1880 and 1950 (RAMESH & MUKHERJEE, 1995). Whereas extrinsic factors, such as famine and epidemics of malaria and influenza, have been suggested to have exacerbated these AVL outbreaks, the major factor that influenced the transmission dynamics was the introduction of antimonial drugs around 1920 (DYE & WOLPERT, 1988). Owing to the extensive DDT spraying by the national malaria eradication campaign, the pattern of intermittent AVL epidemics was interrupted from 1947 onwards. However, this spraying programme was ended in 1963 and a large VL outbreak appeared in Bihar state between 1970 and 1987 (estimated 600 000 cases) and in West Bengal in 1980. PKDL cases were thought to have been the reservoir of infection between the epidemics (ADDY & NANDY, 1992). In 1993, a major epidemic outbreak hit India, causing an estimated 250 000 cases.

In Sudan, since Neave reported the first case of AVL in 1904, the disease had an endemic appearance until the 1940s. The first outbreak reaching epidemic proportions was reported by Stephenson: near the town of Melut in Upper Nile Province, 300 cases occurred with an estimated death rate of 80% (STEPHENSON, 1940). The second major outbreak was reported in 1956 with reportedly 'thousands of cases' surveyed in southern Fung. SATI (1958) investigated and confirmed the outbreak in a community of 5000 persons, the Jum Jum tribe, among which the disease had an attack rate of 10% and a CFR of 41%. AVL has continued unabated in the Sudan ever since, often exacerbated by the cumulative effects of famine and civil war (DE BEER *et al.*, 1991; SEAMAN *et al.*, 1996).

Frequency

The leishmaniases are on the World Health Organization's Tropical Disease Research shortlist of most important tropical diseases. While 61 countries in the world are today concerned by the VL problem, more than 90% of the VL cases are reported from only 4 countries: India, Bangladesh, Brazil, and Sudan (DESJEUX, 1996). The total number of reported cases was 82 000 in 1992 (ASHFORD *et al.*, 1992), but DESJEUX (1992) found a 1:5 ratio of declared to undeclared VL cases in community surveys in India. More recent figures by WHO therefore estimate VL incidence at 500 000 cases per year (MURRAY & LOPEZ, 1996; UNDP/WORLD BANK/WHO, 1997). The majority of these VL cases are AVL, caused by *L. donovani*.

Because VL has a focal distribution, the global figures do not reflect the real importance of VL in certain communities. Reported incidence of kala-azar in endemic areas ranges between 2/1000 person-years in Kenya (SCHAEFER *et al.*, 1995), to 14/1000 person-years in Ethiopia (ALI & ASHFORD, 1994). In a community in

eastern Sudan an incidence of 40/1000 per year was documented (ZIJLSTRA *et al.*, 1994).

Disease gravity

The number of deaths attributable to VL worldwide is difficult to estimate. During 1991, 75 000 persons may have died worldwide from VL (WIJEYARATNE *et al.*, 1994), but the precision of that figure is hard to grasp. Kala-azar has earned the epithet of 'killing disease' (SATI, 1958; DE BEER *et al.*, 1991) for obvious reasons: the first recorded epidemic in Assam province in India is said to have claimed one-third of the population of Nowgong district in 1892–1898 (DYE & WOLPERT, 1988). More recently, SEAMAN *et al.* (1996) described a devastating epidemic in the Sudan where, over the period 1984–1994, an estimated 100 000 VL deaths occurred in a group of 280 000 people living in western Upper Nile province. Forced migration due to the civil war was a major factor in transmission, and the poor nutritional status of the population contributed to the high mortality rates. Whereas both epidemics occurred in immunologically naive populations without any access to treatment, a follow-up study from eastern Sudan documented VL impact in a community with fair access to primary health care. A medical assistant in a health centre in a neighbouring village reportedly administered standard recommended sodium stibogluconate treatment. The CFR was high, 20·5%, probably related to inadequate drug dosage, intermittent administration and/or non-availability of the drug (ZIJLSTRA *et al.*, 1994).

Community perception

ZIJLSTRA (1995) cites over 30 synonyms for the disease in vernacular languages. Kala-azar means 'black sickness', and hyperpigmentation of the skin of the face, hands, feet and abdomen is indeed often observed in Indian cases. Kala, might however refer as well to 'kal', death, and reflect the terrifying effect the disease has on the affected persons (WHO, 1990). The affected communities perceive VL usually as very threatening and their demand for treatment is high. A VL focus does, however, not always capture the attention of press and public health authorities because of the protracted nature of the disease (WIJEYARATNE *et al.*, 1994) and the remoteness of the endemic areas. WIJEYARATNE *et al.* (1994) have drawn attention to the tremendous barrier VL poses to development efforts. In every member of a family where a VL case occurs, many days of productive life are lost due to this severely debilitating disease. The problem is well summarized by DESJEUX (1996): 'The leishmaniases affect mainly people from developing countries. Within these, the affected classes of population are those of lowest socio-economic status, who have minimal political power to influence the decisions of the government and a very limited capacity to assume the costs of the disease.'

Control

TESH (1995) attributed the increasing importance of VL as an emerging parasitic disease to: (i) ecological and demographic changes, most apparent in Latin America, where VL occurs now in many regions where it was not found previously; (ii) the interruption of insecticide-spraying campaigns; (iii) the global AIDS epidemic; and, last but not least, (iv) the relative ineffectiveness of present surveillance and control methods. Historical examples of effective control programmes do, however, exist. Before the founding of the People's Republic of China in 1949, kala-azar was one of the major parasitic diseases of the country. From the areas north of the Yangtze River, about 530 000 cases were reported in 1951 (GUAN & SHEN, 1991). In 1950–58 a nationwide control campaign (mass treatment of patients, killing of infected dogs, use of insecticides) was launched and the disease was largely brought under control in the plains region where the anthroponotic form had reigned. Transmission was, however, not interrupted in the mountainous and desert region where sporadic cases of zoonotic transmission continue to be reported (WHO, 1990). Bihar in India controlled its epidemic through a combination of active case detection and treatment in the community combined with residual spraying in the households (THAKUR *et al.*, 1994; SAXENA *et al.*, 1996). An effective control of the disease seems to hinge upon a combination of reservoir control, vector control and active case detection and treatment (LACERDA, 1994).

What follows is an overview of the several theoretically possible VL control interventions.

Vaccine

At present there is no vaccine available for VL. First- and second-generation vaccines for the prevention of cutaneous leishmaniasis are currently under study. The first-generation vaccines, consisting of killed *Leishmania* organisms mixed with a low concentration of BCG as adjuvant, are subject to field efficacy trials. Second-generation vaccines, which consist of genetically modified *Leishmania* parasites incapable of producing disease, recombinant molecules or their corresponding DNA mixed together in a cocktail vaccine, or recombinant organisms carrying leishmanial genes, are currently undergoing predevelopment research. Animal experiments with an autoclaved *L. major* vaccine for VL are ongoing (UNDP/WORLD BANK/WHO, 1997).

Detection and management of infection

Although serological tests can detect infection in asymptomatic cases, this is no real control option, as the currently available treatment regimens are too toxic, too cumbersome and too expensive to justify their use in healthy persons. Furthermore, current chemotherapy is thought to be unable to eliminate the parasite from the body, so the efficacy of treating infected asymptomatic persons remains doubtful.

Vector control

The sandfly is vulnerable to the same insecticides as the malaria vector. Owing to the large anti-malarial insecticide spraying campaigns, VL almost disappeared from the Indian subcontinent in the 1960s, but reappeared as soon as spraying was discontinued. A community trial in India showed that, after 2 rounds of DDT spraying in a village, no *Phlebotomus argentipes* were found during the peak vector season; in contrast, a large number of these sandflies were collected in the unsprayed comparison village (KAUL *et al.*, 1994). Residual insecticide spraying of houses and animal shelters will have an impact on transmission only when the vector is restricted to the intra- and peridomiciliary area (KAUL *et al.*, 1994), as is the case in India. In Sudan, however, although *P. argentipes* has been regularly found in villages during the past 2 years (EL NAIEM & OSMAN, 1998; EL NAIEM *et al.*, 1999), transmission takes place mainly outside the villages in *Acacia* sp. and *Balanites aegyptiaca* woodlands, and activities such as wood cutting and shepherding expose people to sandflies (HOOGSTRAAL & HEYNEMAN, 1969). Repeated use of ultra-low-volume application of insecticides to the entire community can reduce sandfly numbers but, as this reduction is fast but short-lived, the method is only used in epidemics (WHO, 1990). Insectide paints in slow-release emulsifiable solution were found effective but too expensive compared to other approaches and are no more used. Impregnated bednets have shown their efficacy in field trials for the prevention of cutaneous leishmaniasis (WHO, 1998a) and in eastern Sudan for visceral leishmaniasis (EL NAIEM *et al.*, 1999).

Reservoir control

In zoonotic VL, when dogs are the main reservoir, a control strategy can be based on large-scale serological

screening of dogs. Positive dogs are then eliminated or treated. The latter action has not proved to be very efficient owing to frequent relapse. The testing and culling campaigns have, however, also been criticized, as the eliminated dogs are quickly replaced by susceptible young dogs. Ultimately the best strategy would be to vaccinate the dogs once a vaccine becomes available. Recently, neck collars made of a pyrethroid-impregnated synthetic fabric achieved total protection of the dog for 6 months (KILLICK-KENDRICK *et al.*, 1997), long enough to cover the high transmission season in southern Europe. The product is currently being commercialized.

Case detection and management strategies

Given the absence of a vaccine, and the limited efficacy of vector control in some areas, the single most important strategy for anthroponotic VL control seems case detection and treatment. Current zoonotic VL control strategies target the animal reservoir, but early human case detection and control has gained in importance since the recent emergence of HIV–VL co-infection. In our review we found only 1 published algorithm for the management of VL, based on parasitological diagnosis (MAHMOUD & WARREN, 1977). The reliance on parasitology in endemic areas has, however, been criticized, and since newer serological tests have been developed, we suggest that it is time to revise these test–treatment algorithms.

Diagnostic and therapeutic management of VL

Diagnosis

Sensitive, specific, reproducible, feasible and cheap diagnostic tests are needed if one wants to build an effective and efficient test–treatment strategy (DE RAADT, 1977). Since Leishman identified the parasite in 1901, the definite diagnosis of VL relied on parasitology; demonstrating the organism by microscopy in smear or culture of aspirates or tissue is presumably 100% specific (BRYCESON, 1996). The reported sensitivity of bone-marrow aspirate ranges from 52% to 85% (SIDDIG *et al.*, 1988; ZIJLSTRA *et al.*, 1992; CHOWDHURY *et al.*, 1993a; BRYCESON, 1996). Sensitivity of lymph-node aspirates is lower, 52–58% (SIDDIG *et al.*, 1988; ZIJLSTRA *et al.*, 1992). A novel splenic-aspiration technique was promoted as safe and highly sensitive in eligible patients (KAGER *et al.*, 1983). Kenya's national policy on VL control in 1996 involved the transfer of all suspect patients to a tertiary-care centre in the capital for diagnosis and treatment, which is clearly not the most feasible nor acceptable of control strategies for patients in rural areas. Spleen aspiration can hardly be recommended for rural district hospitals because of the risk of fatal haemorrhage which subsists even with the recently introduced safer procedures (CHULAY & BRYCESON, 1983).

The search for a suitable alternative for splenic aspirates is thus still ongoing. Clinical case definitions have so far not been validated. Though more adapted to primary care their potential validity looks compromised, as the clinical syndrome is quite aspecific. The validity of such case definitions is moreover highly dependent on the local endemicity of characteristics of malaria and other tropical diseases. Pending local validation, clinical criteria can, however, be used as a first screening filter before the application of a second test (BOELAERT *et al.*, 1999a). The older serological tests, such as the formol-gel test, are still in use in India, although their validity is not well documented. In recent years polymerase chain reaction (PCR) techniques for leishmaniases have been developed and applied to visceral disease with contradictory results (ADHYA *et al.*, 1995; NUZUM *et al.*, 1995; BOELAERT & DUJARDIN, 1999). Apart from the fact that PCR does not eliminate the need for tissue sampling, it is not exactly the type of appropriate technology needed in endemic areas. Over the past 20 years, several serological tests have been developed, sharing the feature of a high sensitivity for visceral disease (KAR, 1995). The direct agglutination test (DAT) will be reviewed here in more detail because it was deemed most appropriate for field use (ABDEL HAMEED *et al.*, 1989; CHOWDHURY *et al.*, 1993b; SINGLA *et al.*, 1993; SINHA & SEHGAL, 1994).

In 1985 a Sudanese researcher, Dr Abdallah El Harith, was offered the opportunity to develop a serological test for VL at the Koninklijk Instituut voor de Tropen in Amsterdam. He built upon a technique described by ALLAIN & KAGAN (1975), and on work by MAGNUS *et al.* (1978) who developed a card agglutination test for the diagnosis of African trypanosomiasis. El Harith and colleagues' initial publication on the performance of the DAT in the laboratory reported very high sensitivity and specificity values (EL HARITH *et al.*, 1986) which were corroborated by other authors on laboratory series of banked sera (EL SAFI & EVANS, 1989; HAILU, 1990; CHOWDHURY *et al.*, 1993b; SINGLA *et al.*, 1993; SINHA & SEHGAL, 1994). The ongoing VL epidemic in Sudan (DE BEER *et al.*, 1991) created a pressing demand for better diagnostics, and the DAT was rapidly taken to the field (SEAMAN *et al.*, 1992). Contradictory reports, however, appeared on the specificity of DAT in the clinical setting (ZIJLSTRA *et al.*, 1991; EL MASUM *et al.*, 1995): reportedly, specificity was low in clinical suspects. This uncertainty about DAT specificity resulted in an empirical approach in the field: DAT was used by some clinicians, but in the case of borderline titres they would rely on parasitological tests. Others would abide by parasitology only, and opted for not treating DAT positive–parasitologically negative patients (J. Seaman, personal communication). A freeze-dried DAT antigen was promoted as more stable than the aqueous form (MEREDITH *et al.*, 1995; ZIJLSTRA *et al.*, 1997). Recently SUNDAR *et al.* (1997b) reported high sensitivity and specificity of an rK39 leishmanial antibody dipstick test, but its performance was less good when evaluated in the Sudan (E. E. Zijlstra, personal communication). A re-examination of the DAT validity and reproducibility confirmed its value as a field test (BOELAERT *et al.*, 1999a, 1999b). A cost-effectiveness analysis comparing 4 diagnostic–therapeutic strategies showed recently that a test–treatment strategy based exclusively on the DAT was the best option for the first- and second-line health services in endemic areas (BOELAERT *et al.*, 1999c). The diagnostic algorithm that can be derived from this study is shown in the Figure.

Treatment

Pentavalent antimonials (sodium stibogluconate, Pentostam®; meglumine antimonate, Glucantime®) have been the therapy of choice for more than 50 years now. Current recommended regimen as a first-line drug is a 30-day (20 mg Sb^v/kg daily) parenteral course. Uncomplicated cases may be treated by daily injections at a dispensary or in the home by visiting health workers. Severely wasted patients do need hospitalization (WHO

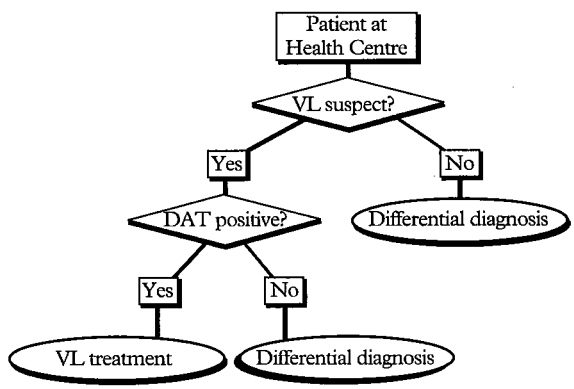

Figure. Diagnostic algorithm for new suspects of visceral leishmaniasis (VL) presenting at health services in endemic areas. DAT, direct agglutination test.

& ODA, 1996) and secondary bacterial infections need to be carefully monitored and treated.

The cardio-, hepato- and nephrotoxic effects of antimonials are well known in high doses (BALLOU *et al.*, 1987). With the standard regimen of 20 mg/kg daily, CHULAY *et al.* (1985) reported electrocardiographic changes and SUNDAR *et al.* (1997c) described cardiotoxicity, while using a generic product. Other side-effects include pancreatitis, arthralgia, myalgia, nausea and vomiting (PEARSON & SOUSA, 1996).Worldwide up to 15% of therapeutic failures have been reported with the pentavalent antimonials, with even higher figures from India. In Bihar, the epicentre of the current Indian epidemic, 37–64% of previously untreated patients infected with *L. donovani* currently fail to respond (SUNDAR *et al.*, 1998). In cases of HIV–VL co-infection, relapse rates are very high as well, as each infection weakens the host response to the other, with high parasite loads and presence of *Leishmania* in unusual cells. Relapse is most likely to occur within 3–12 months after treatment, and in that case, referral to a specialized centre is needed, where second-line drugs as amphotericin B, liposomal amphotericin B, or paromomycin (aminosidine) can be given. The failure of antimony treatment may reflect the failure of some critical element in the individual immune response of the patient rather than actual resistance of the parasite to antimony (KHALIL *et al.*, 1998). Combinations of pentavalent antimony and recombinant gamma-interferon have been used with variable success (BADARO *et al.*, 1990; SQUIRES *et al.*, 1993; SUNDAR *et al.*, 1994; SUNDAR *et al.*, 1997c), but are most probably not the immediate solution for patients in developing countries.

Recently, promising phase-1 and phase-2 trial results of miltefosine, an oral treatment, were published (SUNDAR *et al.*, 1998; JHA *et al.*, 1999). Other perspectives for oral treatment lie in the antifungal azoles but ketaconazole and itraconazole have led to contradictory reports. Atovaquone is still not beyond the stage of animal studies (VAN GOMPEL & VERVOORT, 1997).

The cost of VL treatment to the individual patient and his/her family is high, as treatment is expensive, and often unaffordable. Average cost for specific first-line treatment is estimated at US$ 150 for a WHO-recommended course of 30 days of sodium stibogluconate (WHO & ODA, 1996). In India, average cost for a course with locally produced generic sodium antimony gluconate is US$ 16. The results of an equivalence trial of the generic compound versus the brand drug are forthcoming (H. Veeken, personal communication). Cost of second-line drugs such as amphotericin B, aminosidine sulphate and pentamidine varies between US$ 60 and 600 per treatment course. The total cost of patient care ranges from a reported US$ 4000 per VL patient treated (MARSDEN, 1986) in Brazil, to US$ 394 in the Sudan (GRIEKSPOOR *et al.*, 1999).

Conclusion

The current possibilities for VL control are limited. The highest operational priority should clearly be given to case detection and treatment of VL and PKDL cases, and to vector control through individual protection by impregnated bednets and peri-domestic spraying where indicated by vector characteristics. The currently available diagnostic and therapeutic tools have their limitations, which can seriously compromise the success of this case detection–management strategy. On the diagnostic side, parasitology has a weak sensitivity in lymph-node and bone-marrow aspirates. Splenic aspirates perform better, but are hardly feasible in a district hospital. Serological tests with a very high sensitivity and an acceptable specificity, such as the DAT, have been developed but diagnostic algorithms for their use by the health services need to be introduced on a wider scale. On the therapeutic side, the pentavalent antimonial therapy has an efficacy in the range of 85–95%, but is expensive, relatively toxic and requires a 1 month-long parenteral treatment. The increasing proportion of patients refractory to sodium stibogluconate observed in India adds to this need for increased research efforts. There is thus currently still a clear need for a vaccine and for shorter drug courses that can preferably be administered orally. In the meanwhile, the available means for VL control should be used to the best possible extent, and the DAT and sodium stibogluconate are 2 instruments in that challenge.

References

Abdel Hameed, A. A., El Harith, A. & Abdel Rahim, I. M. (1989). Potential of a direct agglutination test (DAT) for detection of visceral leishmaniasis in a known endemic area in Sudan. *Tropical Medicine and Parasitology*, **40**, 470–471.

Abranches, P., Lopes, F. J., Silva, F. M., Ribeiro, M. M. & Pires, C. A. (1983). Le kala-azar au Portugal. III. Resultats d'une enquête sur la leishmaniose canine realisée dans les environs de Lisbonne. Comparaison des zones urbaines et rurales. *Annales de Parasitologie Humaine et Comparée*, **58**, 307–315.

Addy, M. & Nandy, A. (1992). Ten years of kala-azar in West Bengal, Part I. Did post-kala-azar dermal leishmaniasis initiate the outbreak in 24-Parganas? *Bulletin of the World Health Organization*, **70**, 341–346.

Adhya, S., Chatterjee, M., Hassan, M. Q., Mukherjee, S. & Sen, S. (1995). Detection of *Leishmania* in the blood of early kala-azar patients with the aid of the polymerase chain reaction. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **89**, 622–624.

Ali, A. & Ashford, R. W. (1994). Visceral leishmaniasis in Ethiopia. IV. Prevalence, incidence and relation of infection to disease in an endemic area. *Annals of Tropical Medicine and Parasitology*, **88**, 289–293.

Allain, D. S. & Kagan, I. G. (1975). A direct agglutination test for leishmaniasis. *American Journal of Tropical Medicine and Hygiene*, **24**, 232–236.

Ashford, D. A., Desjeux, P. & De Raadt, P. (1992). Estimation of population at risk of infection and number of cases of leishmaniasis. *Parasitology Today*, **8**, 104–105.

Badaro, R., Jones, T. C., Carvalho, E. M., Sampaio, D., Reed, S. G., Barral, A., Teixeira, R. & Johnson, W. D. J. (1986). New perspectives on a subclinical form of visceral leishmaniasis. *Journal of Infectious Diseases*, **154**, 1003–1011.

Badaro, R., Falcoff, E., Badaro, F. S., Carvalho, E. M., Pedral-Sampaio, D., Barral, A., Carvalho, J. S., Barral-Netto, M., Brandely, M. & Silva, L. (1990). Treatment of visceral leishmaniasis with pentavalent antimony and interferon gamma. *New England Journal of Medicine*, **322**, 16–21.

Ballou, W. R., McClain, J. B., Gordon, D. M., Shanks, G. D., Andujar, J. M., Berman, J. D. & Chulay, J. D. (1987). Safety and efficacy of high-dose sodium stibogluconate therapy of American cutaneous leishmaniasis. *Lancet*, **ii**, 13–16.

Berman, J. D. (1988). Chemotherapy for leishmaniasis: biochemical mechanisms, clinical efficacy, and future strategies. *Review of Infectious Diseases*, **10**, 560–586.

Berman, J. D. (1997). Human leishmaniasis: clinical, diagnostic, and chemotherapeutic developments in the last 10 years. *Clinical Infectious Diseases*, **24**, 684–703.

Boelaert, M. & Dujardin, J. C. (1999). Diagnostic PCR with *Leishmania donovani* specificity. *Tropical Medicine and International Health*, **4**, 789–789.

Boelaert, M., El Safi, S. H., Jacquet, D., de Muynck, A., van der Stuyft, P. & le Ray, D. (1999a). Operational validation of the direct agglutination test (DAT) for diagnosis of visceral leishmaniasis. *American Journal of Tropical Medicine and Hygiene*, **60** , 129–134.

Boelaert, M., El Safi, S. H., Mousa, H. , Githure, J., Mbati, P. A., Gurubacharya, V. L., Shrestha, J., Jacquet, D., de Muynck, A., le Ray, D. & van der Stuyft, P. (1999b). Multicentre evaluation of repeatability and reproducibility of the direct agglutination test for visceral leishmaniasis. *Tropical Medicine and International Health*, **4**, 31–37.

Boelaert, M., Lynen, L., Desjeux, P. & van der Stuyft, P. (1999c). The cost-effectiveness of competing diagnostic–therapeutic strategies for visceral leishmaniasis. *Bulletin of the World Health Organization*, **77**, 667–674.

Bramachari, U. (1922). A new form of cutaneous leishmaniasis—dermal leishmanoid. *Indian Medical Gazette*, **57**, 125–127.

Bryceson, A. (1996). Leishmaniasis. In: *Manson's Tropical Diseases*, 20th edition, Cook, G. C. (editor). London: W.B. Saunders, pp. 1213–1243.

Cahill, K. M. (1964). Leishmaniasis in the Sudan republic.

XXI. Infection in American personnel. *American Journal of Tropical Medicine and Hygiene*, **13**, 794–799.
Cerf, B. J., Jones, T. C., Badaro, R., Sampaio, D., Teixeira, R. & Johnson, W. D. J. (1987). Malnutrition as a risk factor for severe visceral leishmaniasis. *Journal of Infectious Diseases*, **156**, 1030–1033.
Chowdhury, M. S., Al Masum, A., Al Karim, E., Semiao Santos, S., Rahman, K. M., Ar Rashid, H. & El Harith, A. (1993a). Applicability of direct agglutination test (DAT) at a rural health setting in Bangladesh and feasibility of local antigen production. *Archives de l'Institut Pasteur de Tunis*, **70**, 333–344.
Chowdhury, M. S., El Harith, A., Al Masum, A., Al Karim, E. & Al Rahman, A. (1993b). Prevalence of agglutinating anti-*Leishmania* antibodies in two multi-thousand Bengoli communities. *Parasitology Research*, **79**, 444–450.
Chulay, J. D. & Bryceson, A. D. (1983). Quantitation of amastigotes of *Leishmania donovani* in smears of splenic aspirates from patients with visceral leishmaniasis. *American Journal of Tropical Medicine and Hygiene*, **32**, 475–479.
Chulay, J. D., Spencer, H. C. & Mugambi, M. (1985). Electrocardiographic changes during treatment of leishmaniasis with pentavalent antimony (sodium stibogluconate). *American Journal of Tropical Medicine and Hygiene*, **34**, 702–709.
Corkill, N. L. (1948). Activation of latent kala-azar and malaria by battle experience. *Annals of Tropical Medicine and Parasitology*, **42**, 224–228.
De Beer, P., El Harith, A., Deng, L. L., Semiao Santos, S. J., Chantal, B. & van Grootheest, M. (1991). A killing disease epidemic among displaced Sudanese population identified as visceral leishmaniasis. *American Journal of Tropical Medicine and Hygiene*, **44**, 283–289.
De Gorgolas, M. & Miles, M. A. (1994). Visceral leishmaniasis and AIDS. *Nature*, **372**, 734.
De la Loma, A., Alvar, J., Martinez-Galiano, E., Blazquez, J., Alcala-Munoz, A. & Najera, R. (1985). Leishmaniasis or AIDS? *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **79**, 421–422.
De Raadt, P. (1977). Leishmaniasis and world health. In: *Ecologie des Leishmanioses*, Colloques Internationaux du C.N.R.S. (editors). Paris: Editions du C.N.R.S. 239, pp. 313–315.
Desjeux, P. (1992). Human leishmaniases: epidemiology and public health aspects. *World Health Statistics Quarterly*, **45**, 267–275.
Desjeux, P. (1996). Leishmaniasis. Public health aspects and control. *Clinical Dermatology*, **14**, 417–423.
Dye, C. (1992). Leishmaniasis epidemiology: the theory catches up. *Parasitology*, **104 (supplement)**, S7–18.
Dye, C. & Wolpert, D. M. (1988). Earthquakes, influenza and cycles of Indian kala-azar. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **82**, 843–850.
El Harith, A., Kolk, A. H., Kager, P. A., Leeuwenburg, J., Muigai, R., Kiugu, S. & Laarman, J. J. (1986). A simple and economical direct agglutination test for serodiagnosis and sero-epidemiological studies of visceral leishmaniasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **80**, 583–587.
El Masum, M. A., Evans, D. A., Minter, D. M. & El Harith, A. (1995). Visceral leishmaniasis in Bangladesh: the value of DAT as a diagnostic tool. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **89**, 185–186.
El Naiem, D. A. & Osman, O. F. (1998). Evidence for active transmission of visceral leishmaniasis within a village in eastern Sudan. *Acta Tropica*, **71**, 305–309.
El Naiem, D. A., El Nahas, A. M. & Aboud, M. A. (1999). Protective efficacy of lambdacyhalothrin-impregnated bednets against *Phlebotomus orientalis*, the vector of visceral leishmaniasis in Sudan. *Medical and Veterinary Entomology*, **13**, 310–314.
El Safi, S. H. & Evans, D. A. (1989). A comparison of the direct agglutination test and enzyme-linked immunosorbent assay in the sero-diagnosis of leishmaniasis in the Sudan. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **83**, 334–337.
Evans, T. G., Teixeira, M. J., McAuliffe, I. T., Vasconcelos, I., Vasconcelos, A. W., Sousa, A. D., Lima, J. W. & Pearson, R. D. (1992). Epidemiology of visceral leishmaniasis in north-east Brazil. *Journal of Infectious Diseases*, **166**, 1124–1132.
Fernandez, G. M., Aguado, J. M., Buzon, L., Barros, C., Montalban, C., Martin, T. & Bouza, E. (1987). Visceral leishmaniasis in immunocompromised hosts. *American Journal of Medicine*, **83**, 1098–1102.
Griekspoor, A., Sondorp, E. & Vos, T. (1999). Cost-effectiveness analysis of humanitarian relief interventions: visceral leishmaniasis treatment in the Sudan. *Health Policy and Planning*, **14**, 70–76.
Guan, L. R. & Shen, W. X. (1991). Recent advances in visceral leishmaniasis in China. *Southeast Asian Journal of Tropical Medicine and Public Health*, **22**, 291–298.
Hailu, A. (1990). Pre- and post-treatment antibody levels in visceral leishmaniasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **84**, 673–675.
Hashim, F. A., Ali, M. S., Satti, M., El Hassan, A. M., Ghalib, H. W., El Safi, S. & El Hag, I. A. (1994). An outbreak of acute kala-azar in a nomadic tribe in western Sudan: features of the disease in a previously non-immune population. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **88**, 431–432.
Ho, M., Siongok, T. K., Lyerly, W. H. & Smith, D. H. (1982). Prevalence and disease spectrum in a new focus of visceral leishmaniasis in Kenya. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **76**, 741–746.
Hoogstraal, H. & Heyneman, D. (1969). Leishmaniasis in the Sudan republic. 30. Final epidemiological report. *American Journal of Tropical Medicine and Hygiene*, **18**, 1091–1210.
Jha, T. K., Sundar, S., Thakur, C. P., Bachman, P., Karbwang, J., Fischer, C., Voss, A. & Berman, J. (1999). Miltefosine, an oral agent for the treatment of Indian visceral leishmaniasis. *New England Journal of Medicine*, **341**, 1795–1800.
Kager, P. A., Rees, P. H., Manguyu, F. M., Bhatt, K. M. & Bhatt, S. M. (1983). Splenic aspiration: experience in Kenya. *Tropical Geographical Medicine*, **35**, 125–131.
Kar, K. (1995). Serodiagnosis of leishmaniasis. *Critical Reviews in Microbiology*, **21**, 123–152.
Kaul, S. M., Sharma, R. S., Dey, K. P., Rai, R. N. & Verghese, T. (1994). Impact of DDT indoor residual spraying on *Phlebotomus argentipes* in a kala-azar endemic village in eastern Uttar Pradesh. *Bulletin of the World Health Organization*, **72**, 79–81.
Khalil, E. A., El Hassan, A. M.,, Zijlstra, E. E., Hashim, F. A., Ibrahim, M. E., Ghalib, H. W. & Ali, M. S. (1998). Treatment of visceral leishmaniasis with sodium stibogluconate in Sudan: management of those who do not respond. *Annals of Tropical Medicine and Parasitology*, **92**, 151–158.
Killick-Kendrick, R., Killick-Kendrick, M., Focheux, C., Dereure, J., Puech, M. P. & Cadiergues, M. C. (1997). Protection of dogs from bites of phlebotomine sandflies by deltamethrin collars for control of canine leishmaniasis. *Medical and Veterinary Entomology*, **11**, 105–111.
Lacerda, M. M. (1994). The Brazilian Leishmaniasis Control Program. *Memorias del Instituto Oswaldo Cruz*, **89**, 489–495.
Magill, A. J., Grogl, M., Gasser-Ra, J., Sun, W. & Oster, C. N. (1993). Visceral infection caused by *Leishmania tropica* in veterans of Operation Desert Storm. *New England Journal of Medicine*, **328**, 1383–1387.
Magnus, E., Vervoort, T. & van Meirvenne, N. (1978). A card-agglutination test with stained trypanosomes (C.A.T.T.) for the serological diagnosis of *T.b. gambiense* trypanosomiasis. *Annales de la Société Belge de Médecine Tropicale*, **58**, 169–176.
Mahmoud, A. A. F. & Warren, K. S. (1977). Algorithms in the diagnosis and management of exotic diseases. XXIV. Leishmaniases. *Journal of Infectious Diseases*, **136**, 160–163.
Manson Bahr, P. E. C. (1955). A primary skin lesion in visceral leishmaniasis. *Nature*, **175**, 433–434.
Manson Bahr, P. E. C. & Southgate, B. A. (1964). Recent research on kala-azar in East Africa. *Journal of Tropical Medicine and Hygiene*, **67**, 79–84.
Marsden, P. D. (1986). Mucosal leishmaniasis ('espundia' Escomel, 1911). *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **80**, 859–876.
Meredith, S. E., Kroon, N. C., Sondorp, E., Seaman, J., Goris, M. G., van Ingen, C. W., Oosting, H., Schoone, G. J., Terpstra, W. J. & Oskam, L. (1995). Leish-KIT, a stable direct agglutination test based on freeze-dried antigen for serodiagnosis of visceral leishmaniasis. *Journal of Clinical Microbiology*, **33**, 1742–1745.
Montalban, C., Calleja, J. L., Erice, A., Laguna, F., Clotet, B., Podzamczer, D., Cobo, J., Mallolas, J., Yebra, M. & Gallego, A. (1990). Visceral leishmaniasis in patients infected with human immunodeficiency virus. Co-operative Group for the Study of Leishmaniasis in AIDS. *Journal of Infection*, **21**, 261–270.
Murray, C. J. L. & Lopez, A. (1996). *Global Health Statistics*, Volume 2, Murray, C. J. L. & Lopez, A., (editors). Boston: Harvard School of Public Health on behalf of the World Health Organization and the World Bank.
Nandy, A., Addy, M., Maji, A. K., Guha, S. K., Banerjee, D. & Chaudhuri, D. (1998). Recurrence of kala-azar after PKDL: role of co-factors. *Tropical Medicine and International Health*, **3**, 76–78.

Neva, F. A. (1990). Immunotherapy for parasitic disease. *New England Journal of Medicine*, **322**, 55–57.
Nuzum, E., White, F., Thakur, C., Dietze, R., Wages, J., Grogl, M. & Berman, J. (1995). Diagnosis of symptomatic visceral leishmaniasis by use of the polymerase chain reaction on patient blood. *Journal of Infectious Diseases*, **171**, 751–754.
Nyakundi, P. M., Muigai, R., Were, J. B. O., Oster, C. N., Gachihi, G. S. & Kirigi, G. (1988). Congenital visceral leishmaniasis: case report. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **82**, 564.
Pearson, R. D. & Sousa, A. Q. (1996). Clinical spectrum of leishmaniasis. *Clinical Infectious Diseases*, **22**, 1–13.
Procès, S., Assira, A., Ketelslegers, O., Chatelain, B. & Donckier, J. (1997). La leishmaniose viscérale: à propos d'une observation. *Louvain Médical*, **116**, 123–131.
Ramesh, V. (1994). Treatment of post-kala-azar dermal leishmaniasis. *International Journal of Dermatology*, **33**, 153–156.
Ramesh, V. & Mukherjee, H. (1995). Post-kala-azar dermal leishmaniasis. *International Journal of Dermatology*, **34**, 85–91.
Sati, M. H. (1958). Early phases of an outbreak of kala-azar in the Southern Fung. *Sudan Medical Journal*, **1**, 98–111.
Saxena, N. B., Aggarwal, V., Dhillon, G. P., Sharma, R. S. & Rao, J. S. (1996). Visceral leishmaniasis control in India through primary health care system—a successful experiment of district level planning. *Journal of Communicable Disease*, **28**, 122–128.
Schaefer, K. U., Kurtzhals, J. A., Gachihi, G. S., Muller, A. S. & Kager, P. A. (1995). A prospective sero-epidemiological study of visceral leishmaniasis in Baringo District, Rift Valley Province, Kenya. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **89**, 471–475.
Seaman, J., Ashford, R. W., Schorscher, J. & Dereure, J. (1992). Visceral leishmaniasis in southern Sudan: status of healthy villagers in epidemic conditions. *Annals of Tropical Medicine and Parasitology*, **86**, 481–486.
Seaman, J., Mercer, A. J. & Sondorp, E. (1996). The epidemic of visceral leishmaniasis in western Upper Nile, southern Sudan: course and impact from 1984 to 1994. *International Journal of Epidemiology*, **25**, 862–871.
Siddig, M., Ghalib, H. W., Shillington, D. C. & Petersen, E. A. (1988). Visceral leishmaniasis in the Sudan: comparative parasitological methods of diagnosis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **82**, 66–68.
Singla, N., Singh, G. S., Sundar, S. & Vinayak, V. K. (1993). Evaluation of the direct agglutination test as an immunodiagnostic tool for kala-azar in India. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **87**, 276–278.
Sinha, R. & Sehgal, S. (1994). Comparative evaluation of serological tests in Indian kala-azar. *Journal of Tropical Medicine and Hygiene*, **97**, 333–340.
Squires, K. E., Rosenkaimer, F., Sherwood, J. A., Forni, A. L., Were, J. B. & Murray, H. W. (1993). Immunochemotherapy for visceral leishmaniasis: a controlled pilot trial of antimony versus antimony plus interferon-gamma. *American Journal of Tropical Medicine and Hygiene*, **48**, 666–669.
Stephenson, R. W. (1940). An epidemic of kala-azar in the Upper Nile Province of the Anglo-Egyptian Sudan. *Annals of Tropical Medicine and Parasitology*, **34**, 175–179.
Sundar, S., Rosenkaimer, F. & Murray, H. W. (1994). Successful treatment of refractory visceral leishmaniasis in India using antimony plus interferon-gamma. *Journal of Infectious Diseases*, **170**, 659–662.
Sundar, S., Reed, S. G., Sharma, S., Mehrotra, A. & Murray, H. W. (1997a). Circulating T helper 1 (Th1) cell- and Th2 cell-associated cytokines in Indian patients with visceral leishmaniasis. *American Journal of Tropical Medicine and Hygiene*, **56**, 522–525.
Sundar, S., Reed, S. G., Singh, V. P., Kumar, P. C. & Murray, H. W. (1997b). Rapid accurate field diagnosis of Indian visceral leishmaniasis. *Lancet*, **351**, 563–565.
Sundar, S., Singh, V. P., Sharma, S., Makharia, M. K. & Murray, H. W. (1997c). Response to interferon-gamma plus pentavalent antimony in Indian visceral leishmaniasis. *Journal of Infectious Diseases*, **176**, 1117–1119.
Sundar, S., Rosenkaimer, F., Makharia, M. K., Goyal, A. K., Mandal, A. K., Voss, A., Hilgard, P. & Murray, H. W. (1998). Trial of oral miltefosine for visceral leishmaniasis. *Lancet*, **352**, 1821–1823.
Symmers, W. C. (1960). Leishmaniasis acquired by contagion. *Lancet*, **i**, 127–132.
Tesh, R. B. (1995). Control of zoonotic visceral leishmaniasis: is it time to change strategies? *American Journal of Tropical Medicine and Hygiene*, **52**, 287–292.
Thakur, C. P., Sister Frances, Sister Therese, Sister Victoria & Sister Puspa (1994). A kala-azar control programme for remote tribal communities. *World Health Forum*, **15**, 245–247.
UNDP/World Bank/WHO (1997). *Tropical Disease Research: Progress 1995–1996: Thirteenth Programme Report of the UNDP/World Bank/ WHO Special Programme for Research and Training in Tropical Diseases*. Geneva: World Health Organization.
Van Gompel, A. & Vervoort, T. (1997). Chemotherapy of leishmaniasis and trypanosomiasis. *Current Opinion in Infectious Diseases*, **10**, 469–474.
WHO (1990). *Control of the Leishmaniases. Report of a WHO Expert Committee*. Geneva: World Health Organization, Technical Report Series, no. 793.
WHO (1997). Leishmania/HIV co-infection. *Weekly Epidemiological Record*, **72**, 49–54.
WHO (1998a). *A cutaneous leishmaniasis control trial using pyrethroïd impregnated bednets in villages near Aleppo Syria*. Geneva: World Health Organization. WHO/LEISH/97.41.
WHO (1998b). *Leishmania and HIV in gridlock*. Geneva: World Health Organization. WHO/CTD/LEISH/98.9.
WHO & ODA (1996). *Manual on visceral leishmaniasis control*. Geneva: World Health Organization. WHO/Leish/96.40. 1–78.
Wijeyaratne, P. M., Arsenault, L. K. & Murphy, C. J. (1994). Endemic disease and development: the leishmaniases. *Acta Tropica*, **56**, 349–364.
Zijlstra, E. E. (1995). *Kala-azar in the Sudan: epidemiological and clinical studies*. Thesis Universiteit Amsterdam, The Netherlands.
Zijlstra, E. E., Ali, M. S., El Hassan, A. M., El Toum, I. A., Satti, M., Ghalib, H. W. & Kager, P. A. (1991). Direct agglutination test for diagnosis and sero-epidemiological survey of kala-azar in the Sudan. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **85**, 474–476.
Zijlstra, E. E., Ali, M. S., El Hassan, A. M., El Toum, I. A., Satti, M., Ghalib, H. W. & Kager, P. A. (1992). Kala-azar: a comparative study of parasitological methods and the direct agglutination test in diagnosis [see comments]. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **86**, 505–507.
Zijlstra, E. E., El Hassan, A. M., Ismael, A. & Ghalib, H. W. (1994). Endemic kala-azar in eastern Sudan: a longitudinal study on the incidence of clinical and subclinical infection and post-kala-azar dermal leishmaniasis. *American Journal of Tropical Medicine and Hygiene*, **51**, 826–836.
Zijlstra, E. E., El Hassan, A. M. & Ismael, A. (1995). Endemic kala-azar in eastern Sudan: post-kala-azar dermal leishmaniasis. *American Journal of Tropical Medicine and Hygiene*, **52**, 299–305.
Zijlstra, E. E., Osman, O. F., Hofland, H. W., Oskam, L., Ghalib, H. W., El Hassan, A. M., Kager, P. A. & Meredith, S. E. (1997). The direct agglutination test for diagnosis of visceral leishmaniasis under field conditions in Sudan: comparison of aqueous and freeze-dried antigens. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **91**, 671–673.

Received 31 January 2000; revised 31 March 2000; accepted for publication 18 April 2000