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Pesquisa de flebotomíneos (Psychodidae: Phlebotominae) em área urbana de transmissão de leishmaniose visceral no Nordeste do Brasil

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Abstract

Visceral leishmaniasis (VL) is a major public health challenge in Brazil, especially in states where it is endemic. The aim of this study was to investigate the relationship of sand fly population density with environmental variables (temperature, rainfall and relative humidity) in urban areas of the city of Mossoró, Rio Grande do Norte, Northeastern Brazil. Sand flies were captured with Center Disease Control (CDC) traps installed monthly in the intra and peridomicile of three houses. Data analysis was based on the chi-square test and linear regression. A total of 7,347 sand flies were captured, being 93.85% *Lutzomyia longipalpis* and 6.15% *Lutzomyia evandroi*. Sand flies were more commonly found in the peridomicile and there was no difference between the number of males and females. The variables rainy season as well as relative humidity and rainfall, alone or together, did not have an effect on sand fly population density. However, high temperatures had a negative effect. The study of the behavior of sand flies in specific units of endemic areas can provide input to public health authorities for planning appropriate VL vector control measures.

Keywords: *Lutzomyia longipalpis*, *Lutzomyia evandroi*, seasonality, climate, Rio Grande do Norte State.

Resumo

A leishmaniose visceral (LV) é um grande desafio para a saúde pública no Brasil, particularmente nos estados onde é endêmica. O objetivo deste estudo foi verificar a relação da densidade populacional de flebotomíneos com as variáveis ambientais (temperatura, precipitação de chuva e umidade relativa do ar) em bairros urbanos de Mossoró, Rio Grande do Norte. Os flebotomíneos foram capturados com armadilhas CDC instaladas mensalmente no intra e peridomicílio de três casas. A análise dos dados foi baseada no teste Qui-quadrado e na regressão linear. Foram capturados 7.347 flebotomíneos, sendo 93,85% *Lutzomyia longipalpis* e 6,15% *Lutzomyia evandroi*. Os flebotomíneos foram encontrados mais comumente no peridomicílio e nenhuma diferença significativa entre o número de machos e fêmeas foi observada. As variáveis estação chuvosa, bem como a umidade relativa e precipitação de chuva, associadas ou isoladas, não influenciou a densidade populacional dos flebotomíneos. No entanto, a alta temperatura afetou essa densidade de forma negativa. Portanto, este estudo específico em áreas endêmicas é importante, porque as agências de Saúde Pública podem usar essas informações para um planejamento adequado das medidas de controle de vetores LV.

Palavras-chave: *Lutzomyia longipalpis*, *Lutzomyia evandroi*, sazonalidade, clima, Rio Grande do Norte.

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Introduction

In Brazil visceral leishmaniasis (VL) is caused by *Leishmania chagasi* transmitted to humans mainly through the bite of infected females of *Lutzomyia longipalpis* sand flies (PINTO et al., 2010). This disease remains a major public health challenge in Brazil, especially in states where it is endemic (PAULA et al., 2009). Studies focused on the vector and environmental conditions are key for assessing the transmission risk of this tropical disease (MICHALSKY et al., 2009) and can provide input that will help to determine the most appropriate methods of vector control (DIAS et al., 2007).

Sand flies are frequently found in natural ecotopes, such as tree trunks, animal shelters, fallen leaves, cracks in rocks and caves (GALATI et al., 2003), as well as in rural and urban environments characterized by domestic animal shelters and human dwellings, demonstrating the adaptation of these insects to anthropic environments (BARATA et al., 2008). *L. longipalpis* is well adapted to living with humans and domestic animals (REBÊLO, 2001) and can resist adverse conditions and exploit new environments, thereby facilitating VL transmission.

VL is endemic in the state of Rio Grande do Norte, northeastern Brazil, and in recent years there was an increase of reported cases in many municipalities (CORTEZ et al., 2007, XIMENES et al., 2007). However, data on the presence and distribution of *L. longipalpis* in the city of Mossoró has been generally inadequate to draw an eco-epidemiological profile of VL in this municipality. In addition, high prevalence of VL in dogs in several districts of Mossoró (AMÓRA et al., 2006; MATOS et al., 2006) shows the need to assess the sand fly frequency in domiciliary areas. This study aimed to investigate phebotomine sand flies in areas of intense VL transmission in Mossoró and to describe the relationship between the ecology of sand flies and environmental variables.

Material and Methods

1. Study area

The city of Mossoró is located 285 km from the state capital Natal (37° 20' 39" W and 05° 11' 15" S and 16 m altitude above see level). It has an area of 2,110,207 km²; 85% of its population of 234,390 inhabitants live in the urban area (INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA, 2007). The average annual temperature is around 27.5 °C with maximum 36 °C and minimum 21 °C. The average relative humidity is between 59 and 76%. The climate is semi-arid with low rainfall and two well-defined seasons: the rainy season, from January to April and extending as late as June (500 to 700 mm/year) and drought (INSTITUTO DE DEFESA DO MEIO AMBIENTE, 2002).

The entomological investigation was conducted at Abolição, Aeroporto and Rincão districts of Mossoró, classified as areas of intense VL transmission (MINISTÉRIO DA SAÚDE, 2006).

2. Capture and identification

The reasons for choosing domiciliary areas included recent *L. longipalpis* capture history, presence of abundant vegetation, domestic animals and organic matter accumulation in the peridomicile. Three human dwellings with poor sanitary conditions were selected for the study, one from each district. Sand fly captures using CDC light traps were carried out on a monthly basis during four consecutive nights, from 6:00 p.m. to 6:00 a.m., from January to December 2007. One trap was placed in the peridomicile and a second one inside each dwelling, preferably in animal shelters, as proposed by the Brazilian Ministry of Health (MINISTÉRIO DA SAÚDE, 2006).

The insects captured were identified in the Laboratory of Veterinary Microbiology at the Universidade Federal Rural do Semi-Árido (UFERSA). Female specimens were clarified and dissected on slides and covered with slips for observation under an optical microscope (ARANSAY et al., 2000). Sand fly identification was performed according to GALATI (2003).

3. Data analysis

Environmental variables – temperature (°C), relative humidity (%) and rainfall (mm) – were provided by the UFERSA weather station. The male/female ratio, rainy season influence and capture site (intradomicile or peridomicile) were analyzed by a chi-square (χ^2) test. The correlation between environmental variables and sand fly population was tested by multiple or single linear regression with Pearson's coefficient using SigmaStat 3.1.

4. Ethics committee

The study was approved by the Research Ethics Committee of Universidade Estadual do Ceará as part of a research project entitled "Biological control and entomological surveillance of *Lutzomyia longipalpis* in the city of Mossoró, Rio Grande do Norte" (protocol nr. 07465297-4).

Results and Discussion

A total of 7,347 sand flies were captured, 93.85% of which were *L. longipalpis* (χ^2= 2,825.12, df = 1, $p < 0.05$). The remaining sand flies were *Lutzomyia evandroi*. There was no numeric difference between males and females of *L. longipalpis*, but more male *L. evandroi* were caught ($\chi^2 = 1.95$, df = 1, $p < 0.05$). The sand flies were more commonly found in the peridomicile (70.48%) in all districts and months ($\chi^2 = 616.18$, df = 1, $p < 0.05$) (Table 1).

The presence of *Leishmania* vector species explains the active foci of human VL in the area studied, as reported in the municipality of Barreirinhas, Maranhão (REBÊLO et al., 2010). The districts studied are located in the periphery of the city, with abundant vegetation, domestic animals and organic matter, creating favorable conditions for sand fly breeding and population growth, corroborating XIMENES et al. studies (2000). These conditions are known to favor the presence of *L. longipalpis* (MICHALSKY et al., 2009), the predominant phlebotomine species in the city. Similar results were found in a previous monitoring survey by the same research group (AMÓRA et al., 2010).

Lutzomyia evandroi was found in the same ecotopes of *L. longipalpis*. The same was seen in 30 other municipalities in the State of Rio Grande do Norte, where *L. longipalpis* remains

Table 1. Distribution of *Lutzomyia* spp. by species, sex and capture site during the phlebotomine sand fly survey study. Mossoró, Rio Grande do Norte State, northeastern Brazil, 2007.

Sand fly	Sex			Capture site		
	Female	Male	Total	Intradomicile	Peridomicile	Total
L. longipalpis	3,435Aa	3,460Aa	6,895a	2,071Aa	4,824Ba	6,895a
L. evandroi	205Ab	247Bb	452b	98Ab	354Bb	452b
Total	3,640A	3,707A	7,347	2,169a	5,178B	7,347

Capital letters compare the columns and lower case letters the lines. Different letters indicate significantly different values by the chi-square test ($p < 0.05$).

prevalent (XIMENES et al. 1999, 2000; QUEIROZ et al., 2009; AMÓRA et al., 2010). This finding maybe related to male predominance in *L. evandroi* populations and/or that this species is still in process of adaptation with flies preferably feeding on wild animals (XIMENES et al., 1999; QUEIROZ et al., 2009). However, *L. evandroi* has not so far been implicated as a leishmaniasis vector (LAINSON; RANGEL, 2005).

Female sand flies are the transmitting agents of *Leishmania* due to their hematophagous feeding habit and they were consistently found throughout the year. Nevertheless, no numeric difference was found between female and male Lu. *L. longipalpis*, in contrast to previously published studies reporting male predominance (XIMENES et al., 2000; BARATA et al., 2005; MICHALSKY et al., 2009; QUEIROZ et al., 2009). This finding also contrasts with that of a monitoring survey previously performed in Mossoró (AMÓRA et al., 2010) that found a higher number of female flies. It increases the risk of transmission and explains the increase in VL cases in the city.

The significantly higher proportion of sand flies in the peridomicile was also reported in other municipalities in Rio Grande do Norte (XIMENES et al., 2000, 2007), in the city of Mossoró, as mentioned earlier (AMÓRA et al., 2010), and in Montes Claros, state of Minas Gerais, southeastern Brazil, where sand flies were captured near domestic animals (MONTEIRO et al., 2005). These findings can be explained by the devastation of large wild areas for economic exploration, bringing the disease to the periphery of urban centers. Vectors and hosts are forced to migrate to human peridomicile in search of food (BARATA et al., 2005). In this scenario, environment degradation may affect the maintenance of ecological processes and the balance of the enzootic cycle of certain infections, causing a major health public impact, as seen with leishmaniasis in Barreirinhas and the National Park in Maranhão (REBÊLO et al., 2010).

It is also important to note the high number of *L. longipalpis* captured inside the houses, including many females engorged after a blood meal on humans or animals. These data illustrate the endophilic behavior of the vector and emphasize the possibility of VL transmission in the intradomicile (BARATA et al., 2005; MONTEIRO et al., 2005; MISSAWA; DIAS, 2007; XIMENES et al., 2007; MICHALSKY et al., 2009).

Lutzomyia longipalpis was present every month of the year, with peaks in May and July, and monthly captures were significantly different ($p < 0.05$) (Table 2). VL vector showed a slight seasonal distribution, tending to increase during rainy periods. The environmental variables studied were statistically different ($p < 0.05$), especially monthly average rainfall and March was the most humid month. During the study it rained 943.2 mm and the average relative humidity for the rainy period was 74.75% ($\chi^2 = 0.87$, $df = 1$, $p > 0.05$). The temperatures remained almost constant and relative humidity varied slightly (Table 2). The relative humidity and rainfall, alone or together, did not have an effect on sand fly population density ($R \leq -0.189$, $r^2 \leq 0.036$, $p > 0.05$). However, the association of humidity or rainfall with temperature, or the temperature alone, had a negative effect on sand fly density ($R \geq 0.680$, $r^2 \geq 0.462$, $p < 0.05$).

The rainy season did not have an effect on the number of *L. longipalpis* captured ($\chi^2 = 1.22$, $df = 1$, $p > 0.05$). This fact confirms that rainfall and humidity do not have an effect population density. Nevertheless, in the rainy season, more insects were captured in the intradomicile ($\chi^2 = 42.79$, $df = 1$, $p < 0.05$) while in the dry season more insects were captured in the peridomicile ($\chi^2 = 31.71$, $df = 1$, $p < 0.05$) (Table 3).

This study showed that more *L. longipalpis* flies were captured in the intradomicile during the dry season compared to the peridomicile where most of the specimens were collected during the rainy season. Possibly these insects are in search of a more humid environment. Several studies have demonstrated a clear relationship between abiotic factors (temperature, rainfall and humidity) and sand fly population density with interference in adult life cycles or a modification of breeding sites (CHANIOTIS et al., 1971; MONTEIRO et al., 2005; OLIVEIRA et al., 2008; MICHALSKY et al., 2009). The population decrease is expected after the peak rainfall due to destruction of breeding sites in flooded ground and pupa killing in the soil (DIAS et al., 2007). However, the rain and humidity did not significantly affect sand fly density. Similar data were reported in a monitoring survey conducted in 2005–2006 in Mossoró (AMÓRA et al., 2010), and in Várzea Grande, Mato Grosso do Sul, central-west Brazil (MISSAWA; DIAS, 2007). Yet it has been classically reported that rainy periods favor the proliferation and survival of sand flies (DEANE, L. M.; DEANE, M. P., 1955), during (XIMENES et al., 2006; MACEDO et al., 2008; QUEIROZ et al., 2009) or after the rainy months (DIAS et al., 2007).

The temperatures remained constant but high throughout the study. High temperatures alone or associated with other variables had a negative effect on sand flies, probably due to the long rainy season. This inverse correlation between sand flies and high temperature was also reported in the municipality of Nísia Floresta in Rio Grande do Norte (XIMENES et al., 2006), Campo Grande in Mato Grosso do Sul (OLIVEIRA et al., 2008) and Sobral in the state of Ceará (MACEDO et al., 2008). However, in Nísia Floresta, sand flies live at temperatures between 32-36 °C and no

Table 2. *Lutzomyia longipalpis* captured and mean environmental variables, temperature (°C), relative humidity (%) and rainfall (mm), during the phlebotomine sand fly survey study. Mossoró, Rio Grande do Norte State, Northeastern Brazil, 2007.

Months	*Lutzomyia longipalpis*			Environmental variables		
	Female	Male	Total	Temperature	Rainfall	Humidity
January	63A	92B	155a	29.20a	9.70b	72.41ab
February	110A	142B	252b	27.96a	212.20e	77.29ab
March	157A	172B	329c	27.01a	389.30f	80.98b
April	351B	214A	565e	27.43a	141.20d	78.41ab
May	527A	469A	996h	27.07a	110.20d	74.85ab
June	366A	455B	821g	26.71a	42.40c	72.65ab
July	1,080B	791A	1,871i	27.65a	5.00b	63.11ab
August	264A	395B	659f	27.65a	0.00a	55.76a
September	190A	238B	428d	28.46a	0.00a	57.00a
October	112A	154B	266b	28.73a	0.00a	58.47ab
November	133A	182B	315c	28.82a	0.00a	62.94ab
December	82A	156B	238b	28.80a	33.20c	66.63ab

Capital letters compare the columns and lower case letters the lines. Different letters indicate significantly different values by the chi-square test ($p < 0.05$).

Table 3. Distribution of *Lutzomyia longipalpis* by capture site (intra- or peridomicile) dry period (July-Nov) and rainy (Dec-June), during the phlebotomine sand fly survey study. Mossoró, Rio Grande do Norte State, Northeastern Brazil, 2007.

Rainfall	Intradomicile			Peridomicile			Overall
	Female	Male	Total	Female	Male	Total	
Rainy period	639a	607a	1,246a	1,017a	1,093a	2,110a	3,356a
Dry period	361b	464b	825b	1,364b	1,296b	2,660b	3,485a
Total	1,000A	1,071A	2,071	2,381A	2,389A	4,770	6,841

Capital letters compare the columns and lower case letters the lines. Different letters indicate significantly different values by the chi-square test ($p < 0.05$).

significant correlation was found between population density and temperature (XIMENES et al., 2000).

Despite the absence of human VL cases in the dwelling studied, the presence of the vector species in such high numbers associated with a high prevalence of canine VL (AMÓRA et al., 2006, MATOS et al., 2006) is indicative of an area with potential risk of transmission of this zoonosis to humans. Similar data were reported in Janaúba, Minas Gerais (MICHALSKY et al., 2009).

The present study showed that *L. longipalpis* is the predominant species in Mossoró and is present in significant numbers throughout the year. It stresses the difficulty of implementing standard vector control measures due to the particularities of each region. Given the high number of insects caught in the intradomicile, we thus recommend intensification of VL control measures in Mossoró including health education.

In conclusion, the eco-epidemiological profile of VL is complex and shows particularities in each area of transmission. Studies on the behavior of sand flies in specific units of endemic areas can provide input to public health authorities for planning appropriate VL vector control measures.

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