

Contribution to the Sand Fly Fauna (Diptera: Phlebotominae) of Rio Grande do Sul, Brazil and *Leishmania (Viannia)* Infections

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American cutaneous leishmaniasis (ACL) is endemic in the State of Rio Grande do Sul, Brazil. As reports of nearby hospitals suggest, the Parque Estadual do Turvo seems to serve as a source for the disease. During three months from November 1996 to February 1997 we collected, in this park, 2,228 sand flies (10 Lutzomyia species and 2 Brumptomyia species). We applied the polymerase chain reaction to 920 females which belong to the following species: Lu. migonei, Lu. pessoai, Lu. fischeri, Lu. misionensis, Lu. lanei, Lu. neivai, Lu. shannoni, and Lu. monticola, in an attempt to verify natural infection by Leishmania (Viannia), the causative agent of ACL. Le. (Viannia) infections were demonstrated by DNA amplification from two Lu. pessoai and one Lu. misionensis female. Lu. pessoai have been found with leptomonas in the gut believed to be Le. (V.) braziliensis in other endemic areas of northeastern and southeastern Brazil. However, Lu. misionensis has never been found carrying a natural infection of Le. (Viannia).

Key words: sand flies - Phlebotominae - *Leishmania (Viannia)* - polymerase chain reaction - southern Brazil

American cutaneous leishmaniasis (ACL) is endemic in the State of Rio Grande do Sul, Brazil (Forattinni 1973). There are some notified cases of the disease (Lacerda 1994). Hospitals in the area confirmed some cases of cutaneous and mucocutaneous leishmaniasis every year. All patients infected with the disease originated from rural areas of the Parque Estadual do Turvo's marginal forest zone. The park seems to serve as a source for human *Leishmania* infection.

The disease does not seem to be a public health problem therefore the phlebotomine fauna and the dynamics of transmission in this area are still unknown. There is only one report on the biogeographical distribution of phlebotomine species in this area (Dias et al. 1997). Nevertheless, forest clearing and agricultural activities may result in an increase in the number of leishmaniasis cases in the area.

The purpose of the present study was to determine the phlebotomine species of certain areas in the park, and to identify the vector species and their infection rate with *Le. (Viannia)* using the polymerase chain reaction (PCR).

MATERIALS AND METHODS

Study area - The Parque Estadual do Turvo is located in the north of Rio Grande do Sul (27°20' - 27°10' S and 54°10' - 53°40' W). The region represents the most important primary rain forest in southern Brazil and corresponds to an untouched natural ecosystem. It has a wet and hot subtropical climate, with an annual rainfall greater than 1,900 mm. The study was conducted at the Salto Yucumã tourist lodge which sits on the edge of the forest, and is managed by a local family. At the lodge, domestic animals such as cattle, chicken, cats and dogs can be found.

Collection and identification of Phlebotominae - During three months (November 1996 to February 1997) sand flies were caught at weekly intervals. Three ecologically different areas at the lodge were examined: domicile, peridomicile, and forest. The domicile area corresponded to the inside and outside walls of the house, while the peridomicile area was delineated by the walls of the cattle stable and chicken pen. The collection area in the forest was located within a distance of 3 km from the lodge. Sand flies were caught with a manual aspirator in domicile and peridomicile areas, while in the forest, manual aspirators as well as a Shannon trap (Shannon 1939) were used. The specimens were stored in 70% ethylalcohol and brought to the laboratory in Tübingen, Germany for processing. Identification of the sand flies was carried out according to the classification schemes of Young and Duncan (1994) and Martins et al.

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(1978). Females were identified based on the shape of the spermathecae and the number of cibarial teeth and a portion was preserved on slides using Entellan medium. Males were identified by the shape of their genitalia and also preserved on slides using Entellan medium.

Infection rate of the collected sand flies with Le. (Viannia) – PCR was employed to verify if any of 920 females, which belonged to eight species were naturally infected by *Le. (Viannia)*, the causative agent of ACL. Two oligonucleotide primers (GAACGGGGTTTCTGTATGC and TACTCCCCGACATGCCTCTG) were employed to amplify a 70 bp sequence of the conserved region of *Le. (Viannia)* minicircle kinetoplast. *Le. (V.) braziliensis* MHOM/BR/75/M2903 served as positive control while *Le. mexicana* MNYC/BZ/62/M379 was used as the negative. The methods used to apply the PCR were adapted from Arevalo et al. (1993) with some modifications.

After morphological identification the females were immersed individually in 50 µl lysis buffer (Tris-HCl 1M pH 8.0, EDTA 0.5M pH 8.0, SDS 1%) containing Proteinase K and incubated at 65°C for 3 hr for DNA extraction. The PCR master mix was prepared with 5U/µl of Taq Polymerase, 2.5 mM from each of dATP, dCTP, dTTP, dGTP, 70 mM Tris-HCl 0.5M (pH 8.8), 17 mM (NH₄)₂SO₄, 0.1 mM β-Mercaptoethanol, 2 mM MgCl₂, and 1 µM of each oligonucleotide. For the amplification 5 µl of DNA from a pool of 10 females was added to 15 µl of the above-described solution.

The PCR master mix product was prepared shortly before the samples were transferred into the PCR machine, giving better results than using

a product that has been previously frozen. Amplified products were analyzed by electrophoresis at 65 V in NEEO (Roth) agarose gel (3%), stained with ethidium bromide and visualized under UV light.

RESULTS

Phlebotominae fauna - A total of 2,220 *Lutzomyia* specimens and eight *Brumptomyia* specimens were collected and identified. More sand flies were collected in the peridomicile area (1,306/58.6%) than in the domicile (648/29.1%) and forest areas (274/12.3%). We were able to identify 10 species from the *Lutzomyia* genus and two from the genus *Brumptomyia*. Regarding *Lutzomyia*, 18 specimens could not be identified (Table). The predominant species was *Lu. migonei* (domicile 47.8% and peridomicile 62.2%) followed by *Lu. pessoai*, *Lu. fischeri*, *Lu. misionensis*, *Lu. lanei*, *Lu. neivai*, *Lu. shannoni*, and *Lu. monticola*. All species are known to take blood meals on humans. In the forest, all of the above mentioned species were found, with *Lu. misionensis* as the predominant species (32.5%) followed by *Lu. monticola* (16.8%), *Lu. lanei* (14.9%) and *Lu. shannoni* (14.9%). In addition, the species *Lu. correalimai*, *Lu. schreiberi*, as well as *Brumptomyia pinto* and *B. nitzulescui* were found in the forest but these species did not occur in the other two areas. The proportion of males was highest in the domicile and peridomicile areas (60.8%), the females dominated in the forest (85%).

Infection rate - A total of 920 females was analyzed for *Le. (Viannia)* infection. The species were: *Lu. migonei* (200), *Lu. pessoai* (360), *Lu. fischeri*

TABLE

Distribution of *Lutzomyia* and *Brumptomyia* species in Parque Estadual doTurvo from November 1996 to February 1997

Species	Domicile		Peridomicile		Forest		Total	%
	Female	Male	Female	Male	Female	Male		
<i>Lu. migonei</i>	106	204	131	681	5	7	1,134	50.9
<i>Lu. pessoai</i>	154	5	196	20	14	-	389	17.5
<i>Lu. lanei</i>	36	46	63	26	22	19	212	9.5
<i>Lu. neivai</i>	13	48	41	67	2	2	173	7.8
<i>Lu. misionensis</i>	9	-	32	-	89	-	130	5.8
<i>Lu. shannoni</i>	8	-	27	-	41	-	76	3.4
<i>Lu. monticola</i>	-	-	18	-	45	1	64	2.9
<i>Lu. fischeri</i>	17	-	-	-	3	-	20	0.9
<i>Lu. schreiberi</i>	-	-	-	-	-	2	2	0.1
<i>Lu. correalimai</i>	-	-	-	-	-	2	2	0.1
<i>Lu. spp.</i>	2	-	4	-	12	-	18	0.8
<i>B. pinto</i>	-	-	-	-	-	4	4	0.2
<i>B. nitzulescui</i>	-	-	-	-	-	4	4	0.2
Total	345	303	512	794	233	41	2,228	100

(10), *Lu. misionensis* (120), *Lu. lanei* (90), *Lu. neivai* (40), *Lu. shannoni* (50), and *Lu. monticola* (50). Only two (0.6%) *Lu. pessoai* females and one (0.8%) *Lu. misionensis* female were found positive for 70bp of *Le. (Viannia)* DNA. *Lu. pessoai* infected females were collected in the domicile area, and *Lu. misionensis* in the forest (Figure).

DISCUSSION

Five species that were isolated in the park, *Lu. migonei*, *Lu. pessoai*, *Lu. fischeri*, *Lu. neivai* and *Lu. shannoni*, are suspected to be vectors of *Le. (Viannia)* elsewhere in Brazil. They have been found infected with promastigotes in foci of tegumentary leishmaniasis (Young & Duncan 1994 and others).

Two species, *Lu. schreiberi* and *B. pintoii*, have never been isolated in the area before.

Lu. misionensis was found to be the predominant species in the forest, while *Lu. migonei* and *Lu. pessoai* were the predominant species in domicile and peridomicile areas. In contrast, Dias et al. (1997), who studied the sand fly fauna in the park during January to February 1986, identified *Lu. fischeri* and *Lu. intermedia* as the prevalent species in the forest.

Only *Lu. pessoai* and *Lu. misionensis* were found to be infected with *Le. (Viannia)*. In the case of *Lu. misionensis* this is the first report of a natural infection with *Le. (Viannia)*. *Lu. pessoai* is considered as a probable vector of *Le. (Viannia)* in

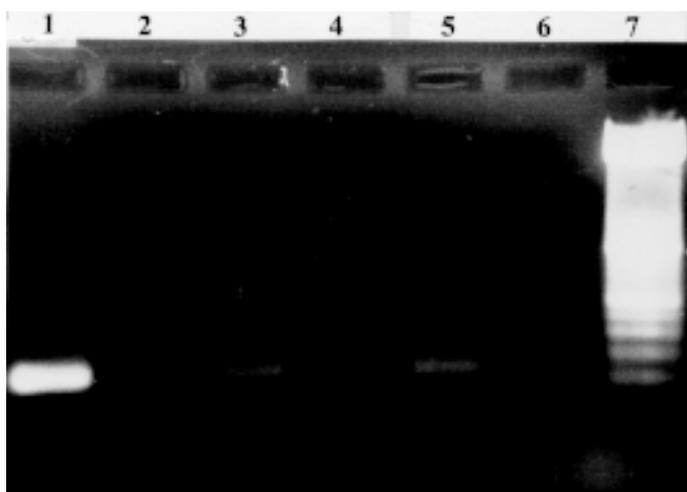
other endemic areas of northeastern and southeastern Brazil.

Infected females were identified in the domicile area and the forest. The infection rate, however, was very low, being 0.6% for *Lu. pessoai* and 0.8% for *Lu. misionensis* respectively. Thus, these two vector species do not appear to play an important role in the transmission of leishmaniasis infection to humans. The infection rate of the total of 920 females investigated was only 0.3%, and in fact the infection of humans with leishmaniasis is rare in the area. In comparison, in Mexico, in the the State of Campeche, an endemic ACL area, Rebollar-Télez et al. (1996) found an infection rate of 2.8% by dissecting 1,288 *Lutzomyia* females.

It is important that further collections of sand flies be made in different ecological areas of the park and in different seasons of the year to obtain more information on the significance of ACL and the dynamics of its transmission in and around the park.

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Polymerase chain reaction based detection of natural infection of Phlebotominae with *Leishmania (Viannia)*. 1: positive control (DNA *Leishmania (V.) braziliensis*); 2: negative control (DNA *Le. mexicana*); 3-4: *Lutzomyia pessoai*; 5: *Lu. misionensis*, 6: negative control (without DNA); 7: marker.

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