Phlebotomine sand flies (Diptera: Psychodidae) in the municipality of Várzea Grande: an area of transmission of visceral leishmaniasis in the state of Mato Grosso, Brazil

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Visceral leishmaniasis (VL) has been naturally transmitted in periurban areas due to the emergence and reemergence of its vectors in such areas. Aimed to further knowledge on ecological aspects affecting the occurrence of phlebotomine sand flies in VL transmission areas in the municipality of Várzea Grande, state of Mato Grosso (MT), Brazil, sand fly captures were carried out. Monthly collections of sand flies were undertaken with CDC light-traps, which were left in both intradomiciliary and peridomiciliary areas of ten residences during four consecutive days between January 2004 and June 2006. Twenty-two species of genus Lutzomyia *and one of* Brumptomyia *were captured. The most abundant species was* Lutzomyia longipalpis *(65.23%), followed by* L. evandroi *(16.26%),* L. lenti *(7.69%),* L. whitmani *(4.92%),* L. sallesi *(2.34%) and* L. termitophila *(1.32%). The highest density of the main VL vector,* L. longipalpis*, was found in peridomiciliary areas, mostly males. No significant correlation was found between environment (temperature, air relative humidity and rain fall) and phlebotomine density; although a slight increase in sand fly density has been observed in the period following rainfalls, particularly* L. longipalpis*. No correlation was observed between distribution and density of* L. longipalpis*, prevalence of human VL cases and the presence of serologically positive dogs. The presence of infected dogs, increased vector density, susceptibility rate and interruption of epidemiological surveillance may raise the risk of VL transmission to man in Várzea Grande.*

Key words: Phlebotomine - *Lutzomyia longipalpis* - Várzea Grande - Mato Grosso

Leishmaniases are regarded as one of the main world zoonosis, and visceral leishmaniasis (VL), particularly, constitutes an important anthroponosis or anthropozoonosis when man acts as a reservoir. The disease is worldwide spread and is found in Americas, Africa, Southern Europe, Asia and Midwestern countries (Lainson & Shaw 1987). In Americas, VL is prevalent from Mexico to Argentina, and Brazil accounts for 90% of the cases in the continent (Soares & Turco 2003). Changes in human behavior and the environment may exert greater impact in the prevalence and pattern of VL transmission.

In Brazil, VL was previously known as a rural disease in several states, including Goiás, Tocantins and Mato Grosso do Sul (MS) (Marzochi & Marzochi 1994), and the first report of the disease in the country was made in 1913 in Mato Grosso (MT) (Alencar & Dietze 1991). Despite the fact that VL prevalence is underestimated, Brazil has recorded increasing incidences of the disease in the last 20 years (Dantas-Torres & Brandão-Filho 2006). Approximately 3,000 clinical cases a year have been recorded in the country with average mortality between 8% and 10% (Gontijo & Melo 2004).

Urbanization conditions of the disease and environmental changes influencing VL transmission can be of natural or anthropogenic origin (Barata et al. 2005, Monteiro et al. 2005), including human activities expansion, habitat fragmentation and deforestation, which have led to an increasing adaptation of the parasite, as well as of phlebotomine sand flies (Marzochi & Marzochi 1994). Thus, this metaxenic disease has been naturally transmitted in periurban areas due to the emergence and reemergence of its vectors in such areas (Tauil 2006). Recent data have confirmed urbanization of the disease in large urban centers (Silva et al. 2001, Bevilacqua et al. 2001, Souza et al. 2004, Monteiro et al. 2005, Barata et al. 2005); add to this the fact that some investigators (Bevilacqua et al. 2001) have suggested that socio-economical factors may be one of the determinant factors for VL transmission in Brazil.

Dogs are regarded as important reservoirs in several VL foci in both rural and periurban areas (Marzochi & Marzochi 1994, Silva et al. 2001). Several reports have correlated human VL with the presence of high canine seroprevalence (Deane & Deane 1955, Lainson & Shaw 1987, Oliveira et al. 2001); in addition, the disease occurrence has been associated with the sheer abundance of vectors (Vieira & Coelho 1998).

In Brazil, VL transmission is associated with a variety of sand fly species, and *Lutzomyia longipalpis* is the main vector species (Lainson & Rangel 2003). *L. cruzi* has been implicated in VL transmission in MS (Santos et al. 1998), being found naturally infected in the municipality of Jaciara, in MT (unpublished observations). Other phlebotomine species, such as

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L. forattini and *L. almerioi*, may also be associated with the disease in MS, both anthropophilic, although has not been evidenced its vectorial ability (Galati et al. 1997, Galati et al. 2006), whereas in the state of Para, *L. antunesi* is considered an alternative VL vector (Lainson & Rangel 2003). In Colombia, *L. evansi* has been reported to be the most likely VL vector (Travi et al. 1990). The VL etiological agent is a protozoan parasite of the family Tripanosomatidae, species *Leishmania (Leishmania) infantum chagasi* (Lainson & Rangel 2005, Shaw 2006).

Most environmental factors affecting the epidemiology of leishmaniases provide evidence on the adaptation of parasites and their vectors to ecological changes due to socio-environmental processes such as deforestation and urbanization. Because these parasites and vectors adapt to modified environmental conditions in order to survive, the epidemiological profile of the disease has also undergone modifications (Marzochi & Marzochi 1994).

Hence, the present study was aimed at furthering knowledge on ecological aspects influencing density, seasonal fluctuation, endophilia and exophilia of phlebotomine sand flies in a VL transmission area in the municipality of Várzea Grande, MT.

MATERIALS AND METHODS

The municipality of Várzea Grande (VG) (15º 32' 30" S 56º 17' 18" W) is known as the industrial town of MT, Brazil. With a population of 214,842 inhabitants (IBGE 2000 apud Ferreira 2001), the town, together with the state capital Cuiabá, comprises the so called Urban Conglomerate of Cuiabá or Metropolitan Region of Cuiabá. VG is at 185m of altitude and has a territorial extension of 949.53km^2. It is in the central-south zone of the Mesoregion of MT, microregion of Cuiabá, with a subhumid tropical climate and annual rainfall of 1,750 mm. The rainy period runs between September and April with greater intensity from January to March. The yearly average temperature is 24ºC, reaching up to 34.1ºC, and absolute maximum temperatures may be over 42ºC. The minimum average temperature in July, the coldest month, is 16.7ºC. The geomorphologic figure of the municipality is mostly represented by Planalto da Casca and Depressão Cuiabana. Low amplitudes predominate in the region with altitudes ranging from 146 to 250 m in the city area. The predominant vegetation is the savannah, from its bushy form to the densest forests along water streams (Piaia 2003). VG has reported both canine and human VL cases since 1998. The transmission area ranges from the districts of Eldorado and São Matheus to Parque Sabiá (Fig. 1). The area under study, including dwellings, was chosen based on previous entomological surveys (Hueb et al. 2000, Ribeiro & Missawa 2002), studies on VL incidence and prevalence rates in man, according to which the municipality was regarded as an area of intense VL transmission in 2003. In the study area, the residents have low socio-economical conditions and dwellings show no basic sanitation, regular garbage collection and electric current. A survey in the area revealed that dogs and chickens were present in 100% of house surroundings, followed by other domestic animals such as ducks, turkeys, pigs, horses, cows, cats, and due to the presence of sites of garbage concentration named "lixões"

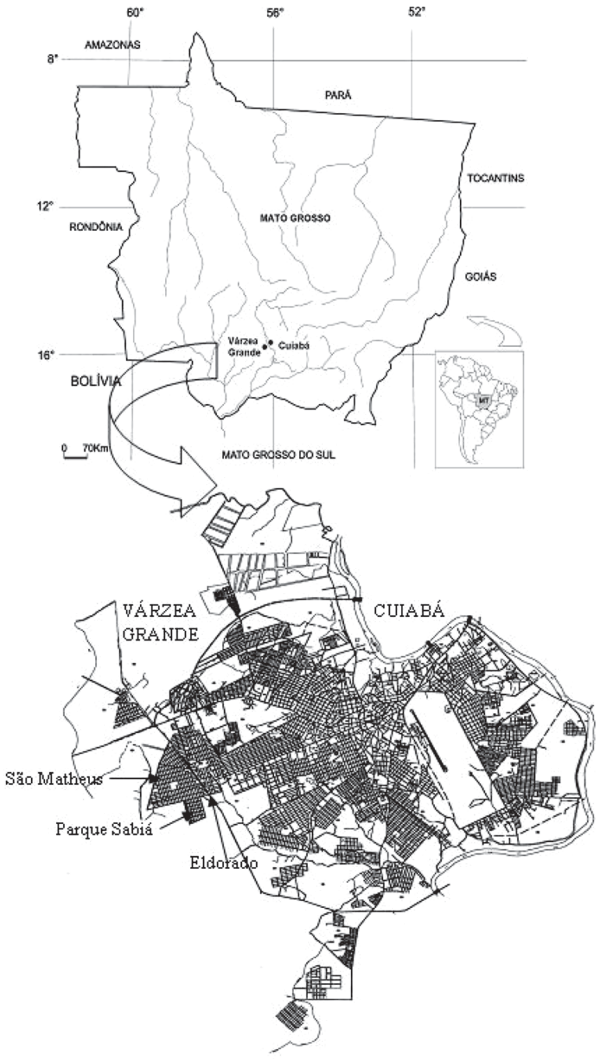

Fig. 1: geographic location and map of the municipality of Várzea Grande, state of Mato Grosso, Brazil, and identification of the districts under study (Eldorado, São Matheus and Parque Sabiá).

surrounding the districts that are close to rural areas, it is very likely that rodents and skunks are present in peridomiciliary areas.

Phlebotomine captures were carried out between January 2004 and June 2006 at VG by means of CDC light traps (Sudia & Chamberlain 1962). The light traps were monthly left in the study sites during four consecutive nights, totaling 12 h of insect collection. Sand fly captures were performed in 10 residences, out of which three were located in the district of Eldorado, four in São Matheus and three in Parque Sabiá. Two CDC light traps were placed in each house, one indoors (bedroom) and the other outdoors (backyard and in animal shelters). During the study period, insecticide substances were not applied both in the study area and in the municipality. The captured insects were killed with ethyl acetate, clarified and identified according to the classification by Young and Duncan (1994) at the Laboratory of Entomology of the state Health Secretary of MT, with posterior

confirmation through reference specimens deposited in the Laboratory of Leishmaniases of René Rachou Institute (Belo Horizonte). Damaged insects could be identified at the level of genus as either *Lutzomyia* spp. or *Brumptomyia* sp.

Bioclimatic data of temperature, air relative humidity (ARH) and precipitation were obtained at the 9th District of Meteorology at VG. Coefficient of determination (R^2) for simple linear regression analysis was used to estimate variables between the environmental and phlebo-tomine density.

RESULTS

The number of 22 species of phlebotomine sand flies of the genus *Lutzomyia* França, 1924 and one species of the genus *Brumptomyia* França and Parrot, 1921 were captured and identified as in Table I. Out of 12,833 captured phlebotomine sand flies, 8,484 were males (66.11%) and 4,349 females (33.89%). Table I depicts the percentage of each species found and the distribution of sand flies captured indoors (1840; 14.34%) and outdoors (10,993; 85.66%). *L. longipalpis* showed to be the predominant species. The relative frequency of species showed to be: *L. longipalpis* (65.23%), *L. evandroi* (16.26%), *L. lenti* (7.69%), *L. whitmani* (4.92%), *L. sallesi* (2.34%), *L. termitophila* (1.32%) and others (2.24%). The insects showed preference for the peridomiciliary environment, whereas *L. longipalpis* was present both indoors and outdoors, most males in both sites. Some of the species were captured every month during the study period, namely: *L. evandroi, L. longipalpis, L. sallesi* and *L. whitmani*.

The distribution of phlebotomine sand flies in each district studied was: 3,037 phlebotomine sand flies in Eldorado (23.66%), 6,303 in São Matheus (49.12%) and 3,493 in Parque Sabiá (27.22%). The insects were more abundant in the peridomiciliary area in São Matheus. The following species were present in the three districts throughout the entire period under study: *L. cerradincola, L. evandroi, L. hermanlenti, L. lenti, L. longipalpis, L. lutziana, L. sallesi, L. saulensis, L. sordellii, L. teratodes, L. termitophila, L. whitmani* and *B. brumpti*.

No significant correlation between dependent (temperature, ARH and precipitation) and independent variables (phlebotomine sand flies) was observed (R^2 = 0.1672, 0.1044 and 0.2653, respectively; $p \geq 0.05$). Nevertheless, a slight increase in the density of insects was observed, especially *L. longipalpis*, following the rainy season (with the highest ARH between October and March), but not significantly correlated (Figs 2 and 3). No significant correlation between distribution and den-

TABLE I

Phlebotomine sand flies belonging to genera *Lutzomyia* and *Brumptomyia* distributed by sex and the place where they were collected in the municipality of Várzea Grande, state of Mato Grosso, from January 2004 to June 2006

Species	Indoors		Outdoors		Total	%
	♂	♀	♂	♀		
L. acantopharynx Martins, Falcão and Silva	4	4	2	3	13	0.10
L. aragaoi (Costa Lima)	-	1	-	-	1	0.01
L. carmelinoi Ryan, Fraiha, Lainson and Shaw	2	-	1	1	4	0.03
L. cerradincola Galati, Nunes, Oshiro and Dorval	1	2	4	1	8	0.06
L. corumbaensis Galati, Nunes, Oshiro and Rego	1	-	-	-	1	0.01
L. dendrophyla (Mangabeira)	-	1	-	2	3	0.02
L. evandroi (Costa Lima &Antunes)	150	162	952	823	2087	16.26
L. flaviscutellata (Mangabeira)	-	3	2	5	10	0.08
L. hermanlenti Martins, Silva and Falcão	1	-	1	3	5	0.04
L. lenti (Mangabeira)	39	36	621	291	987	7.69
L. longipalpis (Lutz & Neiva)	853	264	5142	2112	8371	65.23
L. longipennis (Barreto)	-	-	-	2	2	0.02
L. lutziana (Costa Lima)	1	-	1	2	4	0.03
L. punctigeniculata (Floch & Abonnenc)	-	4	4	7	15	0.12
L. sallesi (Galvão & Coutinho)	46	77	78	99	300	2.34
L. saulensis (Floch & Abonnenc)	-	-	1	6	7	0.05
L. scaffi (Damasceno & Arouck)	-	-	-	1	1	0.01
L. sordellii (Shannon & Del Ponte)	21	21	13	34	89	0.69
L. teratodes Martins, Falcão and Silva	3	10	7	13	33	0.26
L. termitophila Martins, Falcão and Silva	18	30	32	89	169	1.32
L. walkeri (Newstead)	-	-	5	2	7	0.05
L. whitmani (Antunes & Coutinho)	47	22	385	178	632	4.92
Lutzomyia spp.	-	1	3	2	6	0.05
B. brumpti (Larrousse)	6	6	24	23	59	0.46
Brumptomyia sp.	-	3	13	3	19	0.15
Sub-Total	1193 (9.30%)	647 (5.04%)	7291 (56.81%)	3702 (28.85%)	12833	100
Total	1840 (14.34%)		10993 (85.66%)		12833	100

sity of *L. longipalpis*, occurrence of human VL cases and serologically positive dogs was observed (Table II).

DISCUSSION

The predominance of *L. longipalpis* found in the present study showed to be in accordance with Missawa and Lima (2005) in the same municipality and other localities as reported by Oliveira et al. (2003, 2006) for Campo Grande, MS, Resende et al. (2006) and Souza et al. (2004) for Belo Horizonte, state of Minas Gerais (MG), Barata et al. (2004, 2005), and França-Silva et al. (2005) for Porteirinha, MG, Monteiro et al. (2005) for Montes Claros, MG.

The insects' preference for the peridomiciliar area has also been observed in several Brazilian states (Barata et al. 2005, Monteiro et al. 2005, Souza et al. 2004, Resende et al. 2006), which provides evidence of the insect domiciliation influenced by human socio-ecological modifications in the sand fly habitat and the adaptive power of the insects' species (Oliveira et al. 2003, Aguiar & Medeiros 2003, Souza et al. 2004), although Resende et al. (2006) have observed roughly equal proportions of phlebotomine sand flies, mainly *L. longipalpis*, in both intra- and peridomiciliary areas.

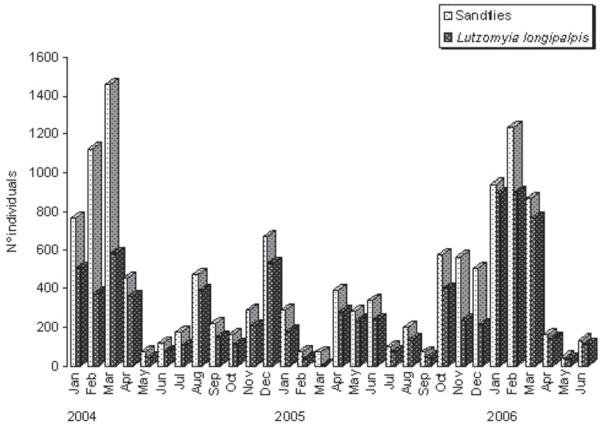

Fig. 2: seasonality of *Lutzomyia longipalpis* captured in Várzea Grande, state of Mato Grosso, Brazil, from January 2004 to June 2006.

TABLE II

Number of *Lutzomyia longipalpis,* human visceral leishmaniasis (VL) cases, prevalence of canine VL per district in Várzea Grande, state of Mato Grosso, from January 2004 to June 2006

District	*L. longipalpis* N	*L. longipalpis* %	Human VL infection	VL Canine prevalence (%)
Eldorado	808	9.65	0	4.55
São Matheus	4958	59.23	1	9.30
Parque Sabiá	2605	31.12	0	21.29
Total	8371	100	1	11.40

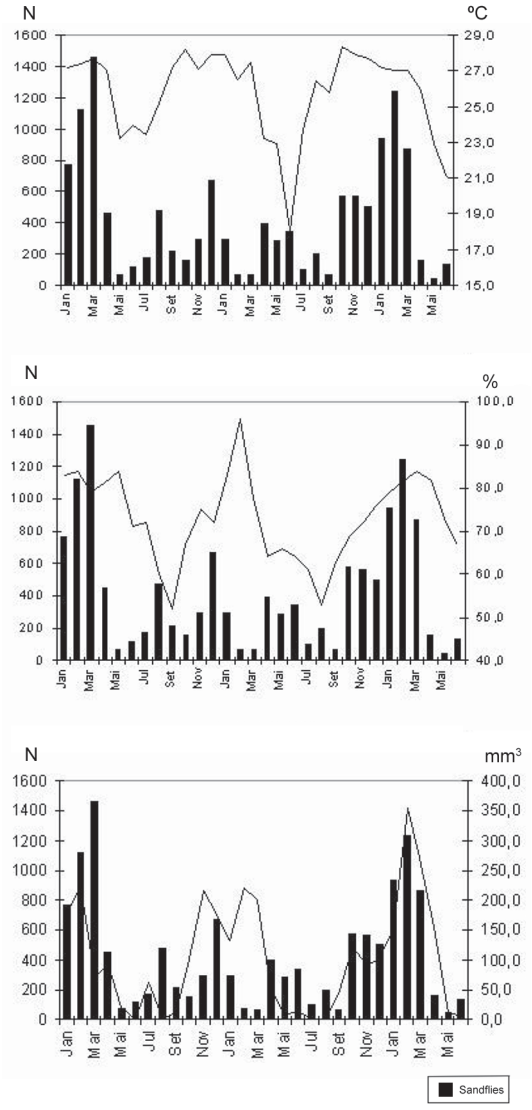

Fig. 3: specimens of phlebotomine sand flies captured correlated with average temperature (ºC), air relative humidity (%) and precipitation (mm³) in Várzea Grande, state of Mato Grosso, Brazil, from January 2004 to June 2006.

The peridomestic distribution of insects observed here may be due to the fact that high rates of sand flies were found inside domestic animal shelters, where phlebotomine sand flies may form abundant aggregates on stationary animals such as dogs and poultry (Lainson & Rangel 2003), probably because of cairomones produced by male insects in order to attract females to feed and copulate.

Considering the fact that *L. longipalpis* is able to adapt to different habitats (Young & Duncan 1994, Barata et al. 2005), one may infer that VL transmission may be taking place into the dwellings and that such species is prone to anthropophily (Rebêlo et al. 1999). The closeness between the houses and the domestic animal shelters may account for the presence of insects indoors together with their capacity of endophilia. The high frequency of male insects found in both intra- and peridomiciliary areas has corroborated data provided by

Oliveira et al. (2003, 2006), Resende et al. (2006), Barata et al. (2004, 2005), evidencing the high adaptation capacity of sand flies enabling the disease transmission. The process of urbanization of insects and, consequently, of the disease is correlated with disorganized urban growth, followed by human migration from rural to urban regions and the environmental destruction. Add to this social crises and environmental changes such as prolonged and periodical droughts enabling new VL foci in urban areas (Vieira & Coelho 1998).

The non-significant correlation between insect density and the bioclimatic variables under study had also not been observed by Souza et al. (2004) in Belo Horizonte, and Rebêlo (2001) in São Luis, state of Maranhão. The non-significant effect of temperature on phlebotomine density was reported by Barata et al. (2004), although Monteiro et al. (2005) have found correlations between high temperatures and peaks of VL transmission. França-Silva et al. (2005) showed no correlation between vector density and the climatic variables, temperature and ARH. On the other hand, Resende et al. (2006) reported an increased number of phlebotomine sand flies, mainly *L. longipalpis*, when the combination of high temperature averages and regular rain periods occurred, as well as Monteiro et al. (2005), Barata et al. (2004) and Oliveira et al. (2003), who have associated phlebotomine density with the first rainy periods and high ARH.

Entomological studies on vectorial occurrence and density seek to provide information both qualitatively and quantitatively on phlebotomine sand flies, VL potential transmitters. They are aimed at further defining prevalence areas and the best period to be surveyed in order to be followed up and assessed in attempts to control sand flies through either physical or chemical methods.

The prevalence of VL-seropositive dogs in the areas under study show that despite VL urbanization, the disease still retains some rural features in areas of animals breeding and subsistence agriculture as observed by Moura et al. (1999) in the neighbor municipality of Cuiabá, MT. The permanence of susceptible animals in the area might have been enabled by canine misdiagnoses provided by low sensitivity and specificity techniques used so far.

According to Resende et al. (2006), the pattern of distribution of *L. longipalpis* coincides with the occurrence of human VL cases, and such correlation has been used to validate autochthony of the cases. Correspondence between human VL cases and high phlebotomine density was not observed between 2004 and 2006 in VG, although Barata et al. (2004) and Souza et al. (2004) have measured correlations between phlebotomine density, prevalence of VL human cases and presence of domestic animals in Porteirinha and Belo Horizonte, MG. Monteiro et al. (2005) and França-Silva et al. (2005) have correlated the presence of high vector density and high VL canine prevalence in Montes Claros and Porteirinha, MG.

The risk factors for *L. (L.) infantum chagasi* infection and the occurrence of human VL cases in urban areas, identified by Moreno et al. (2005), are associated with the environment surrounding residences, mainly with the presence of organic matter, domestic animals in peridomiciliary areas and the contact between susceptible hosts and phlebotomine vectors. Dogs are regarded as infective for phlebotomine vectors even when they are asymptomatic. In symptomatic dogs, the exposure to cutaneous infections and hair loss enable contamination of sand flies, since *Leishmania* has high tropism for the skin. The existence of agricultural practices for soil cultivation, interruption of epidemiological surveys, migratory processes and disorganized urbanization, areas with low living conditions, presence of infected VL dogs, high vector density and infection, and host susceptibility to VL transmission raise the risk of human infection in VG.

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