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Aspects on the ecology of phlebotomine sand flies and natural infection by *Leishmania hertigi* in the Southeastern Amazon Basin of Brazil

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ABSTRACT

The medical and veterinary importance of sand flies (Diptera: Psychodidae) follow as a result of some species ability to transmit the zoonotic protozoa of the genus *Leishmania*. Of all municipalities in the state of Mato Grosso, Sinop ranks first in reported cases of American Cutaneous Leishmaniasis (ACL). Sinop urban zone encompasses three permanent forest preservation areas (APPs) that provide refuge for insects and other vertebrate hosts. We assessed ecological parameters and investigated the natural infection by *Leishmania* spp. of the phlebotomine fauna from four ecotypes with different levels of urbanization in the urban area of Sinop. A total of 62,745 sand flies were collected, of which 52.34% female. Out of 37 species in this study, nine were found to be constant. Sand flies frequency and diversity were highest in APPs (96.85%; 33 species). *Lutzomyia dasypodogeton* was the most frequent species and exhibited the greatest abundance (SISA = 0.977). The neighborhoods around APPs and completely urbanized neighborhoods presented noteworthy ecological similarity. Moreover, eight vector sand fly species with medicalwere identified, and one *L. antunesi* sample pool was found to be naturally infected with *Le. hertigi*. We observed a high frequency and diversity of sand flies, including some species that are known to be major vectors of ACL. Further studies are needed on the natural rates of infection in humans, domestic animals, and sylvatic hosts to better comprehend the leishmaniases dynamics.

1. Introduction

Phlebotomine sand flies (Diptera: Psychodidae) include the primary vectors of protozoa from genus *Leishmania* (Dantas-Torres et al., 2012), the causative agents of leishmaniases. Nearly 900 sand fly species occur worldwide, with more than 500 reported only in the neotropical regions. Circa 260 species occur with notable frequency throughout the Brazilian territory (Sherlock, 2003; Galati, 2003; Shimabukuro and Galati, 2011; Andrade et al., 2013), mainly in the Amazon basin and the northeastern Brazil. In the state of Mato Grosso 114 sand fly species have been reported in the biomes of Amazon rainforest, transition zone, Cerrado and Pantanal (Alves et al., 2011; Amaral et al., 2011; Galati and Ovallos, 2012; Missawa and Maciel, 2007).

Although the transmission of this group of diseases was formerly associated with rural and sylvatic areas, phenomena as climate change, deforestation, and the adaptive capacity of reservoirs and sand flies have been linked to the global urbanization of leishmaniases (Salomón et al., 2012). Anthropogenic environmental changes may disrupt the eco-epidemiology of leishmaniases leading the transmission cycle to become adapted for urban areas (Brazil, 2013; Souza et al., 2014; Ximenes et al., 2007). The interaction among sand flies and hosts in landscapes where forest fragments remain contacting with urban areas is not well known, especially in forested areas that are targets of ecotourism, leisure, education and research.

Sinop municipality experiences the highest pattern of socioeconomic growth in the state of Mato Grosso and has undergone an intensive process of urbanization and land use change for agriculture and cattle-raising. Besides the economic development, the number of American Cutaneous Leishmaniasis (ACL) cases in Sinop is also on the rise. Currently, the municipality ranks first in the reported cases of ACL

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in the state of Mato Grosso (Ministério da Saúde, 2016). Despite ACL importance in the municipality, the potential for transmission remains unknown. Thus, investigation of the sand fly fauna composition and natural infection rate is decisive for understanding the disease transmission cycle attempting to identify possible vectors.

To better describe the transmission cycle of leishmaniasis in a particular region it is of great importance to understand how sand flies natural behavior regulates their populations, due to the fact that each sand fly species interact in a specific way with their habitat (Marco, 1997). In addition, the implementation of control measures and prevention require the comprehension of the transmission process. Therefore, studies involving the fauna ecology and composition, and data about their natural infection are fundamental. In this context, the present study evaluated the ecological parameters and the natural infection by *Leishmania* spp. in sand flies fauna from the urban area of Sinop, state of Mato Grosso, in four different ecotypes.

2. Material and methods

2.1. Study area

Sinop is known to have the fourth largest population in the state of Mato Grosso (133,000 inhabitants) and is located on the highway BR-163, approximately 500 km (311 miles) north from the capital city of Cuiabá (IBGE, 2017). Sinop have been settled in the southern Amazon rainforest biome (11°51′27.1″S – 55°29′52.2″W), in the Arc of Deforestation. Current vegetal composition merges residual open ombrophilous forest and savanna patches. Flat to gently undulating relief covers the territorial extension of 3,942,229 km^2. This region has tropical wet and dry climate, consisting of approximately six months of dry season, rainfall circa 2500 mm with high proportion in the six months of wet season, average temperature of 26 °C (ranging between 20 °C and 40 °C). The bulk of the rainfall in the region falls from October to April (INMET, 2016). Sinop has three permanent forest preservation areas (APPs) within its urban region, namely Forest Park, Unemat Forest and Botanical Garden. These sites receive innumerous visits especially on weekends, given that they serve as leisure areas for walking on the trails and contemplation of the wide-ranging current fauna.

2.2. Fieldwork

The current study forms part of a comprehensive project on the ecology and transmission dynamics of leishmaniases in the northern state of Mato Grosso. Data collection and sand flies captures were previously conducted from May 2014 to April 2015 (Thies et al., 2016). In summary, we surveyed nine neighborhoods with distinct levels of urbanization (points 1–18) and the three APPs (points 19–24) (Fig. 1). In order to select the ecotypes, we adopted the following criteria: three neighborhoods situated in completely urbanized areas (CUN); three neighborhoods presenting forest fragments (NFF); three neighborhoods located around the APPs (NAA); and the three APPs, therefore comprising four ecotypes at all. The geographical positioning of the sites was obtained by using GARMIM-ETREX® GPS device. Captures were carried out by using CDC light traps (Sudia and Chamberlain, 1962) that were placed at shadowed spots, one trap in the peridomicile of two dwellings (with domestic animals) within each neighborhood, and two traps in each APP. Sand flies were collected from 6 p.m. to 7 a.m. on the following day, during three consecutive nights, always in the first week of each month.

2.3. Identification of sand flies

After counting under the stereo microscope, male sand fly specimens collected were preserved in microtubes by adding 70% ethanol until identification, while females were dissected and stored in microtubes containing 6% DMSO at −20 °C until processing. For identification, male flies were mounted in Berlese's liquid on microscope slides under coverslips. Female sand flies were identified after dissection of heads and the last three abdominal segments. Subsequently female sand flies were pooled in microtubes containing one to 10 specimens, separated by species, month, and collection point. These pools were later used for DNA isolation. Identification of sand flies was conducted in accordance with Young and Duncan (1994). Specimens that could not be identified due to missing or incomplete characters were considered *Lutzomyia* spp.

2.4. Leishmania detection by nested PCR

DNA isolation from female sand flies were made by using the illustra™ tissue & cells genomicPrep Mini Spin kit (GE Healthcare Life Sciences, USA) following manufacturer instructions. Reliability of the DNA extraction was verified by the amplification of *Lutzomyia* cacophony IVS6 region, using genus-specific primers previously described by Lins et al. (2002). PCR for detection of *Leishmania* DNA in female sand flies was carried out by *Leishmania* nested PCR (LnPCR), specifically targeting the SSUrRNA gene (Cruz et al., 2002, 2006; Guillaume et al., 1992). The positive sample for *Leishmania* DNA displayed a 353 bp fragment profile in 2% agarose gel electrophoresis during observation under UV light. DNA extracted from *Le. infantum* strain MHOM/BR74/PP75 was used as positive control in all assays.

2.5. Sequencing of positive samples

Amplicons were purified from agarose gels by using the QIAquick® Gel Extraction Kit (Qiagen, Germany) and sequenced bi-directionally with the BigDye® Terminator v3.1 Sequencing Kit (Applied Biosystems, France) in the automated sequencer ABI 3730 (Life Technologies). The nucleotide sequences were analyzed using BioEdit tools (http://www.mbio.ncsu.edu/bioedit/bioedit.html), afterward subjected to the basic local alignment search tool (BLAST) (www.ncbi.nlm.nih.gov/blast) and submitted to GenBank (https://www.ncbi.nlm.nih.gov/nuccore).

2.6. Minimum infection rate in sand flies

Leishmania minimum infection rate in sand flies was calculated as the number of positive pools divided by the number of specimens analyzed, multiplied by 100 (Paiva et al., 2007).

2.7. Diversity indices and abundance

Simpson's index was used in order to calculate species dominance, Shannon-Wiener's index (H') (Hill, 1973) to calculate the diversity, Pielou's evenness measure (J') (Pielou, 1969) was used to calculate the equitability, similarity was calculated by measure of Jaccard index (Ludwig and Reynolds, 1988), and constancy was calculated according to Uramoto et al. (2005). Species abundance was evaluated by the standardized index of species abundance (SISA) (Roberts and Hsi, 1979). Jackknife's estimator was calculated as stated by Smith and van Belle (1984).

2.8. Statistical analysis

Because the trapping data were not normally distributed, assessed by Shapiro-Wilk test (p-value < 0.001) (Shapiro and Wilk, 1965), we proceeded with the nonparametric equivalent statistical test. Mann-Whitney's and Kruskal-Wallis' tests (Hollander et al., 2014) were used to compare the total number of sand flies to qualitative variables. Spearman's correlation (Hollander et al., 2014) was employed to compare the total number of sand flies to quantitative variables. Furthermore, the Nemenyi's test (Nemenyi, 1963) was used for multiple comparisons.