Major Article

Ecology of phlebotomine sand flies in a Brazilian area with recent leishmaniasis transmission (Itaúna, in Minas Gerais state)

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Abstract

Introduction: Leishmaniasis constitutes a serious but neglected tropical disease. Recently, socio-environmental, biological and physical changes have altered the range of leishmaniasis, causing it to spread into urban areas. In Minas Gerais, the disease is endemic, exhibiting regional differences and reaching urban centers. The purpose of this study was to evaluate entomological aspects related to the ecoepidemiology of leishmaniasis in Itaúna.

Methods: Monthly catches with HP traps were carried out from June 2017 to May 2018, in three ecological areas (urban, rural, and forest). The adaptability of the species to anthropic environments was assessed using the synanthropy index (SI).

Results: We collected 1306 specimens of phlebotomine sand flies. Of the species of medical importance, Lutzomyia longipalpis, the vector of Leishmania infantum, represented 90.4% of the specimens identified at species level (n=1260). Nyssomyia whitmani, the vector of Leishmania braziliensis, represented 1.6% of the total. Lu. longipalpis displayed an SI of +95.8, a value that denotes a marked preference for human environments. For Ny. whitmani, the SI was -25, expressing the tendency of this species to occupy uninhabited areas. The population of the three most numerous species captured increased with rain, high temperatures, and high relative humidity. Although captured at low numbers, Ny. whitmani showed a different profile concerning the climate variables analyzed.

Conclusions: Understanding the epidemiology of the disease may assist the health services in formulating effective control measures for improving community health and contributing to the establishment of a dynamic relationship and a global awareness of the health/disease process.

Keywords: Leishmaniasis. Phlebotominae. Synanthropy index. Lutzomyia longipalpis. Nyssomyia whitmani.

INTRODUCTION

Leishmaniases are anthropozoonotic infectious diseases transmitted by the bite of Leishmania-infected female phlebotomine sand flies. In the American continent, their two basic forms are visceral leishmaniasis (VL) and cutaneous leishmaniasis (CL). Prevalently, leishmaniases were associated with rural and non-anthropic areas, where human beings could accidentally get infected by passing through or colonizing infected areas. Over the years, this pattern has changed, and currently the transmission cycle also occurs in urban centers.

Epidemiological studies have shown that changes in the transmission profile and increasing urbanization of leishmaniases can be due to deforestation and the haphazard growth of cities, which reduces and modifies the natural habitats of these insects, restricting their environment and causing species adaptation. Nuorteva named the ability of certain species to adjust to human-created or modified environmental conditions, which generally result from urbanization, synanthropy. The synanthropy index (SI) measures the degree of adaptability of species to urbanized environments and has been studied from an ecological perspective to evaluate the results of human influence on the original fauna of an environment.

Studies have shown that climatic factors, such as temperature, rainfall and relative humidity can also correlate with the occurrence of phlebotomine sand flies and their population density. An understanding of the ecology of Leishmania vectors and their monthly distribution associated with these climate variables...
can contribute to a better comprehension of the epidemiology of leishmaniases and their dynamics and possibly improve the effectiveness of control strategies in transmission areas.

The Ministry of Health (MS) has recently classified Itaúna (Minas Gerais) as an area of sporadic transmission of VL. The municipality is concerned because of the increased number of human and canine cases, the presence of the competent vector (Lu. longipalpis) and Itaúna’s proximity to endemic areas. Thus, in order to elucidate the ecology of the phlebotomine sand flies in relation to the epidemiology of leishmaniases, we started a project in the municipality of Itaúna. This involved a survey of the sand fly fauna, calculation of the synanthropic index of each species captured and evaluation of the influence of climate on the population density of these insects. These data may contribute to the establishment of a dynamic relationship and a global understanding of the health/disease process, leading to more effective and integrated measures for efficient disease control in Itaúna.

**METHODS**

**Study Area**

This study was conducted in Itaúna (20°4ʹ26ʺS, 44°34ʹ24ʺW), a municipality in the state of Minas Gerais, Brazil. Itaúna is located in the midwestern region of the state, 76 km from Belo Horizonte. According to the Brazilian Institute of Geography and Statistics the municipality comprises an area of 495,769 km², with approximately 72 neighborhoods and an estimated population of 92,561 inhabitants. The region is hilly with a clayey soil and a tropical climate with hot summers and dry winters. Average altitude is 893 m with minimum of 711 m and maximum of 1321 m. Itaúna’s biome is cerrado and Atlantic Forest.

**Capture and Identification of Phlebotomine Sand Flies**

The entomological captures were made in three ecologically distinct areas: urban, rural, and forest. The favorable criteria considered for the selection of capture points were the presence of fruit trees, abundant vegetation, accumulation of organic matter in the soil, and presence of chicken-coops and domestic animals. Thus, the urban site selected for this study was a residence located in a neighborhood called "Chácara do Quitão" (20°03ʹ26ʺS, 44°35ʹ55ʺW, altitude 864 m), which is predominantly residential and has a dense human occupation, located near the city center, with the presence of chickens, pigs and dogs. The rural area selected for this study was a farm in Piaguassu neighborhood (20°02ʹ26ʺS, 44°36ʹ55ʺW, altitude 881 m), with few dwellings and the presence of pasture, gardens, chickens, pigs, cattle, goats, dogs, and cats. The selected forest area belongs to the Zoonoses Control Center (ZCC), located in the Industrial District (20°02ʹ26ʺS, 44°36ʹ54ʺW, altitude 844 m), at a distance of 10 km from the urban center of Itaúna, which has a vast area of transitional forest (cerrado and semi-deciduous seasonal forest) with wild birds and mammals, a small perennial stream, and low solar incidence. In the forest area, the ZCC was taken as reference for placement of the traps. To minimize any possible bias due to sporadic and intermittent presence of horses and dogs in ZCC facility, the traps were mounted at about 300 m from the facility.

The captures were performed monthly, during three consecutive nights, from June 2017 to May 2018, using three HP light traps for each ecological area. In each area, the traps were placed at 300 m from each other. The male specimens were stored in 70% alcohol for subsequent taxonomic identification, and the females were conditioned in 6% DMSO and stored at -20° C for further dissection (taxonomic identification). Identification at species level was performed according to the classification proposed by Galati with abbreviation of the generic names as proposed by Marcondes.

**Climate data**

Monthly climate data, including temperature (°C), relative humidity (%), wind speed (m/s), and rainfall (mm³), were obtained from the nearest weather station in the municipality (OMM83635), District of the Brazilian Institute of Meteorology of Divinópolis, through the National Institute of Meteorology. Divinópolis is located approximately 43 km from Itaúna (20°8ʹ21ʺS 44°53ʹ17ʺW).

**Synanthropy index**

The synanthropy index (SI) was calculated according to Nuorteva formula. The index ranges from +100 (maximum) to -100 (minimum), with positive values representing the highest degree of association with man and negative values indicating aversion to the urban environment.

\[
SI = \frac{2a + b - 2c}{2}
\]

where

\[
a = \text{percentage of a species captured in urban areas}
\]

\[
b = \text{percentage of the same species captured in rural areas}
\]

\[
c = \text{percentage of the same species captured in forest areas}
\]

**RESULTS**

During the 12 months of the study period, 1306 phlebotomine sand flies were captured, among which 1260 were identified at level species. The sand flies captured belonged to six genera (Lutzomyia, Evandromyia, Pintomyia, Nyssomyia, Psathyromyia and Brumptomyia), and included 11 species: Brumptomyia brumpti (Larrousse, 1920), Evandromyia cortezezzii (Brêthes, 1923), Evandromyia evandroi (Costa Lima & Antunes, 1936), Evandromyia lenti (Mangabeira, 1938), Evandromyia sallesi (Galvão & Coutinho, 1940), Evandromyia tertiphitlla (Martins, Falcão & Silva, 1964), Lu. longipalpis (Lutz & Neiva, 1912), Nyssomyia whitmani (Antunes & Coutinho, 1939), Pintomyia pessoai (Coutinho & Barreto, 1940), Psathyromyia brasiliensis (Costa Lima, 1932), and Psathyromyia lutziana (Costa Lima, 1932) (Table 1). Among them, 1099 were males (87.5%) and 161 females (12.5%), with an M/F ratio of 7.0. Thirty-eight females were identified as belonging to the cortezezzii complex because female specimens of Ev. cortezezzii and Ev. sallesi are indistinguishable. Forty-six specimens were considered unidentified (3.5%) because the morphological characters necessary for their identification at
TABLE 1: Distribution of male and female of phlebotomine sand fly species (n = 1260) captured in Itaúna, Minas Gerais state, Brazil. June 2017 to May 2018.

| Species                  | Urban area       | Rural area       | Forest area       | Total |
|--------------------------|------------------|------------------|-------------------|-------|
|                          | ♂      | ♀      | Both genders | ♂      | ♀      | Both genders | ♂      | ♀      | Both genders | ♂      | ♀      | Both genders | %     |
| Br. brumpti              | 1      | 2      | 3           | -      | 1      | 1           | -      | 1      | 1           | 4      | 5      | 0.40      |
| cortezezii complex       | -      | 28     | 28          | -      | 6      | 6           | -      | 4      | 4           | -      | 38     | 38        | 3.02  |
| Ev. cortezezii           | 5      | -      | 5           | -      | -      | -           | -      | -      | -           | -      | 5      | 5        | 0.40  |
| Ev. evandroi             | 5      | 5      | 10          | -      | -      | -           | -      | -      | -           | -      | 5      | 5        | 0.79  |
| Ev. lenti                | 8      | 13     | 21          | -      | 5      | -           | -      | 1      | 1           | 8      | 19     | 27        | 2.14  |
| Ev. sallesi              | 1      | -      | 1           | -      | -      | -           | -      | -      | -           | -      | 1      | 1        | 0.08  |
| Ev. termitophila         | -      | 1      | 1           | -      | 1      | 1           | 1      | -      | 1           | 2      | 3      | 0.24      |
| Lu. longipalpis          | 977    | 73     | 1050        | 84     | 3      | 87          | 1      | 1      | 2           | 1062   | 77     | 1139      | 90.40 |
| Ny. whitmani             | 2      | 1      | 3           | 3      | 3      | 6           | 6      | 5      | 11          | 11     | 9      | 20        | 1.59  |
| Pi. pessoai              | -      | -      | -           | 3      | -      | 3           | 1      | 4      | 5           | 4      | 4      | 8         | 0.63  |
| Pa. brasiliensis         | -      | -      | -           | -      | -      | -           | -      | 1      | 1           | -      | 1      | 1         | 0.08  |
| Pa. lutziana             | 1      | -      | -           | -      | -      | -           | -      | 2      | 2           | 1      | 2      | 3         | 0.24  |
| Total                    | 1000   | 123    | 1023        | 90     | 19     | 109         | 9      | 19     | 28          | 1099   | 161    | 1260      | 100.00 |

Species level were missing. The main species of medical importance were Lu. longipalpis (VL vector), which represented 90.4% of the specimens captured, and Ny. whitmani (CL vector), 1.6%.

During the period studied, monthly rainfall ranged from 0 mm to 332.5 mm. The average temperature and relative humidity were 21.9 (standard deviation [SD] 2.6 °C and 64.3 (SD 11.3%), respectively. The dry season extended from Apr. to Sept. and the rainy season from Oct. to Mar. The overall profile of the three main climate variables was similar to that of the previous two years (Figure 1). The population curve of the predominant species in our study (Lu. longipalpis) is shown in Figure 2. A populational peak was observed in the warm and rainy season, when relative humidity increases. Although captured at much smaller numbers compared to Lu. longipalpis (Table 1), population curves were also constructed for the cortelezii complex, Ev. lenti, and Ny. whitmani. Whereas populations of the first two species oscillated in a similar way to Lu. longipalpis, Ny. whitmani showed a populational peak in the dry season.

Throughout the study period, 1123 (89.1%) specimens were captured in the urban area, 109 (9.7%) in the rural area, and 28 (2.2%) in the forest. Ev. cortezezii, Ev. evandroi and Ev. sallesi were caught only in the urban area, and Pa. brasiliensis only in the forest. The other species were found in at least two of the three ecological areas, in various proportions. Regarding species richness, nine species were captured in the urban area, six species in the rural area and eight species in the forest. These results are represented by a logic diagram (Figure 3). A greater number of males was captured in urban and rural areas, while a higher number of females was captured in the forest, all of them identified at species level (Table 1).

Regarding the synanthropy index, Ev. evandroi showed absolute synanthropy (SI +100) and Pa. brasiliensis showed absolute asynanthropy (SI -100). Ev. lenti and Lu. longipalpis showed a high degree of adaptability to the urban environment, with SI +83.4 and SI +95.8, respectively (Table 2). Considering that the cortelezii complex included only females and that Ev. cortezezii and Ev. sallesi were only represented by male specimens, it was not possible to calculate their synanthropy index.

**DISCUSSION**

The geographical distribution of leishmaniases in the country indicates that its epidemiology is influenced by a great range of environmental, climatic and socioeconomic aspects7,14-16. Moreover, the emergence of new risk factors has favored human-vector contact, leading to new epidemiological scenarios and increasing the incidence of leishmaniases. These new epidemiological scenarios might demand improvements in the control program17-19.

Over recent years, Minas Gerais has experienced a significant increase in the number of reported cases of VL, representing 71% of the total cases recorded in the southeast of Brazil20. During our study in the municipality of Itaúna in Minas Gerais state, 1306 specimens were captured, with a fauna of 11 species, distributed in six genera (Table 1). Among the species, we highlight the presence of Lu. longipalpis and Ny. whitmani, which are acknowledged vectors of Le. infantum and Le. braziliensis. These results are similar to those found in another study conducted in the municipality, where the same genera and almost all the same species were captured21.

As climatic factors may influence the population dynamics of phlebotomine sand flies, it is fundamentally important to understand...
The occurrence of a particular sand fly species in a given ecological area (urban, rural or forest) is related to its adaptation to environmental conditions, resource availabilities and its competitive interaction with other species[30]. In the present study, the richness and abundance of species varied between the localities, confirming that environmental or geographical factors may determine a different configuration of phlebotomine sand fly populations. When analyzing the density of sand fly species in each ecological area, it was observed that the smallest number of specimens captured occurred in the forest area (2.1%) (Figure 2). Other studies performed in Brazil also found peaks of Lu. longipalpis during hot and rainy months[21,22,23,24,25,26,27,28,29]. The distinct profile found for Ny. whitmani may be regarded with caution due to the small number of specimens (20 specimens) captured in our study (Figure 2).

In the present study, a high density of the predominant species (Lu. longipalpis) was found in the urban environment (92.2%) with SI +95.8 (Table 2). The abundance and frequency of this species in the urban area of Itaúna resemble those recorded by several other studies in Brazil[37-41, including a recent entomological fauna survey developed by our group in Itaúna[21]. According to Melo[42], among all sand fly species, the most adapted to the anthropic environment is Lu. longipalpis. Its high degree of adaptation to the peridomestic environment of anthropic areas is mainly influenced by the presence of domestic animals[21,22,23,24,25,26,27,40,43]. In the forest area, ZCC was taken as reference for the placement of traps. The low number of Lu. longipalpis captured in the area (two specimens only) indicated that the distance between the traps and ZCC was adequate. The sporadic presence of horses and dogs in the ZCC did not bias the results.

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**Figure 1:** Historical series of main climate variables (rainfall, temperature and relative humidity) in Itaúna, Minas Gerais state, Brazil. Legend: black circle - 2015/2016, black square 2016/2017; white circle - 2017/2018 (present study).

**Figure 2:** Synanthropy index (SI) for phlebotomine sand fly species captured in three areas (forest, rural and urban) of Itaúna, Minas Gerais state, Brazil, from June 2017 to May 2018.

| Species         | SI     |
|-----------------|--------|
| Br. brumpti     | +50.0  |
| Ev. evandroi    | +100.0 |
| Ev. lenti       | +83.4  |
| Ev. termitophila| +16.6  |
| Lu. longipalpis | +95.8  |
| Ny. whitmani    | -25.0  |
| Pi. pessoai     | +95.8  |
| Pa. brasiliensis| -100.0 |
| Pa. lutziana    | -33.4  |
FIGURE 2: Population curves of the four most numerous species of phlebotomine sand flies captured in Itaúna, Minas Gerais state, Brazil. Months with climate variables above the averages in the studied period are marked by lines below the abscissa: rainfall - continuous line; temperature - short dashed line; relative humidity- long dashed line. June 2017 to May 2018.

FIGURE 3: Distribution of phlebotomine sand fly species in forest, rural, and urban areas. Itaúna, Minas Gerais, Brazil. June 2017 to May 2018
Some sand fly species may be in the early stages of adaptation to the urban environment and, although apparently rare and without epidemiological importance, they would be adaptable and able to perform vector functions. Despite previous studies on sand fly biology and behavior, some gaps related to the synanthropy of these insects remain to be clarified.

Our results emphasize the importance of studying the ecology/biology and behavior of phlebotomine sand flies, demonstrating their epidemiological role in the transmission of leishmaniases in urban centers. Thus, the potential of some species of phlebotomine sand flies as vectors of Leishmania, along with their habits and synanthropy, should be considered by the competent health agencies.

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AUTHORS’ CONTRIBUTIONS

NCLP, EMM, AJVP, JVL and DMP participated in the collection of phlebotomines; FOLS, ESD and RSL were responsible for specimen identification; FOLS, ESD, EMM and NCLP participated in taxonomic discussions; CLFD was responsible for the statistical analysis of the results; NCLP, EMM, ESD and CLFD prepared the manuscript. All authors have read and approved the final version of the article.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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