Phlebotomine sand flies (Diptera, Psychodidae) in an American tegumentary leishmaniasis transmission area in northern Espírito Santo State, Brazil

Fauna de flebotomíneos (Diptera, Psychodidae) em área de transmissão de leishmaniose tegumentar americana no norte do Estado do Espírito Santo, Brasil

Abstract

American tegumentary leishmaniasis is endemic to the Espírito Santo State, Brazil, where it is widely distributed. The composition of the phlebotomine sand fly fauna in an American tegumentary leishmaniasis focus was determined by monthly sampling, using Shannon light traps in an Atlantic Forest reserve and adjacent habitat that had been modified by human activity. Seasonal fluctuations in numbers of the most abundant species were also monitored from June 2004 to May 2006. Of the 6,176 specimens collected, 47.4% were captured in the forest and 52.6% in the disturbed habitat. Although Lutzomyia daviisi (60.8%) predominated in specimens from the forest, those captured near human dwellings consisted almost entirely of Lu. choti (72%) and Lu. intermedia (24.3%). All three species occurred throughout the year. Based on our findings, Lu. intermedia probably acts as the principal domestic Leishmania vector in the study area.

Leishmaniasis; Psychodidae; Vector Ecology

Introduction

American tegumentary leishmaniasis is considered one of the six most important parasitic infectious diseases worldwide (World Health Organization. http://www.who.int, accessed on 10/ Oct/2006). The disease constitutes a major public health problem in Brazil, where it occurs in all 26 States. American tegumentary leishmaniasis is endemic to the State of Espírito Santo, where it is widely distributed, threatening a significant proportion of the rural population.

Northern Espírito Santo was covered by Atlantic Forest until the mid-20th century, and American tegumentary leishmaniasis does not appear to have constituted a serious public health problem for the original settlers. Furthermore, there are no records of cases of mucocutaneous leishmaniasis resulting from old infections with Leishmania braziliensis in the region.

Beginning in the 1980s, the suspension of insecticide use for malaria control may have favored invasion of houses by sand flies and coincided with the appearance of the first American tegumentary leishmaniasis cases in northern Espírito Santo. The study area was selected in order to obtain information to help elucidate the American tegumentary leishmaniasis epidemiological profile in the region, where human cases have been observed for about 20 years. A total of 2,132 cases were reported from 1986 to 2003.
The phlebotomine sand fly *Lutzomyia intermedia* (Lutz & Neiva, 1912) predominates in peridominciliary captures from old established rural communities. This species has been incriminated as the main vector of *L. braziliensis* in Southeast Brazil 6,7,8, although *Lu. whitmani* (Antunes & Coutinho, 1939) and *Lu. migonei* (França, 1920) are also frequently found biting humans in the peridomicile 9.

The lack of studies on the phlebotomine sand fly fauna and potential *Leishmania* vectors in northern Espírito Santo motivated this study, the objective of which was to determine the composition of the phlebotomine sand fly fauna in an Atlantic Forest reserve and adjacent habitat disturbed by human activity. Seasonal population fluctuations of the main species in both environments were also monitored, providing information to help clarify the sylvatic and domiciliary American tegumentary leishmaniasis transmission cycles of *Leishmania* in the study area.

**Material and methods**

**Study area**

Two distinct habitats were chosen for sampling:

- Environment I: forest in the Sooretama Biological Reserve (18º33’-19º05’S; 39º55’-40º15’W), comprising an area of approximately 24,000ha, bordered by the municipalities (counties) of Linhares, Sooretama, Jaguaré, and Vila Valério. The principal vegetation type is Atlantic Rainforest, harboring a fauna that is similar in many ways to that of the Amazon region, as well as large numbers of endemic species 10. The climate is hot, humid, and tropical, with a mean annual temperature of 23°C. Together with the Vale do Rio Doce Reserve, this area constitutes the largest remnant of the Atlantic Forest in the State of Espírito Santo 10.
- Environment II: the disturbed habitat surrounding human dwellings in Jurama (18º59’S; 40º15’W), a rural area in the municipality of Vila Valério (Figure 1). This locality is situated at least 800m from the reserve and has steep hillsides largely occupied by coffee plantations and pasture. Most of the houses are rustic and surrounded by pigsties, henhouses, and cowsheds.

**Phlebotomine sand fly captures**

Modified Shannon light traps 11 were installed within the forest and peridomincile. Monthly sampling was performed simultaneously in the two habitats during the first 3 hours after sunset, from June 2004 to May 2006. In total, 72 hours of sampling per trap were performed in each habitat.

**Mounting and identification of phlebotomine sand flies**

Specimens were mounted using the Barreto & Coutinho technique 12 and identified as to species according to the Young & Duncan taxonomic criteria 13. Specimens whose taxonomic characters were damaged or obscured were identified to the generic level. Sample material was deposited in the entomology collection of the Department of Tropical Medicine at the Federal University in Espírito Santo.

**Climate data**

Climate data (mean temperature, rainfall, and relative humidity) were obtained from the weather station of the National Institute of Meteorology located in the Espírito Santo State Institute for Research, Technical Assistance, and Rural Extension (INCAPER) in Linhares municipality, approximately 30km from the study area.

**Statistical analysis**

Spearman’s non-parametric test was used to determine the correlation between the climate data and the most abundant species in the different habitats. The Mann-Whitney test was used to verify differences between numbers of specimens in the most abundant species collected in the two habitats. Differences were considered significant at p < 0.05.

**Results**

A total of 6,176 specimens from genera *Brumptomyia* and *Lutzomyia* were captured during the study. The species captured were: *B. figueiredoi* Mangabeira & Sherlock, 1961; *Lu. ayrozai* (Barreto & Coutinho, 1940); *Lu. callipygaa* Martins & Silva, 1965; *Lu. choti* (Floch & Abonnenc, 1941); *Lu. davisi* (Root, 1934); *Lu. ferreirana* (Barreto, Martins & Pellegrino, 1956); *Lu. fischeri* (Pinto, 1926); *Lu. davisi* (Root, 1934); *Lu. ferreirana* (Barreto, Martins & Pellegrino, 1956); *Lu. fischeri* (Pinto, 1926); *Lu. intermedius* (Lutz & Neiva, 1912); *Lu. laei* (Barreto & Coutinho, 1941); *Lu. lentii* (Mangabeira, 1938); *Lu. lutzi* (Costa Lima, 1932); *Lu. matosi* (Barreto & Zago, 1956); *Lu. migonei* (França, 1920); *Lu. pascalei* (Coutinho & Barreto, 1940); *Lu. pelloni* (Sherlock & Alencar, 1959); *Lu. pestanae* (Barreto & Coutinho, 1941); *Lu. quinquefer* (Dyar, 1929); *Lu. schreiberi* (Martins, Falcon & Silva, 1975); *Lu. shannoni* (Dyar, 1929), and
Young & Porter, 1972. In all, 2,925 (47.4%) of the phlebotomines were captured in the forest and 3,251 (52.6%) from the adjacent disturbed habitat. More species (n = 16) were found in the forest.

When numbers from the two habitats were pooled, the most abundant species was *Lu. choti*, with 3,341 specimens (54.1% of the total), or 29.9% in the forest and 70.1% in the disturbed habitat. The second most abundant species was *Lu. davisi*, with 1,806 specimens (29.2%), almost exclusively (98.5%) from the forest, followed by *Lu. intermedia*, with 804 specimens (13%) almost exclusively (98.1%) from the disturbed habitat.

There was no significant difference in the number of *Lu. choti* specimens captured in the forest and the disturbed habitats. However, significantly more *Lu. davisi* specimens were captured in the forest and more *Lu. intermedia* in the disturbed habitat (p < 0.05).

The predominant species in the forest were *Lu. davisi* (60.8%) and *Lu. choti* (34.1%).

Source: Espírito Santo State Integrated System of Georeferenced Databases (GEOBASES).
male/female ratios for these two species were approximately 1:4 and 1:1, respectively (Table 1).

Two species predominated in the disturbed habitat: *Lu. choti* and *Lu. intermedia* (72% and 24.3%, respectively). Males predominated in both species (approximately 2:1 for *Lu. choti* and 3:1 for *Lu. intermedia*) (Table 2).

In the forest, *Lu. davisi* was most abundant in the months of April, May, and June 2005, while *Lu. choti* reached its peak in October 2004 and February and May 2006. In the disturbed habitat, *Lu. choti* showed clear peaks in March and April 2005 and March and May 2006, while *Lu. intermedia* was most abundant in December 2004. Figures 2 and 3 show the monthly distribution of the three main species in the two habitats, together with mean monthly relative humidity and temperature. Statistical analysis did not show any significant correlation between monthly rainfall or temperature and seasonal patterns of abundance of any of these species in either habitat (Figures 4 and 5).

When mean monthly relative humidity was correlated with numbers of the most abundant species in the different habitats, significant negative values were obtained for *Lu. choti* in the forest (R = -0.50, P = 0.01) and for *Lu. intermedia* in the disturbed habitat (R = -0.52, P = 0.00). A significant positive correlation was found for *Lu. davisi* in the forest (R = 0.42, P = 0.03), while there was no correlation in the disturbed habitat for *Lu. choti* (R = 0.03, P = 0.88).

**Discussion**

The high frequency of phlebotomine sand flies in the disturbed habitat confirms observations by other authors in different regions of Brazil. In Espírito Santo, Barros et al. suggested that the construction of human dwellings in the middle of banana groves creates a favorable environment for these insects by providing both shelter and abundant blood meal sources. However, the current study showed a greater wealth of species in the forest, probably due to the greater variety of resting sites provided by plant cover.

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**Table 1**

<table>
<thead>
<tr>
<th>Species</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. figueiredoi</em></td>
<td>2</td>
<td>-</td>
<td>2</td>
<td>0.07</td>
</tr>
<tr>
<td>Brumptomyia sp. *</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>0.03</td>
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<tr>
<td><em>Lu. ayrozai</em></td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>0.14</td>
</tr>
<tr>
<td><em>Lu. callipyga</em></td>
<td>5</td>
<td>-</td>
<td>5</td>
<td>0.17</td>
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<tr>
<td><em>Lu. choti</em></td>
<td>426</td>
<td>573</td>
<td>999</td>
<td>34.15</td>
</tr>
<tr>
<td><em>Lu. davisi</em></td>
<td>350</td>
<td>1,429</td>
<td>1,779</td>
<td>60.82</td>
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<tr>
<td><em>Lu. ferreirana</em></td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>0.07</td>
</tr>
<tr>
<td><em>Lu. fischeri</em></td>
<td>6</td>
<td>33</td>
<td>39</td>
<td>1.33</td>
</tr>
<tr>
<td><em>Lu. intermedia</em></td>
<td>10</td>
<td>5</td>
<td>15</td>
<td>0.51</td>
</tr>
<tr>
<td><em>Lu. lutziana</em></td>
<td>13</td>
<td>-</td>
<td>13</td>
<td>0.45</td>
</tr>
<tr>
<td><em>Lu. matosi</em></td>
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<td>-</td>
<td>1</td>
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</tr>
<tr>
<td><em>Lu. migonei</em></td>
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<td>4</td>
<td>6</td>
<td>0.21</td>
</tr>
<tr>
<td><em>Lu. pascalei</em></td>
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<td>-</td>
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</tr>
<tr>
<td><em>Lu. quinquefer</em></td>
<td>-</td>
<td>4</td>
<td>4</td>
<td>0.14</td>
</tr>
<tr>
<td><em>Lu. schreiberi</em></td>
<td>16</td>
<td>8</td>
<td>24</td>
<td>0.82</td>
</tr>
<tr>
<td><em>Lu. shannoni</em></td>
<td>3</td>
<td>2</td>
<td>5</td>
<td>0.17</td>
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<tr>
<td><em>Lu. yuilli yuilli</em></td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>0.03</td>
</tr>
<tr>
<td>Lutzomyia spp. **</td>
<td>1</td>
<td>22</td>
<td>23</td>
<td>0.79</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>839</td>
<td>2,086</td>
<td>2,925</td>
<td>100.00</td>
</tr>
</tbody>
</table>

* Brumptomyia sp. probably refers to *B. avellari* (Costa Lima, 1932), *B. cunhai* (Mangabeira, 1942), or *B. nitzulescui* (Costa Lima, 1932);
Table 2


<table>
<thead>
<tr>
<th>Species</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lu. choti</td>
<td>1,655</td>
<td>687</td>
<td>2,342</td>
<td>72.03</td>
</tr>
<tr>
<td>Lu. davisi</td>
<td>12</td>
<td>15</td>
<td>27</td>
<td>0.83</td>
</tr>
<tr>
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<td>1</td>
<td>-</td>
<td>1</td>
<td>0.03</td>
</tr>
<tr>
<td>Lu. fischeri</td>
<td>20</td>
<td>17</td>
<td>37</td>
<td>1.14</td>
</tr>
<tr>
<td>Lu. intermedia</td>
<td>607</td>
<td>182</td>
<td>789</td>
<td>24.30</td>
</tr>
<tr>
<td>Lu. lanei</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>0.03</td>
</tr>
<tr>
<td>Lu. lenti</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>0.03</td>
</tr>
<tr>
<td>Lu. lutziana</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>0.03</td>
</tr>
<tr>
<td>Lu. migonei</td>
<td>30</td>
<td>7</td>
<td>37</td>
<td>1.13</td>
</tr>
<tr>
<td>Lu. pelloni</td>
<td>3</td>
<td>-</td>
<td>3</td>
<td>0.09</td>
</tr>
<tr>
<td>Lu. pestanai</td>
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<td>2</td>
<td>2</td>
<td>0.06</td>
</tr>
<tr>
<td>Lu. schreiberi</td>
<td>3</td>
<td>3</td>
<td>6</td>
<td>0.18</td>
</tr>
<tr>
<td>Lu. shannoni</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>0.09</td>
</tr>
<tr>
<td>Lu. yuilli yuilli</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>2,333</td>
<td>918</td>
<td>3,251</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Figure 2

Association between relative humidity and mean temperature and sand fly population fluctuations in forest in the Municipality of Sooretama, Espírito Santo State, Brazil, June 2004 to May 2006.
The abundance of *Lu. choti* in the study area and its occurrence in both habitats suggests that the species has eclectic feeding habits. *Lu. choti* is the only one of the three most abundant sand fly species that is not a suspected *Leishmania* vector, since it has not been recorded in the principal ATL-endemic areas of Espírito Santo. Furthermore, there are no reports of this species’ natural infection with the parasite. However, Brandão-Filho et al. suggested that *Lu. choti* may be an ATL vector, based on its predominance in the Zona da Mata region of the Brazilian State of Pernambuco.

Although highly anthropophilic, *Lu. davisi* does not appear to be involved in ATL transmission in the study area, based on the absence of reported cases from the period when deforestation occurred. It is probably a sylvatic species and is common in humid regions of the Amazon. This species is involved in the transmission of *L. (Viannia) naiiffi* Lainson & Shaw, 1989 in the Brazilian state of Rondônia, Grimaldi Jr. et al. isolated *L. (Vi.) braziliensis* from naturally infected *Lu. davisi* in the Amazon region. There is no clear evidence of sylvatic infection of humans by *Leishmania* in Espírito Santo, since the disease attacks adults and children of both sexes, characteristic of an intradomiciliary transmission pattern. Since this species belongs to a group (*Psychodopygus*) including several species incriminated as vectors, it deserves close and thorough observation in the area.

Given its high prevalence in the disturbed habitat, *Lu. intermedia* can be considered the main vector in the study area, confirming observations by other authors in Espírito Santo. This sand fly is abundant in American tegumentary leishmaniasis foci in Southeast Brazil and is the suspected vector of *L. (V.) braziliensis* in the area, specimens having been found naturally infected with the parasite.

Destruction of primary forest in northern Espírito Santo from the 1940s onwards appears to have favored increased density of *Lu. intermedia* in disturbed habitats, indicating that this species is able to adapt to deforested environments. Lima suggested that *Lu. intermedia* was pre-adapted to open habitats, drawing on humans and domestic mammals as blood meal sources.
The adaptation of *Lu. intermedia* to the disturbed habitat would explain changes in the epidemiological profile of American tegumentary leishmaniasis in Espírito Santo, where intradomesticary transmission of *Leishmania* is known to occur. The low frequency of this species in the forest supports this hypothesis.

Although *Lu. intermedia* is highly anthropophilic, it is also strongly attracted to dogs and horses, which may act as secondary reservoirs of *L. (V.) braziliensis*. The species *Lu. choti*, *Lu. davisci*, and *Lu. intermedia* occurred in almost all months of the study period. *Lu. intermedia* frequently presents peaks in hotter, more humid months. In the present study, low numbers of this species were recorded during and after periods of high rainfall, suggesting that intense rain is unfavorable to the development of immature forms, since breeding sites in disturbed habitats would suffer greater impact from extreme weather conditions. More detailed studies are needed to understand the seasonal population fluctuations of the main species. To meet all the requirements of Killick-Kendrick studies on natural infection are needed to expand knowledge on the role of some vector species.

Finally, the higher frequency of *Lu. intermediam* in the disturbed habitat confirms its epidemiological importance, highlighting the risk of geographic expansion of American tegumentary leishmaniasis as this species occupies new areas from which native vegetation has been removed.
Resumo

A leishmaniose tegumentar americana é endêmica e amplamente distribuída no Estado do Espírito Santo, Brasil. Com o objetivo de avaliar a composição da fauna de flebotomíneos e verificar a flutuação sazonal, foram realizadas capturas mensais com armadilhas Shannon modificadas em reserva florestal de Mata Atlântica (Reserva Biológica de Sooretama) e ambiente antrópico adjacente, no período de junho de 2004 a maio de 2006. Foram capturados 6.176 espécimes, dos quais 47,4% ocorreram no ambiente florestal e 52,6%, no ambiente antrópico. Lutzomyia davisi (60,8%) predominou no ambiente florestal e Lu. choti (72%) seguida de Lu. intermedia (24,3%) predominaram no ambiente antrópico. As três espécies ocorreram ao longo de todo o ano. Os dados indicam que Lu. intermedia provavelmente atue como principal vetor domiciliar de Leishmania na área de estudo.

Leishmaniose; Psychodidae; Ecologia de Vetores

Contributors

T. M. Virgens participou no projeto de pesquisa, coleta de moscas de óleo, identificação de espécimes, análise e interpretação de dados, e na redação do manuscrito. C. B. Santos participou na coleta de moscas de óleo, identificação de espécimes, e na revisão final do manuscrito. I. S. Pinto participou na coleta de moscas de óleo, identificação de espécimes, análise de dados, e na revisão final do manuscrito. K. S. Silva e F. C. Leal participaram na coleta de moscas de óleo, coleta de dados, e na revisão final do manuscrito. A. Falqueto coordenou o projeto e a revisão final do manuscrito.

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