Phlebotominae distribution in Janaúba, an area of transmission for visceral leishmaniasis in Brazil

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In Brazil, visceral leishmaniasis (VL) is caused by Leishmania chagasi *parasites that are transmitted to man through the bites of infected females of* Lutzomyia longipalpis *sand flies. In order to evaluate transmission risk and to clarify the epidemiology of this tropical disease, studies focused on the vector and favorable environmental conditions are of fundamental importance. In this work, we surveyed the phlebotomine sand fly fauna in Janaúba, a Brazilian municipality that is endemic for VL. During a two-year period, entomological captures were performed monthly in 15 districts with high, moderate and low profiles of VL transmission. A total of 14,591 phlebotomine sand flies were captured (92%* L. longipalpis*), with a predominance of males. Most specimens were captured in the peri-domicile setting, although the number of specimens captured in the intra-domicile setting emphasises the anthropophilic behaviour of this insect. The population density of* L. longipalpis *was modulated by climate variations, particularly with clear increases immediately after the rainy season. However, the pattern of distribution did not coincide with the occurrence of human or canine cases of VL. This suggests that the eco-epidemiology of VL is particular to each area of transmission and must be taken into account during the design of public health control actions.*

Key words: *Lutzomyia longipalpis* - visceral leishmaniasis - eco-epidemiology - Janaúba

Visceral leishmaniasis (VL) is a zoonotic disease that is endemic in many of the tropical countries around the globe. This disease has been considered an emerging disease that is still in expansion worldwide, and it has also been placed among the seven endemics of priority by the World Health Organization (WHO 2003). VL occurs in Asia, Europe, the Middle East Africa and America, with 90% of the overall number of cases occurring in Brazil, Bangladesh, India and Sudan (Soares & Turco 2003). Approximately 147 million people are exposed to a possible risk of infection, and 100,000 individuals are currently infected (WHO 2007).

In Latin America, VL has been described in 12 countries, including Brazil, where human cases have been reported in every geographical region from the Northern state of Pará to the Southern state of Paraná, including the Central states of Minas Gerais (MG), Goiás and Mato Grosso (MS 2006). Between 2001-2006, 20,530 cases of human VL were notified to the Brazilian Ministry of Health (SINAN/SVS 2004). The state of MG accounted for about 12% of the total number of cases.

Typically regarded as a sylvatic disease of rural areas, the epidemiological profile of VL has changed with time to become a major urban concern (Tauil 2006). A decrease in the availability of wild animals, mainly caused by environmental modifications associated with human migration from endemic areas to urban peripheries, has transformed domestic dogs and humans into tempting feeding alternatives for the phlebotomine sand fly vectors of VL. Both parasites and phlebotomine vectors have become adapted to the environmental changes caused by anthropisation (Ashford 2000). In Brazil, 65.5% of the notified cases of VL between 2001-2006 were from urban areas (SINAN/SVS 2004). In MG, this percentage reached upwards of 84% during this same time period (SINAN/SVS 2004). The disease was noted in the periphery of cities with varying sizes such as Porteirinha (39,000 inhabitants), Montes Claros (355,000 inhabitants) and the state's capital of Belo Horizonte (2,500,000 inhabitants) (Silva et al. 2001, Barata et al. 2004, Sousa et al. 2004, França-Silva et al. 2005, Monteiro et al. 2005). In Janaúba, a city with 65,500 inhabitants in the Northern region of MG, 12 human cases of VL were notified in 2001 (SINAN/SVS 2004). This number increased to 18 in 2002, 35 in 2003 and 39 in 2004 (SINAN/SVS 2004). Due to the steady increases, this area was considered as an area of high VL transmission (MS 2006). However, data regarding the presence and distribution of VL vectors and phlebotomine sand flies species were generally insufficient to draw the eco-epidemiological profile of the disease in this locality.

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In an effort to clarify the vectorial aspects of VL, we performed a survey of the phlebotomine fauna in Janaúba. Due to the known susceptibility of phlebotomine sand flies to abiotic factors such as climate variables, the modulation of the phlebotomine population density as it related to these variables was also evaluated in this study.

MATERIALS AND METHODS

Study area - Janaúba (15°47’50″S, 43°18’31″W) is located in the Northern region of MG, Brazil (Fig. 1). The municipality occupies an area of 2,160 km^2, which corresponds to 0.36% of the total state area. The state is divided into 25 districts that are distributed in six regions. The city of Janaúba has 65,500 inhabitants and it is located 547 km from the state’s capital, Belo Horizonte. Janaúba reaches a maximum altitude of 516 m and the climate is semi-arid.

Selection of capture sites **-** The entomological survey was conducted in the following 15 districts of the Janaúba municipality: Algodões, Centro, Cerâmica Cowan, Dente Grande, Gameleira, Novo Paraíso, Padre Eustáquio, Piranhas, Ribeirão do Ouro, Rio Novo, Santo Antônio, Santa Cruz, Saudade, Vereda and Vila Isaías. Based on the number of human VL cases (n), districts with high ($n \geq 4.4$), moderate ($2.4 \leq n < 4.4$) and sporadic ($n < 2.4$) transmission profiles have been included. This classification followed criteria adopted by the Brazilian Ministry of Health (MS 2006). Data concerning the prevalence of canine VL in these districts were obtained from the Department of Health of Janaúba, in the first semester of 2006.

Captures were performed during three consecutive nights in the last week of each month, in one house per district during a period of two years (April 2005-March 2007). The houses were selected based on the occurrence of human and/or canine cases of VL in the neighbourhood, as well as on the environmental conditions that favour the rearing of phlebotomine sand flies. Each point of capture was geo-referenced (Fig. 2). Two HP light traps (HP Biomédica Ltda, Belo Horizonte, MG), developed by Pugedo et al. (2005), were mounted in the peridomicile and intra-domicile areas of the houses. The sand flies captured in each three-night period were pooled and are considered to be representative of the corresponding month. All specimens were preserved in 70% ethanol for identification according to the taxonomical classification of Young and Duncan (1994).Specimens with missing or incomplete characters that impaired their identification were considered as *Lutzomyia* spp.

Climate data **-** Monthly climate data (temperature, humidity, rainfall and wind speed) were collected by a metereological station of the Fifth District of Meteorology (5th DISME) of the Brazilian Institute of Meteorology, which is located in Janaúba.

Statistical analysis - The distribution of monthly captured *Lutzomyia longipalpis* was tested for normality using the Kolmogorov-Smirnov test. The average mean and standard deviations (s.e.) were calculated using SigmaStat version 3.1.1 (Systat software Inc, USA). This software was additionally employed for multiple regression analysis using best subset regression. All tests were performed with 95% confidence.

Fig. 1: geographical localization of Janaúba in the state of Minas Gerais, Brazil.

Fig. 2: distribution of the points of entomological capture (▲) in the districts of Janaúba, state of Minas Gerais, Brazil, April 2005-March 2007.

RESULTS

A total of 14,591 phlebotomine sand fly specimens were captured in Janaúba. Of the captured specimens, 10,824 (74%) were males (M) and 3,767 (26%) were females (F), giving an overall M/F ratio for the captures of 2.9. The phlebotomine fauna consisted of eight species: *Brumptomyia brumpti* (0.01%), *Lutzomyia intermedia* (0.74%), *Lutzomyia lenti* (4.14%), *L. longipalpis* (92.39%), *Lutzomyia cortelezzii* (0.63%), *Lutzomyia sordellii* (0.03%), *Lutzomyia termitophila* (0.05%) and *Lutzomyia whitmani* (0.03%). Besides, 1.99% was grouped as *Lutzomyia* spp.

L. longipalpis was captured in large numbers (13,480 specimens) and was present in all districts during the vast majority of capturing months (data not shown). Interestingly, 42% of these specimens were captured in Algodões (Table). Although no human case of VL had been notified there until 2006, the high prevalence (28.3%) of canine VL (Table) and the presence of the vector species

TABLE

Epidemiological data of visceral leishmaniasis (VL) in districts of Janaúba, state of Minas Gerais (MG), Brazil

Levels of transmission[a]	District	Number of inhabitants	*Lutzomyia longipalpis* captured				Canine VL[b]	Human VL[c]
			Total	%	M/F	P/I	%	n
High	Cerâmica Cowan	2,678	75	0.6	1.8	2.9	16.3	12
	Dente Grande	3,463	1,184	8.8	3.2	5.7	14.6	7
	Gameleira	3,713	565	4.2	2.5	5.4	15.4	15
	Padre Eustáquio	3,960	1,451	10.8	3.0	3.0	19.5	18
	Ribeirão do Ouro	3,317	566	4.2	2.7	2.6	15.4	14
	Rio Novo	2,977	1,043	7.7	1.8	1.7	11.0	12
	Santo Antônio	3,875	607	4.5	3.3	3.3	15.3	8
	Saudade	2,304	289	2.1	3.7	4.7	9.2	9
	Vila Isaías	2,348	66	0.5	1.0	0.9	13.2	7
Moderate	Centro	3,358	697	5.2	3.2	9.4	16.6	5
	Novo Paraíso	1,753	179	1.3	1.8	1.2	14.2	6
Sporadic	Algodões	914	5,667	42	3.7	10.2	28.3	0
	Piranhas	785	93	0.7	1.3	1.0	8.7	0
	Santa Cruz	2,124	123	0.9	1.6	0.9	20.3	3
	Vereda	2,273	875	6.5	2.8	8	22.1	2
	Total	39,842	13,480	100.0	-	-	-	118

a: MS 2006; *b*: data from the Department of Health of Janaúba, MG, Brazil (1st semester of 2006); *c*: cases of human VL notified between 2002-2006 (SINAN/SVS 2004); M/F: male/female ratio; P/I: ratio between the number of *Lutzomyia longipalpis* captured in the peri-domicile (P) and intra-domicile (I). The levels of transmission of human VL (MS 2006) were coded as follows: high: dark grey background; moderate: light grey background; sporadic: white background.

in such high numbers indicate that this area likely has a high potential risk for transmission to humans. Padre Eustáquio was another district with high *L. longipalpis* numbers (10.8%). In this district, in addition to a high prevalence of canine VL (19.5%) the district also reported a high number of human VL cases (18). On the other hand, Vila Isaías, an area of high transmission of VL, had the smallest number of *L. longipalpis* specimens (66) in addition to a high prevalence of canine VL.

The M/F ratio of *L. longipalpis* varied from 1.0 in Vila Isaías, a district of high transmission of VL, to 3.7 in Algodões (Table). The distribution of *L. longipalpis* between peri-domicile (P) and intra-domicile (I) locations also varied according to district (Table). The highest P/I ratio (10.2) was observed in Algodões (sporadic transmission), whereas the lowest values (0.9) were noted in Vila Isaias (high transmission) and Santa Cruz (sporadic transmission).

The population density of *L. longipalpis* during the period of our study followed a normal distribution with a calculated average mean of 561 ± 96 (± s.e.). For the majority of months, levels oscillated between ± four standard errors (s.e.), except for February, April and May of 2006 and March of 2007, when exceptionally high numbers of specimens were captured (Fig. 3). This increase appeared to be the result of low rainfall, high humidity and low wind speed, as indicated by a tendency of the corresponding spots to clusterise under these conditions (Fig. 3). Temperature appeared to be less important when compared to these three other variables. None of the climate variables appeared to account for the ability to predict the population density of *L. longipalpis* ($p < 0.05$), meaning that no statistical correlation was found between the population density per month and any climate variable individually or in any combination. Closer examination of the weather conditions before those specific months with increased population showed that all four areas had a single characteristic in common: the increased population had been had been immediately preceded by intense rainfall (Fig. 4).

DISCUSSION

The density of phlebotomine vectors and their rate of infection by *Leishmania*, in addition to low socio-economic level, modified environmental conditions and the presence of domestic animals in high numbers near households have been considered determinant conditions for the installation and transmission of VL (Foratini 1960, Sherlock & Guitton 1969, Sherlock 1996, Vieira & Coelho 1998). In Janaúba, the majority of houses in many districts are poor, contain bad sanitation conditions and have precarious garbage collection. In addition, high numbers of domestic animals contribute to the accumulation of organic matter. These conditions are known to favour the presence of *L. longipalpis*, the predominant phlebotomine species in the city. In fact, a predominance of *L. longipalpis* was also observed in previous studies conducted by our group in other endemic areas for VL in Brazil (Sousa et al. 2004, Barata et al. 2004, 2005, França-Silva et al. 2005, Monteiro et al. 2005, Missawa & Dias 2007). In Várzea Grande, an area of transmission in the state of Mato Grosso with semi-arid climate, the

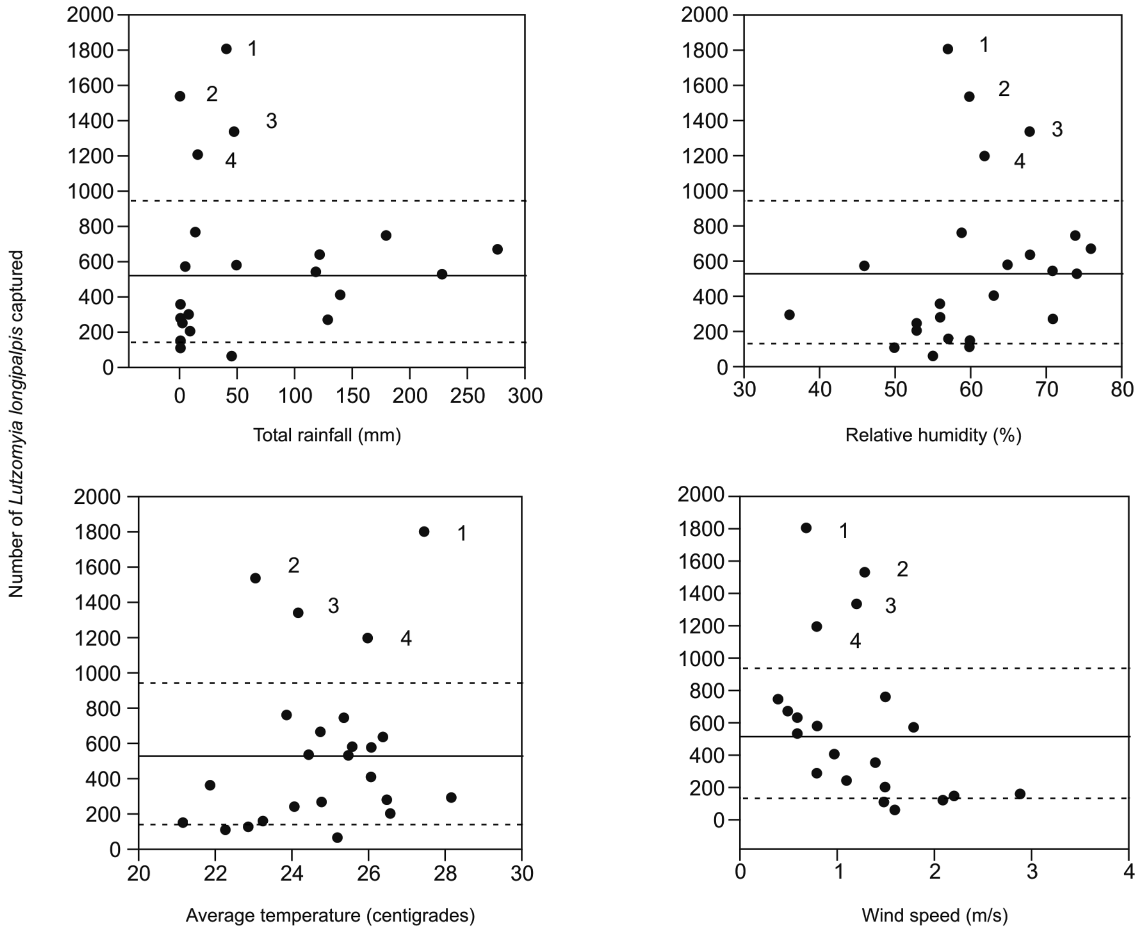

Fig. 3: populational density of *Lutzomyia longipalpis* in Janaúba, state of Minas Gerais, Brazil, as a function of individual climate variables. Each dot represents the number of specimens captured in a given month. The average mean (continuous line) ± four times the standard deviation (dashed line) calculated for the whole period (April 2005-March 2007) are indicated. The numbered months are as follows: 1: February 2006; 2: May 2006; 3: April 2006; 4: March 2007.

percentage of *L. longipalpis* specimens was not relatively high (65.23%) (Missawa & Dias 2007). The average number of phlebotomine sand flies captured per night in Várzea Grande was 134, whereas in Janaúba this number was 203 during the two-year sampling period. Although the population density in Janaúba was 34% higher, the phlebotomine fauna in Várzea Grande was more diverse, comprising 22 species of *Lutzomyia* and one species of *Brumptomyia* compared to the total of eight species that were found in Janaúba.

It is worth noting that an extremely high percentage of *L. longipalpis* specimens were captured in Algodões, a district with no reported cases of human VL until 2006. This district is located in the periphery of the city, has abundant vegetation, domestic animals and organic matter providing excellent conditions for phlebotomine sand fly breeding and population growth. Despite the absence of human VL cases, the presence of the vector species in such high numbers associated with the high prevalence (28.3%) of canine VL (Table) is indicative of an area with potential risk of transmission to humans.

Females are the transmitting agents of *Leishmania* due to their haematophagous feeding habit, and they were consistently found as a minority in every district, except for Vila Isaías, where both sexes were present in equal numbers (Table). In fact, the predominance of *L. longipalpis* males in field captures is in accordance with previously published studies (Oliveira et al. 2003, 2006, Barata et al. 2004, 2005, Resende et al. 2006).

Although the great majority of *L. longipalpis* specimens were captured in the peri-domicile setting, it is worth noting the high numbers of *L. longipalpis* captured inside of houses. These data illustrate the endophilic behaviour of the vector and emphasise the possibility of VL transmission in the intra-domicile setting (Santos et al. 2003, Sousa et al. 2004, Barata et al. 2004, 2005, França-Silva et al. 2005, Monteiro et al 2005, Resende at al. 2006, Missawa & Dias 2007).

Several studies have demonstrated a clear relationship between abiotic factors, including temperature, rainfall and humidity to the population density of phlebotomine sand flies, due to the interference in adult life

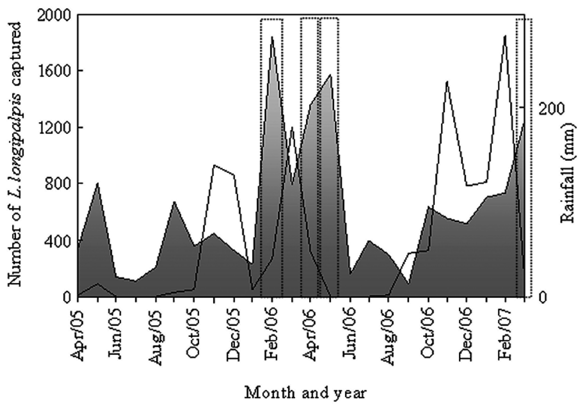

Fig. 4: populational density of *Lutzomyia longipalpis* in Janaúba, state of Minas Gerais, Brazil (grey area), from April 2005-March 2007 and local rainfall (continuous line) in the same period. The four months with unusually high densities are indicated by dotted lines.

cycles or to a modification in breeding sites (Scorza et al. 1968, Chaniotis et al. 1971, Roberts 1994, Monteiro et al. 2005). Since their pioneer studies, Deane and Deane (1955) have noted the interference of weather seasons on the populational density of *L. longipalpis.* They report that humid rainy periods favour the proliferation and survival of the species. After this initial report, several authors have observed high increases in the populational density of these phlebotomine sand flies either during rainy months (Gomes et al. 1980, Aguiar & Soucasaux 1984, Gomes & Galati 1987, Salómon et al. 2002, Barata et al. 2004) or after the rainy period (Sousa et al. 2004, Dias et al. 2007). In fact, the increased humidity that accompanies rainfall periods promotes vegetation sprouting, which in turn provides adequate sheltering and breeding conditions for the sand flies. This relationship between population density of sand flies and the rainfall period was not very clear at Várzea Grande, despite this climate's similarity with that of Janaúba. Taking into account the four peaks of rainfall during the two-year period of our Janaúba study (Fig. 4), only the third peak (with a maximum index in November of 2006) was not followed by a populational increase of *L. longipalpis*. However, in that period, the rainy season extended from October 2006-February 2007. December 2006 and January 2007 were still rainy months, a condition that may have impaired any population increase that might have been expected after the rainfall peak in November 2006.

The rate of *Leishmania* infection in the vector is usually low in nature, even in endemic areas, and the massive presence of *L. longipalpis* has been considered crucial to an increased risk for VL transmission (Deane & Deane 1955, Sherlock & Miranda 1992). The population density of *L. longipalpis* in Janaúba did not appear to be related with either the transmission profiles of the human VL or the prevalence of canine VL, in agreement with the results reported for Várzea Grande (Missawa & Dias 2007). Nevertheless, we cannot disregard a clear limitation of these studies: the comparison of cumulative variables (number of human cases and prevalence of canine VL in a given period of time) with a sampling variable (population density of *L. longipalpis*). However, using this same approach in other endemic localities that are located in the Northern region of MG (Porteirinha and Montes Claros, respectively), a positive correlation was found between the population density of *L. longipalpis* and canine cases of VL (França-Silva et al. 2005, Monteiro et al. 2005).

In conclusion, the eco-epidemiological profile of VL is complex and displays particularities in each area of transmission. For this reason, it is highly important to develop studies in specific endemic localities. A clear example of this is in Janaúba, where our results point to a main modulation of *L. longipalpis* density by rainfall. Public Health Agencies should take this type of information into account during the planning of adequate VL vector control measures.

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