

Animal reservoirs for visceral leishmaniasis in densely populated urban areas

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Abstract

Leishmaniasis is a zoonotic disease of major public health and veterinary importance, affecting 88 countries with up to 2 million cases per year. This review emphasizes the animal reservoirs and spreading of visceral leishmaniasis (VL) in urban areas, particularly in two Brazilian metropolitan areas, namely São Luis and Belo Horizonte, where the disease has become endemic in the past few years. Urbanization of visceral leishmaniasis in Brazil during the last decades has created favorable epidemiological conditions for maintenance of the disease, with dense human populations sharing a tropical environment with abundant populations of the mammalian reservoir and the invertebrate vector, facilitating transmission of the disease.

Key Words: visceral leishmaniasis, *Leishmania*, animal reservoir.

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Introduction

Leishmaniasis is a zoonotic disease of major public health and veterinary importance. According to the World Health Organization, the visceral form of the disease is endemic in 88 countries distributed in four continents, with 1.5 to 2 million cases per year and 350 million people under risk of infection (WHO; <http://www.who.int/tdr/index.html>). Human leishmaniasis in the Americas can be classified into two broad categories, namely American tegumentary leishmaniasis (TL) and American visceral leishmaniasis (VL) also known as “kala-azar”. American TL may have several clinical manifestations, including (i) cutaneous leishmaniasis, characterized by skin lesions, which may heal spontaneously or progress to chronic lesions with severe scarring; (ii) mucocutaneous leishmaniasis, characterized by ulcerative and destructive lesions in mucous membranes and mucocutaneous junctions; and (iii) diffuse cutaneous leishmaniasis, characterized by non-ulcerative nodular lesions. Conversely, the visceral form of the disease is chronic and

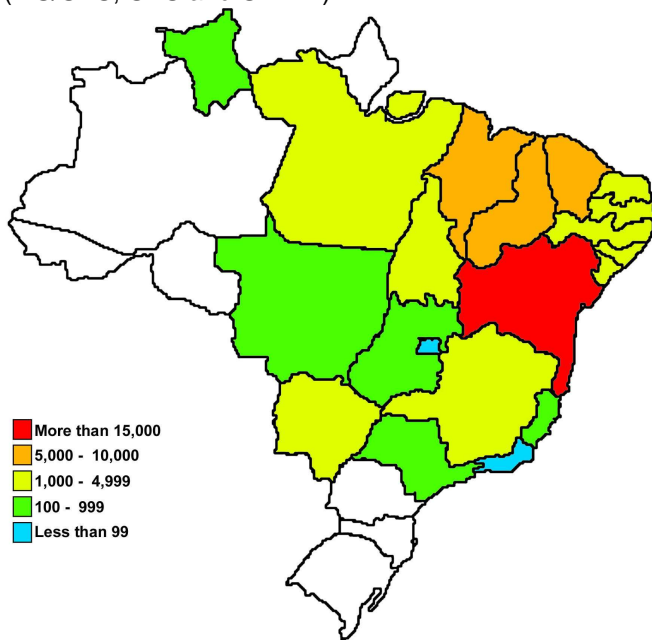
progressive, affecting several organs including the spleen, liver, bone marrow, lymph nodes, and skin.

Human VL has a wide distribution throughout Latin America, extending from Mexico to Argentina [1]. American VL is rapidly spreading in several regions of Brazil, and it is currently considered an urban disease. Migration of rural populations to periurban areas led to urbanization of the disease [2], since it created conditions in which the human population shared an environment with high densities of canine and invertebrate vector populations [3]. Environmental changes resulting in habitat destruction may have a significant impact on vector populations, with some species disappearing, and others becoming more abundant as some species can colonize and adapt to anthropogenic environmental conditions [2].

The distribution of American VL in Brazil includes the states of Alagoas, Bahia, Ceará, Distrito Federal, Espírito Santo, Goiás, Maranhão, Mato Grosso, Mato Grosso do Sul, Minas Gerais, Pará, Paraíba, Pernambuco, Piauí, Rio de Janeiro, Rio Grande do Norte, Roraima, Sergipe, São Paulo, and Tocantins [1] (Figure 1). There is a

broad variation in the prevalence of VL among different regions in Brazil, with higher prevalence in the Northeastern part of the country (Figure 1). According to the Brazilian Ministry of Health (Fundação Nacional de Saúde - FUNASA; www.funasa.gov.br), from 1980 to 2005, there were 60,954 cases of VL registered in Brazil, with the highest number of cases in the States of Bahia (16,635) and Maranhão (8,484). Official records also indicate over 1,000 new human cases of VL yearly in Brazil. In the State of Maranhão, human and canine cases concentrate in the Island of São Luis (Figure 2), where there is a large urban area. In the last five years, 239 human cases of VL that led to 13 deaths and 7,682 canine cases were recorded (Center for Zoonosis Control of São Luis, unpublished data).

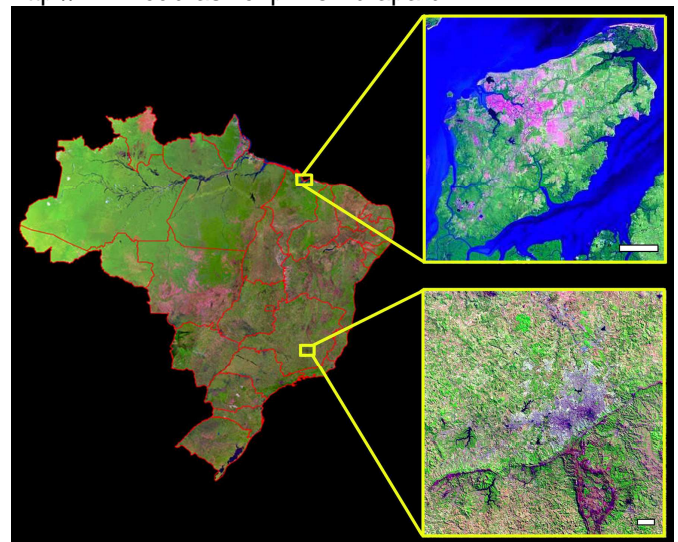
Figure 1. Distribution of human visceral leishmaniasis in Brazil. Cumulative number of cases from 1980 to 2005. Source of data: Brazilian Ministry of Health (MS/SVS, SES and SINAN).



According to the Health Department of the State of Minas Gerais (SES/MG), 2,018 cases of human VL were confirmed in the state, resulting in 188 deaths (average lethality rate of 10.8%) from 2000 to 2005. The cities of Belo Horizonte, Ribeirão das Neves and Santa Luzia (all part of the metropolitan area of Belo Horizonte), as well as Montes Claros and Janaúba (in the northern part of the State), and Paracatu (in the western part of the State) accounted for 51% of the cases.

The metropolitan area of Belo Horizonte (Figure 2), with an estimated population of more than three million people and an area of approximately 6,000 Km², had the first case of human VL in 1994. After that first case, the disease spread quickly with 345 human cases of VL confirmed in that area between 1994 and 1999 [4].

Figure 2. Satellite view of the metropolitan areas of São Luis (State of Maranhão) and Belo Horizonte (State of Minas Gerais). Bar = approximately 7 Km. Source: MIRANDA, E.E.; COUTINHO, A.C. (Coord.). Brasil Visto do Espaço. Campinas: Embrapa Monitoramento por Satélite, 2004. Available at: <http://www.cdbrasil.cnpem.embrapa.br>.



Here we review the literature and present some unpublished data with emphasis on animal reservoirs and the spread of VL in urban areas, particularly in two Brazilian metropolitan areas, namely São Luis and Belo Horizonte (Figure 2).

Etiology of visceral leishmaniasis

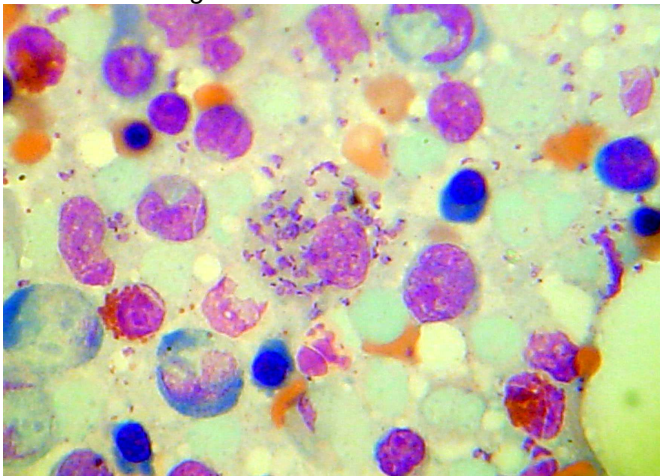
VL is caused by flagellated protozoa belonging to the order Kinetoplastida, family Trypanosomatidae and genus *Leishmania*. Human visceral leishmaniasis is caused by *Leishmania donovani* and *Leishmania infantum* in the Old World and *Leishmania chagasi* in the New World [reviewed by 5 and 6]; however, there is currently a tendency to consider *L. infantum* and *L. chagasi* as one single species [7].

Leishmania is a parasite that has heteroxeny during its life cycle so it has two hosts, one vertebrate (canids, rodents, and humans) and the other invertebrate sandfly (Order Diptera, Family

Psychodidae, Sub-family Phlebotominae) belonging to the genus *Phlebotomus* in the Old World and the genus *Lutzomyia* in the New World, with one vector species, *Lutzomyia longipalpis*, in Brazil [1,8,9].

Leishmania has distinct forms during its life cycle. Promastigotes and paramastigotes are flagellated and mobile, and they are present in the digestive tract of the invertebrate host, whereas the non-flagellated amastigotes (Figure 3), are found within phagocytic cells in the vertebrate host [10]. The infective form, named metacyclic promastigote is smaller with a longer flagellum when compared to the procyclic or non-infective form. Upon infection of the vertebrate host, the promastigotes undergo phagocytosis and become amastigotes, multiplying intracellularly until the host cell ruptures, releasing the parasite, which then infects other macrophages [11, 12]. The different clinical manifestations of the disease are determined by the species of the parasite as well as the immune condition of the host [11].

Figure 3. Bone marrow smear from a dog with a macrophage containing a myriad of amastigotes of *Leishmania chagasi*.



Natural hosts and reservoirs of visceral leishmaniasis in metropolitan areas

VL used to be restricted to rural and peri-urban areas in Brazil. However, with emergence of foci of the disease in urban areas, visceral leishmaniasis has assumed a major role in public health [13]. The domestic dog is the most important reservoir of VL in metropolitan areas. The dog develops an intense cutaneous parasitism, favoring infection of sandflies, thereby playing an important role in the epidemiological chain of VL [14]. Importantly, most

dogs remain asymptomatic for long periods of time, which contribute to the maintenance and transmission of the disease, since the absence of clinical signs delays the diagnosis favoring the maintenance and transmission of VL. Indeed, it has been demonstrated that there is a significant correlation between the spatial distribution of seropositive dogs and human cases of VL in the metropolitan area of Belo Horizonte [15]. Furthermore, the geographic distribution of the first cases of canine VL in Belo Horizonte from 1993 and 1997 coincided with the emergence of human VL. Apparently under those conditions the establishment of human VL was preceded by infection of dogs in a given area [16].

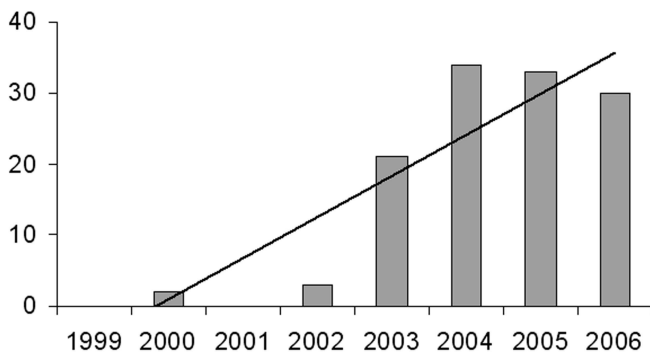
There are no differences in predisposition among dogs of different ages or sexes. However, breed predisposition has been documented, with Cocker and Boxers having a higher risk when compared to other breeds [17]. Furthermore, dogs with short hair are predisposed when compared to those with long hair. The role of other species as reservoirs is not clear. For instance, natural infection of domestic cats with *Leishmania* has been documented [18]. In Europe, in addition to dogs, rodents are also identified as reservoirs, whereas humans and domestic cats are considered accidental hosts [19,20].

Environmental degradation associated with intensive migration of human populations from rural into urban areas favored urbanization of VL in Brazil. In the Island of São Luis, the first urban cases of human VL were identified in 1982 [21]. Concomitantly, the first cases of VL in dogs were reported in that region [22]. Initially, human and canine VL occurred in the periphery of the city of São Luis, but it gradually spread to other municipalities on the island (Paço do Lumiar, São José de Ribamar, and Raposa). The first cases of VL coincided with marked environmental changes and migration of rural human populations into the urban area. In addition, large industrial plants were established at that time, which stimulated influx of immigrants from other Northeastern Brazilian States where VL was endemic [23, 24]. Currently there are areas of the metropolitan region of São Luis with an extremely high seroprevalence in dogs, reaching 25% in one of the districts [25]. These high seroprevalences are associated with poor sanitation and housing conditions in areas previously occupied by the natural vegetation [26].

An ecoepidemiological study is currently underway in the Island of São Luis to identify risk factors for visceral leishmaniasis in that particular area.

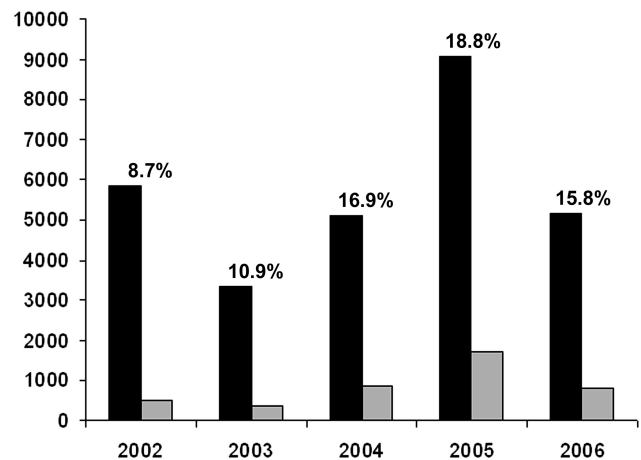
Similarly, the first case of VL in the metropolitan area of Belo Horizonte was diagnosed in 1994, and was followed by a fast spreading of the disease with 345 confirmed human cases from 1994 to 1999 [4]. In 2001, there were 31 cases of human VL reported, resulting in six deaths (Secretaria Municipal de Saúde de Belo Horizonte; <http://www.pbh.gov.br/smsa/>). In 2004, the number of human cases reached 128 with 21 deaths in the metropolitan area of Belo Horizonte, whereas in the whole State of Minas Gerais there were 587 cases with 49 deaths during the same period. Taking as an example Ribeirão das Neves, which is part of the Belo Horizonte metropolitan area, the first autochthonous case of VL was confirmed in 2000, with no cases reported in 2001, but with a marked increase in the number of cases after 2002 (Figure 4). Interestingly, the increase in human cases of VL coincided with increases in seroprevalence in the canine population in the same area (Figures 4 and 5).

Figure 4. Number of confirmed cases and trend line of human visceral leishmaniasis in Ribeirão das Neves (metropolitan area of Belo Horizonte, State of Minas Gerais) from 1999 to 2006.



Although the dog is recognized as the major source of infection for the invertebrate vector and therefore potential transmission to humans [15, 27-30], several species of wild canids, marsupials, and rodents have been incriminated as reservoirs in endemic areas [5]. However, the suitability of these species as reservoirs as well as their role in the epidemiology of VL remain to be investigated [1].

Figure 5. Number of samples of canine serum processed (black columns) and number of seropositive dogs (gray columns) from 2002 to 2006. The percentages indicate the frequency of seropositive dogs per year.



Several wildlife species from the Amazon rain forest in Brazil, including rodents, marsupials, procyonids, canids, and edentates have been surveyed for *Leishmania* infection. The parasite was detected only in the crab-eating fox (*Cerdocyon thous*) by culturing samples of the spleen and liver, and by inoculation in hamsters [31]. However, *L. chagasi* has been previously isolated from two opossums, *Didelphis albiventris*, captured in an endemic area in Jacobina (State of Bahia, Brazil), but the rate of infection was very low [32,33]. Furthermore, *L. chagasi* has been cultured from spleen, liver, and skin of the opossum (*Didelphis marsupialis*) in Colombia [34, 35]. In addition, infection of the rodent *Proechimys canicolus* has been demonstrated by PCR [36].

In order to be considered an effective reservoir of a parasite that is transmitted by a blood-suckling insect, in addition to playing a role in the maintenance and dissemination of the parasite under natural conditions, it is essential to demonstrate that the putative reservoir species effectively infects the invertebrate vector during the blood meal [1]. Considering these criteria, only *Cerdocyon thous* and *Didelphis marsupialis* are considered natural wild reservoirs of *L. chagasi* [35, 37].

Didelphis sp. is present in both São Luis and Belo Horizonte metropolitan areas where there is a high canine seroprevalence [25, 26]. However, although there is some evidence indicating that the

presence of opossums is a predisposing factor for canine infection [38], the epidemiological role and the impact of the opossum as a reservoir of VL in urban areas is still not clear.

In spite of the fact that the dog is the major reservoir for human VL, other animal species may play a significant role in the maintenance of the disease. These include chickens, pigs, cattle, and horses kept around the residences in urban areas, which favor proliferation of the vector and therefore transmission of the disease [39, 40]. Importantly, evaluation of the intestinal contents of *L. longipalpis* indicated that 24.4% (n=547) fed on vertebrate blood, including avian (87.9%); rodent (47.2%); human (42.4%); canine (27.6%); opossum (26.6%); and equine (22.5%) blood [41].

Transmission

Transmission of VL generally takes place between the invertebrate vector and a mammalian host. A female phlebotominae sandfly vector is infected by ingesting amastigotes present in the dermis during the blood meal. The amastigotes transform into promastigotes within the digestive tract of the insect from 24 to 48 hours after the blood meal. The promastigotes then proliferate in the intestine and migrate to the esophagus and pharynx, and are regurgitated into the mammalian host during the blood meal [42]. Soon after inoculation the promastigotes are phagocytosed by macrophages and locate inside a vacuole that fuses with lysosomes, named the parasitophorous vacuole [10, 43-45]. The promastigotes then transform into amastigotes that replicate intracellularly eventually causing the rupture of the macrophage and thereby infecting other phagocytic cells [10, 46]. Thus, control programs for VL must take into account the vectors, reservoirs, and susceptible hosts. Understanding the circumstances in which parasites are transmitted from dogs to sandflies is essential for implementing sound prophylactic measures [3].

The role of *L. longipalpis* as vector of AVL was first suspected in the 1930s when liver samples from patients suspected of yellow fever were diagnosed with VL. This first study performed in the Northeastern Brazilian State of Sergipe resulted in the description of the first clinical case of VL in a patient whose house had large numbers of *L. longipalpis* both indoors and outdoors [47]. Later on, other cases of human and canine VL

were also linked to *L. longipalpis* [48]. However, the role of this sandfly as a vector for VL was initially characterized in the 1950s, when promastigotes of *L. chagasi* were detected in *L. longipalpis*, and infection occurred after exposure of foxes to infected sandflies [49-51]. At that time, the dog was identified as the main reservoir of the disease since *L. longipalpis* often became infected after feeding on infected dogs [27]. Subsequently, transmission of *Leishmania* was demonstrated in hamsters via the bite of experimentally infected laboratory-bred *L. longipalpis* [52]. The role of *L. longipalpis* was definitely established after identification of this sandfly as the only species present in the environment where cases of human VL were diagnosed, with sandflies from this particular area being able to infect hamsters [53, 54].

L. longipalpis was considered to be the only biological vector of *L. chagasi* for several years, but cases of VL began to be diagnosed in regions where this sandfly species was absent, indicating that other species could be transmitting the parasite to vertebrate hosts. Indeed, *Lutzomyia evansi* infected with *L. chagasi* was identified as the major vector for VL in certain areas of Colombia [55, 56] and Venezuela [57]. *L. evansi* is present in several Latin America countries including Costa Rica, Honduras, El Salvador, Guatemala [58] and Mexico [59], but it has not been detected in Brazil. Although there is evidence supporting the notion that *Lutzomyia cruzi* may act as an alternative vector for VL in Brazil [60], this view is still debatable [1].

L. longipalpis is the predominant sandfly species in São Luis, where it is abundant in the peridomestic environment [61-64]. This behavioral feature of *L. longipalpis* may partially explain the higher rates of infection in dogs than in humans [1]. Importantly, *L. longipalpis* has been captured indoors and outdoors in all areas with reported cases of human VL in São Luis. *L. longipalpis* is also the predominant species of sandfly in the metropolitan area of Belo Horizonte [65].

Although VL is largely recognized as an insect-borne disease, there are several reports of transmission in the absence of the vector. For instance, the transmission through blood transfusions in human patients has been reported [66] as well as transmission by common use of needles among drug addicts [67]. Vertical

transmission of *Leishmania* has been documented in humans [68]. Although a previous study failed to demonstrate vertical transmission in dogs [72], amastigotes of *Leishmania* have been detected in the uterus of a bitch with evidence of vertical transmission [69] and has been suggested by other investigators [70, 71]. Venereal transmission of VL has been documented between an infected man and his wife in an area completely free of the insect vector [73]. This form of transmission is also likely to occur in dogs [27]. We have recently demonstrated that dogs with VL develop specific genital lesions, namely epididymitis and balanopostitis, with an abundance of intralesional amastigotes. These lesions are associated with shedding of *Leishmania* in the semen, further supporting the notion of venereal transmission in dogs [74]. We are currently investigating whether venereal transmission actually occurs in dogs. Although the epidemiological significance of venereal transmission in dogs is merely speculative at this point, this form of transmission may have a significant impact in densely populated urban areas, particularly in Brazil where the vast majority of the canine population is not neutered.

Impact of vaccination in dogs

According to the World Health Organization, control measures for VL include reducing the population of the insect vector by massive application of insecticides, elimination of seropositive dogs, and early diagnosis and treatment of human cases [75]. In Brazil, the control of VL is heavily based on elimination of positive dogs, and although this measure does have some effect on the number of human cases, it is not sufficient for eradication of the disease [75, 76]. In 2003, the Brazilian Ministry of Health made new recommendations for controlling VL, including the control of the vector base on entomological surveys. Thus, application of insecticide that was previously performed within 300 meters of each confirmed human case, now is performed in areas classified as moderate or high risk of transmission, whereas areas free of the vector are not treated with insecticide and remain under entomological surveillance [77].

The control of canine VL is based on the elimination of seropositive dogs; serological surveillance in areas with high or moderate risk of transmission; the use of collars impregnated with

4% deltamethrin; the use of kennels with screens; and control of the population of stray dogs. However, treatment of canine VL is not recommended since it results only in improvement of clinical signs, but treated dogs remain a reservoir of the disease [77, 78].

A commercial vaccine against canine VL is currently available in Brazil [79]. This vaccine has been approved by the Brazilian Ministry of Agriculture, which is responsible for evaluation and approval of therapeutic and prophylactic animal health products. However, the Brazilian Ministry of Health does not recommend vaccination of dogs for prevention of human VL, and proposes the elimination of seropositive dogs in areas with transmission of the disease even if the dog has been vaccinated ("Nota Técnica", May 09, 2005).

Recently, LiESAp-MDP, which is an experimental but not commercially available vaccine, had an efficacy of 92% in experimentally and naturally infected dogs in France, with protection lasting for 24 months [80]. The vaccine commercialized in Brazil has 79% efficacy after 12 months of vaccination [79]. In Iran, another experimental vaccine that is a precipitate of *Leishmania* major associated with aluminum hydroxide and the BCG vaccine resulted in an efficacy of 69% [81].

Although in theory vaccination of dogs may have a significant impact in the occurrence of human VL, a drawback of the vaccine currently available in Brazil is that it does not allow for serological differentiation between infected and vaccinated dogs, which may eventually compromise the efficacy of the official programs for controlling VL.

Future perspectives

Human VL in Brazil affects mostly the low-income class. Therefore, improving socioeconomic conditions is an essential step for effectively controlling the disease. According to Link and Felan [82], socioeconomic deprivation might be a "fundamental cause" of disease because it favors multiple risk factors. In fact, in the State of Maranhão, which has a low Human Development Index (HDI) and is the poorest state in Brazil (<http://www.undp.org.br>), has a high number of cases (Figure 1). VL has a complex correlation with poverty, involving factors such as poor housing and sanitation conditions, economically

driven migration, poor nutrition, and the predisposition to other infectious diseases, which are features of the most heavily affected populations [reviewed by 83].

Poor sanitation is a factor that contributed to maintenance and dissemination of VL in two districts of São José do Ribamar, the metropolitan area of São Luis [25]. Cases of human VL tend to have the same spatial pattern as irregular land occupations due to migratory fluxes [24]. Generally, the habitations are built near pre-existing forests or non-developed areas, where there is a tendency for an abundance of the vector and reservoirs [26].

Other factors that may have a significant impact in controlling VL in the future include the development of an insecticide with lower environmental impact and the development of new drugs that require a shorter therapeutic scheme and that are less toxic [84]. In addition, improvement of the currently available serological tests reducing the occurrence of false-negative results may also influence the efficacy of control programs. This could be achieved by using immunodominant antigens for serological detection, which may favor standardization of the assay and improvement of its sensitivity and specificity, particularly during the early stages of canine VL.

Urbanization of VL in Brazil during the last decades has created a very favorable epidemiological condition for maintenance of the disease, with dense populations of reservoirs, vector and humans sharing the same tropical environment that is extremely favorable for transmission of the disease. Thus, controlling VL under these conditions is an enormous challenge.

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