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New challenges in the epidemiology and treatment of visceral leishmaniasis in periurban areas

Kathryn M. Dupnik^{1,2}, Eliana L. Nascimento^{2,3}, Joao F. Rodrigues-Neto⁴, Tatjana Keesen⁴, Maria Zélia Fernandes^{5,6}, Iraci Duarte⁷, and Selma M.B. Jeronimo^{2,4,5}

¹Division of Infectious Diseases, Weill Cornell Medical College, New York, NY, USA

²Instituto Nacional de Ciência e Tecnologia de Doenças Tropicais, INCT-DT, Brazil

³Department of Infectious Diseases, Universidade Federal do Rio Grande do Norte, Natal, RN, Brazil

⁴Department of Biochemistry, Universidade Federal do Rio Grande do Norte, Natal, RN, Brazil

⁵Health Post-Graduate Program, Universidade Federal do Rio Grande do Norte, Natal, RN, Brazil

⁶Department of Internal Medicine, Universidade Federal do Rio Grande do Norte, Natal, RN, Brazil

⁷Fundação Nacional de Saúde, Secretaria de Saúde do Estado do Rio Grande do Norte, Natal, RN, Brazil

Abstract

Visceral leishmaniasis [VL] represents a major public health problem in many areas of the world. This review focuses on the impact of periurbanization on the epidemiology and treatment of VL, using Brazil as an example. VL continues to be mostly a disease of poverty with impact on families. However, the disease has expanded in Latin America, with foci reported as far south as Argentina. There is an increasing overlap of *Leishmania infantum chagasi* and HIV infections and other immunosuppressive conditions, resulting in VL emerging as an opportunistic infection. This new setting poses new challenges for VL disease control and patient management.

Keywords

Visceral leishmaniasis; urbanization; HIV; Cancer

Introduction

Leishmaniasis is a complex of diseases caused by infection with one of several species of *Leishmania* parasites, transmitted to humans by phlebotomine sand flies. Leishmaniasis is currently endemic in eighty-eight countries worldwide, and the number of people at risk for *Leishmania* infection is over 300 million [Hotez et al. 2008; Desjeux 2001]. An estimated 12

million people are infected with *Leishmania*, but only a percentage of these will progress to clinical disease, approximately 1 million people yearly [Desjeux 2001]. There are multiple clinical forms of disease including cutaneous, mucocutaneous, diffuse, disseminated, and visceral types.

Visceral leishmaniasis (VL) is fatal if left untreated. Ninety percent of VL cases occur in India, Bangladesh, Sudan, Nepal and Brazil. Post-kala-azar dermal leishmaniasis (PKDL) is a complication of VL, usually developing after therapy [Pearson et al., 1999]. VL is commonly caused by the *Leishmania donovani* complex. *L. donovani* causes most of the VL in India, Bangladesh, and Sudan [Kumar et al., 1999]. In Europe, northern Africa, and parts of China, VL is caused by *L. infantum*. In the Americas, VL is caused by *L. chagasi*, which genetically seems to be indistinguishable from *L. infantum*, suggesting its introduction by Europeans during colonization in the sixteenth century [Mauricio et al., 1999, Shaw, 2006]. We will refer to *Leishmania chagasi* as *L. i. chagasi* in this review. In addition, cases of VL due to other species of *Leishmania* have been documented, as in Brazil where *L. amazonensis* has been isolated from patients with clinical VL [Barral et al. 1991; Aleixo et al. 2006].

VL continues to be a neglected tropical disease with a significant economic burden for the individuals, families, and communities it affects, as it mostly affects underprivileged groups. VL incidence increases during wars, droughts, and socioeconomic changes, as has occurred in Sudan and other countries [Boelaert et al. 2009; Seaman et al. 1996]. For over 80% of VL patients in Bihar state, India, their families had to obtain a loan to pay for VL treatment [Meheus et al. 2006; Boelaert et al. 2009]. The largest cost burdens were in the form of medication purchases and lost household income related to illness and hospitalization [Meheus et al. 2006].

Changing epidemiology of visceral leishmaniasis

VL in the Old World including the Mediterranean, Middle Eastern Asia, Indian subcontinent, and East African regions can be an anthroponosis or an anthroponosis [Desjeux, 2001]. Indian VL seems to be a true anthroponosis. A high density of *Phlebotomus argentipes* sand flies in an area with high prevalence of asymptomatic infection and clinical VL can increase the transmission of VL in India [Topno et al. 2010]. VL in Latin America is an anthroponosis [Evans et al., 1990].

The contribution of human asymptomatic infection to maintaining the endemicity of *Leishmania* is not completely understood. Recent changes resulting from peri-urbanization of VL may help to elucidate the dynamics of transmission of *Leishmania* to humans. In Brazil and other countries in Latin America, VL was a disease of rural communities and of children [Deane and Deane 1962; Badaro et al. 1986; Evans et al. 1992]. However, over the past three decades, VL has emerged as a focally epidemic disease in the outskirts of many expanding Brazilian cities [Costa 2008; Nascimento et al. 2008; Albuquerque et al. 2009; Dantas-Torres and Brandao-Filho 2006; Arias et al. 1996] including Natal in the state of Rio Grande do Norte [Jeronimo et al., 2004]. It has also spread to southeast Brazil, Argentina, and Paraguay [Salomon et al. 2008; Salomon et al. 2009].

Dogs were traditionally considered the principal reservoirs of *L. i. chagasi* in rural areas of Brazil [Evans et al. 1990, Nascimento et al. 2008, Queiroz et al. 2009]. In foxhounds in the US, vertical and horizontal transmissions of *Leishmania* have been shown [Duprey et al., 2006], but these transmission modalities are more difficult to confirm in areas with *Leishmania* sand fly vectors. In Brazil, sand flies are a crucial part of the transmission cycle [Mukhopadhyay et al., 1998]. Due to peri-urbanization, canine VL often precedes human VL [Camargo-Neves et al., 2001]. Studies in a focal endemic area in the state of Rio Grande do Norte, the area surrounding the state capital of Natal, showed a 32.6% *Leishmania* infection rate in dogs. Asymptomatic *Leishmania* infection in humans from the same area was also high, with 24.6% of individuals positive for anti-*Leishmania* antibodies and 38.6% had positive delayed hypersensitivity responses to *Leishmania* antigens [DTH] [Jeronimo et al, 2004]. The rate of *Leishmania* infection in this area was similar in all age groups, indicating that people of all ages and sex were exposed to *Leishmania* infection (unpublished results).

The presence of human VL in a household is associated with an increase in the number of people infected by *Leishmania* in that household, as measured by presence of anti-*Leishmania* antibodies or DTH responses (Table 1). This may indicate that humans can be a reservoir for *Leishmania* in highly populated areas or that all members of the household have the same high-risk exposures. Controlled studies need to be conducted to better clarify the different components of the chain of transmission and to assess the role of asymptomatic humans in *Leishmania* transmission.

The extent of asymptomatic *Leishmania* infection in some areas of Brazil may also result in a change in the epidemiological profile of visceral leishmaniasis. Individuals with asymptomatic infection may develop clinical disease when they become immunocompromised. Thus, with a growing population of immunocompromised patients in areas with large numbers of at-risk asymptomatic *Leishmania*-infected individuals, the burden of *Leishmania* disease may increase further [Daher et al. 2009; Oliveira et al. 2008; Nascimento et al, 2011]

Complicated VL cases pose additional challenges for treatment and post-treatment management [Werneck et al. 2003; Sundar, 2003]. Organ transplants are becoming more widespread in cities that are endemic for visceral leishmaniasis in Brazil, [Broeckeaert-van et al. 1979; Sirvent-von et al. 2004; Oliveira et al. 2008] as in Natal and Fortaleza in the Northeast region and Belo Horizonte in the Southeast [Costa 2008; Albuquerque et al. 2009; Jeronimo et al. 1994]. A brief review of human immune response to *Leishmania* will help to illustrate why immunocompromised hosts have an increased risk of developing symptomatic VL.

Immune response in symptomatic and asymptomatic visceral leishmaniasis

The manifestations of human *L. i. chagasi* infection vary. The majority of infected subjects experience asymptomatic, self-resolving infection [Badaro et al. 1986; Evans et al. 1992; Holaday et al. 1993; Jeronimo et al. 2000]. Control of infection and resistance to re-infection

requires *Leishmania*-specific Type 1 CD4⁺ T cell responses. The presence of protective immunity can be determined by a delayed type hypersensitivity (DTH) skin test response to *Leishmania* antigens [Montenegro test] or by assessment of production of interferon-gamma by peripheral blood mononuclear cells in response to leishmanial antigens *in vitro* [Carvalho et al. 1981; Carvalho et al. 1988; Bacellar et al. 1991; Bacellar et al. 1996]. *Leishmania*-infected individuals initially develop increase in anti-leishmanial antibodies and later develop positive DTH response; the latter immune response is protective against development of VL symptoms but does not indicate clearance of the *Leishmania* organisms. Symptomatic VL has no cellular immune response to *Leishmania* antigens [Mayrink et al. 1989; Carvalho et al. 1985; Reed et al. 1986; Carvalho et al. 1988; Carvalho et al. 1988]. The immunosuppression is specific to *Leishmania* antigens, and reverses months after successful chemotherapy [Jeronimo et al. 2000, Evans et al. 1995]. A positive DTH response, either after asymptomatic infection or following successful treatment, is an indication that an effective type 1 cellular immune response has developed. This type of response is long lasting [Jeronimo et al. 2000]. Individuals with *Leishmania* asymptomatic infection who are unable to mount an effective type 1 cellular immune response are at increased risk for relapse and recurrence of VL [Nascimento et al., 2008].

Clinical manifestations of visceral leishmaniasis

The incubation period for visceral leishmaniasis is variable, ranging from 2–14 months [Evans et al., 1992]. In rural areas of Brazil, where *Leishmania* is endemic, the ratio of asymptomatic to symptomatic human infections with *L. i. chagasi* varies with age, from approximately 6 – 8:1 in children to 18:1 in adults [Badaro et al., 1986]. These ratios have increased in Brazil, over the same time period as general improvement in health conditions and socioeconomic status [Maciel et al. 2008; Andrade et al. 2009]. Sterile cure is not thought to occur after *Leishmania* infection, whether self-resolving or treated [Mendonca et al., 2004]. Visceral leishmaniasis has developed in former residents of endemic areas who have become immunocompromised several years after having left the endemic area [Badaro et al., 1986].

The onset of VL symptoms is often insidious and difficult to date. The disease usually has a subacute or chronic course, but in some cases, there is a more abrupt onset. Symptoms include fever, malaise, anorexia, weight loss, and abdominal enlargement. Splenomegaly and hepatomegaly are common findings of classic visceral leishmaniasis. The spleen is firm and non-tender and frequently becomes massively enlarged. Case reports of VL in immunosuppression have reported absence of hepatosplenomegaly. Wasting can be pronounced in chronic infection. Hyperpigmentation has been reported in patients in India and the Sudan. Jaundice is occasionally present. Late in the course epistaxis, gingival bleeding, and petechiae can be present. Edema and ascites may also develop due to hypoalbuminemia [Thakur 1984; Evans et al., 1985]. In most endemic areas, the diagnosis of visceral leishmaniasis is based on clinical symptoms and some laboratory findings. Parasite confirmation can be obtained by examination of stained material or culture from specimens collected from bone marrow or splenic aspirates. Anemia, thrombocytopenia, neutropenia, and hypergammaglobulinemia are common. The white blood cell count may be as low as 1,000 per cubic millimeter; eosinopenia is common and is reversed within first

week of successful treatment. The erythrocyte sedimentation rate and C-reactive protein are elevated [Maciel et al., 2008]. The levels of gamma globulin are markedly increased because of polyclonal B-cell activation, with inversion of the normal albumin to globulin ratio [Miles et al., 2005]. The levels of total globulin can be as high as 10 g per deciliter. Circulating immune complexes, autoantibodies and rheumatoid factors are present in many patients [Carvalho et al., 1983, Pearson et al., 1986, Atta et al., 2007]. Glomerulonephritis may develop, but renal failure is rare [Dettwiler et al., 2010].

Immune suppression of various etiologies increases the risk of developing visceral leishmaniasis after infection with *Leishmania* parasites and changes the course of disease. Infection severity, response to therapy, and probability of relapse are worse in this group. HIV-VL co-infection was initially studied in Europe, where VL reemerged parallel to the HIV pandemic [Alvar et al. 2008], but has been described worldwide [Montalban et al., 1989; Desjeux, 1995; Wolday et al., 2001, Daher et al., 2009]. There are case reports and case series that suggest a clinical course similar to those with HIV for individuals who are post-solid organ transplant or on immunomodulatory medications or chemotherapy [Morales et al., 2003; Broeckaert-van et al., 1979; Sirvent-von et al., 2004].

In nonendemic areas, VL can manifest as an opportunistic infection in patients who have emigrated from or visited an endemic area [Bogdan et al., 2001; Schonian et al., 2008]. A study in Basel, Switzerland assessing leishmaniasis cases from 1999 to 2005 found 11 VL cases with underlying immunosuppression [Weisser et al., 2007]. Four cases had HIV, two hematologic malignancies, one was on steroids for granulomatous hepatitis, one had diabetes, and one had mononucleosis. The mortality rate within this group was over 30%. Fernandez-Guerrero et al. [2004] examined all cases of VL in immunocompromised patients at their center in Madrid, Spain from 1991 – 2001. They found no difference in presentation of VL between patients with AIDS and other immunosuppressive conditions. The numbers in this study were small, but the clinical failure rate was higher for non-HIV-infected patients and relapse rates were similar. Although not listed as an AIDS-defining illness by the US Centers for Disease Control, visceral leishmaniasis is considered an opportunistic infection and an indication for prompt initiation of anti-retroviral therapy [Pasquau et al., 2005]. An increase in organ transplant, immunosuppressive medication administration, and HIV in areas where *Leishmania* is endemic creates a potential for more severe cases of leishmaniasis with recurrence. We will review VL with such co-morbidities.

The challenges of treating leishmaniasis

Treatment recommendations for visceral leishmaniasis vary by region [Thornton et al., 2010]. Pentavalent antimonial compounds were considered the treatment of choice for decades, but the development of resistance among *L. donovani* isolates in countries such as India and serious side effects have limited their use in many areas [Berman, 2005; Herwaldt et al., 1992]. Even so, it is still effective and remains standard therapy in many areas of the world, including Brazil [Pearson et al, 1999; Murray et al, 2005]. The recommended dose is 20 mg pentavalent antimony/kg body weight per day given intravenously or intramuscularly. This dose should not be exceeded, because of increased risk of toxicities, including prolonged QT-interval and ventricular tachycardia and pancreatitis. The recommended

duration of treatment is 20–28 days [Sundar, 2001; Berman, 2003]. Longer periods of treatment have been used in patients for whom the first course of therapy is unsuccessful or for the treatment of PKDL [Thakur and Kumar, 1990; Thakur, 1984].

Liposomal amphotericin B for the treatment of visceral leishmaniasis in the United States is a drug approved by the Food and Drug Administration [Pearson et al., 1999, Murray et al, 2005], and is the drug of choice in most industrialized countries and more recently in other countries as its cost has decreased [Sundar et al., 2008; Mueller et al., 2006]. The recommended dosage for liposomal amphotericin B in immunocompetent people is 3mg/kg body weight daily on days 1 through 5, 14, and 21. The cure rate decreases with shorter courses and lower doses, but single dose amphotericin was shown to be effective for VL in Bihar state, India [Sundar et al., 2010; Sundar et al., 1998]. For immunocompromised people, the recommendation is 4mg/kg body weight on days 1 through 5, 10, 17, 24, 31, and 38 [Molina et al., 2007].

Miltefosine (hexadecylphosphocholine), the first oral drug for the treatment of leishmaniasis, is a treatment option in areas where resistance to pentavalent antimonial drugs is widespread and has been approved for use in India [Jha et al., 1999]. However, parasites resistant to miltefosine have been documented [Berman et al., 2006; Choudhury et al., 2008]. It has teratogenic potential and cannot be used in pregnant women. In a study comparing miltefosine and sodium stibogluconate in Ethiopia, miltefosine had a higher initial treatment failure rate (8%) overall compared to 1% of the sodium stibogluconate (SSG) group. There was also a higher rate of initial treatment failure and higher rate of relapse in HIV-positive patients who received miltefosine [Ritmeijer et al., 2006; Berman, 2008].

Paromomycin is an aminoglycoside that is effective against *Leishmania* [Sundar et al., 2007; Hailu et al., 2010]. A randomized controlled trial comparing paromomycin at a dose of 11 mg/kg body weight intramuscularly daily for 21 days with amphotericin B at a dose of 1 mg/kg body weight intravenously every other day for 30 days, in India, showed that paromomycin was as effective as amphotericin B. However, patients on paromomycin developed more adverse events including transient elevation of aspartate aminotransferase levels, transient reversible ototoxicity and more injection-site pain [Sundar et al., 2007; Sundar et al., 2011]. In an open label, randomized control trial in Africa, paramomycin at 15 mg/kg/day for 21 days was ineffective [Hailu et al., 2010, Musa et al, 2010], however the efficacy of higher doses and combination therapy warrant further studies.

Combination therapy for the treatment of VL has been considered with goals of decrease the risk of resistance to the few available agents and for potential decrease in treatment duration. Recently, the efficacy of short-course combination treatments was compared with the standard monotherapy regimen in India [Sundar et al., 2011]. The trial compared liposomal amphotericin B with miltefosine or paromomycin, or miltefosine and paromomycin. All combinations were non-inferior to the standard treatment and patients had fewer adverse events than did those with monotherapy. Cost effectiveness analysis of combination therapies for VL showed that the combination miltefosine-paromomycin was the most cost-

effective treatment strategy, followed by liposomal amphotericin B with paromomycin [Sundar et al., 2011].

There are few additional drugs in the pipeline for treatment of visceral leishmaniasis. Drugs with potential anti-leishmanial activity are detailed in recent reviews [Thornton et al., 2010; Golgher et al., 2011]. Adjunctive immunomodulatory therapy has been studied, but is not part of standard care [Auyeung et al., 2010]. The sequences of different *Leishmania* genomes and proteomic analyses of the different *Leishmania* species should aid identification of potential new pathways and targets for treatment of leishmaniasis [Ivens et al., 2005; El-Sayed et al., 2005; Herrera-Najera et al., 2009].

The complexity of visceral leishmaniasis in the presence of immunosuppression Leishmaniasis and solid organ transplant

Patients who have received solid organ transplants may develop VL post-transplant in the setting of impaired cell-mediated immunity associated with immunosuppression [Oliveira et al., 2008]. They may have reactivation of a previously latent infection or become newly infected. There have been reports of atypical presentations of VL in patients who have received an organ transplant. One case report described a patient on cyclosporine and prednisone after renal transplant who presented with fever, hepatosplenomegaly, leukopenia, and anemia and was found to have amastigotes of *Leishmania v. braziliensis* in bone marrow and skin lesions with later detection in aqueous humor after development of bilateral endophthalmitis during a VL relapse [Antinori et al., 2008]. Araujo et al., [2010] reported a case of ileal *L. i. chagasi* in a patient who had received a cadaveric liver transplant and was maintained on tacrolimus and prednisone. In addition to liver and kidney, VL has been reported in patients post-transplant for lung, heart, and combined kidney-pancreas [Larocca et al., 2007; Morales et al., 2003; Torregrosa et al., 1993]. Campos-Varela et al. [2008] reported on nine cases of VL in liver transplant patients. They found that these VL patients less frequently manifested fever and hepatosplenomegaly, possibly due to iatrogenic immunosuppression [Campos-Varela et al., 2008]. Secondary prophylaxis has been suggested, but there have been no studies to document efficacy of this approach for decreasing recurrence and relapse rates.

Renal transplant is the most commonly reported type of solid-organ transplant in patients with post-transplant VL [Dettwiler et al., 2010]. Of 800 renal transplants conducted at two centers in Fortaleza, Brazil from 2001–2007, there were eight cases of VL, three of whom had relapse 2–7 months after treatment [Oliveira et al., 2008]. Interstitial nephritis related to *Leishmania* infection of kidneys has been reported and can be a cause of recurrent renal failure. A case series from Italy reported five renal transplant patients who developed VL during the early post-transplant period, all of whom had associated graft dysfunction. One patient with graft failure and occlusion of graft vasculature had graft explantation with *Leishmania* amastigotes seen on pathology. After 2004, this center implemented recombinant K39 (rK39) testing as part of pre-transplant screening with monthly assays in the first three months post-transplant followed by testing for any signs and symptoms of visceral leishmaniasis. Two consecutive positive rK39 tests were considered diagnostic and treatment for VL was initiated [Veroux et al., 2010]. The rK39-based ELISA is a sensitive

and specific for diagnosis of symptomatic VL in immunocompetent individuals and can differentiate progressive from self-resolving infection [Braz et al., 2002]. At a hemodialysis center in Natal, Brazil where *Leishmania* is endemic, 22% of patients tested [excluding patients with history of leishmaniasis, malaria, and Chagas disease] were positive for *L. i. chagasi* by Indirect Fluorescent Antibody Technique (IFAT) [Souza et al., 2009]. As the availability of organ transplant increases and more transplant centers and recipients are located in VL-endemic areas in Brazil and elsewhere, we can expect the rate of transplant-associated VL to increase; new guidelines for management of a significant percentage of patients at risk for development of VL in the post-transplant period are needed.

Fitzpatrick et al [2010] described an expanded pre-transplant infectious diseases screening program that includes *Leishmania* antibody tests with clinical follow up for patients with positive antibody results and over two years, they detected two patients with positive *Leishmania* serology pre-transplant. The guidelines of the American Society of Transplantation Infectious Diseases Community of Practice for parasitic infections in solid organ transplant patients were published in 2009 [Kotton and Lattes, 2009]. They recommend SSG, antimony, or amphotericin for first-line treatment of “new world leishmaniasis” and SSG or antimony for “old-world.” They offer a series of recommendations based on grade 3 evidence for solid organ transplant: screening for *Leishmania* antibody pre-transplant, reducing immunosuppression early in treatment for VL, and mention secondary prophylaxis but acknowledge lack of strong evidence for recommending it [Kotton and Lattes, 2009].

Visceral leishmaniasis during usage of immunomodulatory agents and steroid therapy

There has been a number of case reports of VL developing as an opportunistic infection in patients treated with immunosuppression for rheumatologic diseases. It is unclear how common VL presents as an opportunistic infection in this population. One retrospective cohort study in Barcelona, Spain analyzed 94 patients who had been treated with infliximab from 1999 to 2004 and found one case of VL [Garcia-Vidal et al., 2009]. VL has been reported in two patients with rheumatoid arthritis, one receiving infliximab, [Fabre et al. 2005] and the other receiving etanercept [Bagalas et al., 2007]. The latter patient later died of a disseminated fungal infection. Pittalis et al. [2006] reported three cases of leishmaniasis in patients treated with steroids [Pittalis et al., 2006]. These patients experienced relapses of VL despite therapy with amphotericin. VL was diagnosed in a woman with lupus on steroids who developed VL five years after living in Hong Kong and was suspected of having activation of latent disease in the setting of immunosuppression [Wallis and Clark, 1983].

Visceral leishmaniasis and HIV infection

HIV/VL coinfection has been reported in 35 of the 88 countries with visceral leishmaniasis and accounts for 2–12% of the world's VL cases [WHO Report 2007]. The coinfection rate, however, varies and in Ethiopia approximately thirty percent of VL cases are coinfecting with HIV. Southern Europe has reported that 70% of patients with VL also have HIV. From 2001 to 2005 in Brazil, 16,210 cases of VL were reported, 176 associated with an AIDS

diagnosis and 315 with HIV without AIDS. In 2003, Thakur et al [2003] reported that 1.5% of patients with VL at a center in Bihar who consented to be tested for HIV were HIV-positive, compared to 4.1% of controls matched for age and sex.

The original descriptions of VL in patients with HIV pre-dated the use of HAART (Highly Active Antiretroviral Therapy, a regimen combining three or more drugs e.g., two nucleoside reverse transcriptase inhibitors (NRTIs) and a protease inhibitor (PI), two NRTIs and a non-nucleoside reverse transcriptase inhibitor (NNRTI) or other combinations) and nearly all patients had extremely low CD4 counts. There are numerous case reports of visceral leishmaniasis with detection of parasites in atypical locations, such as GI tract, lungs, tonsils, ascitic fluid, duodenum, and rectum in patients with HIV and low CD4 counts [Rosenthal et al., 2000; Pasquau et al., 2005].

Kubar et al [1998] looked at the risks for VL development in patients with HIV in Alpes-Maritimes, France. They found a similar level of *L. infantum* antibody positivity in HIV positive patients and the overall community (approximately 13%), but a higher rate of VL in HIV positive people compared to the community as a whole, which at that time had less than one case of VL per 100,000 inhabitants. They retrospectively and prospectively checked the CD4 count and *L. infantum* serologies of HIV-positive patients in their area and found that VL related to primary *Leishmania* infection occurred at a higher CD4 count than VL from reactivation [Kubar et al., 1998].

Bourgeois et al. [2008], found that introduction of HAART decreased the risk of relapse after treatment of primary infection in their population in France [Bourgeois et al., 2008; 2010]. Twenty-seven HIV positive patients with VL were followed prospectively from 1995 to 2004; sixteen patients had a total of 38 relapses. Predictors for relapse in this study were CD4 < 100 and not on antiretroviral therapy at the time of initial VL diagnosis. They noted no relapses when the CD4 was over 200, although it was variable which patients were maintained on secondary prophylaxis and when this intervention was stopped. A decrease in cases of VL in patients with HIV after introduction of HAART was observed in France in 1996. The population studied was 55,626 HIV patients entered into a national clinical database in which 165 cases of VL were detailed. They found that incidence rate of VL decreased from 11.6/10,000 person-years before 1996 to 6.3 per 10,000 person-years after 1996. The relative hazard for VL in patients on three or more HIV medications was 0.41 with CI 0.26–0.65 [del Giudice et al., 2002].

Despite improvements following the introduction of HAART, HIV/VL coinfection remains a significant problem. Initiation of HAART therapy is not a guarantee that a person with HIV will not develop VL primary infection or post-therapy relapses. A more recent study looked at twenty-three HIV/VL coinfecting patients identified by chart review [Alexandrino-de-Oliveira et al., 2010]. They found that 43% were diagnosed with VL and HIV at the same time. Of the remaining patients, 35% were on antiretroviral therapy at the time of diagnosis; however, only one patient had a HIV viral load suppressed to less than 1000 copies/mL. VL was the first opportunistic infection in 60% of the patients. Of the six patients with six-month follow up, two had VL relapses. These six patients did not have significant increase in CD4 count over those six months despite therapy with HAART. Bourgeois et al. recently

published a series of ten coinfecting HIV/VL patients followed for up to ten years with recurrent VL episodes despite secondary prophylaxis. They propose a distinct entity that they call “active chronic visceral leishmaniasis” to describe this occurrence [Bourgeois et al., 2010].

Outcomes for patients with HIV and VL from 2003– 2006 were studied in Humera, Ethiopia where HAART was introduced in 2004. Approximately 356 coinfecting patients were followed over 2928 patient-months of follow-up with development of 256 VL episodes and 39 deaths. Identified risk factors for relapse in this population were CD4 < 100, TB coinfection, and previous episode of VL, but potential reinfection could not be excluded in this endemic area. About of 28% of first VL relapses in patients on ART occurred with a CD4 of greater than 200, although only 5% had had a CD4 greater than 200 for 6 months. For those who developed VL after starting ART, the mean time to diagnosis of VL was 3 months after starting ART with a mean CD4 of 130 [Collin et al., 2004]. Colomba et al [2009] screened 145 HIV-positive patients for exposure to *L. infantum* by looking for kDNA by PCR (24/145 positive) and for IgG antibody [2/145 positive]. Among the two *Leishmania* antibody-positive patients, one had a history of VL. They found that kDNA positivity was associated with elevated viral load, but not CD4 count [Colomba et al., 2009].

Visceral leishmaniasis has also been described as a manifestation of the immune reconstitution syndrome (IRIS) occurring after initiation of antiretroviral therapy, but these are limited to case reports [Auyeung et al., 2010]. Neither maintenance therapy nor secondary prophylaxis have been proven effective for eliminating or decreasing VL relapse episodes, although some studies have suggested benefit from prophylaxis. In a multicenter, open-label randomized clinical trial conducted from 1997– 1999, seventeen HIV-positive patients with VL were randomized to lipid complex amphotericin or no treatment every twelve days for one year. The odds ratio for not developing relapse was 3.5 favoring amphotericin, but with a confidence interval of 0.3– 52 [Lopez-Velez et al., 2004].

Molina et al [2007] studied seventeen HIV-positive patients from 2001–2005 who had had at least one prior episode of VL and who all were receiving liposomal amphotericin as secondary prophylaxis. Nine patients had 21 episodes of VL. The probability of remaining relapse-free at 6 months was 90% but at 36 months was only 56%. The numbers in this study were small, but the authors noted a significant increase in CD4 in the non-relapsing group and no significant increase in the relapsing group [Molina et al., 2007]. Another study in Spain reported on 228 VL episodes in 155 coinfecting HI/VL patients from 1988– 2001. Of the patients who had VL relapse, 30% of the patients were on HAART and 42% were on secondary prophylaxis for VL at the time of relapse, although secondary prophylaxis was considered to be protective for relapse [Pasquau et al., 2005].

VL relapses in HIV-positive patients do not appear to be related to development of resistance to amphotericin. Lachaud et al [2009] evaluated 10 patients coinfecting with HIV and *L. infantum* in France followed for a mean of 36 months. They received lipid formulation amphotericin B for treatment of primary episode, followed by secondary prophylaxis with liposomal amphotericin. Of these patients with recurrent VL, three of ten

were reinfections and seven of ten were relapses, none of which demonstrated decreased susceptibility to amphotericin by an assay developed by the investigators.

Visceral leishmaniasis and malignancy

Visceral leishmaniasis at time of diagnosis of malignancy and during chemotherapy treatment has been reviewed [Kopterides et al., 2007]. VL can be an opportunistic infection in patients with malignancy, but the literature on malignancy and VL is restricted to case reports and small case series. Domingues et al. [2009] described a 15-year-old Brazilian boy with Hodgkin's Disease in his second remission when biopsy of an enlarged lymph node showed both *Leishmania* amastigotes and pathologic findings consistent with Hodgkin's disease. Fakhar et al. [2008] reported on a 12 year-old girl in Iran with acute lymphocytic leukemia and biopsy-confirmed VL. VL has also been described during treatment for solid tumors, including head and neck and colon cancers [Kopterides et al., 2007].

Pregnancy

Despite a decrease in cell-mediated immunity during pregnancy, women do not seem to have a higher incidence of disease during pregnancy. However, pregnant women seem to have severe VL disease. A retrospective analysis comparing ambisome and sodium stibogluconate (SSG) for 39 pregnant women with VL in Sudan was reported by Mueller et al [2006]. Prior to September 2004, pregnant women with VL were treated with SSG (n=23), but with ambisome and SSG for clinically severe VL (n=4). From October 2004, all pregnant women received ambisome (n=12). The authors report clinical cure for all women but one in the SSG group who defaulted on therapy. There were thirteen spontaneous abortions in the SSG group. Women were discharged without follow up after therapy was completed, so data on relapse, recurrence, and outcome for delivered infants are incomplete. In another study from eastern Sudan conducted from 2006- 2009 [Adam et al., 2009], women were treated with sodium stibogluconate for 30 days. They were followed up every 2 weeks until delivery and for twelve months after delivery. Infants born to these mothers were followed for one year. All women were HIV negative and all received blood transfusions for severe anemia. Four women died of hepatic encephalopathy during treatment. One infant developed failure to thrive, fever and hepatosplenomegaly and died during the first week of treatment. No note was made of recurrence or treatment failures in the women who were treated for VL. Figueiro-Filho et al. [2008] described a case series of five women treated for VL during pregnancy, four with ambisome and one with antimony for VL detected post-delivery died of hemorrhage post-delivery. No relapses in women or VL in delivered babies were reported.

Conclusions

Visceral leishmaniasis is a disease with significant mortality and economic burden. The epidemiology of the disease is changing and in Brazil the impact on periurban areas is increasing. Expansion of geographic distribution coincides with concomitant increase in populations in endemic areas at risk for symptomatic VL such as those with HIV, post-organ transplant, and on immunosuppressive regimens. Individuals currently living in or who have

visited or emigrated from endemic areas are at risk for development of clinical VL during immunosuppression. Guidelines exist for management of people at risk for VL peri-transplant [Kotton and Lattes, 2009] and for people who are coinfecting with HIV and VL. However, further research is needed to manage visceral leishmaniasis with comorbidities.

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Number of *Leishmania*-infected subjects in a household considering the percentage of subjects residing in the same household with a history of VL.
Leishmania infection is greater with increasing number of family members with history of visceral leishmaniasis

Table 1

<i>Leishmania</i> infection *	Per cent of VL cases in the household									
	0		0-10		10-20 %		>20		Total	
	N	[%]	N	%	n	%	N	%	n	%
Negative	215	59.2	303	51.8	297	39.3	108	26.0	923	43.5
Positive	148	40.8	282	48.2	459	60.7	308	74.0	1197	54.5
Total	363	17.1	585	27.6	756	35.7	416	19.6	2120	100

Leishmania infection was considered as either presence of anti-*leishmania* antibodies or positive LST. M-L Chi square [Chi square 112.9, df=3, p<0.0001]