

Phlebotominae Sand Flies in Paraguay. Abundance Distribution in the Southeastern Region

Oscar D Salomón⁺, Gustavo C Rossi*, Blanca Cousiño**, Gustavo R Spinelli***, Antonieta Rojas de Arias**, Delfin G López del Puerto**, Arnaldo J Ortiz**

Centro Nacional de Diagnóstico e Investigación en Endemo-Epidemias, Av. Paseo Colón 568, 1063, Buenos Aires, Argentina
*Centro de Estudios Parasitológicos y de Vectores, La Plata, Argentina **Servicio Nacional de Erradicación del Paludismo-IICS, UNA, Asunción, Paraguay ***Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, La Plata, Argentina

From September 1993 to August 2001, 7,190 phlebotomine were collected with CDC light trap in an endemic area for human leishmaniasis, in the departments of Misiones and Itapúa, Paraguay. Eleven species were identified: *Lutzomyia neivai* (93.7%), *L. whitmani* (4.1%), and *L. fischeri*, *L. shannoni*, *L. migonei*, *L. misionensis*, *L. cortelezzi*, *L. pessoai*, *L. alphabetica*, *Brumptomyia avellari* and *B. guimaraesi* (less than 1%). The last three species are new records for the country. The biodiversity and phlebotomine abundance were associated with the proximity to primary forest or gallery forest, but *L. neivai* was also found in peridomestic periurban environment. *L. neivai* was found throughout the year, and showed a period of higher activity from September to April (spring to fall) with a unimodal or bimodal pattern in relation to the annual rainy peaks during the summer. Background literature about phlebotomine from Paraguay has been reviewed.

Key words: Phlebotominae - *Lutzomyia* - vector ecology - Paraguay

The first reported outbreak of tegumentary leishmaniasis in Paraguay was in 1934 due to the Chaco war (González & Oliveira y Silva 1939, González & Arce Queirolo 1955b). Boggino and Mass (1945) recorded the first autochthonous case of visceral leishmaniasis. However, leishmaniasis had been already known in Paraguay for many decades (Migone 1913a,b, González & Arce Queirolo 1955a). *Leishmania (Viannia) braziliensis* was isolated from cutaneous cases, while visceral and scarce cutaneous diffuse cases were ascribed to *L. (L.) chagasi* and *L. (L.) amazonensis* respectively (Grimaldi Jr et al. 1989). The incidence of tegumentary leishmaniasis ranged from 110 to 200 cases/year, but 900 to 1,600 cases were reported in 1982, 1985-1987, and 1991 periods (Arias et al. 1996). An outbreak of canine visceral leishmaniasis took place close to Asunción, the capital city in 1997 (Samudio et al. 1997, Canese 2000). Thus, leishmaniasis is an increasing public health problem in Paraguay.

Phlebotominae sand flies were reported by sporadic captures mostly in connection with leishmaniasis' foci. Seventeen species were identified, and *Lutzomyia whitmani* was found naturally infected by *L. (V.) braziliensis* (França 1920, Duret 1950, Del Ponte 1960, Martins et al. 1978, Rojas de Arias & Ayala 1989, Inchausti et al. 1990, Inchausti & Rojas de Arias 1991, Hashiguchi et al. 1992, Young & Duncan 1994, Galati 2000).

This study is the first report of serial Phlebotominae collections in Paraguay. It was carried out in the departments of Misiones and Itapúa, between 1993 and 2001. Previous and current results are discussed in order to understand sand fly abundance associations, with the purpose of contributing to the future design of surveillance strategies based on entomological data.

MATERIALS AND METHODS

Phlebotominae were collected at ten sites along the Paraná river, in the departments of Misiones (Coratei) and Itapúa (Ayolas, San Cosme-Damián, Carmen del Paraná, Quiteria, Aguapey, Encarnación, Bella Vista, Capitán Meza, Mayor Otaño), Paraguay (Fig. 1). Two CDC miniature light traps were used in parallel per site, both with CO₂ (500 ml/h), placed 1.5 m above the ground. Captures were carried out monthly during 24 h, from September 1993 to August 2001. Environmental characteristics of the sites are described in the discussion. Phlebotominae were processed and identified according to Young and Duncan (1994) and Marcondes (1996). Meteorological data were obtained from EBY Ituzaingó (Argentina). Fisher or χ^2 tests were used for statistical analysis. All statistical tests were considered significant with 0.01 or less associated probabilities.

RESULTS

Considering 332 days of capture (141 positive) 7,190 specimens were collected (Table I). There were 8 out of the 17 species previously recorded for Paraguay, besides *L. alphabetica*, *Brumptomyia avellari* and *B. guimaraesi*, which represent new records for the country (Table II). The ecological environment of Ayolas, San Cosme-Damián, Encarnación, Quiteria, Mayor Otaño, Capitán Meza, and Carmen del Paraná are similar and their phlebotomine abundance was low (82.9% of collections with five or less sand flies), so the results of these sites were grouped (Table I). Encarnación had a maximum cap-

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OD Salomón and GR Spinelli are members of "Carrera del Investigador Científico", Conicet.

+ Corresponding author. Fax: +54-11-4331.2536. E-mail: danielsalomon@hotmail.com

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Fig.1: Phlebotominae collection sites with CDC light trap from 1993 to 2001, Paraguay.

ture of 16 phlebotomine/day and 64 *L. neivai* in the overall collection. No phlebotomine was obtained at the Carmen del Paraná site.

On the other hand, 97.1% of the sand flies were collected at the Coratei, Aguapey and Bella Vista sites. The total number of days when traps have been placed did not differ among the three stations, but the days with one or more sand flies collected were significantly different between Coratei (33.3%) and Bella Vista (71.4%), while Aguapey showed an in-between figure (49.2%).

The predominant species was *L. neivai* in all the sites of capture. *L. neivai* Williams means in the three sites with largest collections were 0.66 in Coratei, 3.59 in Aguapey and 5.23 in Bella Vista. However, *L. neivai* female: male sex ratio in Bella Vista and Aguapey ranges from 1.4 to 1.6, significantly different from the 2.1 ratio in Coratei. The relative abundance of species was different among the sites (Table I), *L. neivai* were collected with other species in Coratei, Aguapey and Bella Vista 4, 3 and 19 times respectively. In Bella Vista the lower relative abundance obtained in a single capture for *L. neivai* was 72.7%, and it was found together with *L. whitmani* (23.5%), *L. fisheri* (3.5%) and *L. migonei* (0.3%). The four species with the highest captures besides *L. neivai* considering the overall collection were *L. whitmani*, *L. fisheri*, *L. shannoni* and *L. migonei*, their female: male sex ratios

were 1.5, 1.5, 0.1 and 0.5, and they were found in 10, 9, 2 and 9 times respectively.

L. neivai was collected throughout the year, but the highest captures were obtained between September and February. In Coratei and Aguapey a significant peak was observed in the 1999-2000 season (maximum November). In Bella Vista there were two peaks, the 1999-2000 peak (maximums December and February), but also a higher one during the 2000-2001 season (maximums October and February) (Fig. 2). *L. neivai* highest collection in a single capture were 258,666 and 922 sand flies for Coratei, Aguapey and Bella Vista respectively (ratio 1: 2.3: 3.6). *L. neivai* collections from September to February during the year with highest peaks were 358, 1,670 and 2,007 sand flies (ratio 1: 4.7: 5.6). The other species were usually found together with *L. neivai* peaks.

DISCUSSION

Phlebotomine species diversity and relative abundance were similar among the collections in Misiones and Itapúa, Paraguay, and those reported previously from the Argentinean side of the common border (Salomón et al. 2002). In the latter, scarce captures of *L. longipalpis* were obtained (Salomón et al. 2001b), while *L. alphabetica* and *L. cortezzi* were collected after the cited report (unpublished data).

TABLE I

Phlebotominae species and species relative abundance (%), collected with CDC light trap in Coratei, Aguapey, Bella Vista, and the remaining capture sites (Others), Paraguay, September 1993-February 2001

| Species | Coratei (%) | Aguapey (%) | Bella Vista (%) | Others | Total (%) |
|-----------------------|-------------|-------------|-----------------|------------|-------------|
| <i>L. neivai</i> | 470 (97.3) | 2527 (99.7) | 3541 (89.3) | 198 (96.1) | 6736 (93.7) |
| <i>L. pessoai</i> | 0 | 0 | 8 (0.2) | 0 | 8 (0.1) |
| <i>L. fischeri</i> | 2 (0.5) | 0 | 52 (1.3) | 0 | 54 (0.7) |
| <i>L. migonei</i> | 2 (0.5) | 0 | 24 (0.6) | 4 (2.0) | 30 (0.4) |
| <i>L. shannoni</i> | 0 | 0 | 32 (0.8) | 0 | 32 (0.4) |
| <i>L. misionensis</i> | 0 | 0 | 4 (0.1) | 0 | 4 (< 0.1) |
| <i>L. whitmani</i> | 2 (0.5) | 6 (0.2) | 287 (7.2) | 2 (1.0) | 297 (4.1) |
| <i>L. cortelezzi</i> | 0 | 2 (< 0.1) | 2 (< 0.1) | 1 (0.5) | 5 (< 0.1) |
| <i>L. alphabetica</i> | 0 | 0 | 3 (< 0.1) | 0 | 3 (< 0.1) |
| <i>B. avellari</i> | 2 (0.5) | 0 | 0 | 1 (0.5) | 3 (< 0.1) |
| <i>B. guimaraesi</i> | 5 (1.3) | 0 | 13 (0.3) | 0 | 18 (0.2) |
| Total | 483 (100) | 2535 (100) | 3966 (100) | 206 (100) | 7190 (100) |

L.: *Lutzomyia*; *B.*: *Brumptomyia*

The Bella Vista capture site was located at the dense forest near the Paraná river, the Coratei site was at modified vegetation close to residual patches of the Paraná gallery forest, and the Aguapey site was at the residual forest, 300 m from a water stream and 30 m from a house. Therefore, phlebotomine density and collection frequency correlated to the density of the vegetation coverage. The species diversity was positively associated with the vicinity of primary forest, as 10 out of the 11 species identified were in Bella Vista. Bella Vista had also the lowest *L. neivai* female: male ratio probably due to the closeness to the resting/breeding places. The low captures obtained in highly modified environments such as those grouped

herein as “others” showed that sand flies might reach peridomestic habitats. Thus, the risk of human-phlebotomine effective contact is possible even in the vicinity of cities such as Encarnación. *L. neivai*, *L. whitmani*, and *L. migonei* were associated with peridomestic tegumentary leishmaniasis foci in Argentina (Salomón et al. 2001a,c-e, 2002).

L. neivai seasonal abundance showed activity from August-September to April-March (spring to fall) with a single peak or a bimodal pattern. Consistently, phlebotomine populations usually showed the highest density peaks during the warm-humid months, and the lowest in the cold-dry season, both in Brazil and Argen-

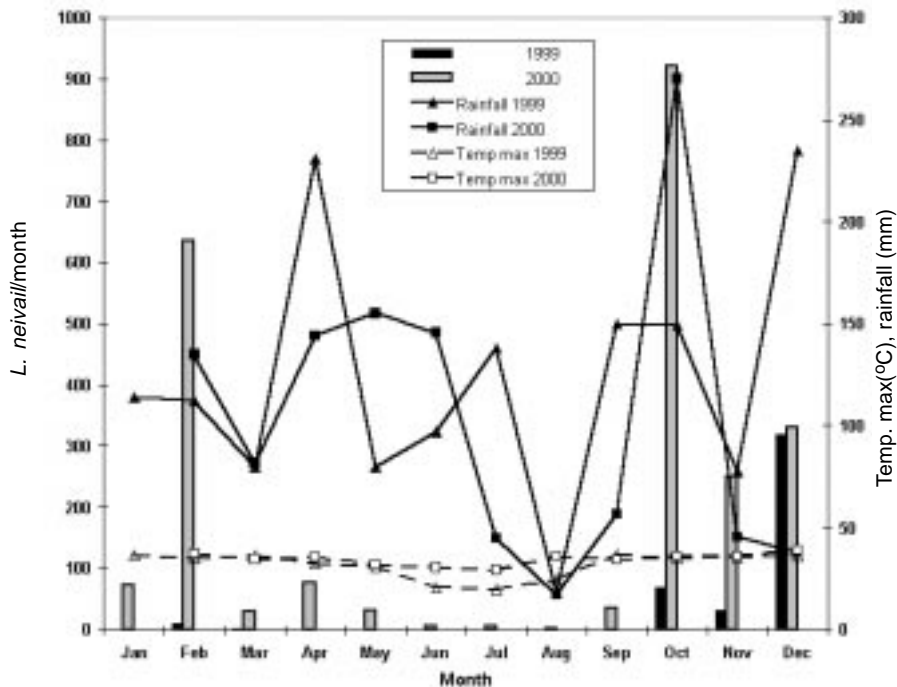


Fig. 2: *Lutzomyia neivai* abundance, maxima temperatures (°C) and rainfall (mm) by month during the year 1999 and 2000. Bella Vista, Itapúa department, Paraguay.

tina (Aguiar & Soucasaux 1984, Gomes & Galati 1987, Salomón et al. 2002), although the density is not always correlated with the rainfall and the temperature (Condino et al. 1998). The Bella Vista 2000-2001 peak may be due to the fact that sand flies were collected there during the winter of year 2000, captures not observed in other sites or years. Phlebotomine abundance peaks were associated with rainfall peaks. The 1999-2001 peaks were also observed in the captures at the Argentinean equivalent sites, and it was attributed to favorable climatic conditions (Salomón et al. 2001b, 2002).

Paraguay is a landlocked country ecologically divided in three main regions: (1) the densely wooded hills of the east and the Paraná river basin, with over 2,000 mm of rainfall per year; (2) the grassy plains, woody patches and marshes of the center and the Paraguay river basin, with 1600 mm of rainfall per year; (3) the dry forest and thorny scrub of the large arid region of the west (Chaco), with less than 800 mm of rainfall per year. Data from the Chaco region are scarce and in connection with the southern marshes of the Pilcomayo river basin. West to 61°W, 150 cases of tegumentary leishmaniasis were reported in 1934 during military activities carried out in the woods (Cañada del Carmen) (González et al. 1939, González & Arce Queirolo 1955b).

The central region involves 20% of the territory and 80% of the country population and the highest number of leishmaniasis cases. Phlebotominae of this region were reported in the departments of San Pedro, Guaira, Caazapá, Caaguazú and Central (Table II). *L. longipalpis* was cap-

tured recently during an outbreak of canine visceral leishmaniasis with Shannon traps during the evening (Samudio et al. 1997).

The eastern region belongs to the Amazonian domain (departments of Itapúa, Misiones, and Alto Paraná) and showed the greatest phlebotomine biodiversity. *L. alphabetica*, *B. avellari* and *B. guimaraesi* reported here are the first records for the country. Between 1984 and 1985 an outbreak occurred in a recently settled locality (Tavapy II) in Alto Paraná, three man-biting sand fly species were identified *L. intermedia s.l.* (78.6%), *L. migonei* (12.8%) and *L. whitmani* (5.6%) (Rojas de Arias & Ayala 1989). Outbreaks of tegumentary leishmaniasis were also reported in recently settled communities at Limoy, Alto Paraná (Hashiguchi et al. 1991). At the Argentinean border an outbreak was also reported in 1997-1998 (Salomon et al. 2001d).

L. neivai (*L. intermedia* complex) was the prevalent species in all the sites sampled in the departments of Itapúa and Misiones. *L. intermedia* complex was recently differentiated in two species, *L. intermedia sensu strictu* and *L. neivai* (Marcondes 1996, Marcondes et al. 1998, Marcondes & Borges 2000). The previous captures are referred here as *L. intermedia s.l.*, although the specimens captured in Paraguay could be attributed to *L. neivai* considering the latitude and longitude where they were collected. *L. intermedia s.l.* and *L. whitmani* were both incriminated as vectors of *L. braziliensis*. *L. whitmani* is the most important species in residual forests or historical collections within the forest, while the relative abun-

TABLE II
Phlebotominae species by department or region (Chaco) reported in Paraguay ^a

| | San Pedro | Guaira | Caaguazú | Caazapá | Itapúa | Misiones | Alto Paraná | Central | Amambay | Chaco |
|---|-----------|--------|----------|---------|--------|----------|-------------|---------|---------|-------|
| <i>Lutzomyia alphabetica</i> | | | | | x | | | | | |
| <i>Lutzomyia aragaoi</i> | x | | | | | | | | | |
| <i>Lutzomyia cortelezzi</i> | | | | | x | | x | | | |
| <i>Lutzomyia fischeri</i> | x | | x | x | x | x | | | | |
| <i>Lutzomyia intermedia s.l.</i> ^b | x | x | x | x | x | x | x | | | x |
| <i>Lutzomyia lanei</i> | x | | x | | | | | | | |
| <i>Lutzomyia longipalpis</i> | x | x | | | | | | x | | |
| <i>Lutzomyia longispina</i> | | | | | | | x | | | |
| <i>Lutzomyia lutziana</i> | x | | | | | | | | | |
| <i>Lutzomyia migonei</i> | x | | x | x | x | x | x | x | | x |
| <i>Lutzomyia misionensis</i> | | | | | x | | | x | | |
| <i>Lutzomyia monticola</i> | x | | x | | | | | | | |
| <i>Lutzomyia pessoai</i> | x | | x | | x | | | | | x |
| <i>Lutzomyia sallesi</i> | x | | | | | | | | | |
| <i>Lutzomyia shannoni</i> | | x | x | x | x | | x | | x | |
| <i>Lutzomyia teratodes</i> | | | | | | | | | | |
| <i>Lutzomyia walkeri</i> | | | | | | | x | | x | |
| <i>Lutzomyia whitmani</i> | x | x | x | x | x | x | x | | | |
| <i>Brumptomyia avellari</i> | | | | | x | x | | | | |
| <i>Brumptomyia guimaraesi</i> | | | | | x | x | | | | |

^a: in the literature there are two more species that requires confirmation: *Lutzomyia correlimai* (Galati 2000), and *Lutzomyia teratodes* (Young & Duncan 1994); ^b: *L. intermedia sensu lato/L. neivai*

Sources: Duret 1950, Martins et al. 1978, del Ponte 1960, Hashiguchi et al. 1991, 1992, Young & Duncan 1994, this report.

dance of *L. intermedia s.l.* was higher in peridomestic habitats in Brazil, in the state of Rio de Janeiro (Rangel et al. 1984, 1990, Oliveira-Neto et al. 2000, Souza et al. 2001), in the state of São Paulo (Forattini et al. 1972, Gomes et al. 1990, Gomes 1994, Tolezano 1994, Stolf et al. 1993, Casanova 2001) and in the state of Paraná (Teodoro & Kuhl 1997), and in Argentina (Salomón 2001a-e, 2002). However, in a eastern primary forest reserve in the state of Paraná, Brazil, *L. ayrozai* and *L. geniculata* were the predominant species (Marcondes et al. 2001). On the other hand, *L. whitmani* was the prevalent species in peridomestic habitats of the state of Paraná, Brazil (Luz et al. 2000), and in human bait collections in Alto Paraná department, Paraguay (Hashiguchi et al. 1992), although in this department, in the Monday river, Rojas de Arias and Ayala (1989) found a higher proportion of *L. intermedia s.l.* biting humans near dwellings.

In conclusion, in the Southeastern region of Paraguay 11 phlebotomine species were identified. *L. neivai* was the prevalent species followed by *L. whitmani*. The proportion of the latter and the size of the overall collections increased in the vicinity of the residual primary forest or the secondary gallery forest. The sand fly population had peaks related to the warm-rainy months, when suspected vectors of *L. braziliensis* were found in peridomestic habitats and periurban collections. The species biodiversity is greater in this region in other areas of Paraguay. Any surveillance strategy or control design in the area should take into account the different geographical lineage of sand fly complexes as that of *L. intermedia-L. neivai*. That lineage may have different degrees of peridomestic adaptation, and therefore different *Leishmania* transmission associated risk.

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