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Monitoring of *Lutzomyia longipalpis* Lutz & Neiva, 1912 in an area of intense transmission of visceral leishmaniasis in Rio Grande do Norte, Northeast Brazil

Monitoramento de *Lutzomyia longipalpis* Lutz & Neiva, 1912 em área de transmissão intensa de leishmaniose visceral no Rio Grande do Norte, Nordeste do Brasil

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Abstract

Urban increase of visceral leishmaniasis (VL) in Brazil is associated with the adaptation of its vector, *Lutzomyia longipalpis*, to environments modified by humans. The present study reports the results of an entomological monitoring of *L. longipalpis* and the effect of environmental variables on its population density. Sandflies were captured in the municipality of Mossoró, State of Rio Grande do Norte, Northeastern Brazil, from January 2005 to December 2006. Two CDC light traps were placed monthly for four consecutive nights in the peridomicile of selected households. Data analysis was based on the chi-square test and linear regression. A total of 2,087 sandflies were captured, 99.86% of which were *L. longipalpis*. A higher proportion of females were captured ($p < 0.05$). Monthly analysis of the variables temperature, relative humidity and rainfall did not show a significant influence on population density. However, there were seasonal differences: approximately 70% of sand flies were captured during the rainy season ($p < 0.05$). The predominant species, *L. longipalpis*, is present in substantial number, representing a public health risk. Therefore, because of higher prevalence during the rainy season, we recommend intensified VL control measures before and during this season to reduce the risk of disease transmission.

Keywords: *Lutzomyia longipalpis*, seasonality, epidemiology, vector control, visceral leishmaniasis.

Resumo

No Brasil, o crescimento urbano da leishmaniose visceral (LV) está associado com a adaptação do seu vetor, *Lutzomyia longipalpis*, aos ambientes modificados pelo homem. Este estudo relata a vigilância entomológica de *L. longipalpis* e os efeitos das variáveis ambientais sobre a sua densidade populacional. Os flebotomíneos foram capturados no município de Mossoró, Rio Grande do Norte, no Nordeste do Brasil, a partir de janeiro de 2005 a dezembro de 2006. Duas armadilhas tipo CDC foram colocadas mensalmente durante quatro noites consecutivas no peridomicílio das casas escolhidas. A análise dos dados foi baseada no teste Qui-quadrado e regressão linear. Um total de 2.087 flebotomíneos foram capturados, dos quais 99,86% foram *L. longipalpis*. Mais fêmeas do que machos foram capturados ($p < 0,05$). Na análise mensal das variáveis ambientais a temperatura, umidade relativa e a chuva não tiveram impacto significativo sobre a densidade populacional de *L. longipalpis*. No entanto, houve diferenças sazonais: aproximadamente 70% dos flebotomíneos foram capturados durante a estação chuvosa ($p < 0,05$). Assim, *L. longipalpis*, a espécie predominante, representa um risco à saúde pública. Portanto, devido à maior prevalência no período chuvoso, recomendamos intensificar as medidas de controle da LV antes e durante este período para reduzir o risco de transmissão da doença.

Palavras-chave: *Lutzomyia longipalpis*, sazonalidade, epidemiologia, controle vetorial, leishmaniose visceral.

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Introduction

The importance of visceral leishmaniasis (VL) as a public health concern has increased significantly due to the urbanization process and changes in the natural environment (TRAVI et al., 2002). In Brazil, *Lutzomyia longipalpis*, remain the major vector in all regions including the southern region (SECRETARIA ESTADUAL DE SAÚDE DO RIO GRANDE DO SUL, 2009) and is found in all States of the Northeastern, Southeastern and Central-western region as well as in the capital Brasília (BOLETIM EPIDEMIOLÓGICO DO DISTRITO FEDERAL, 2009). Sand flies have a major role in the dynamics of VL transmission requiring in-depth investigation of their ecology to help formulating effective vector control measures, especially in regions where the disease is endemic (TEODORO et al., 2003).

Environmental factors such as temperature, relative humidity, and rainfall have different effect on sand fly population density (DIAS et al., 2007). Studies investigating seasonal variation of phlebotomine fauna can help better understand the interaction of each species with its habitat, as well as leishmaniasis transmission dynamics (MACEDO et al., 2008; MICHALSKY et al., 2009).

VL is endemic in the State of Rio Grande do Norte, Northeastern Brazil, and the transmission has steadily spread to other areas, as evidenced by an increasing number of municipalities reporting VL cases in recent years (AMÓRA et al., 2006; DUARTE et al., 2008; QUEIROZ et al., 2009). The purpose of the present study was to investigate the presence of *L. longipalpis* in an intense VL transmission area and to correlate the ecology of sand flies with environmental variables in this area.

Materials and Methods

1. *Study area*

The municipality of Mossoró is located 285 km from Natal, the capital of the State of Rio Grande do Norte. Its coordinates are 37° 20' 39" W Longitude and 5° 11' 15" S Latitude. Occupying an area of 2,110.207 km², 85% of Mossoró population (234,390) is concentrated in an urban area (IBGE, 2007). The average annual temperature is approximately 27.5 °C. The average relative humidity is between 59 and 76%. The climate is semiarid and characterized by low rainfall and two well-defined seasons: a rainy season, from January to April and extending as late as June (500 to 700 mm/year), and a dry season (IDEMA, 2002).

An entomological monitoring was conducted in a Mossoro neighborhood, Rincão, classified as subject to intense VL transmission based on mean number of human VL cases ≥ 4.4 in the last five years (BRASIL, 2006).

2. *Capture and identification*

The study inclusion criteria for domiciles included recent history of *L. longipalpis* capture, presence of abundant vegetation in the peridomicile, domestic animals, and organic matter accumulation. The selected domiciles also had poor sanitary conditions. Captures were carried out monthly from January 2005 to December 2006 over four consecutive nights from 6:00 p.m. to 6:00 a.m. using CDC light traps. Two traps were placed at each domicile, one inside the household and the second one in the peridomicile area, preferably in animal shelters, as proposed by the Brazilian Ministry of Health (BRASIL, 2006).

The captured insects were identified at the Entomology Laboratory of the State Health Secretariat of Rio Grande do Norte. They were washed with saline solution and males were separated from females. Female specimens were then clarified and dissected on slides and covered with slips for observation under an optical microscope (ARANSAY; SCOULICA; TSELENTIS, 2000). Sandfly identification was performed according to Galati (2003).

3. *Data analysis*

Environmental variables including temperature (°C), relative humidity (%), and rainfall (mm) information was obtained from the Universidade Federal Rural do Semi-Árido weather station. Male/female ratio and capture site were analyzed using the chi-square (χ^2) test. The correlation between environmental variables and sandfly population density was modeled using single or multiple linear regression analysis and the strength of the association was assessed by Pearson's correlation coefficient at $p < 0.05$ using SigmaStat 3.1 software (2004).

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 n. 07465297-4).

Results

A total of 2,087 sand flies were captured, 99.86% of which were *L. longipalpis* ($\chi^2 = 1{,}037.51$, df = 1, $p < 0.05$). The species was present in all months studied with a peak in April and July (Table 1). The remaining sandflies were *Lutzomyia evandroi* (COSTA LIMA; ANTUNES, 1936). During the study period, about 60% of *L. longipalpis* specimens captured were females, which was significantly higher than male specimens ($\chi^2 = 75.78$, df = 1, $p < 0.05$).

The relative humidity ranged from 62 to 82%, air temperature ranged from 26 to 29 °C and rainfall was between 0.0 and 242 mm/month. Environmental variables were statistically different only for monthly average rainfall. March and April were the wettest months ($\chi^2 = 3.37$, df = 1, $p < 0.05$) (Table 1).

The temperature remained almost constant during the study period. Nevertheless, humidity and insect capture peaks were irregularly distributed (Table 1). The analysis of monthly variables (temperature, relative humidity, and rainfall) did not show an effect on population density ($p > 0.05$). However, a comparison of the rainy and dry seasons showed that 78.45% of sandflies were

Table 1. Entomological monitoring conducted in an area of intense transmission of visceral leishmaniasis in the State of Rio Grande do Norte, Northeastern Brazil, during 2005 and 2006.

Months	*Lutzomyia longipalpis*			Environmental variables		
	Female	Male	Total	Temperature (°C)	Rainfall (mm)	Humidity (%)
January	49A	31B	80f	29.43a	1.15f,g	65.36a
February	103A	48B	151d,e	28.96a	66.4d	69.93a
March	34A	22A	56g	28.22a	242.35a	75.42a
April	320A	198B	518a	27.55a	188.5b	82.09a
May	94A	46B	140d,e	27.05a	94.05c	79.56a
June	142A	74B	216c	26.28a	88.75c,d	78.41a
July	230A	129B	359b	27.13a	23.80e	67.62a
August	114A	56B	170d	27.70a	19.80e	63.61a
September	123A	83B	206c	28.22a	4.85f	65.07a
October	41A	11B	52g	28.78a	0.00g	62.50a
November	12A	6A	18h	28.92a	0.00g	74.55a
December	61A	57A	118e	29.12a	0.00g	66.99a

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

Table 2. Distribution of *Lutzomyia longipalpis* during the dry and the rainy seasons in 2005 and 2006 in an area of intense transmission of visceral leishmaniasis in the State of Rio Grande do Norte, Northeastern Brazil.

Rainfall	Female	Male	Total
Rainy period	1,042A,a	593B,a	1,635a
Dry period	281A,b	117B,b	168b
Total	1,323A	761B	2,084

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

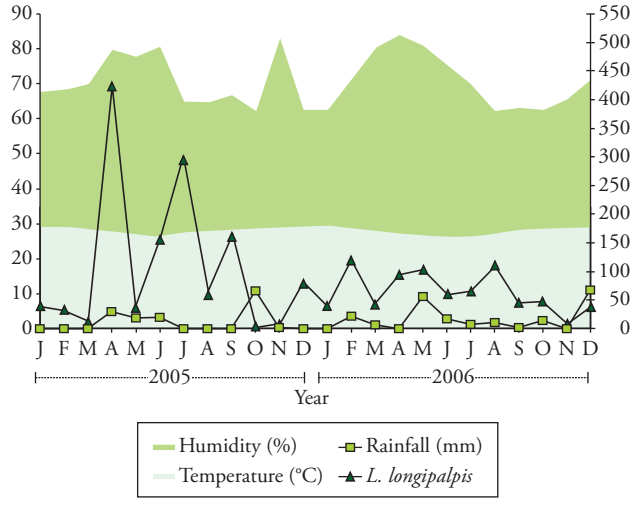

Figure 1. Influence of relative humidity and rainfall on *Lutzomyia longipalpis* population during an entomological monitoring in an area of intense visceral leishmaniasis transmission in the State of Rio Grande do Norte, Northeastern Brazil, from 2005 to 2006.

captured during the rainy season ($\chi^2 = 596.81$, df = 1, $p < 0.05$) (Table 2).

Figure 1 shows sandfly temporal variation and environmental variables. This vector assumes a characteristic seasonal distribution with increased density during rainy periods.

Discussion

Sandflies were captured in Mossoró monthly during the study period. The same pattern holds for other Brazilian States: Maranhão (REBÊLO, 2001), Minas Gerais (MONTEIRO et al., 2005) and Mato Grosso do Sul (OLIVEIRA et al., 2008). Ximenes et al. (2007) conducted a study in Mossoró and other municipalities in the State of Rio Grande do Norte, and demonstrated clear adaptation of *L. longipalpis* to the anthropic environment. *L. evandroi* has been found in the same ecotopes as *L. longipalpis*, but so far it has not been implicated as a leishmaniasis vector (LAINSON; RANGEL, 2005).

The same predominance of VL vector among captured sandflies in our study has also been demonstrated in several Brazilian States such as Ceará (DEANE, 1956), Pará (LAINSON et al., 1985), Amazonas (RIBEIRO; MISSAWA, 2002), Minas Gerais (MONTEIRO et al., 2005), Rio Grande do Norte (XIMENES et al., 2007; QUEIROZ et al., 2009), and Mato Grosso do Sul (OLIVEIRA et al., 2008) and even in other countries where the main VL vector is another species of sandfly, such as *Lutzomyia cruzi* in Mato Grosso do Sul (RIBEIRO; MISSAWA; ZEILHOFER, 2007), *Lutzomyia evansi* in Colombia (CORTÉS; FERNANDÉZ, 2008) and *Lutzomyia pseudolongipalpis* in western Venezuela (FELICIANGELI et al., 2006).

The finding of a higher proportion of females captured in Mossoró is similar to that described by Silva et al. (2008) in Paraná, Brazil, but contrasts with that reported in most other

studies in Brazil (BARATA et al., 2008; OLIVEIRA et al., 2008; QUEIROZ et al., 2009; MICHALSKY et al., 2009).

Our results are similar to those of Barata et al. (2004), which showed climatic factors influencing sandfly population in the Brazilian municipality of Porteirinha, State of Minas Gerais, indicating a significant correlation between the number of sandflies and rainfall. Other studies have shown that the rainy period significantly affects insect population, with increased density also seen in the post-rainy period when humidity is high (GALATI et al., 2003; BARATA et al., 2004). Studies conducted in Campo Grande, State of Mato Grosso do Sul, Central-western Brazil (OLIVEIRA et al., 2008), and in Nísia Floresta, State of Rio Grande do Norte, Northeastern Brazil (XIMENES et al., 2006), as well as in Central-western Venezuela (FELICIANGELI et al., 2006), show that the highest *L. longipalpis* population density coincided with the rainy period.

Sandfly density increased during the rainy season, which was also reported in a study conducted in Belo Horizonte, State of Minas Gerais (RESENDE et al., 2006) and Rondônia (GIL et al., 2003). The rainy season is short and irregular in most Northeastern States in Brazil (XIMENES et al., 2006). During this season, moderate rain levels may favor the development of sandflies, but breeding sites are likely destroyed with ground flooding, killing the pupae in the soil. This suggests that sandfly seasonality is associated to rain distribution patterns, which act by modifying microenvironmental conditions (DIAS et al., 2007). In Belo Horizonte, the largest number of *L. longipalpis* were seen during the highest rainfall combined with the highest mean temperatures (RESENDE et al., 2006). These data corroborate our study, where the temperature remained relatively high and constant throughout the period studied.

The monthly analysis of the correlation between climatic factors and *L. longipalpis* population density in the present study did not show significant differences probably due to the lack of variation in relative humidity and temperature over the months studied, demonstrating that sandfly population increases are associated with rainfall. This has already been evidenced in other Brazilian cities such as Belo Horizonte, State of Minas Gerais (MARGONARI et al., 2004), São Luís, State of Maranhão (REBÊLO, 2001) and Campo Grande, State of Mato Grosso do Sul (OLIVEIRA et al., 2008).

In the present study, *L. longipalpis* insects are present in substantial number throughout the year, representing a public health risk. There are seasonal variations in the vector's distribution with increased density during rainy periods. Thus, we recommend intensified VL control measures in Mossoró before and during the rainy season to reduce the risk of disease transmission.

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