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Entomological survey of sandflies (Diptera: *Psychodidae*: *Phlebotominae*) in the City of Concórdia, Brazil and its importance as a reservoir for *Leishmania* spp.

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ABSTRACT

Some sandflies are vectors for *Leishmania*sis, therefore knowing the fauna of the region is important in combating the disease. Sandflies were captured in Concórdia, SC, Brazil, and *Leishmania* spp. in captured females. Thirty-eight specimens of six different species were collected, one of which (*Brumptomyia carvalheiroi*) is reported for the first time in the south of the country. However, there were no samples of sandflies positive for *Leishmania* spp. Despite reports of allochthonous cases in the region studied, the agent is not yet circulating in vectors, but these represent a potential risk when new cases occur.

Introduction

*Leishmania*sis is one of the most neglected diseases in the world and mainly affects developing countries (PAHO, 2017). It is historically known as a rural endemic disease, however the transmission pattern has changed, and it has expanded throughout the country (Seva *et al*., 2017), as is the case in the southern region, where there is a tendency to expand to unaffected municipalities (Dias *et al*., 2022).

Bearing in mind the scarcity of studies on vectors in the western region of Santa Catarina (Orlandin *et al*., 2019) and their importance for epidemiological monitoring, the present work carried out a survey of sandflies in the city of Concórdia, as well as a survey of *Leishmania* spp. in females of captured vectors.

Materials and methods

The study was conducted in the city of Concórdia (27°14'3" S, 52°1'43" W), located in southern Brazil, which does not present an endemic situation for the disease, but has records of allochthonous cases. Six areas (A to F) were selected, corresponding to different districts of the municipality, where the frequency of the characteristic observed in the areas is reported below: proximity to forest (100%), presence of domestic animals (83.3%), presence of production birds (16.7%), recent deforestation (16.7%), report of *Leishmania*sis in Cavia porcellus (16.7%) and proximity to the central area (66,7%).

To capture the insects, CDC (Centers for Disease Control) light bait traps were used, installed indoors and outdoors in six research areas. During the period of one year (April 2021 to March 2022), the traps were turned on at the beginning of sunset and remained on for 12 hours, for three nights of each month. The captured sandflies were transferred to microtubes and frozen at -20ºC and subsequently submitted to species identification, following the classification adopted by Galati (2018). After identification, the females were placed in microtubes containing 70% alcohol and kept again at -20ºC until the molecular test was performed.

After identification, the females were submitted to the modified DNA extraction technique of Michalsky *et al*. (2011). For this, the insects were macerated individually and 50 µL of lysis buffer (100mM Tris-HCL, 100mM NaCl, 25mM EDTA, 25mM EDTA, 0.5% SDS, pH 8.0), 0.5 µL of proteinase K (20 mg/mL) and incubated at 37ºC overnight. Subsequently, 70 µL of ultrapure water and 120 µL of buffer-saturated phenol were added. Then, treatment was carried out with 120 µL of chloroform: isoamyl alcohol (24:1) and the DNA obtained from the supernatant was precipitated at -20º overnight, adding 20 µL of 3M sodium acetate (pH 5.2) and 200 µL of ice-cold absolute ethanol. The precipitate was washed with 100 µl of ice-cold 70% ethanol, resuspended in 10 µl of TE and stored at -20°C. The DNA concentration and degree of purity were estimated by reading in a spectrophotometer (NanoDropTM Lite, ThermoFischer Scientific).

For the PCR assay, primers LITSR 5'-CTGGATCATTTTCCGATG-3' and L5.8S 5'-TGATACCACTTATCGCACTT-3' were used, which amplify a product of 300 - 350 bp from the internal transcript spacer region of the ITS1 ribosomal gene (SSU rRNA and 5.8S rRNA) from *Leishmania* (El Tai *et al*., 2000). The reactions were conducted in a thermocycler (BIORAD T100™ Thermal Cycler), using the conditions described by Schonian *et al*. (2003). After amplification, the PCR products were subjected to electrophoresis in a 1% agarose gel, stained with ethidium bromide and visualized using a transilluminator.

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The climatic variables were obtained from the Agricultural Research and Rural Extension Company of Santa Catarina (Epagri, 2020) to assess the correlation between them, and the number of sandflies captured. For this purpose, Spearman's non-parametric correlation test was used. To evaluate the sex ratio inside and outside the home, Fisher's Exact test was used. The tests were performed with a significant level of 5% using the statistical software Jamovi.

Results

In all, 38 sandflies were collected throughout the sample period (Table 1) and discriminated according to species, sex, and location (indoor and outdoor). In the Fisher's Exact test, the sex ratio inside and outside the home was not statistically significant (P= 0.729).

Table 1. Species, sex, and location of captured sandflies.

Species	Females	Males	Hous[e]	Peri[b]	Total	
					n	%
Migonomyia migonei	14	11	7	18	25	65,8
Pintomyia fischeri	-	2	-	2	2	5,3
Pintomyia monticola	1	-	1	-	1	2,6
Psathyromyia serie lanei	3	-	2	1	3	7,9
Brumptomyia carvalheiroi	2	3	1	4	5	13,2
Brumptomyia sp.	2	-	-	2	2	5,3
Total	22	16	11	27	38	-
	57,89	42,11	28,95	71,05	-	100

[a]Household, [b]Peridomiciliary

As for the seasons, spring represented 52.63% of captures, followed by summer (31.57%), autumn (13.15%) and winter (2.63%). The months with the highest number of captures were October and January, both with 23.68%. In the Shapiro-Wilk test, sandflies did not present a normal distribution for the climatic variables (P= 0.044).

Figure 1 shows the number of sandflies captured in relation to environmental variables: temperature, relative humidity, and precipitation. Spearman's correlation test revealed a moderate positive correlation between temperature and the number of sandflies (P= 0.049 and correlation coefficient = 0.577). Precipitation and humidity showed no significant correlation (P= 0.366 and 0.645, respectively).

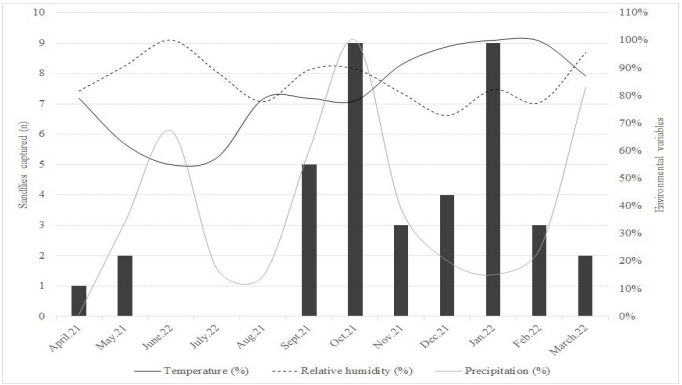

Fig. 1. Number of sandflies captured (bars) in relation to environmental variables: temperature, relative humidity, and precipitation (lines). To build the graph, the highest monthly value obtained from the environmental variable was considered 100% and the other values were calculated proportionally to this value.

As for the sampled areas, only four of the six areas were captured. The percentage of captured sandflies was 31.6%, 15.6%, 26.3%, 26.3%, 0% and 0% for areas A, B, C, D, E and F, respectively.

The DNA samples showed concentrations (≥11.9 ng/μL) and degree of purity (≥1.5) suitable for the PCR technique, however all samples were negative for *Leishmania* spp research.

Discussion

This survey provides a description of the sand fly fauna in the city of Concórdia, located in western Santa Catarina. Although the municipality is not endemic, there are records of imported cases of the disease (Santa Catarina, 2020). No sandflies positive for *Leishmania* spp. were identified, which corroborates the work carried out in five municipalities close to the researched region (Dias *et al*., 2022).

The entomological survey carried out by Board of Epidemiological Surveillance of Santa Catarina (DIVE-SC), carried out between 2005 and 2015, recorded the capture of the species *M. migonei* in Concórdia (DIVE, 2015). This was the most prevalent species in the present research, corresponding to 65.79% of collections. The species *P. serie lanei*, *Pi. Fischeri, Br. Carvalheiroi, Brumptomyia* sp. and *Pi. Monticola* are being reported for the first time in the municipality and the species *Br. Carvalheiroi* is being reported for the first time in the southern region of the country, which demonstrates the dispersion of these insects in the south and the importance of entomological monitoring in the region. Since the species *Br. Carvalheiroi* had its first description in 2007, in the state of São Paulo (Shimabukuro *et al*., 2007). The presence of the species *M. migonei* and *Pi. Fischeri* can be considered a risk factor due to its vectorial capacity (Galvis-Ovallos *et al*., 2017). In view of this, infected allochthonous animals that settle in the municipality can lead to a new focus of the disease. A suitable measure to be carried out by veterinarians working in the municipality is to guide tutors from endemic regions regarding the use of topical insecticides with repellent action in their dogs, to avoid the blood meal, in addition to requesting screening tests on the animals. Although the genus *Brumptomyia* spp. have wild behavior (Oliveira *et al*., 2003), the present study carried out the capture in peridomicile, in addition to a record in the household area, which suggests the adaptation of the species to the domestic environment.

When analyzing the seasonality of sandflies according to climatic variables, greater abundance was observed during and after months with greater precipitation, as well as greater abundance in the warmer months. In view of these results, it is possible to create future strategies of preventive measures, which can be directed to these periods of greater vector abundance, which occurred in the spring and summer seasons.

Conclusion

Among the six species captured, two have vector capacity, indicating a risk of a possible outbreak of *Leishmania*sis if infected allochthonous animals enter the municipality. Although the studied region has allochthonous cases of *Leishmania*sis, there were no sandflies positive for *Leishmania* spp., which indicates that the number of contaminated animals and vectors in the region is possibly directly related to the spread of the disease. However, the adoption of preventive measures is still valid for controlling the disease, such as carrying out screening tests and using topical insecticides with repellent action in animals from endemic areas. In addition, periodic entomological surveillance should be maintained, in addition to educational measures with the population.

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Conflict of interest

The authors declare that there is no conflict of interest.

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