SAND FLIES (DIPTERA: PSYCHODIDAE) IN AN ENDEMIC AREA OF LEISHMANIASIS IN AQUIDAUANA MUNICIPALITY, PANTANAL OF MATO GROSSO DO SUL, BRAZIL

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SUMMARY

The Aquidauana municipality is considered an endemic area of leishmaniasis and an important tourist site in Mato Grosso do Sul State. The aim of this study was to investigate the sand fly fauna in the city of Aquidauana. Captures were carried out twice a month, from April 2012 to March 2014 with automatic light traps and active aspiration, in the peridomicile and domicile of six residences. A total of 9,338 specimens were collected, 3,179 and 6,159 using light traps and active aspiration, respectively. The fauna consisted of: Brumptomyia brumpti, Evandromyia aldaefalcaoeae, Ev. evandroi, Ev. lent, Ev. orcyi, Ev. sallusi, Ev. termotheila, Ev. walkeri, Lutzomyia longipalpis and Psathyromyia bigeniculata. The most abundant species captured was Lutzomyia longipalpis, present in all the ecotopes, predominantly in peridomicile areas, and mainly males. Leishmania DNA was not detected in the insects. It was observed the abundance of the sand fly fauna in the region, as well as the high frequency of Lu. longipalpis, the main vector of L. infantum. The results of this study show the need to increase the monitoring and more effective control measures. It is noteworthy that the studied region presents several activities related to tourism and recreation, increasing the risk of transmission of leishmaniasis to this particular human population.

KEYWORDS: Phlebotomine sand fly; Lutzomyia longipalpis; Fishing tourism.

INTRODUCTION

Sand flies (Diptera: Psychodidae: Phlebotominae) present medical importance because they are vectors of protozoans of the genus Leishmania, which are the etiological agents of leishmaniasis. These diseases are considered a major worldwide public health issue due to their high incidence and lethality rates. In Mato Grosso do Sul State, leishmaniasis are rapidly expanding; cases of visceral leishmaniasis (VL) and cutaneous leishmaniasis (CL) have been reported in 56 and 72 municipalities, respectively. From 1999 to 2011, 162 cases were registered; in 2013, seven cases were confirmed, and one death reported, and the city is now considered an area of intense VL transmission. Regarding CL, Aquidauana is an area of moderate transmission, with five confirmed rural cases in 2013.

Approximately 900 species of sand flies are known worldwide. In Brazil, about 235 species have been reported and 66 of these were registered in Mato Grosso do Sul. Sand flies are found in different climate conditions, in the wild, rural areas and often urban centers. Therefore, studies regarding the fauna of these insects and their behavior are determinant aspects in the epidemiology of leishmaniasis. They are also of major importance in endemic areas as they provide basic information to design control measures of the disease by health services.

Thus, this study aimed to investigate the fauna, as well as the distribution and the abundance of sand flies in the urban area of the municipality of Aquidauana, Mato Grosso do Sul State, Brazil.

MATERIAL AND METHODS

Study Area

The municipality of Aquidauana (20°28’15”S, 55°47’13”W and altitude of 149 m) is located 139 km from the state capital, Campo Grande, in the western region of Mato Grosso do Sul. According to the 2014 Brazilian Institute of Geography and Statistics census, the city...
population was 45,614 inhabitants. *Aquidauana* is situated at the edge of the plain area of the *Pantanal* biome. The city presents geomorphological characteristics of *Maracaju* plateau and the *Aquidauana/ Bala Vista* valley, forms the border of the territory. The river *Aquidauana* and the streams *João Dias* and *Guanandy* are located in the urban area of the municipality. The climate, according to the Köppen Climate Classification System is AW, defined as a humid tropical climate. The daily temperature average starts to increase in August and reaches its peak in December.

Six neighborhoods were selected, *Bairro Alto*, *Pinheiro*, *São Francisco*, *São Cristovão*, *Guanandy* and *Paraíso* (Fig. 1). In each of them, one residence was chosen to install two light traps, in both, the peridomicile and the domicile areas. The selection of neighborhoods followed the following criteria: notified cases of VL and/or CL human/canine cases, together with the presence of domestic animals shelters such as kennels, hen houses and pig pens.

The selected residences, as well as the neighboring houses, had fruit trees in the backyards. *Bairro Alto* is located in the central region with no streams nearby. *Pinheiro*, *São Cristovão*, *São Francisco*, *Guanandy* and *Paraíso* are located in the outskirts of the city and are delimited by the streams *João Dias* and *Guanandy*. In these neighborhoods, the traps were installed about 200 m away from the remaining riparian forest, except for the selected residence in *Guanandy*, which also had a pig pen.

**Sand flies captures**

The collections were carried out twice a month with *Falcão* modified light traps placed both in the domicile and the peridomicile area of the selected residences, from April 2012 to March 2014. The captures were always conducted from 6 pm to 7 am even during the period of daylight saving time. In order to increase the females sample size, captures were also performed by means of an electric manual aspirator inside animal shelters in each of the selected residences. These collections began...
in November 2012 and lasted 10-15 minutes. Captured insects were identified based on the classification described by Galati\(^3\) and genera abbreviations followed the recommendation of Marcondes\(^3\).

**Molecular analysis**

After identification, females were grouped in pools of up to 10 insects, according to species, location area and date of capture. Later, samples were sent to the Laboratory of Molecular Biology at the Federal University of Mato Grosso do Sul to investigate the presence of Leishmania spp. using the polymerase chain reaction (PCR) assay. DNA samples were extracted with 5% Chelex\textregistered resin solution (Sigma-Aldrich, St. Louis, USA) and PCR was carried out according to the method of El Tai et al.\(^3\).

**Data analysis**

The Standardized Index of Species Abundance (SISA) was calculated to determine the most abundant species, according to the spatial distribution, for which 1.00 corresponds to the most abundant species\(^4\). For the diversity analysis, the Shannon’s diversity index (H)\(^4\) was calculated and the measure of evenness or dominance of species was obtained using the Pielou’s Index (J)\(^4\). Dispersion data, symmetry and outliers were described by using the box plot graphic. The Mann-Whitney U-test was used to compare the male-female ratio between the peri and the intradomicile areas.

**RESULTS**

After 7,488 hours of collection using the light traps and 72 hours of collection using the active aspiration, 9,338 sand flies were captured, distributed among the genera: Brumptomyia, Evandromyia, Lutzomyia and Psathyromyia. The fauna comprised ten species: Brumptomyia brumpti (Larrousse, 1920), Evandromyia aldafalcaoae (Santos, Andrade-Filho and Honer, 2001), Evandromyia evandroi (Costa Lima, 1932), Evandromyia lenti (Mangabeira, 1938), Evandromyia orcyi (Oliveira, Sanguinetti, Almeida and Andrade Filho, 2015), Evandromyia sallesi (Galvão and Coutinho, 1939), Evandromyia termiotiphila (Martins, Falcão and Silva, 1964), Evandromyia walkeri (Newstead, 1914), Lutzomyia longipalpis (Lutz and Neiva, 1912) and Psathyromyia bigeniculata (Floh & Abonnenc 1941).

Through the use of light traps, 3,179 specimens were captured, from which 2,780 (87.45%) were males and 399 (12.55%) were females. Table 1 shows the predominance of Lu. longipalpis, with 2,957 (93.02%) specimens, followed by Ev. aldafalcaoae with 31 (0.98%) and others species totaling 56 specimens (1.76%). Although eight out of the ten species reported in this study were captured in the Guanandy neighborhood with Shannon’s diversity index of 0.6046, in São Francisco, where fewer species were caught, there was a higher Shannon’s index of 1.3103, with a Pielou equitability of 0.7312 (Table 1).

**Lutzomyia longipalpis** was the most abundant species (SISA = 1.00), ranking first in the classification (Table 2). The species was captured in all the sampled areas, especially in the peridomicile, except for the São Francisco neighborhood (Fig. 2).

**Evandromyia aldafalcaoae** was the second most abundant species and presented a SISA = 0.95, despite having fewer collected specimens compared to **Ev. walkeri** (SISA = 0.89, the third in the ranking). **Ev. aldafalcaoae** was more abundant in the neighborhood Pinheiro (n = 21),

| Species                  | Pinheiro M | Peri M | São Francisco M | Intra | Peri | São Cristovão M | Intra | Peri | Guanandy M | Peri | Paráso M | Intra | Peri | Bairro Alto M | Total M | Female F | Total F | n (%) |
|--------------------------|------------|--------|-----------------|-------|------|-----------------|-------|------|-------------|------|----------|-------|------|----------------|---------|----------|---------|------|
| Br. brumpti              | -          | -      | 1               | -     | -    | -               | -     | -    | -           | -    | -        | -     | -    | -              | 1       | -        | 1       | 0.03 |
| Ev. aldafalcaoae         | 3          | 3      | 12              | 3     | 1    | 1               | 1     | 2    | 1           | 2    | -        | 1     | -    | 1              | 21      | 10       | 31      | 0.98 |
| Ev. evandroi             | 1          | -      | 1               | -     | -    | 1               | -     | 8    | 2           | 1    | -        | -     | -    | 1              | 15      | 15       | 0.47   |
| Ev. lenti                | 1          | -      | -               | -     | -    | -               | -     | 2    | -           | 1    | -        | -     | -    | 3              | 1       | 4        | 0.13   |
| Ev. orcyi                | 1          | -      | -               | -     | -    | 1               | 1     | 3    | 2           | -    | -        | -     | -    | 3              | 3       | 6        | 0.19   |
| Ev. sallesi              | -          | -      | -               | -     | 1    | 1               | -     | 6    | 1           | -    | 6        | -     | -    | 6              | 8       | 14       | 0.44   |
| Ev. termiotiphila        | -          | -      | 1               | -     | -    | -               | -     | -    | 1           | -    | -        | -     | -    | 1              | 1       | 1        | 0.03   |
| Ev. walkeri              | -          | -      | 4               | -     | 1    | 1               | -     | 23   | 65          | 29   | -        | 5     | 3    | -              | 105     | 30       | 135     | 4.25 |
| Lu. longipalpis          | 90         | 15     | 670             | 72    | 4    | 1               | 3     | -    | 278         | 41   | 522      | 62    | 12   | 688           | 75      | 1        | 45      | 116  |
| Ps. bigeniculata         | 2          | -      | -               | -     | -    | 1               | 3     | 2    | 2           | 3    | -        | -     | 1    | -              | 9       | 6        | 15      | 0.47 |
| Total                    | 96         | 19     | 682             | 76    | 11   | 1               | 8     | 1    | 279         | 44   | 524      | 66    | 38   | 667           | 123     | 3        | 3       | 50   |
| Shannon Diversity Index  | 0.1530     | 1.3103 | 0.0726          | 0.6046| 0.7257| 0.0822          |       |      |             |      |          |       |      |                |         |          |         |      |
| Pielou Eveness Index     | 0.0950     | 0.7312 | 0.0405          | 0.2907| 0.4509| 0.0592          |       |      |             |      |          |       |      |                |         |          |         |      |

Intra: intradomicile; Peri: peridomicile; M: male; F: female; Br.: Brumptomyia; Ev.: Evandromyia; Lu.: Lutzomyia; Pa.: Psathyromyia.
Figueiredo HR, Santos MFC, Casaril AE, Infran JOM, Ribeiro LM, Fernandes CES, Oliveira AG. Sand flies (Diptera: Psychodidae) in an endemic area of leishmaniasis in Aquidauana municipality, Pantanal of Mato Grosso do Sul, Brazil. Rev Inst Med Trop Sao Paulo. 2016;58:87.

where a hen house was present in the peridomicile; while Ev. walkeri \((n = 135)\) had the highest number of individuals collected in the pig pen of the residence located in Guanandy. The species Ev. evandroi, Ev. lenti, Ev. orcyi, Ev. sallesi, Pa. bigeniculata and Evandromyia orcyi were only slightly present (Table 2).

In total, both in the peridomicile and domicile areas, more males were captured than females, with a ratio of 6.97. Lu. longipalpis presented a ratio of 8.07 and Ev. walkeri 3.5. However, the species Ev. sallesi had more females than males, with a ratio of 0.75 and Ev. evandroi presented only females.

Figure 3 shows the male-to-female ratio of Lu. longipalpis correlating it to the peridomicile and domicile areas of the neighborhoods. Only in Guanandy, a statistically significant difference \((p = 0.001)\) was observed between the two ecotopes.

### Aspiration

Seven species totaling 6,159 specimens were collected, with 5,120 (83.13%) males and 1,039 (16.87%) females: Ev. aldafaocaee, Ev. evandroi, Ev. sallesi, Ev. walkeri, Lu. longipalpis, Pa. bigeniculata and Ev. orcyi.

Table 3 describes the species captured in each ecotope and Table 4 shows their SISA. Lutzomyia longipalpis was the most frequent species (97.68%) and was present in all the neighborhoods, ranking first (SISA = 1.00). Evandromyia walkeri ranked second and Ev. sallesi ranked third. Other species accounted for 0.16% of the total.

The total male-to-female ratio was 4.9; 5.1 for Lu. longipalpis and, on the contrary, Ev. sallesi presented a female predominance (0.36) (Table 3). By analyzing the male-to-female ratio of Lu. longipalpis captured using light traps, there was a significant difference between the neighborhood Bairro Alto and the neighborhoods Guanandy, Paraíso and São Cristóvão (Fig. 4).

From the total, 82.96% of the insects were caught in the neighborhoods of Guanandy, Bairro Alto and São Cristóvão (Fig. 5).

Comparing the peridomicile ecotopes, the pig pen located in Guanandy presented the highest yield and the greatest variety, with eight out of the ten species collected (Fig. 6 and Table 1).

### Leishmania DNA detection in the females

No Leishmania DNA was detected in the 418 females sand flies assessed through the molecular analysis.
DISCUSSION

The epidemiology of leishmaniasis is complex and thus requires flexible control strategies suitable for each region and local occurrences. Therefore, knowledge about vectors is a fundamental tool to understand the transmission dynamics in order to plan preventive and control measures. This information is especially important in the city studied, where cases of visceral and cutaneous leishmaniasis are increasing. Considering that Aquidauana is a part of the Brazilian Pantanal and ecotourism tours and fishing activities are developed in this region of the state, the contact between vector and human population has been favored.

Several methods of sand flies capture have been used, but among these, light traps stand out and are widely used in fauna studies, as they are easy to use and inexpensive; although they may interfere in both the quality and the quantity of specimens collected. On the other hand, by standardizing the collections for fortnightly captures during two consecutive years, associated with another capture technique, it was possible to increase the number of specimens collected, providing data to understand the behavior of the species present in the urban area of the city.

Considering the limitations of this type of capture, active aspiration was also performed in order to increase the number of captured females to investigate the presence of flagellates. After analyzing the performance of the two techniques, it was observed that the aspiration obtained the highest number of insects in less time gathering (72 h). In contrast, in 7,488 hours, light traps captured fewer specimens, but presented more variety. This amount could be explained by the dynamics of the capture technique; while the sand fly is attracted by light and host in one method, by in the other, the collection is active, and did not give the insect a chance to choose.

The wider variety of species observed in light traps collection has already been reported by other authors. Actually, this high diversity has also been demonstrated in other municipalities located in the Pantanal. Casaril et al. found 12 species in a total of 7,370 specimens in the city of Corumbá, MS. In Caceres, state of Mato Grosso, despite the lower frequency of insects, 28 different species were observed. In Aquidauana, during the study period, ten species of sand flies were captured in a total of 9,338 individuals, differing from a previous study.
The four most abundant species in this study were *Lu. longipalpis*, which had the highest number of individuals captured in both techniques used, followed by *Ev. aldafalcaoae*, *Ev. walkeri* and *Ev. sallesi*.

An increased frequency of *Lu. longipalpis* was noted when compared to data reported by Almeida et al.13. It is known that a ability of a species to adapt to an environment can be influenced by environmental conditions, abiotic factors, food supply and interspecific competitive interactions49,50, therefore *Lu. longipalpis* could be exerting a greater selective pressure over the local species48,49,50,51, besides that, this species seems to be more anthropized41,42,44,46,52,53,54,55,56,57,58.

The urbanization of the vector strongly indicates its anthropophilic behavior and points out its important role in local transmission of the parasite, since Aquidauana is considered an area of intense VL transmission. Previous studies have demonstrated the association of a high frequency of *Lu. longipalpis* in higher prevalence regions 6,54,59,60,61. This fact reinforces the species’ importance of connecting the transmission to the etiological agent.

The species *Ev. aldafalcaoae* was found in all the ecotypes, and appears in second place in the ranking of the most abundant species. A higher frequency of this species was observed in Pinheiro neighborhood. *Aquidauana* is the type locality of this species’ male62. *Ev. aldafalcaoae* has already been reported in other regions of Pantanal, such as Corumbá63, Neocôndida64 and Caceres in Mato Grosso65. In addition to these locations, Dorval et al.66 found a single male in the domicile of a residence in Bela Vista, Mato Grosso do Sul.

*Evandromyia walkeri* was the third most abundant species however this species ranked second in the active aspiration. Although it has been reported in areas of forest and wood48,50,61, this species was captured in the peridomicle and domicile of almost all the sampled sites. The highest number of *Ev. walkeri* specimens was collected in the neighborhood of Guanandy, a fact that could be explained by the proximity of this location.

### Table 4

Classification of the sandflies species captured using aspiration according to Standardized Index of Species Abundance (SISA) in Aquidauana, MS, from April 2012 to March 2014

| Species           | Ranking | SISA |
|-------------------|---------|------|
| *Ev. aldafalcaoae*| 6°      | 0.07 |
| *Ev. evandroi*    | 4°      | 0.21 |
| *Ev. orcyi*       | 5°      | 0.09 |
| *Ev. sallesi*     | 3°      | 0.30 |
| *Ev. walkeri*     | 2°      | 0.33 |
| *Lu. longipalpis* | 1°      | 1.00 |
| *Pa. bigeniculata*| 7°      | 0.04 |

| Br.: Brumptomyia; Ev.: Evandromyia; Lu.: Lutzomyia; Pa.: Psathyromyia. |

### Table 3

Distribution of sandfly species captured using aspiration in peridomicle according to neighborhoods and gender in Aquidauana. Mato Grosso do Sul, Brazil, from April 2012 to March 2014 (n = 6159)

| Species             | Pinheiro M | F | São Francisco M | F | São Cristóvão M | F | Guanandy M | F | Paraíso M | F | Bairro Alto M | F | Total |  |
|---------------------|------------|---|-----------------|---|-----------------|---|-------------|---|------------|---|---------------|---|-------|---|
| *Ev. aldafalcaoae*   | -          | - | -               | - | -               | - | 2           | - | -          | - | -              | - | 2     | 2 |
| *Ev. evandroi*       | -          | - | -               | - | 2               | - | -           | - | 1          | - | 3               | - | 3     | 0,05|
| *Ev. orcyi*          | -          | 4 | 4               | 2 | 4               | - | -           | - | -          | - | 4               | - | 4     | 0,06|
| *Ev. sallesi*        | -          | 5 | 16              | 39| -               | - | -           | - | 16         | 44| 60              | - | 97,68 |
| *Ev. walkeri*        | -          | 3 | 66              | 4 | -               | - | -           | - | 69         | 4 | 73              | - | 1,19 |
| *Lu. longipalpis*    | 578        | 145| 18             | 1 | 1375            | 110| 1374        | 497| 155        | 7 | 1534           | 222| 5034 | 982 |
| *Pa. bigeniculata*   | -          | 1 | 1               | - | 1               | - | 1           | - | 1          | - | 1               | - | 1     | 0,02|
| Total               | 578        | 145| 18             | 1 | 1378            | 117| 1457        | 546| 155        | 7 | 1534           | 223| 5120 | 1039| 6159 | 100,00|
| Índice de Diversidade de Shannon (H) | 0.0000 | 0.0464 | 0.3060 | 0.0000 | 0.0043 |
| Equitabilidade de Pielou (J) | 0.0000 | 0.0334 | 0.1707 | 0.0000 | 0.0062 |

Peri: peridomicle; M: male; F: female; Br.: Brumptomyia; Ev.: Evandromyia; Lu.: Lutzomyia; Pa.: Psathyromyia.
to the insect’s natural habitat. This neighborhood is inserted into a riparian area of the Guanandy stream, and it is located about 100 meters away from the confluence with the Aquidauana river. The presence of Ev. walkeri at various sites in the city, even in small numbers, indicates an adaptation process to the urban and anthropic environment, considering that Aquidauana is in expansion process and natural ecotypes are decreasing.

It is highly important to know the behavior of Ev. walkeri, as it has been found naturally infected by Leishmania (Viannia) spp. Data reported by Oliveira et al. from April 2012 to March 2014, and from November 2012 to March 2014, respectively, indicated a predominance of males compared to females. The male-to-female ratio of Lu. longipalpis was 8.07:1 using light traps and 5.1:1 through manual aspiration. Comparing both capture methods, a higher yield of females was observed in aspiration, as there was a decline of captures made by aspiration reflected a different pattern regarding the abundance index. The ranking of species had Lu. longipalpis in first place and a variation between Ev. walkeri and Ev. sallesi. Despite being in third place, Evandromyia sallesi presented a low yield in the collection, fact that was also reported by Nunes et al. in the municipality of Bonito, which borders the Aquidauana municipality, where a sporadic low density presence of this species was reported. Although there is no report of anthropophilic behavior, DNA from L. infantum has been found in this species, enhancing its epidemiological significance.

The Guanandy neighborhood had the highest number of specimens and variety of species caught (eight out of ten) in both techniques of capture; however this site showed a predominance of Lu. longipalpis, therefore Pielou’s and Shannon’s indexes of this neighborhood occupied the third place among the sampled ecotypes, a tendency that has also been observed in other studies performed in the state. On the other hand, the São Francisco neighborhood was the ecotope where the highest abundance and evenness indexes of species caught through light trap was registered. Despite the low frequency in capture and the presence of only seven of the ten species collected in the city, it appears that species are in equilibrium in this site, without the predominance of one of them. Guanandy is located in the outskirts of the city, therefore its surroundings presented rural characteristics, including the presence of synanthropic animals near the residences according to the residents.

The second lowest Shannon’s index was observed in the Bairro Alto neighborhood, situated downtown in the city. This result was similar to data reported by Oliveira et al. in Campo Grande. In general, central areas of cities have modified environments caused by human action; most terrain features constructions and vegetal biomass are reduced, which probably disfavors the presence of several species of sand flies. However, species able to adapt to these modified environments demonstrate an anthropophilic tendency and the possibility of transmitting pathogens.

Regarding the sex ratio, irrespective of the type of capture, a predominance of males compared to females was noted. The male-to-female ratio of Lu. longipalpis was 8.07:1 using light traps and 5.1:1 through manual aspiration. Comparing both capture methods, a higher yield of females was observed in aspiration, as there was a decline of
the male-to-female ratio. Ximenes et al.\textsuperscript{18} observed a ratio of 18.26 and 4.62 using light traps and manual aspiration, respectively.

Through the use of light traps, it was observed that, comparing male-to-female ratios of peridomestic and domicile areas, only the Guanandy neighborhood showed no significant differences among the ecotopes. The domicile presented a greater equilibrium between males and females, probably due to the presence of dogs living inside the houses, which may have contributed to the encounter of more females in this environment\textsuperscript{45,75,76,77}. Other authors have previously reported differences among the peridomestic and domicile ratios, however a higher proportion of males in all the ecotypes was reported\textsuperscript{4,11}.

The predominance of males is unanimous, even in studies performed in the state, like Bonito with a ratio of 5.7\textsuperscript{45}, Ponta Porã with 4.8\textsuperscript{99} and 2.95 in Campo Grande\textsuperscript{99}. This behavior may also have been influenced by the male characters related to hatching before females\textsuperscript{19,36,79}.

Moreover, the observed predominance can be explained by the lekking behavior described for Lu. longipalpis\textsuperscript{79,90,91,92}, in which males are attracted by kairomones released by the hosts, leading the males to release pheromones that attract females\textsuperscript{80,83,85}, providing an environment where sand flies can copulate, and females can feed in animals. Because of the weight of the females after feeding, they could seek refuge in shelters with less luminosity to perform digestion, thus becoming less attracted to the traps\textsuperscript{86}. It is possible that the presence of large amounts of males and the attraction of females close to a host is an adaptive process to increase reproductive success of the species\textsuperscript{6,79,81}.

Regarding the peridomestic and domicile environments, a significant difference was noted between them. The peridomestic was the ecotope with greater quantity and diversity of species, except for the São Francisco neighborhood, where the total amount of insects collected was so low that it was impossible to infer on this behavior. Another neighborhood that showed a large quantity of insects in the domicile was São Cristovão. It is important to note the proximity of this site to the hen house, since the house wall bordered the enclosure of the hen.

Other studies that reported a higher yielding of Lu. longipalpis in the domicile suggested that this behavior may be possible related to unfavorable weather conditions, lack of hosts in the peridomestic, and in some cases the presence of domestic animals living inside the houses, which highlights their endophilic capacity and the possibility of increasing the parasite transmission in this environment\textsuperscript{86,86,87}.

Therefore, the greater number of specimens captured in the peridomestic is probably related to the presence of animals. This behavior, as described in several other reports, shows the sand flies' preference for environments with the presence of domestic or farmed animals\textsuperscript{82,84,88,90,91,92,93,94}. Actually, some authors emphasize the insects' preference for farmed animals with respect to animals in natural habitats\textsuperscript{95,96}.

Regarding the Lu. longipalpis feeding habits, several studies reported the eclective habit of this species\textsuperscript{45,46,75,76,97,98}, however Morrison et al.\textsuperscript{99} suggested that it may act as an opportunist and feed on the closest animals to its breeding site. A higher attraction for birds, especially chickens, and dogs have also been demonstrated\textsuperscript{52,75,76,98}. Other domestic mammals such as cats, equines, goats, cattle and swine have also been evidenced as part of these insects diet\textsuperscript{52,75,76,95,98}.

The pig pen was an environment where the amount of collected insects was very significant. This result is consistent with the studies of Galati et al.\textsuperscript{15} and Carvalho et al.\textsuperscript{100} who have also observed a greater attractiveness of sand flies in this ecotope. It can be noted that the presence of large amounts of organic matter, produced by feces and food scraps in precarious hygienic environments, in addition to the shading of the area by a remnant of riparian vegetation, were probably important to create and maintain the insects at this site. This can be justified by other studies that have already shown the presence of organic matter as a predisposal factor to the finding of these insects in environments with such characteristics\textsuperscript{91,93,94,100}. Alexander et al.\textsuperscript{100} reported other factors that could influence the attractiveness by different host species that must be considered, such as the biomass difference, heat loss, CO2 production and the odor released by the animal, besides blood nutritional value.

According to Gomes\textsuperscript{99}, this eclectic behavior is a precondition for the ability of a species to evolve to synanthropy. In domiciliation for example, the species tendency of using humans or domestic animals as food supply and artificial ecotopes as shelters is clear. This fact evidences the survivability of species after the destruction of their natural ecotopes.

Additional studies on feeding habits and breeding sites of sand flies are certainly needed for a better understanding of leishmaniasis transmission cycle in the municipality.

In the present study, no sand fly was detected naturally infected with Leishmania in a total of 418 females analyzed by PCR. This result corroborates those from Souza et al.\textsuperscript{92}, who found no positivity among 318 samples. In several studies, the occurrence of natural infection caused by Leishmania among sand fly populations has been described as low. In Brazil, the infection estimated rates were 0.4\% in Bahia and Maranhão\textsuperscript{101,102}, 0.7\% in Mato Grosso\textsuperscript{103}, 0.9\% in Minas Gerais\textsuperscript{100}, 1.1\% in Corumbá\textsuperscript{106} and 2\% in Rio de Janeiro\textsuperscript{104}.

Infection rates can be influenced by vectors’ ecological factors such as intraspecific behaviors, diet and host population of each region. The selection of the molecular technique employed can also influence these rates, as it may lead to lower rates of natural infection\textsuperscript{52,105,106,107,108}.

In the municipality of Aquidauana, the maintenance of leishmaniasis, especially VL is likely to be related to the high prevalence of canine infection, as only in the year 2013, 1,666 dogs were diagnosed and 883 were euthanized, among suspected and confirmed cases\textsuperscript{109}.

In the present study, it was possible to observe the sand flies adaptation to the urban area of the municipality of Aquidauana, which was confirmed by the presence of several species both in the peridomestic and the domicile of all the ecotopes. The abundance of sand fly fauna in the region and the high frequency of Lu. longipalpis, a L. infantum vector, was noted, demonstrating the need to intensify the monitoring and to establish more efficient control measures. It is noteworthy that the studied region is involved in several recreational activities, thus representing an even greater risk factor for the transmission of leishmaniasis.
CONFLIT OF INTEREST

The authors declare that there are no conflicts of interest.

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