Article/Artigo

Sandfly fauna in an area endemic for visceral leishmaniasis in Aracaju, State of Sergipe, Northeast Brazil

Fauna flebotomínica em área endêmica de leishmaniose visceral em Aracaju, Estado de Sergipe, Nordeste do Brasil

Verónica de Lourdes Sierpe Jeraldo[1], Marco Aurélio de Oliveira Góes[2], Claudio Casanova[3], Claudia Moura de Melo[1], Edilson Divino de Araújo[4], Sinval Pinto Brandão Filho[5], Danilo Esdras Rocha Cruz[1] and Mara Cristina Pinto[6]

ABSTRACT

Introduction: In recent years, visceral leishmaniasis, a major public health problem, has been spreading from the rural to urban areas in many areas of Brazil, including Aracaju, the capital of the State of Sergipe. However, there are no studies of the sandfly fauna in this municipality or its variation over the year. **Methods:** Phlebotomine sandflies were collected from a rural area of Aracaju from September 2007 to July 2009. Modified CDC ultra-violet (UV) light traps were used to evaluate sandfly monthly distribution and their presence in the domestic and peridomestic environments. **Results:** The most abundant species was *Lutzomyia longipalpis* (90.4%) followed by *Evandromyia lenti* (9.6%). A chicken shed trap site had the highest proportion of *L. longipalpis* (51.1%) and large numbers of *L. longipalpis* were also collected in the houses closest to the chicken shed. There was a positive correlation between monthly rainfall and *L. longipalpis* abundance. **Conclusions:** *Lutzomyia longipalpis* is the most abundant species and is probably the main vector of the visceral leishmaniasis agent in the rural area of Aracaju. An increase in *L. longipalpis* frequency was observed during the rainy season. The peridomicile-intradomicile observations corroborate the importance of chicken sheds for the presence of *L. longipalpis* in the peridomestic environment. The great numbers of *L. longipalpis* inside the houses confirm the endophilic behaviour of this species and the possibility of visceral transmission in the intradomicile.

Keywords: Phlebotominae. Sandfly. *Lutzomyia.* Sergipe.

RESUMO

Introdução: Nos últimos anos, a leishmaniose visceral, um importante problema de saúde pública, vem apresentando expansão das áreas rurais para as urbanas de muitas regiões do Brasil, incluindo-se Aracaju, capital do Estado de Sergipe. No entanto, não existem estudos sobre a fauna de flebotomíneos presente nesse município ou de sua distribuição ao longo do ano. **Métodos:** As coletas de flebotomíneos foram realizadas em uma área rural de Aracaju, capital do Estado de Sergipe no periodo de setembro de 2007 a julho de 2009. Armadilhas CDC modificadas acopladas com luz ultravioleta (UV) foram utilizadas para avaliar a distribuição mensal e a presença de flebotomíneos nos ambientes doméstico e peridoméstico. **Resultados**: *Lutzomyia longipalpis* foi a espécie mais abundante (90,4%), seguida por *Evandromyia lenti* (9,6%). Os locais com as maiores quantidades de *L. longipalpis* (51,1%) foram um galinheiro e as casas mais próximas a ele. Houve uma correlação positiva entre a precipitação mensal e a abundância de *L. longipalpis*. **Conclusões**: *Lutzomyia longipalpis* é a espécie mais abundante e, provavelmente, o principal vetor do agente da leishmaniose visceral na área rural de Aracaju. Foi observado um aumento na frequência de *L. longipalpis* nos meses chuvosos. As coletas realizadas no ambiente externo e interno dos domicílios reforçam a relevância dos galinheiros para a presença de *L. longipalpis* no ambiente peridoméstico. O grande número de *L. longipalpis* dentro das casas confirma o comportamento endofílico dessa espécie e a possibilidade de transmissão intradomiciliar da leishmaniose visceral.

Palavras-chaves: Phlebotominae. Flebotomíneos. *Lutzomyia.* Sergipe.

1. Laboratório de Doenças Infecciosas e Parasitárias, Instituto de Tecnologia e Pesquisa de Sergipe, Aracaju, SE. 2. Vigilância Epidemiológica, Secretaria de Estado da Saúde do Estado de Sergipe, Aracaju, SE. 3. Laboratório de Mogi Guaçu, Superintendência de Controle de Endemias, Secretaria da Saúde do Estado de São Paulo, São Paulo, SP. 4. Departamento de Biologia, Universidade Federal de Sergipe, Aracaju, SE. 5. Centro de Pesquisas Aggeu Magalhães, Fundação Oswaldo Cruz, Recife, PE. 6. Faculdade de Ciências Farmacêuticas, Universidade Estadual Julio de Mesquita Filho, Araraquara, SP.

Address to: Dra. Verónica de Lourdes Sierpe Jeraldo. LDIP/ITP. Av. Murilo Dantas 300, Bairro Farolândia, 49032-490 Aracaju, SE, Brasil.
Phone: 55 79 3218-2190
e-mail: veronica_sierpe@hotmail.com
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INTRODUCTION

Visceral leishmaniasis, an importante public health problem, is found in most regions of Brazil and is in the process of expanding into new areas. The first clinical report of visceral leishmaniasis caused by *Leishmania infantum chagasi* Cunha & Chagas 1937 was made in the State of Sergipe by Chagas in 1936[1]. Chagas also observed that the most frequent vector of *L. chagasi* was the sandfly species *Lutzomyia longipalpis* (Lutz & Neiva 1912), a species widely distributed from Mexico to Argentina[2]. In recent years, visceral leishmaniasis has been showing progressive expansion from rural to urban areas of many important cities and regions of Brazil, including Aracaju, the capital of Sergipe. However, there are no studies on the sandfly fauna present in this municipality or its monthly variation.

Such studies carried out in areas endemic with leishmaniasis are essential for any control measures that aim to reduce disease transmission[3-5]. The objectives of this study are to evaluate the sandfly fauna in a rural area of Aracaju, their monthly distribution and presence in intra and peridomiciliary environments.

METHODS

Study area

Aracaju is the capital of the State of Sergipe and is located on the coast of northeast Brazil. According to Köppen´s climate classification, the region is As (tropical rainy type with dry season in the summer). The mean annual rainfall is 1,400mm, and the average annual temperature is 26°C.

The study was conducted in the rural area of Mosqueiro (11°03´S 37°08´W), where canine and human visceral leishmaniasis cases have been recorded. This locality occupies 43.4% of the area of Aracaju municipality (73.54km^2) and had a population of 18,544 inhabitants in 2008[6], having undergone considerable population growth. The

area was originally native forest land which underwent a process of intense deforestation preceding the establishment of smallholdings for subsistence farming and houses with gardens containing bushes and fruit trees (e.g. cashew trees). Most of these properties are also home to various domestic animals, including chickens, dogs and rabbits. The shade of the trees and the presence of organic matter (leaves and fruit) in the areas surrounding the houses protect the ground from sunlight.

Monthly sandfly distribution

The sandflies were sampled with modified Centers for Disease Control (CDC) ultra-violet (UV) light traps installed during two consecutive nights per month, from September 2007 to July 2009, from 18h to 7h, at five different sites (one chicken shed and four domiciles).

Houses 1 and 2 share the same plot of land, on which two dogs live, and are 15 and 30m, respectively, away from the same chicken shed. Houses 3 and 4 are about 100m from houses 1 and 2 and do not have chicken sheds. There are two dogs in house 3 and one in house 4 (**Table 1**).

The monthly collection was not undertaken in June 2009. The sandfly species' names are in accordance with the classification system of Galati[7].

Monthly rainfall data were obtained from the Centro de Meteorologia de Sergipe (CEMESE).

Peridomicile-intradomicile observation

To evaluate the relationship between the number of *L. longipalpis* in the peridomicile and intradomicile, nine houses - including three used in the previous observation (monthly sandfly distribution) - were investigated using two UV light traps, one inside (living room) and the other outside the domiciles (porch) from 18h to 7h. For comparison, one trap was kept in the chicken shed used in the previous observation, and one was placed in the backyard of the nearest house (15m away from the chicken shed). These observations were carried out between July 2008 and July 2009, except in June 2009.

Statistical analysis

The sandfly catches were log transformed (number of sandflies +1) so that analysis of variance (ANOVA) could be used in the statistical analysis. However, in those cases where the catches were not normally distributed, non-parametric tests, such as Kruskal-Wallis or Mann-Whitney, were applied. The Williams' means are presented to clarify the interpretation of the data in view of the great differences in the numbers of sandflies caught at the various sites. The Spearman method was used to estimate possible correlations between the rainfall means and the number of sandfly specimens.

TABLE 1 - Numbers of specimens and Williams' mean (Wm) by sex and percentage of both sexes of *Lutzomyia longipalpis* and *Evandromyia lenti,* by capture site in Mosqueiro, Aracaju municipality, State of Sergipe, Brazil, from September 2007 and July 2009.

	Lutzomyia longipalpis						*Evandromyia lenti*					
	male		female		both sexes		male		female		both sexes	
Site	**N**	**Wm**	**N**	**Wm**	**N**	**%**	**N**	**Wm**	**N**	**Wm**	**N**	**%**
Chicken shed	710	9.8[a]	313	4.6[a]	1,023	51.1	13	0.3[a]	20	0.6[a]	33	15.6
House 1	282	5.0[a]	124	2.2[a]	406	20.3	23	0.4[a]	47	1.2[a]	70	33.0
House 2	231	3.0[b]	111	1.6[a]	342	17.1	24	0.5[a]	29	0.7[a]	53	25.0
House 3	85	2.1[b]	45	1.4[a]	130	6.5	14	0.3[a]	15	0.5[a]	29	13.7
House 4	71	1.6[b]	31	0.7[a]	102	5.1	8	0.2[a]	19	0.6[a]	27	12.7
Total	**1,379**		**624**		**2,003**	**100.0**	**82**		**130**		**212**	**100.0**

N: number; **Wm:** Williams' mean; **%:** percentage; numbers in the same columns with the same letters are not significantly different by Kruskal-Wallis test (p>0.05).

RESULTS

Monthly sandfly distribution: a total of 2,215 sandflies were collected between September 2007 and July 2009 at 5 locations in a rural area of Aracaju using a total of 220 traps over 44 nights. *Lutzomyia longipalpis* was the most abundant species with 2,003 specimens collected (1,379 males and 624 females), followed by *Evandromyia lenti* (Mangabeira, 1938) with 212 specimens collected (82 males and 130 females). The sex ratio was male biased in *L. longipalpis* (1:0.3) and female biased in *E. lenti* (1:0.6) (**Table 1**).

The chicken shed trap site had the highest number of *L. longipalpis* (51.1%). William´s means also showed that the chicken shed and the house closest to it were the sites with the highest numbers of *L. longipalpis* males (p=0.003). There was no statistically significant difference between the numbers of females collected at the trap sites (p>0.05), although more females were collected in the chicken shed than at the other sites. The catches of *E. lenti* males and females presented a more uniform distribution among the sites with a predominance of females (p>0.05).

The *L. longipalpis* distribution over the months showed a similar pattern in both years, with peaks from April to July 2008 and in April and May 2009 (**Figure 1**). There was a positive correlation between the monthly rainfall and the number of *L. longipalpis* males (rs=0.58; p=0.005) and females (rs=0.59 p=0.004).

Peridomicile-intradomicile observations: a total of 1,258 *L. longipalpis* sandflies were collected in the two environments; 398 (32%) specimens were collected in the intradomicile and 860 (68%) in the peridomicile. The percentages of intradomicilary sandflies were calculated for each house and varied from 7% to 44% (**Table 2**).

The trap used for comparison, inside the chicken shed, collected the highest number of sandflies (646 specimens; Williams' means = 31.5 specimens/trap) and the trap in the backyard of the nearest house (15m away from the chicken shed) collected a total of 104 specimens (Williams' mean = 7.6 specimens/trap).

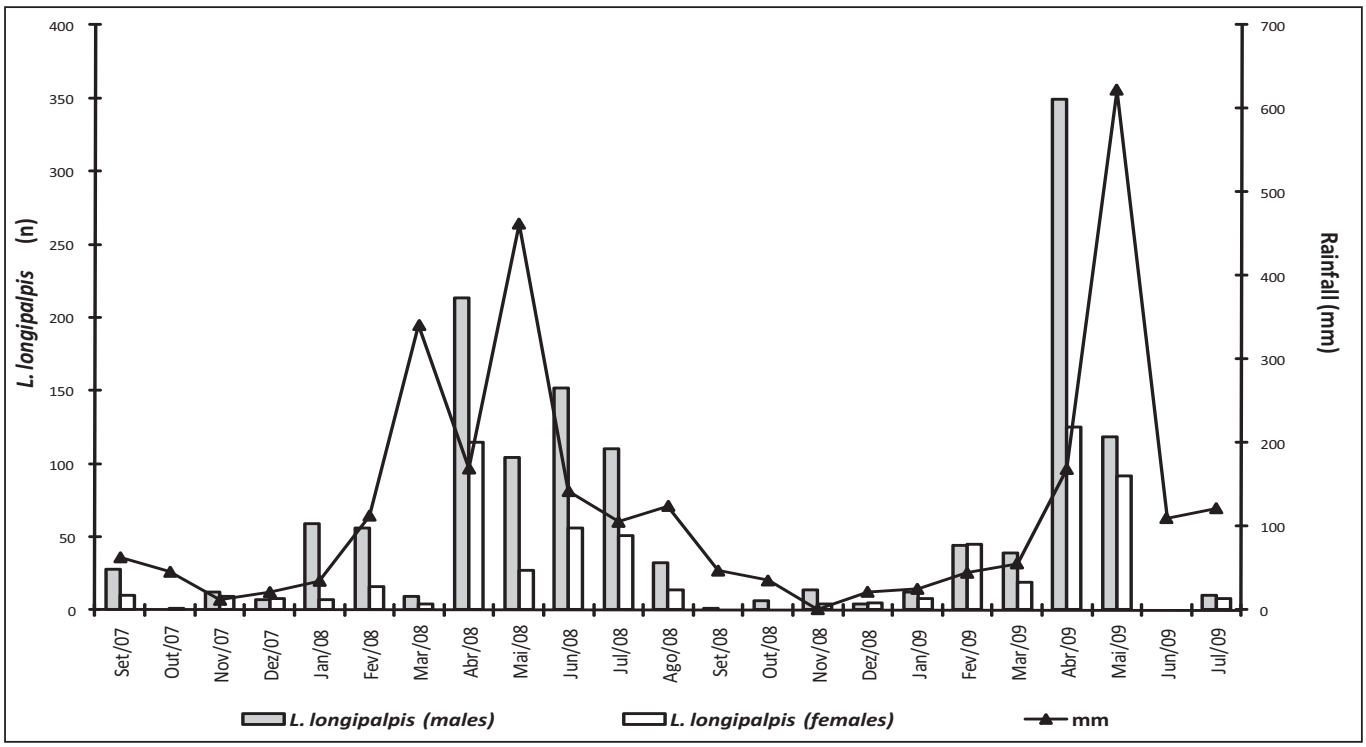

FIGURE 1 - Monthly rainfall and number of *Lutzomyia longipalpis* males and females captured in Mosqueiro, a rural area of Aracaju, State of Sergipe, Brazil, from September 2007 to July 2009.

TABLE 2 - Numbers of specimens, Williams' means and percentages of *Lutzomyia longipalpis* captured in intradomiciles of nine collection sites and the presence of animals in Mosqueiro, Aracaju municipality, State of Serigipe, Brazil, from July 2008 and July 2009.

Site	Animals	Peridomicile* (Williams' means)	Intradomicile* (Williams' means)	Intradomicile (%)
House 1**	chicken shed; two dogs	226[a] (7.3)	176[a] (5.9)	44.0
House 2	caged birds	92[a] (4.4)	70[a] (5.1)	43.0
House 3	no animals	4[a] (0.3)	3[a] (0.2)	43.0
House 4	two dogs	244[a] (12.6)	96[a] (4.1)	28.0
House 5	no animals	40[a] (1.4)	12[a] (0.6)	23.0
House 6	rabbits	61[a] (4.5)	16[b] (0.9)	21.0
House 7**	two dogs	124[a] (7.6)	19[b] (1.2)	13.0
House 8	cats	56[a] (3.3)	5[b] (0.3)	8.0
House 9**	one dog	13[a] (0.9)	1[b] (0.1)	7.0
Total		**860**	**398**	**32.0**

*numbers in the same line with the same letters are not significantly different by Mann-Whitney test (p>0.05); **houses 1, 7 and 9 correspond to houses 2, 3 and 4 in the previous observation.

DISCUSSION

Despite the fact that 1,769 cases of visceral leishmaniasis were reported between 1990 and 2009 in Sergipe, Brazil[8], there are no published studies on the vectors present in that state. This is the first evaluation of the sandfly fauna and its monthly distribution in the rural area of the capital of the State, Aracaju. *L. longipalpis* is the main vector of the visceral leishmaniasis agent in Latin America and is probably responsible for its transmission in Sergipe. The species *E. lenti* is not considered a proven vector of leishmaniasis agents, although it is found in areas of American cutaneous and visceral leishmaniasis transmission[9-11]. *Lutzomyia longipalpis* (90.4%) and *E. lenti* (9.6%) were the only species captured in the present study; however, it is noteworthy that in a previous pilot study carried out in the area, four specimens of *Nyssomyia intermedia* were collected (unpublished data).

Our results demonstrating a male-biased sex ratio for *L. longipalpis* and a female-biased one, or at least an equal distribution, for *E. lenti* were in agreement with other studies that used light traps near animal shelters[5,11]. One hypothesis suggested for such distinct patterns of aggregation is that the distribution depends on the distance at which each species mates from its hosts. Males of species that approach animals to mate would be more male-biased[11]. It is well known that this insect vector's behavior can be mediated by chemical communication. For some sandfly species, the confirmed presence of pheromones combined with host odors is thought to be important for the aggregation of males and females[12-14].

Pheromones of *L. longipalpis* males were detected[15,16], but *E. lenti* pheromones were only detected in males collected in the Southern region but not in those collected in Northeastern Brazil[17]. There are no data on the male pheromones of *E. lenti* from Aracaju. Another hypothesis suggested is that when light traps are used different aggregation patterns may occur due to the distinct phototropism of the sandfly sexes[18]. We should also note that the proportion of females/males of *L. longipalpis* (31.2%) was higher than those found in other surveys, for instance, Dias et al.[19] (19.6%), Nunes et al.[20] (12.3%) and Colla-Jacques et al.[5] (14.8%). Such differences could be due to the UV light used in CDC traps in the present study rather than the incandescent light used in those other studies. A previous pilot study in the same area has shown that UV light traps attract a higher number of sandflies and a greater proportion of females than incandescent light traps[21].

The increased frequency of *L. longipalpis* during the rainy season occurred during both years of the study. In other Brazilian states such as Mato Grosso do Sul[3,20], São Paulo[5], Maranhão[22] and Rio Grande do Norte[23], a similar positive correlation between rainy periods and sandfly frequencies has been observed. It is likely that long periods of drought lead to the dry conditions which hinder the development of both immature stages and adults. Probably the combined effects of rainfall, air temperature, evapotranspiration and soil water balance affect the quality of the sandfly's breeding habitats in such a way as to cause fluctuations in the adult populations[5,24,25].

The great numbers of *L. longipalpis* in animal shelters, especially chicken and pigsties, in the peridomiciliary environment are remarkable, although the choice of host is probably due to its availability (number and size) rather than to specific attractiveness[26]. These results reinforce the observation that *L. longipalpis* moves into peridomiciliary environments when domestic animals and their shelters are present[13,27].

Our data suggest that other animals such as dogs or rabbits are also important for the presence of sandflies in the peridomicile and houses without animals presented the lowest number of sandflies. Because approximately 30% of the sandflies captured in this study were collected indoors and there was variability between the catches in the houses (7% - 44%), it does not seem that *L. longipalpis* presents strictly exophilic habits in this rural area. Such differences are likely due to the presence of animals in the peridomicile and the different accessibility of each house for sandflies, as shown by Quinnel & Dye[28]. During the peridomicile-intradomicile observations, the highest sandfly catches inside the chicken shed (646 specimens; 2.6-fold greater than the highest catch in the backyards of the houses, 244 specimens) bring out the importance of chicken sheds, as highlighted by Lane[29] for many species of sandfly, including *L. longipalpis*. Probably, the proximity of the chicken sheds to the dwellings is a risk factor for human infection, as observed by Alexander[30], due to the higher numbers of sandflies near or inside the dwellings, as suggested for the species involved in cutaneous leishmaniasis transmission in Paraná, Brazil[31].

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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