

Phlebotomine sand flies and canine infection in areas of human visceral leishmaniasis, Cuiabá, Mato Grosso

Flebotomíneos e infecção canina em áreas de leishmaniose visceral humana, Cuiabá, Mato Grosso

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Abstract

Visceral leishmaniasis is a systemic infectious disease that can cause to a severe, potentially life-threatening chronic condition in humans. Risk factors for infection in urban areas have been associated with poor living conditions, the presence of sand fly vectors and infected pets. This study aimed to describe sand fly and canine infection in the neighborhoods of human visceral leishmaniasis occurrence in the city of Cuiabá, Mato Grosso State, central-western Brazil, reported between January 2005 and December 2006. A total of 1,909 sand flies were collected. They were predominantly males and the most frequent species were *Lutzomyia cruzi* (81.25%), *Lutzomyia whitmani* (13.88%) and *Lutzomyia longipalpis* (2.62%). The sand fly density was not significantly correlated with the variation of environmental factors. The prevalence of canine visceral leishmaniasis in the neighborhoods studied was 26.82% and it was found that areas with high density of vectors coincided with areas of high prevalence of dogs and those with the highest rates of human cases. The study of vectors and other potential hosts are essential for a good understanding of visceral leishmaniasis and the related public health concerns, aiming at the prevention and control of leishmaniasis in the city of Cuiabá, Mato Grosso State.

Keywords: Visceral leishmaniasis, *Lutzomyia cruzi*, *Lutzomyia longipalpis*, dog, Mato Grosso.

Resumo

A leishmaniose visceral é uma doença infecciosa sistêmica, de evolução crônica grave, potencialmente fatal para o homem. Os fatores de risco para a infecção em áreas urbanas têm sido associados às precárias condições de moradia, à presença de flebotomíneos vetores e de animais domésticos infectados. O presente trabalho objetivou descrever a fauna flebotomínica e infecção canina nos bairros de ocorrência da leishmaniose visceral humana no município de Cuiabá, Mato Grosso, notificados no período de janeiro de 2005 a dezembro de 2006. Foram coletados 1.909 flebotomíneos, as espécies mais frequentes foram *Lutzomyia cruzi* (81,25%), *Lutzomyia whitmani* (13,88%) e *Lutzomyia longipalpis* (2,62%). A densidade de flebotomíneos não apresentou correlação significativa com a variação dos fatores ambientais. A prevalência de leishmaniose visceral canina nos bairros foi de 26,82% e observou-se que as regiões com grande densidade de vetores coincidem com áreas de alta prevalência em cães e áreas nas quais foi detectado o maior número de casos humanos. O estudo de vetores e outros possíveis hospedeiros são imprescindíveis para um bom entendimento da doença a fim de gerar benefícios para a saúde pública, visando à prevenção e o controle das leishmanioses no município de Cuiabá e no Estado de Mato Grosso.

Palavras-chave: Leishmaniose visceral, *Lutzomyia cruzi*, *Lutzomyia longipalpis*, cão, Mato Grosso.

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†In memoriam

Introduction

Visceral leishmaniasis (VL) is a systemic infectious disease that can cause a severe, potentially life-threatening chronic condition in humans (BRASIL, 2006). In the Americas, *Leishmania (Leishmania) chagasi* (Cunha; Chagas, 1937) is the causative agent of human and canine VL (FRANÇA-SILVA et al., 2003).

The World Health Organization (WHO) considers VL the sixth leading tropical disease, given its high incidence, high mortality in malnourished children and untreated individuals and emergence in HIV-infected individuals. There are reported each year about 500,000 new cases of VL, with 90% occurring in India, Bangladesh, Nepal, Sudan and Brazil (WHO, 2010).

In Latin America, VL has been described in at least 12 countries. Ninety percent of VL cases occur in Brazil being reported in 19 of the 27 Brazilian states. According to the Brazilian Ministry of Health, in the last ten years, there was an average of 3,156 cases annually, 54% of them among children under 10 years and mean mortality rate of 8%, mainly affecting rural areas and suburbs of large municipalities (BRASIL, 2006). Poor living conditions and presence of infected domestic animals have been identified as risk factors for *L. (L.) chagasi* infection in urban areas (MORENO et al., 2005).

The transmission of *L. (L.) chagasi* occurs through the bite of female dipteran insects belonging to the Psychodidae family, Phlebotominae subfamily; *Lutzomyia longipalpis* (Lutz, Neiva, 1912) is the main vector species of VL in the Americas (ARIAS et al., 1996). They have crepuscular and nocturnal habits and are well adapted to the peridomestic environment, feeding on a wide variety of vertebrate hosts, including humans (MORENO et al., 2005). Another species, *L. cruzi* (Mangabeira, 1938) was also described as a vector of VL in Corumbá, Mato Grosso do Sul, central-western Brazil (GALATI et al., 1997; SANTOS et al., 1998) and Jaciara/MT (MISSAWA et al., 2011).

The first VL cases in Cuiabá were reported in 2005. There were five native cases during the year, with two deaths. In the first half of 2006, three more cases and one death were reported in the capital Cuiabá. It is noteworthy that among the eight patients with confirmed VL in Cuiabá, five were living in urban areas, mainly concentrated in the northern district of the city (MESTRE, 2006).

According to data from the Coordination Office for Environmental Surveillance of the State Health Department of Mato Grosso (SES-MT) in the period 1998 to 2005, entomological surveys were conducted in 68 municipalities in Mato Grosso, and *L. chagasi* vectors species were identified in 32 of them. The isolated presence of *L. longipalpis* and *L. cruzi* has been recorded in nine and eight municipalities, respectively. Of the 18 municipalities where concurrent transmission of human and canine VL has been reported, disease vectors were found in 14 (MESTRE; FONTES, 2007).

The entomological surveys conducted in Cuiabá by the Animal Disease Control Center in partnership with SES-MT laboratory of entomology identified vector species of VL in the neighborhoods of the capital, and they were even present in areas of simultaneous occurrence of human and canine VL (MESTRE; FONTES, 2007). The present study aimed to describe sand fly and canine infection in the city of Cuiabá, Mato Grosso State, Brazil.

Materials and Methods

1. Study area

Mato Grosso is located in the central-western region of Brazil and its capital city is Cuiabá. The capital is situated on the left bank of the Cuiabá River, at an average altitude of 165 m above sea level, covers an area of 3,362,755 km² and has a population of 551,098 inhabitants (Brazilian Institute of Geography and Statistics, IBGE, 2010). The climate is tropical hot, with approximately five months of drought from May to September. The mean annual rainfall is 1750 mm, with maximum intensity in December, January and February. The maximum temperature during the summer months is around 43 °C, with an average annual of 27 °C. The predominant vegetation is "cerrado" (savanna) (PIAIA, 1999).

The neighborhoods were selected based on the occurrence of human VL cases reported in the Information System for Notifiable Diseases of the Municipal Health Cuiabá (BRASIL, 2007), from January 2005 to December 2006 (Figure 1).

The neighborhoods Jardim Três Poderes, Jardim Itapuã, Jardim União e Barreiro Branco are in the northern district in the area of Centro Político Administrativo and have an estimated population of 7,500 inhabitants. São Roque is a subdivision from Bela Vista and it is contiguous areas belonging to the eastern district, with an approximate population of 8,500 inhabitants (Institute for urban research and development, IPDU, 2009). There are relatively new areas of irregular urban settlement consisting primarily of low-income families and are characterized by lack of basic infrastructure and strong anthropogenic actions.

2. Sand flies capture and identification

Sand flies were captured using the US Centers for Disease Control and Prevention (CDC) light traps (SUDIA; CHAMBERLAIN, 1988) installed around 10 households and used between 5:00 PM and 7:00 AM. They were georeferenced using the Global Positioning System (GPS). Samples were collected during three consecutive nights per month from January 2008 to April 2009. Two traps were installed in Jardim Itapuã, two in Jardim Três Poderes, two in Jardim União, two in the neighborhood of Barreiro Branco, one trap was installed in the Novo Milênio ranches, and two in Bela Vista neighborhood, including the set of São Roque households.

Insects collected were killed with ethyl acetate and sent to the laboratory of entomology at the Institute of Biology at Universidade Federal de Mato Grosso (UFMT) for sex determination and confirmation as proposed by Young and Duncan (1994). All specimens are deposited in the laboratory.

According to Rangel and Lainson (2003), *L. cruzi* female is structurally indistinguishable from *L. longipalpis* female, although the two species can be differentiated by small morphological characteristics of males. The same applies to the species *L. cortelezzii* and *L. sallesi* and *L. dreisbachi* and *L. hermanlenti*. Thus, in the present study, when females of these species were captured together and, in the absence of males for sex differentiation, they were classified as *Lutzomyia* spp.

3. Serological analysis of canine VL

The canine sample size was calculated using Epi Info version 3.2. The estimated canine population in endemic areas was 1,290 animals for 8.4% prevalence of canine VL in Cuiabá (MESTRE, 2006) totaling 343 dogs in the neighborhoods studied. Blood samples were obtained between October and November 2008, and collected by veterinarians of the research team.

Dog sera were divided into two aliquots, one was sent to the study laboratory for screening by enzyme immunoassay (ELISA) and one was sent to the leishmaniasis laboratory of UFMT Veterinary Hospital for immunofluorescence assay (IFA). Positive sera were those showing a positive reaction at dilutions above the cutoff of 1:40. After diagnosis, all seropositive dogs were gathered authorized by their owners and killed at Cuiabá Animal Disease Control Center.

4. Statistical analysis

The correlation between environmental variables and sand fly density and the prevalence of dogs in the neighborhoods was assessed using the Pearson's correlation coefficient (r) which requires the assumption of a linear relationship between the variables analyzed, indicating the direction and strength of the correlation ($-1 \leq r \leq +1$).

5. Weather data

The data on temperature, relative humidity and rainfall in Baixada Cuiabana during the study period were obtained from UFMT Experimental Weather Station.

Results and Discussion

From January 2008 to April 2009, captures were carried out for 48 nights, totaling 576 hours, with the collection of 1,909 sand flies belonging to the genus *Lutzomyia* in 1924 including nine species: *L. carmelinoi* Ryan, Fraiha, Lainson and Shaw, 1986; *L. cruzi*, *L. davis* (Root, 1934); *L. evandroi* (Costa Lima, Antunes, 1936); *L. lenti* (Mangabeira, 1938); *L. longipalpis*, *L. termitophila* Martins, Falcão and Silva, 1964; *L. sordellii* (Shannon, Del Ponte, 1927), *L. whitmani* (Antunes; Coutinho, 1939). Thirteen specimens of *Lutzomyia* spp. were not identified due to damaged taxonomic structures or because they consisted of a complex of *cortezii*, *dreisbachi* or *longipalpis*. We identified a single specimen from genus *Brumptomyia* França and Parrot, 1921 and *Brumptomyia* sp.

The most frequent species were *L. cruzi* (81.25%), *L. whitmani* (13.88%) and *L. longipalpis* (2.62%), and they were predominantly males (Table 1).

The ratio of males to females captured was approximately 3:1. If the method of collection is reflecting the population dynamics of these insects, it suggests that female and male insects have different attraction patterns. Aguiar et al. (1985), in a study, concluded that one of the advantages of light traps is that they attract males in greater number; or either it could indicate that the

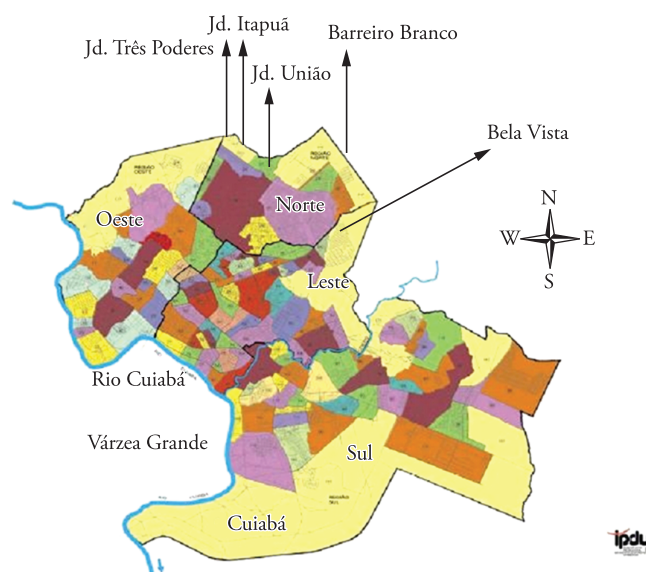


Figure 1. Urban area of Cuiabá and neighborhoods with occurrence of human visceral leishmaniasis, 2005-2006. Source: IPDU (2009), Brasil (2007).

Table 1. Species of phlebotomine sand flies in the city of Cuiabá, Mato Grosso State, January 2008 to April 2009.

Species	Males		Females		Total	
	Nº	%	Nº	%	Nº	%
<i>L. carmelinoi</i>	2	0.10	5	0.26	7	0.37
<i>L. cruzi</i>	1,138	59.61	413	21.63	1,551	81.25
<i>L. davis</i>	-	-	1	0.05	1	0.05
<i>L. evandroi</i>	1	0.05	1	0.05	2	0.10
<i>L. lenti</i>	6	0.31	6	0.31	12	0.63
<i>L. longipalpis</i>	44	2.30	6	0.31	50	2.62
<i>L. sordellii</i>	-	-	1	0.05	1	0.05
<i>L. termitophila</i>	1	0.05	3	0.16	4	0.21
<i>L. whitmani</i>	190	9.95	75	3.93	265	13.88
<i>Lutzomyia</i> spp.	1	0.05	12	0.63	13	0.68
<i>Brumptomyia</i> sp.	3	0.16	-	-	3	0.16
Total	1,386	72.60	523	27.40	1,909	100

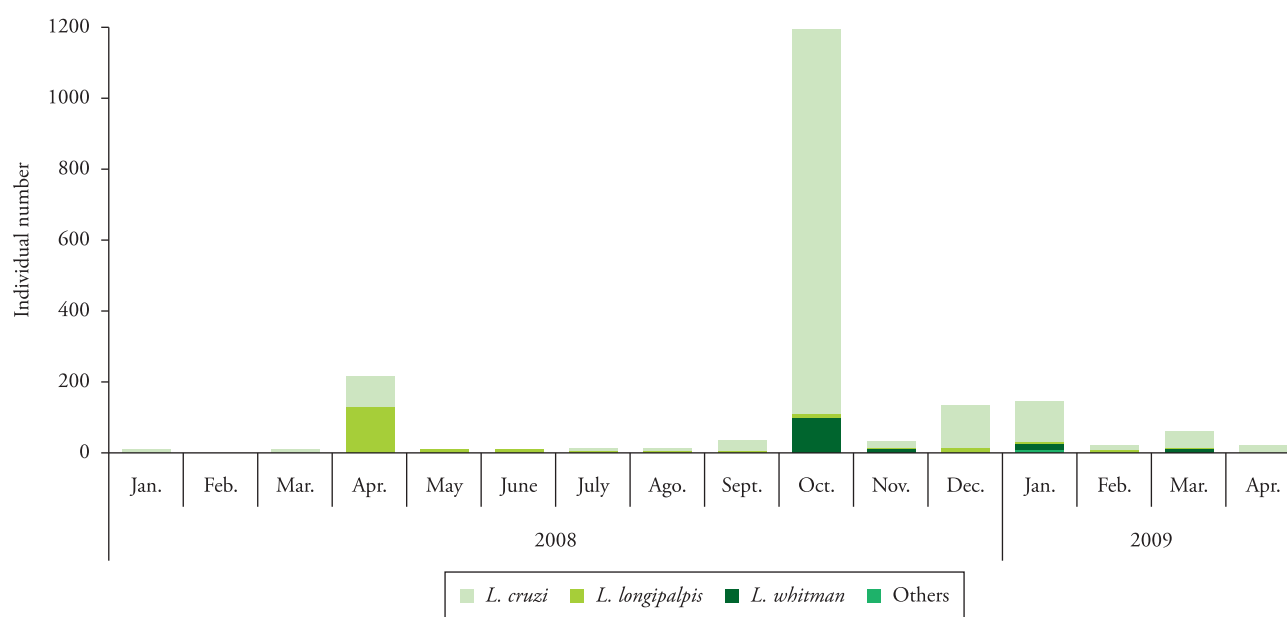
traps were placed near mosquito breeding sites as males emerge first. There is also the possibility that males are attracted to traps when monitoring females for mating; according to Feliciangeli (1987), male sand flies form aggregates on females for mating.

Table 2 shows the frequency of sand flies in the neighborhoods studied. *L. cruzi* were collected in all neighborhoods surveyed, and the highest density was seen in Jardim Três Poderes, accounting for 68.47% of sand flies captured. *L. whitmani* occurred in four neighborhoods, and was more frequently seen in Barreiro Branco. *L. longipalpis* was captured in three neighborhoods with greater frequency in Barreiro Branco, an urban-savanna transition area.

Figure 2 shows the number of sand flies collected per month. The month of October 2008 showed the highest density with 1,208 (63.28%) sand flies captured. Among the species captured in this month, *L. cruzi* was the most common (1,100 specimens). This species was captured more frequently in the forest and henhouse,

Table 2. Frequency of phlebotomine sand flies species obtained by CDC trap capture, Cuiabá, Mato Grosso State, January 2008 to April 2009.

Species	Jardim Itapuã	Jardim Três Poderes	Jardim União	Barreiro Branco	Bela Vista	Total
<i>L. carmelinoi</i>	-	6	-	1	-	7
<i>L. cruzi</i>	6	1,307	27	204	7	1,551
<i>L. davisii</i>	-	-	-	1	-	1
<i>L. evandroi</i>	-	-	-	2	-	2
<i>L. lenti</i>	1	4	1	6	-	12
<i>L. longipalpis</i>	-	15	1	34	-	50
<i>L. sordellii</i>	-	-	-	1	-	1
<i>L. termitophila</i>	1	-	1	2	-	4
<i>L. whitmani</i>	4	122	-	138	1	265
<i>Lutzomyia</i> sp.	1	4	6	2	-	13
<i>Brumptomyia</i> sp.	3	-	-	-	-	3
Total	16	1,458	36	391	8	1,909
%	0.84	76.38	1.89	20.48	0.42	100

**Figure 2.** Monthly variation of the main species of phlebotomine sand flies in the city of Cuiabá, Mato Grosso State, January 2008 to April 2009.

demonstrating a process of adaptation of sand flies to the human environment, as described in other areas of Brazil (REBÊLO et al., 1999; FRANÇA-SILVA et al., 2003).

The presence of *L. whitmani* throughout the year was significant as it occurred in almost all months, except for January and February 2008 and April 2009. Souza et al. (2004) observed the occurrence of this species in different months of the year and found a significant correlation between *L. whitmani* density and rainfall in the city of Belo Horizonte, southeastern Brazil.

Although few specimens of *L. longipalpis* were captured during the study period, it is significant that this species was present in almost all months of the year, mainly in the bordering areas between urban and rural neighborhoods of Barreiro Branco and Novo Milênio. Missawa et al. (2008) in a study conducted in the municipality of Várzea Grande near Cuiabá showed that *L. longipalpis* females fed preferentially on birds (30.8%) and

rodents (21.2%) but also fed on blood from humans, opossums, cows, horses and dogs, demonstrating the opportunistic nature of this species.

As shown in Figures 3 and 4, sand fly density showed a weak correlation with temperature variation ($r = 0.346$). The relative humidity peaked in October, implying that this period could be important for the emergence of winged forms (OLIVEIRA et al., 2003), a variable that was not correlated with sand fly density ($r = -0.180$).

Sand fly density increased in the month of April, coinciding with the end of the rainy season when soil moisture remains high due to rainfall in the previous month. During the rainy season, soil flooding prevents the development of immature stages of sand flies. There was also a peak in sand fly density following the first rain falls in October (Figure 5), but it was not statistically significant.

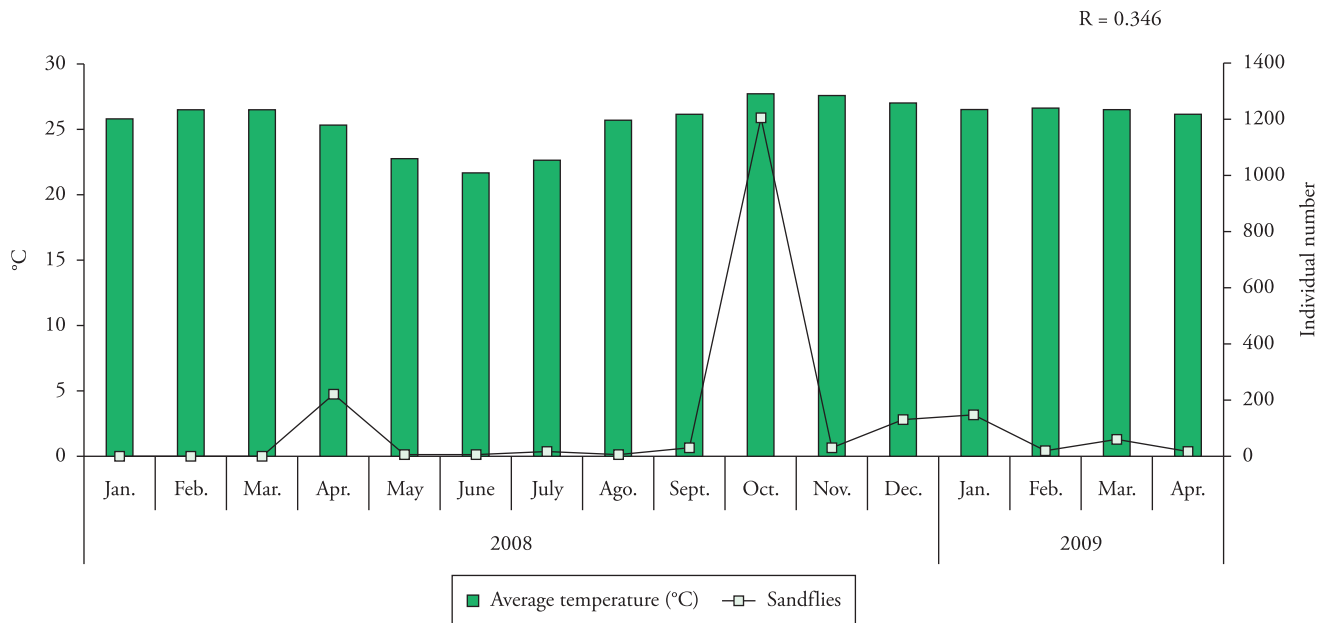


Figure 3. Phlebotomine sand flies density and average monthly temperature (°C) in Cuiabá, Mato Grosso State, January 2008 to April 2009.

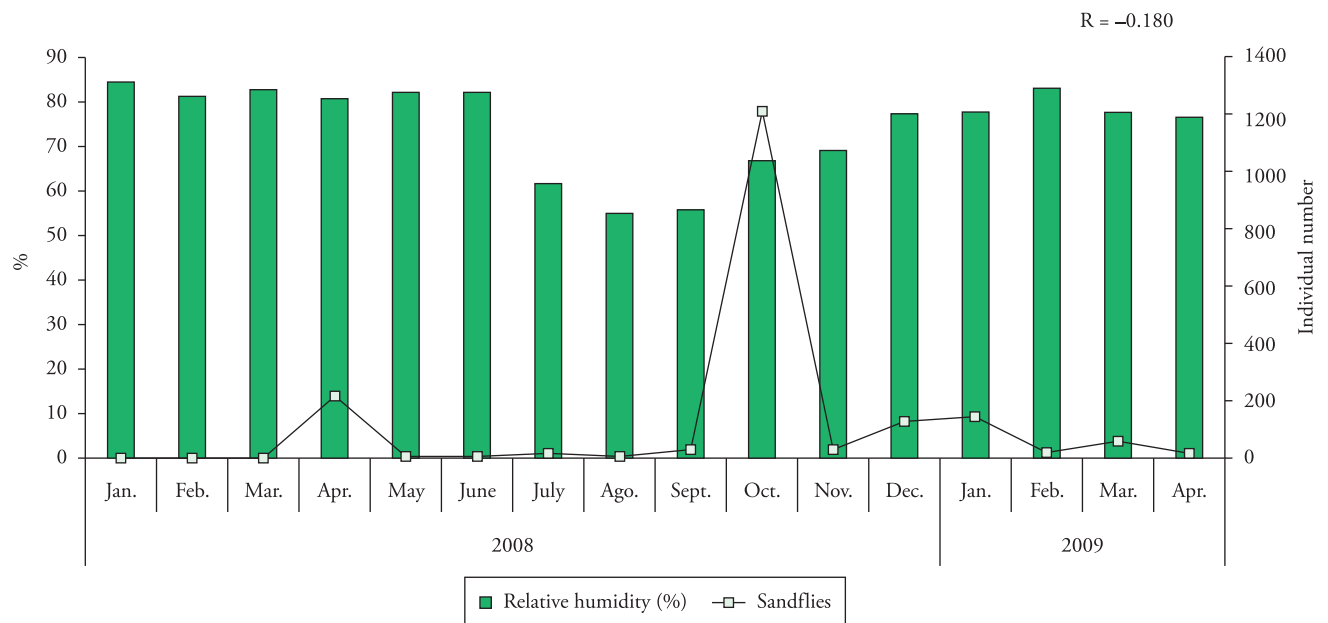


Figure 4. Phlebotomine sand flies density and relative humidity (%) in Cuiabá, Mato Grosso State, January 2008 to April 2009.

In other words, there was no significant correlation between sand fly population density and rainfall in Cuiabá ($r = -0.068$).

The serological survey included 343 dogs, of which 92 were positive for leishmaniasis on IFA. The prevalence of canine VL was high in all the neighborhoods studied with a mean prevalence of 26.82% (Table 3). Areas with high density of VL vectors coincided with those areas of high prevalence of VL in dogs. It is noteworthy that Jardim Três Poderes had a canine prevalence of 25.00%, high density of *L. cruzi*, presence of *L. longipalpis* and *L. whitmani* and reported human cases in 2005 and 2006. Barreiro Branco, located in an urban-savanna transition area, showed a high prevalence of

canine VL (48.84%), the presence of *L. cruzi*, *L. longipalpis* and *L. whitmani* vectors, and a reported VL human case in 2005.

There was no significant correlation between the prevalence of dogs and *L. cruzi* vector density ($r = 0.107$) in the neighborhoods studied but there was a strong correlation with *L. longipalpis* ($r = 0.838$) and *L. whitmani* density ($r = 0.683$), although these species have not been involved in canine VL.

The high number of dogs positive for leishmaniasis may explain the high endemicity of VL in the neighborhoods studied, since canine infection may precede human disease and is closely associated to both spatial and temporal dissemination of human

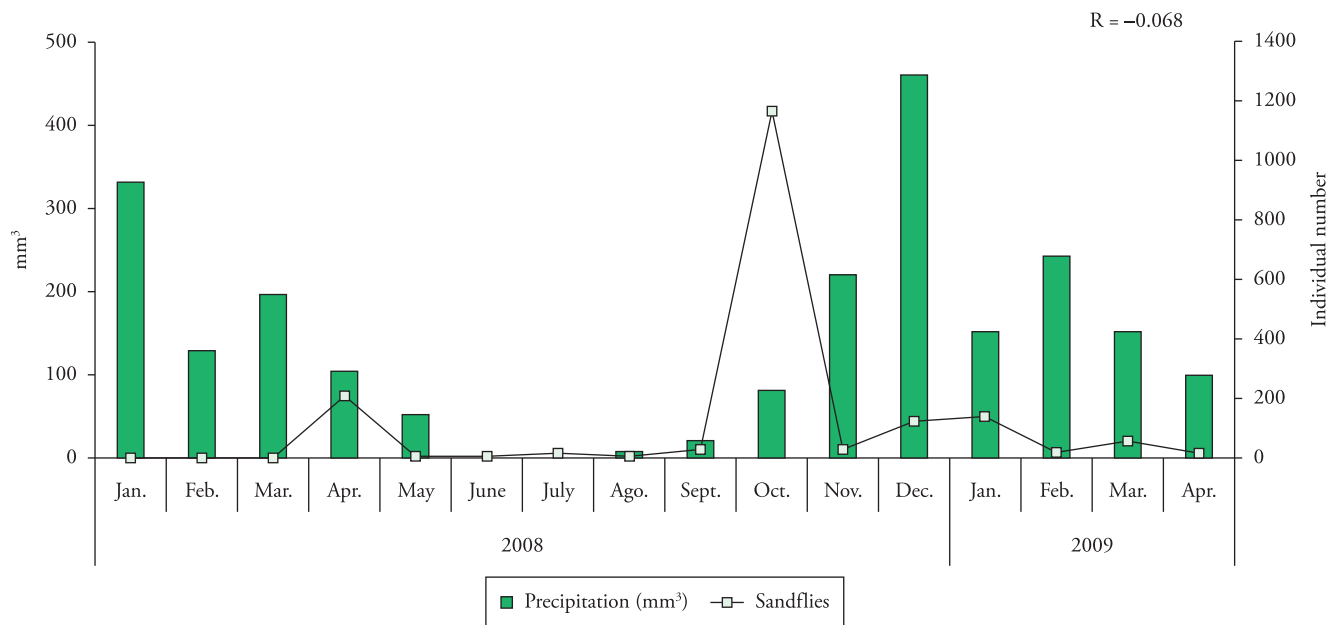


Figure 5. Phlebotomine sand flies density and cumulative monthly rainfall (mm³) in Cuiabá, Mato Grosso State, January 2008 to April 2009.

Table 3. Seroprevalence in dogs and a list of phlebotomine sand flies vectors in Cuiabá, Mato Grosso State, January 2008 to April 2009.

Neighborhoods	Examined dogs	Positive dogs	CVL %	<i>L. cruzi</i>	<i>L. longipalpis</i>	<i>L. whitmani</i>	Human VL cases*
Jardim Itapuá	65	5	7.69	6	-	4	1 (2006)
Jardim Três Poderes	56	14	25.00	1,307	15	122	1 (2005)
Jardim União	77	24	31.17	27	1	-	1 (2005)
Barreiro Branco	94	41	48.84	204	34	138	1 (2005)
Bela Vista	51	8	15.69	7	-	1	1 (2006)
Total	343	92	26.82	1,551	50	265	6 cases

*Reported human VL cases. VL = Visceral leishmaniasis; CVL = Canine visceral leishmaniasis; *L.* = *Lutzomyia*.

disease (ALVES; BEVILACQUA, 2004). This association has been well demonstrated in study on VL epidemic in Belo Horizonte (OLIVEIRA et al. 2001).

The results found in the neighborhoods studied indicate great potential for the occurrence of the two clinical forms (visceral and cutaneous) of human leishmaniasis due to a large number of serologically positive vectors captured in dogs.

Data analysis by geographic area has increasingly gained importance in health because it can provide input for planning and developing actions based on the spatial distribution of diseases, location of health services and environmental risk among others (BARCELLOS; BASTOS, 1996). The epidemiological and spatial evolution of VL allows to inferring that human and canine cases are possibly spreading to other neighborhoods in the city.

The study of circulating *Leishmania* species, other potential hosts and vectors is essential for a good understanding of the disease. At the same time, partnerships between multidisciplinary professional groups and the public, researchers and community should be promoted and strengthened to develop strategies for prevention and control of VL statewide in Mato Grosso.

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