Article/Artigo

Analysis of sandflies (Diptera: Psychodidae) in Barra do Garças, State of Mato Grosso, Brazil, and the influence of environmental variables on the vector density of *Lutzomyia longipalpis* (Lutz & Neiva, 1912)

Análise da fauna flebotomínica (Diptera: Psychodidae) em Barra do Garças, Estado de Mato Grosso, Brasil, e a influência das variáveis ambientais na densidade vetorial de *Lutzomyia longipalpis* (Lutz & Neiva,1912)

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ABSTRACT

Introduction: Leishmaniasis is an infectious and parasitic zoonotic, non-contagious, vector-borne disease caused by protozoa of the genus *Leishmania*. In Brazil, the major vector of *Leishmania* (*Leishmania*) *infantum chagasi* (Cunha & Chagas, 1934) is *Lutzomyia longipalpis*. Barra do Garças, State of Mato Grosso, was designated as a priority area by the Brazilian Ministry of Health for american visceral leishmaniasis, and it is important to identify the vector species present in this municipality. Our objective was to raise sandflies and study the influence of environmental variables on the vector density of *Lutzomyia longipalpis*. **Methods**: We performed entomological monitoring in 3 districts using Centers for Disease Control and Prevention (CDC) light traps and recorded human cases of american visceral leishmaniasis in the city. We calculated the relative frequency and richness of sandflies and applied a transfer function model to the vector density correlate with relative humidity. **Results:** The sandfly population was composed of 2 genera and 27 species, totaling 8,097 individuals. Monitoring identified *Lutzomyia longipalpis* (44%), followed by *Lutzomyia lenti* (18.9%), *Lutzomyia whitmani* (13.9%), *Lutzomyia carmelinoi* (9.1%), *Lutzomyia evandroi* (5.1%), *Lutzomyia termitophila* (3.3%), *Lutzomyia sordellii* (1.9%), and 20 other species (<4%). The male:female ratio was 3.5:1. We observed high species diversity (Dα = 6.65). *Lutzomyia longipalpis* showed occurrence peaks during the rainy season; there was a temporal correlation with humidity, but not with frequency or temperature. **Conclusions**: The presence of *Lutzomyia longipalpis* in the urban area of Barra do Garças underscores the changing disease profile, which was previously restricted to the wild environment.

Keywords: Sandflies. Leishmaniasis. Time Series. *Lutzomyia longipalpis*.

RESUMO

Introdução: Leishmanioses são doenças infecciosas e parasitárias de caráter zoonótico, não-contagiosas, transmitidas por vetores, causada por protozoários do gênero *Leishmania*. No Brasil, o principal vetor da *Leishmania* (*Leishmania*) *infantum chagasi* (Cunha & Chagas, 1934) é o *Lutzomyia longipalpis*. Barra do Garças, no Estado de Mato Grosso, é prioritária pelo Ministério da Saúde para a leishmaniose visceral americana, sendo importante conhecer as espécies de vetores presentes no município. O objetivo foi levantar os flebotomíneos e estudar a influência das variáveis ambientais sobre a densidade vetorial de *L. longipalpis*. **Métodos**: Realizou-se um monitoramento entomológico em três bairros com registro de casos humanos de leishmaniose visceral americana. Utilizando armadilhas luminosas CDC, calculou-se a frequência relativa e riqueza de flebotomíneos e aplicou-se um modelo de função de transferência para verificar a correlação da densidade vetorial com a umidade relativa do ar. **Resultados**: A fauna de flebotomíneos foi composta por 2 gêneros e 27 espécies, totalizando 8.097 indivíduos. No monitoramento prevaleceu *L. longipalpis* (44%), seguida por *L. lenti* (18,9%), *L. whitmani* (13,9%), *L. carmelinoi* (9,1%), *L. evandroi* (5,1%), *L. termitophila* (3,3%), *L. sordellii* (1,9%) e outras vinte espécies (abaixo de 4%). A proporção de machos para fêmeas foi 3,5:1. Observou-se uma alta diversidade de espécies (Dα=6,65). *L. longipalpis* apresentou picos de ocorrência durante a estação chuvosa; não havendo correlação temporal entre sua frequência e temperatura, mas sim com a umidade relativa do ar. **Conclusões**: A presença de *L. longipalpis* na área urbana de Barra do Garças, MT destaca a mudança do perfil da doença, antes restrita ao ambiente silvestre.

Palavras-chaves: Flebotomíneos. Leishmanioses. Séries temporais. *Lutzomyia longipalpis*.

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INTRODUCTION

American visceral leishmaniasis (AVL) is an infectious parasitic disease that is zoonotic in nature, with dogs as its main urban reservoir. In Brazil, AVL is caused by *Leishmania* (*Leishmania*) *infantum chagasi* (Cunha & Chagas, 1934). Transmission is performed by sandflies (order: Diptera, family: Psychodidae: sub-family: Phlebotominae) with *Lutzomyia longipalpis* (Lutz & Neiva, 1912) and *Lutzomyia cruzi* (Mangabeira, 1938) as the main vectors.

According to estimates by the World Health Organization, leishmaniasis is prevalent in 4 continents and endemic in 88 countries[1]. AVL is endemic in Brazil, reaching 5 regions, with the occurrence of human cases in 24 states.

In 1913, AVL was first described in Brazil from an autopsy of a patient from Boa Esperança, State of Mato Grosso[2]. The disease has rapidly expanded into the interior of Mato Grosso (MT) following the process of uncontrolled urban expansion, coupled with the intense migratory flow between the towns of the central-south with the north and southeast of the State[3].

From 2001-2006, the municipality of Barra do Garças-MT recorded 19 human cases of AVL, 2 of whom progressed to death[4]. The municipality is classified as a priority area for AVL, alternating between areas of moderate and/or sporadic transmission[5]. The presence of *L. longipalpis* and *L. cruzi* in the municipality of Barra do Garças-MT was reported between 1996 and 2004[6]. Seropositive dogs have also been reported[3] with average positive rates of 12%.

Given the above, and the increasing expansion of AVL in Mato Grosso, it became crucial to monitor the sandfly species present around residences in Barra do Garças-MT.

The objective of this study was to monitor the sandflies in neighborhoods of the City of Barra do

Garças-MT with a record of human cases of AVL. We also studied the influence of environmental variables, i.e., temperature, relative humidity, and rainfall, on the vector density of *L. longipalpis*.

METHODS

Barra do Garças-MT has a population of 56,423 inhabitants and a land area of 9,079 km^{2}[7], and is located along the Araguaia and Garças rivers (**Figure 1**), forming an important tourist resort, especially during June-August by offering extensive freshwater beaches that are visited by tourists from Mato Grosso, Goiás, Distrito Federal, among others. It is situated at latitude 15°89'00" and longitude 52º25'66" and is located at 318 m above sea level[8]. Its climate is Aw (hot and humid), and the dry season is from May-October and the rainy season is from November-April[9]. The average annual rainfall is 1,750mm and the mean annual temperature is 24°C (minimum 0°C, maximum 40°C)[10]. The predominant vegetation type is savanna, with small portions of forest and a transition zone[6].

FIGURE 1 - Geographical location of the municipality of Barra do Garças, State of Mato Grosso, Brazil.

The collection of sandflies was carried out monthly in the neighborhoods of Novo Horizonte, São José, and Vila Maria. These locations were chosen because of the presence of human cases of AVL, and these are peripheral areas, located southwest of Barra do Garças-MT. Samples were collected for 4 consecutive nights between 17:00-07:00 for 24 months from September 2004 to August 2006, thereby covering different seasons.

The sandflies were collected[11] from peridomestic locations with the use of Centers for Disease Control and Prevention (Atlanta, USA) light traps, with a total of 10 traps, distributed as follows: 3 in the Novo Horizonte neighborhood with the following geographic coordinates (15º52'509" 52º19'336"; 15º52'305" 52º19'138"; and 15º52'242" 52º19'458"), 4 in São José (15º52'803" 52º19'291"; 15º53'112" 52º19'736"; 14º53'066" 51º06'156"; and 15º53'204" 52º19'683"), and 3 in Vila Maria (15º53'211" 53º19'736"; 15º53'600" 52º19'683"; ad 15º53'741" 52º19'761").

The homes were chosen to maintain favorable environmental characteristics for the life cycle of sandflies, as there were organic matter in soil (leaves, fruit, rotting logs, and feces/manure), abundant vegetation (fruit trees), livestock (dogs and chickens), and market gardens. We measured the following variables: temperature (°C), relative humidity (%), rainfall (mm), and geographic coordinates. The monthly rainfall averages for the period studied were obtained from the Brazilian Company of Airport Infrastructure from the station based in Barra do Garças-MT.

The trapped insects were killed with ethyl acetate, sorted, and stored in plastic vials containing 70% alcohol for further clarification and identification at the species level by the Laboratory of Entomology, Central Level of the State Secretariat of Health of Mato Grosso (SES/MT), using phase contrast optical microscopy and a taxonomic key[12]. From February 2005, we also identified the sex of the collected individuals.

The relative frequency of the sandflies was calculated using percentages, and the Margalef's index (Dα) was used to calculate wealth, translated from faunal composition[13].

The influence of temperature and relative humidity on the number of sandflies was assessed using the transfer function model[14]. We also used analysis of variance (ANOVA) to determine whether significant differences occurred between the vector density of *L. longipalpis* and rainfall in different seasons - winter and summer - in the years studied. To check whether there was a correlation between relative humidity and rainfall, we applied the Bartlett correlation test[14], and this was tested for up to a 2-month lag.

RESULTS

In the neighborhoods of the City of Barra do Garças-MT in the period from September 2004 to August 2006, the sandfly population was composed of 2 genera and 27 species, totaling 8,097 sandflies collected from peridomestic locations; of these, 44% were *L. longipalpis*. From February 2005, when we began to sex the species, the proportion of males to females of *L. longipalpis* was approximately 3.5:1 (**Table 1**).

The index of species richness (Dα) was 6.65, where Dα values > 5 indicate high diversity. *L. longipalpis* was the most abundant species, being collected in all months surveyed (**Figure 2**).

In the period studied, the temperature varied from 16-40°C, and the relative humidity ranged from 24-92% while maintaining the standard of Mato Grosso, Brazil.

All cross-correlations between the frequency of *L. longipalpis* with temperature at different time periods were statistically zero ($p > 0.05$). However, there was a significant correlation between the frequency of *L. longipalpis* with the relative humidity in the same ($p < 0.07$) and previous ($p < 0.05$) month.

How had a significant correlation between the relative frequency of *L. longipalpis* and humidity can apply a transfer function model for a time. To better explain the model, the monthly relative humidity (X_t), the monthly frequency of *L. longipalpis* (Y_t), and the unit of time (t) for each month are considered.

The model transfer function that relates these 2 series is given by:

$Y_t = 1.17Y_{t-1} - 0.31Y_{t-2} + 2.86X_t - 1.14X_{t-1} - 0.765\alpha_{t-1} + \alpha_t$, indicating that the monthly frequency of *L. longipalpis* (Y_t) depends on the frequency of *L. longipalpis* in the previous month (Y_{t-1}) and in the

TABLE 1 - Sandflies collected with CDC type light traps from September 2004 to August 2006 in Barra do Garças-MT, by species and sex after February/2005.

Species	From September 2004 to August 2006		From February 2005 to August 2006	
	frequency	relative frequency	female	male
Brumptomyia brumpti	44	0.54	20	17
Lutzomyia acanthopharynx	53	0.65	25	11
Lutzomyia brasiliensis	6	0.07	3	3
Lutzomyia carmelinoi	734	9.07	104	137
Lutzomyia davisi	2	0.02	2	-
Lutzomyia evandroi	416	5.14	80	48
Lutzomyia flaviscutellata	3	0.04	-	-
Lutzomyia goiana	1	0.01	1	-
Lutzomyia hermanlenti	26	0.32	12	7
Lutzomyia inflata	4	0.05	1	-
Lutzomyia lenti	1,532	18.92	274	408
Lutzomyia longipennis	50	0.62	24	6
Lutzomyia longipalpis	3,560	43.97	556	1,582
Lutzomyia lutziana	2	0.02	2	-
Lutzomyia punctigeniculata	2	0.02	2	-
Lutzomyia runoides	2	0.02	-	2
Lutzomyia sallesi	54	0.67	16	19
Lutzomyia saulensis	3	0.04	3	-
Lutzomyia scaffi	1	0.01	1	-
Lutzomyia shannoni	2	0.02	1	1
Lutzomyia shawi	1	0.01	1	-
Lutzomyia sordellii	150	1.85	70	28
Lutzomyia teratodes	16	0.20	4	3
Lutzomyia termitophila	269	3.32	69	59
Lutzomyia walkeri	35	0.43	10	21
Lutzomyia wellcomei	1	0.01	-	1
Lutzomyia whitmani	1,128	13.93	368	466
Total	**8,097**	**100.00**	**1,649**	**2,819**

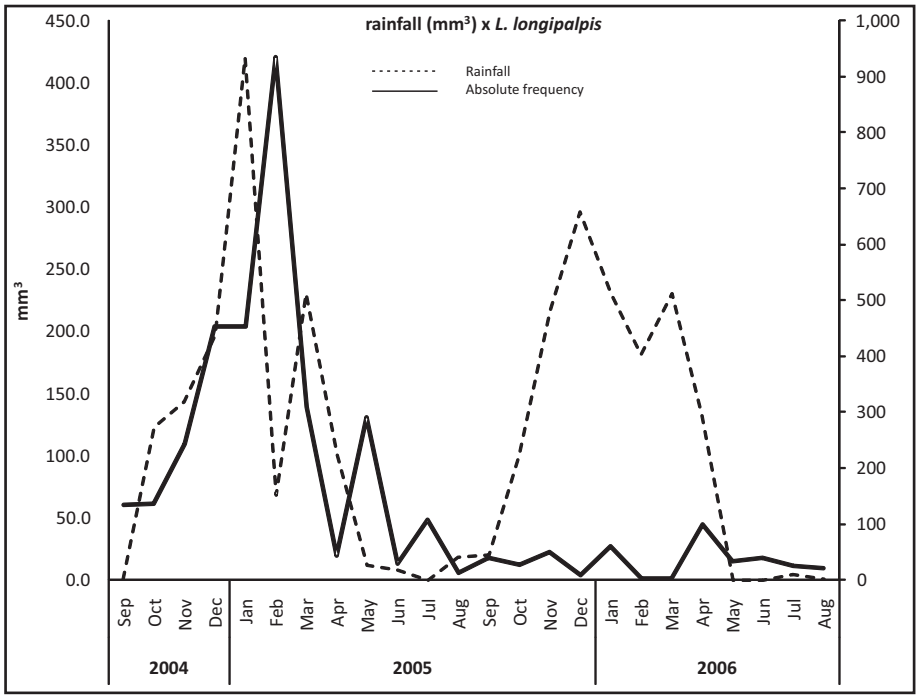

FIGURE 2 - Relationship between the vector density of *Lutzomyia longipalpis* collected with CDC type light traps and the average rainfall from September 2004 to August 2006 in the town of Barra do Garças-MT.

second preceding month (Y_{t-2}) and on the relative humidity in the same month (X_t) and in the previous month (X_{t-1}). The corresponding weights are given by the numerical values of the model. Moreover, the same monthly frequency of *L. longipalpis* (Y_t) also depends on other random factors, and the same month and the previous month are represented by α_t and α_{t-1}, respectively.

The association between the frequency of *L. longipalpis* captured and the variation of rainfall is represented in **Figure 2**. The annual rainfall was 1,668.9 mm, 1,489.6 mm, and 1,406.3 mm for the years 2004, 2005, and 2006, respectively.

ANOVA applied to verify if the seasons, i.e., dry and wet, interfere with the vector density of *L. longipalpis* provided a p value of 0.00058 ($p < 0.05$), indicating that there were significant differences for various periods, and the vector density of *L. longipalpis* was highest in the rainy season from November 2004 to March 2005.

When testing the influence of rainfall on relative humidity, there was a significant correlation between rainfall with relative humidity in the same month ($r = 0.72$, $p < 0.05$) and in the subsequent 2 months ($r = 0.60$, $r = 0.50$, $p < 0.05$).

DISCUSSION

The richness of sandfly species in the neighborhoods studied in the City of Barra do Garças-MT was high ($D\alpha = 6.65$), and featured a very diverse population that was comprised of 2 genera and 27 species. The most abundant species were *L. longipalpis* (44%), *Lutzomyia lenti* (Mangabeira, 1938) (18.9%), *Lutzomyia whitmani* (Antunes & Coutinho, 1939) (13.9%), and *Lutzomyia carmelinoi* (Ryan Fraiha, Lainson & Shaw, 1986) (9.1%). Noteworthy is the finding of 4 species of sandflies of importance to public health, i.e., *L. longipalpis*, *L. whitmani*, *Lutzomyia flaviscutellata* (Mangabeira, 1942), and *Lutzomyia wellcomei* (Fraiha, Shaw & Lainson, 1971). However, the species *L. lenti* and *L. carmelinoi* are not considered as vectors of leishmaniasis.

The third most abundant species, *L. whitmani*, is the main vector of american tegumentary leishmaniasis (ATL) in the State of Mato Grosso and in Barra do Garças-MT, being found in peridomestic and indoor areas (V Correa, GBM Lima, MFM Queiroz: unpublished data). This species is in the process of domestication and is widely distributed with greater adaptation to man-made changes to the environment[15].

Despite the low density of *L. flaviscutellata* and *L. wellcomei* in the urban area, their presence is of concern given the importance they represent to public health. *L. flaviscutellata* is a common species of forest and half-domestic environments. Since they are vectors of *Leishmania* and arboviruses, their preferred habitats are chicken coops, pig pens, corrals, and other attachments of domestic animals and the external and internal walls of human homes[15].

L. wellcomei, another vector for ATL, has been reported for the first time in Mato Grosso. The presence of this species

was reported[15] in the States of Amazonas, Pará, Ceará, Maranhão, and Pernambuco, and is essentially a forest species. Therefore, its presence in urban areas suggests their possible urbanization, and it is thus important to study their biology and ecology.

The altered environments favors adaptation of some species of sandflies especially in peridomiciliary, thereby favoring the transmission of the disease, as evidenced in this study, with the presence of *L. longipalpis* in the urban areas surveyed in Barra do Garças-MT, confirming the findings of some authors[16-18].

Our results suggest that the transmission pattern of AVL may be changing from its previous association with occupational or recreational activities, and this may also be associated with its transmission in the peridomestic environment, corroborating the findings of other authors[19-21].

The transmission of AVL is also influenced by poor sanitation, lack of environmental management, the movement of people and wildlife reservoirs between wild and urban environments, and the existence of dogs infected with *Leishmania* (*Leishmania*) *infantum chagasi*[22].

The peripheral districts studied have a predominantly low-income population. In addition, 90% of the households surveyed raise chickens. As mentioned earlier, the presence of chickens was one of the criteria for choosing the properties in the present study because this promotes the accumulation of organic matter, thereby favoring the breeding of sandflies, and being one of their preferred food sources[23,24]. The presence of chickens in large quantities in the areas surrounding homes appears to be of epidemiological significance, in that it works as a decoy for the vector, keeping it in this environment[23]. Our results confirmed that chickens were a risk factor for the presence of *L. longipalpis*.

Another important condition for the maintenance of AVL in urban areas is the presence of dogs, which are reservoirs of the disease[23], and 70% of the households surveyed kept a dog. It is important to emphasize that chickens and dogs promote the continuity of the vector and the etiologic agent of the disease, thereby bringing the disease to humans[22,23].

The differentiation of *L. longipalpis* by sex showed a higher capture rate for males, corroborating the findings of other authors[17,25]. This higher ratio can be explained by the natural behavior of the sandflies because males follow females to ensure fertilization.

Factors such as temperature, relative humidity, and rainfall have an influence on sandfly population density, depending on the area analyzed[25]. In the present study, temperature had no influence on the frequency of *L. longipalpis*. However, relative humidity influenced the vector density of *L. longipalpis*, corroborating the findings of other authors[20,26,27].

Moreover, the time series model showed that the relative humidity in the same and previous month influences the population density of *L. longipalpis*, the same effect was observed for the frequency of the species population, and that the interference was even greater as it persisted from the second preceding month.

By comparing the relative frequency of *L. longipalpis* in the dry and rainy seasons, we noticed a significant increase in the frequency of *L. longipalpis* during the rainy season, as reported by other authors[12,17,18,28-31].The rainy season increases air humidity and favors the growth of vegetation that provides shelter and adequate conditions for the reproduction of sandflies[32].

Peak capture and rainfall did not coincide for the 2 years studied, suggesting that rainfall encourages the growth of the sandfly population, but not excessively so, as reported by other authors[25,26]. The direct effect of large amounts of rain can change the biological cycle of the sandfly. It is possible that excessive rainfall alters the availability of the organic matter that they use for the deposition of eggs and for their larvae to feed on; however, it could also kill the immature flies. Therefore, there seems to be a threshold level of rainfall that is favorable for the reproductive cycle of sandflies.

In the first year of entomological monitoring (September 2004-August 2005), we observed a large number of *L. longipalpis* in the study area; however, this was followed by a decline in the second year. This fact can also be associated with environmental management (e.g., tree pruning, removal of organic matter, and eliminating some chickens and pigs) that was conducted in the peridomestic environment, as confirmed by other authors[5,33].

The presence of *L. cruzi* in the urban area was previously reported in Barra do Garças-MT[6], but this species was not collected during the 2 years of monitoring. The occurrence of a species in a local community is partially determined by its adaptation to the conditions and available resources and partly by competitive interactions with other species[34]; as these species are very close phylogenetically, both must use the same ecological resources, and competition must occur[18]. Therefore, interspecific competition could explain the absence of *L. cruzi* during this period.

The results indicated a worrying situation for the urban area of Barra do Garças-MT, as *L. longipalpis* was the most frequent and abundant species, being collected in every month of the year and found in all households surveyed, since it is the main vector of *Leishmania* (*Leishmania*) *infantum chagasi* in Brazil[19,35]. Entomological knowledge about the seasonality and population dynamics of *L. longipalpis* in Barra do Garças-MT allows the targeting of prevention and control activities for AVL, optimizing financial resources and actions, and thus promoting the strengthening of public health.

The importance of detecting *L. longipalpis* is the fact that this species is more adapted to the epidemiology of the disease, is widely distributed throughout the country, and is present in areas where AVL is a constant concern of public health management programs[36].

The high richness of sandflies found reinforces the importance of further studies in order to raise more qualitative and quantitative information on the transmission cycle of leishmaniasis in the municipality of Barra do Garças-MT.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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