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Phlebotomines in an area endemic for American cutaneous leishmaniasis in northeastern coast of Brazil

Flebotomíneos em uma área endêmica para Leishmaniose Tegumentar Americana no litoral do Nordeste do Brasil

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Abstract

Phlebotomines have worldwide distribution with many species present in Brazil, including the northeastern region, where the fauna is very diverse. The aim of this study was to identify the sandfly fauna in an area endemic for American cutaneous leishmaniasis (ACL) in the state of Pernambuco. Sandflies were caught on three consecutive nights every month from October 2015 to September 2016, from 5 pm to 5 am, using seven light traps of Centers for Disease Control (CDC) type. Females were identified and used for molecular *Leishmania* detection. A total of 2,174 specimens belonging to ten species were collected: *Lutzomyia choti* (88.2%; 1,917/2,174) was the most abundant species, followed by *Lutzomyia whitmani* (8.1%; 176/2,174) and *Lutzomyia sordellii* (1.5%; 33/2,174). The majority of the specimens were collected in peridomestic areas (64.1%; 1,394/2,174) and during the rainy period. All the samples examined were negative for *Leishmania* spp. The presence of *Lutzomyia whitmani* indoors and in peridomestic areas indicates that the inhabitants of this area are exposed to the risk of infection by the parasites responsible for ACL.

Keywords: *Lutzomyia*, *Leishmania*, vectors.

Resumo

Os flebotomíneos apresentam uma ampla distribuição mundial com muitas espécies presentes no Brasil, inclusive na região Nordeste, onde a fauna é bastante rica. O objetivo desse estudo foi identificar a fauna de flebotomíneos em uma área endêmica para Leishmaniose Tegumentar Americana (LTA), no estado de Pernambuco. As capturas foram realizadas mensalmente, durante três noites consecutivas das 17h às 5h, utilizando sete armadilhas luminosas tipo CDC, no período de outubro de 2015 a setembro de 2016. As fêmeas identificadas foram utilizadas para análise molecular para detecção de *Leishmania*. Um total de 2.174 espécimes pertencentes a dez espécies foram coletadas: *Lutzomyia choti* (88,2%; 1.917/2.174) a espécie mais abundante, seguida por *Lutzomyia whitmani* (8,1%; 176/2.174) e *Lutzomyia sordellii* (1,5%; 33/2.174). A maioria dos espécimes foi coletada no peridomicílio (64,1%; 1.394/2.174) e no período chuvoso. Todas as amostras avaliadas foram negativas para *Leishmania* spp. A presença de *Lutzomyia whitmani* no intradomicílio e peridomicílio indica que a população residente nesta área está exposta ao risco de infecção por parasitos causadores de LTA.

Palavras-chave: *Lutzomyia*, *Leishmania*, vetores.

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Introduction

Phlebotomines (Diptera: Psychodidae: Phlebotominae) are insects of great public health importance, given that they are responsible for transmission of viruses, bacteria and parasites, which include protozoa belonging to the genus *Leishmania* (Kinetoplastida: Trypanosomatidae). Indeed, it is believed that 98 phlebotomines species are proven or suspected vectors of human leishmaniasis (MAROLI et al., 2013).

These dipterans are distributed around the world, with major abundance in Neotropical regions (SHIMABUKURO & GALATI, 2011). Among the most important species, the ones belonging to the genus *Lutzomyia* (sensu YOUNG & DUNCAN, 1994) are responsible for transmission of leishmaniasis in the New World (KILLICK-KENDRICK, 1999). The geographical spread of American cutaneous leishmaniasis (ACL) has been directly correlated with the distribution of sandfly species. This is influenced by environmental and climatic changes due to human activity, thus resulting in adaptation of wild species to modified environments, such as inside domestic animal shelters and in areas surrounding them (KOVATS et al., 2001; PATZ et al., 2004; COSTA et al., 2007).

In Brazil, several species are involved in transmission of ACL: *Lutzomyia whitmani*, *Lutzomyia intermedia*, *Lutzomyia wellcomei*, *Lutzomyia complexa*, *Lutzomyia neivai*, *Lutzomyia fischeri* and *Lutzomyia migonei* (MAROLI et al., 2013). Particularly in the northeastern region, the fauna is very diverse. For instance, in the state of Pernambuco, the diversity of species is lower than in the states of Maranhão and Bahia, but it is much greater than in the states of Alagoas, Ceará, Paraíba, Piauí, Rio Grande do Norte and Sergipe (DANTAS-TORRES et al., 2010). Studies conducted in the state of Pernambuco have identified species of *Lutzomyia* transmitters of ACL (LUCENA et al., 1984; BRANDÃO-FILHO et al., 1998; SILVA & VASCONCELOS, 2005; BALBINO et al., 2005; ANDRADE et al., 2005; DANTAS-TORRES et al., 2010; GUIMARÃES et al., 2012; MIRANDA et al., 2015; AGRA et al., 2016).

In Pernambuco, ACL occurs in all regions of the state (ANDRADE et al., 2005), and some areas are endemic for ACL, such as the Três Ladeiras district of the municipality of Igarassu. In this area, 62 cases of human ACL were reported between 2008 and 2013 (RAMOS, 2015). Nevertheless, knowledge of the phlebotomine fauna in some endemic areas is still limited, which makes it difficult to control these vectors. Therefore, the present study aimed to contribute knowledge of the phlebotomine fauna in an area endemic for ACL in northeastern coast of Brazil.

Materials and methods

Study area

This study was conducted in the district of Três Ladeiras, which is endemic for ACL. It is located in the municipality of Igarassu (7°50'00" S and 34°54'30" W), in the metropolitan region of Recife, Pernambuco, Brazil. The district of Três Ladeiras has a total area of 102.3 Km^2 which represents 33.44% of the total area of the municipality of Igarassu (2000). The study area was located in the rural zone, where the main economic activity consists of sugar cane crops. In addition, it has vegetation cover formed of rain forest fragments. The climate is tropical (warm and humid), with rainy periods ranging from autumn to winter (March-September), average temperature of 25 °C and average rainfall of 2000 mm (IGARASSU, 2000). Climatic information was obtained from the Pernambuco Technology Institute (ITEP).

Collection and identification of phlebotomines

Specimens were collected on three consecutive nights every month from October 2015 to September 2016, from 5 pm to 5 am, using seven light traps of the Centers for Disease Control (CDC) model, with a mean distance of 900 m among them. Each trap was installed at a height of 1.5 m above the ground and in different ecotopes: indoors (P1 and P2), peridomestic areas (P3-stable, P4-kennel and P5-hen house) and forested areas (P6 and P7) (Figure 1). The specimens thus caught were identified in accordance with the dichotomous key of Young & Duncan

Figure 1. Spatial distribution of collection points in Igarassu, PE, Brazil.

(1994). The thorax and abdomen of specimens that had been identified as female (except for the three last segments, which had been used for morphological identification) were then conserved in 70% alcohol for subsequent molecular *Leishmania* analysis.

DNA extraction and polymerase chain reaction test (PCR)

DNA was extracted from 490 phlebotomines (*L. choti* = 405; *L. whitmani* = 73; *L. sordellii* = 10; *L. evandroi* = 1 and *L. longispina* = 1), which were divided into 100 pools containing approximately five specimens each. For DNA extraction, the Purelink™ Genomic DNA mini-kit (Invitrogen, USA) was used, in accordance with the manufacturer's recommendations. The DNA samples were analyzed by PCR using the primers L1 (5'-GGG GAG GGG CGT TCT GCG AA- 3') and L2 (5'-GGC CCA CTA TAT TAC ACC AAC CCC-3') for the genus *Leishmania* (MICHALSKY et al., 2002). DNA extracted from blood of dogs infected with *Leishmania infantum* was used as the positive control, and DNA from animals in non-endemic areas that had been proven to be uninfected was used as the negative control.

Data analysis

The results were analyzed by descriptive statistics. The Lilliefors test was used to verify the normality of the data. The relationship between the number of phlebotomines collected and the climatic variations was evaluated through the Friedman test. This test was also used to evaluate the presence of males and females in the environment, along with the month variations in the number of species collected. The chi-square (x^2) test with Yates correction was used to compare occurrences of phlebotomine species in different ecotopes. The significance level was taken to be 5%. The BioEstat software, version 5.3, was used to perform the statistical calculations.

Results

A total of 2,174 phlebotomines were caught, and females predominated (58.3%; 1,267/2,174) over males (41.7%; 907/2,174). Thus, the sex ratio (M:F) was 1:1.40, but there was no statistical difference (Fr = 1.3333; p = 0.2482).

Ten species of phlebotomines were identified. Among them, *Lutzomyia choti* predominated (88.2%; 1,917/2,174), followed by *Lutzomyia whitmani* (8.1%; 176/2,174) and *Lutzomyia sordellii* (1.5%; 33/2,174), which together represented more than 90% of the specimens collected (Table 1). *L. choti*, *L. whitmani* and *L. sordellii* were also the only species found at all collection points. All collected specimens were deposited in an entomological collection (protocol number: 98/2016) of the Unidade Acadêmica de Garanhuns, Universidade Federal Rural de Pernambuco.

The majority of the phlebotomines were caught in peridomestic areas, which accounted for 1,394 specimens (64.1%), followed by forest areas with 733 (33.7%) and lastly indoors with 47 (2.2%) (Table 1). The largest proportion of the specimens of *L. choti* (824/1,917) were collected in a peridomestic area (P5), from a hen house next to a tree trunk. The next largest proportion was from a forested area (481/1,917) (P3), (x^2 = 37,402; p = 0.0000). *L. whitmani* (108/176) was also most frequently found in a peridomestic area, but in this case it was in a stable (P3). Very few samples of this species were found indoors (9/176). *L. sordellii* (15/33) was collected in a forested area (P6) (Table 1). *L. choti* predominated at points P3 and P6, compared with *L. whitmani* (x^2 = 40,272; p = 0.0000). It is important to highlight that *L. complexa*, *L. longispina* and *L. wellcomei* were found in the peridomestic environment (Table 1).

Phlebotomines were found in all the months of the study, corresponding to a mean of 181 sandflies a month. Nevertheless, the monthly density was higher in January 2015 (395) and May 2016 (802). In May, the temperature was lower than in January, whereas the humidity and rainfall were higher (Figure 2). Surprisingly, climatic variations in temperature (Fr = 3.0000;

Table 1. Phlebotomines species collected of Igarassu - Pernambuco, from October 2015 to September 2016, using light traps of the Centers for Disease Control (CDC) model.

SPECIES	MALE	FEMALE	DOMICILE		PERIDOMICILE			FOREST	
			P1	P2	P3	P4	P5	P6	P7
					AF (RF%)				
L. choti	741 (38.7)	1176 (61.3)	26 (1.3)	4 (0.2)	397 (20.7)	30 (1.6)	824 (43)	481 (25.1)	155 (8.1)
L. whitmani	103 (58.5)	73 (41.5)	7 (4.0)	2 (1.1)	108 (61.4)	2 (1.1)	8 (4.5)	38 (21.6)	11 (6.3)
L. sordellii	23 (69.7)	10 (30.3)	2 (6.1)	1 (3.0)	2 (6.1)	2 (6.1)	6 (18.2)	15 (45.5)	5 (15.2)
L. quinquefer	12 (100)	0	1 (8.3)	0	0	0	0	11 (91.7)	0
L. wellcomei	12 (100)	0	0	0	5 (41.7)	0	1 (8.3)	4 (33.3)	2 (16.7)
L. evandroi	10 (90.9)	1 (9.1)	2 (18.2)	0	0	0	4 (36.4)	3 (27.3)	2 (18.2)
L. longispina	2 (66.7)	1 (33.3)	0	0	1 (33.3)	0	1 (33.3)	0	1 (33.3)
L. brasiliensis	2 (100)	0	0	1 (50)	0	0	0	0	1 (50)
L. complexa	1 (100)	0	0	0	0	0	1 (100)	0	0
L. naftalekatzi	1 (100)	0	0	0	0	0	0	1 (100)	0
***Lutzomyia* spp.**	0	6 (100)	1 (16.7)	0	2 (33.3)	0	0	2 (33.3)	1 (16.7)
Total	907 (41.7)	1267 (58.3)	39 (1.8)	8 (0.4)	515 (23.7)	34 (1.6)	845 (38.9)	555 (25.5)	178 (8.2)

AF – Absolute Frequency; RF – Relative Frequency.

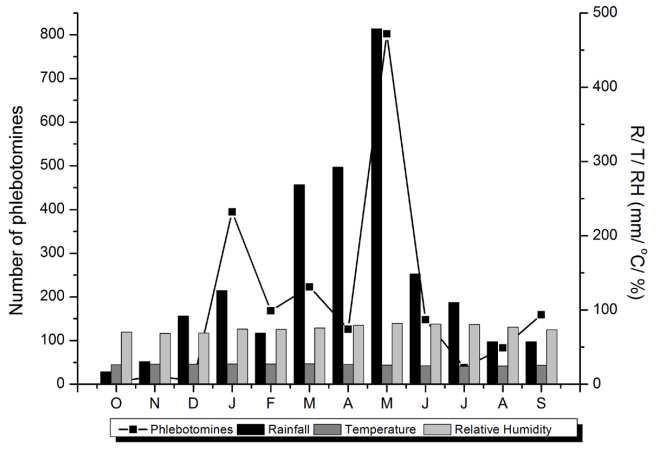

Figure 2. Occurrence of phlebotomines in Igarassu, PE, Brazil and climatic conditions observed in the study from October 2015 to September 2016.

p = 0.0833), relative humidity (Fr = 1.3333; p = 0.2482) and rainfall (Fr = 0.333; p = 0.5637) were not found to have any influence on the abundance of phlebotomines. In relation to species, *L. choti* presented peaks in its population density in January 2015 and May 2016, and was the only species caught in all months. *L. whitmani* was most abundant in May 2016 and was absent only in October and November 2015 (Table 1).

All samples were found *Leishmania* negative in the molecular analysis.

Discussion

The phlebotomine fauna was evaluated in a rural area endemic for ACL, located in the northeastern region of Brazil. A wide diversity of species was observed in the present study (*L. choti*, *L. whitmani, L. sordellii*, *L. quinquefer*, *L. wellcomei*, *L. evandroi*, *L. longispina*, *L. brasiliensis*, *L. complexa* and *L. naftalekatzi).* These findings are important regarding the epidemiology of leishmaniasis in the study area, given that an increased number of species was observed in comparison with studies conducted in the 1980s, in which three species were identified: *L. whitmani*, *L. evandroi* and *L. squamiventris* (LUCENA et al., 1984). It is likely that, with the destruction of natural habitats and the dispersion of wild animals that are food sources for sandflies, these dipterans have been undergoing modification of their behavior, such that they have sought new targets on which to feed (i.e. dogs and humans), thus coming closer to peridomestic areas.

Lutzomyia choti was the most frequent species. Although little is known about this species, it has often been observed in areas endemic for ACL (ANDRADE et al., 2005; MIRANDA et al., 2015). Therefore, its role in transmission of protozoa belonging to the genus *Leishmania* deserves to be better investigated.

The species *L. choti*, *L. sordellii* and *L. whitmani* were collected in all the environments studied. This demonstrated the capacity of these species to adapt to houses, especially those located next to rain forest fragments. The location with the highest number of specimens caught was the peridomestic area, and this finding corroborates previous studies (BARATA et al., 2005; SILVA et al., 2010). It is important to highlight that in the present study, animal shelters (hen houses, stables and a piggery) were found in peridomestic areas, and their presence may have influenced the number of specimens collected.

It is known that climatic and other factors (such as the presence of vegetation) have an influence on the incidence of phlebotomines (COSTA et al., 2013). In addition, some species may present different seasonal patterns in the same geographic area, due to climatic variations (GUIMARÃES et al., 2012). In the present study, the month with the highest numbers of specimens and species collected was the month with the highest relative humidity and rainfall. These factors favor growth of vegetation and accumulation of organic matter in the soil, thus making it possible for breeding sites to appear (DIAS et al., 2007). Although representative correlations between sandfly numbers and rainfall and relative humidity have been reported (GUIMARÃES et al., 2012), climatic variation did not have any influence on the abundance of specimens in the present study.

All the specimens molecularly analyzed were negative for *Leishmania*. It is likely that the lower number of specimens analyzed did not allow detection of *Leishmania* spp. DNA. Therefore, studies with larger numbers of samples should be performed in order to elucidate the possible roles of species that were proven to transmit ACL in the present study area or were suspected of this.

The present study contributed towards knowledge of the phlebotomine fauna in an area endemic for ACL in the state of Pernambuco. The abundant presence of *L. choti* reveals the importance of further studies to evaluate its role as a possible vector. In addition, the presence of *L. whitmani* makes it possible for outbreaks to occur. Lastly, the rainy period was considered to be the time of highest risk in the study area, given the greater presence of phlebotomines.

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