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DIVERSITY AND SPECIES COMPOSITION OF SAND FLIES (DIPTERA: PSYCHODIDAE) IN A VENEZUELAN URBAN FOCUS OF CUTANEOUS LEISHMANIASIS

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ABSTRACT. The present study examined the spatial and temporal abundance and diversity of phlebotomine sand flies in an area of Venezuela that is an ancient focus of leishmaniasis. The study was conducted in 6 stations in urban localities in Trujillo City, located in northwestern Venezuela (9°22′24″N, 70°26′08″W), which is located in a mountain range in the Andean ecoregion (altitude = 600–1,010 m). During 1995–99, entomological surveys were conducted after and before the rainy season. Shannon light traps were operated from 1800 to 2000 h in peridomestic site trap locations. Twelve species were captured, and *Lutzomyia youngi, L. ovallesi, L. scorzai. L. gomezi, L. lichyi,* and *L. shannoni* occurred at all localities in each year. The abundance of these species showed low variation over time but high variation between localities. The Sørensen similarity index, used to compare diversity between years within each locality, ranged from 0.60 at Carmona to 0.84 at La Hacienda. Sand fly communities exhibited annual variation in species richness and diversity. Variations were affected more by changes in species abundance than by changes in species composition. *Lutzomyia ovallesi, L. lichyi,* and *L. scorzai* had the highest coefficient of variation between years (63, 38, and 23%, respectively).

KEY WORDS Sand fly, Psychodidae, leishmaniasis

INTRODUCTION

In endemic areas, human cutaneous leishmaniasis occurs in foci that have both a natural source of infection (reservoirs) and vector sand flies. Human cases are commonly rural or sylvan, although very often they are reported close to human dwellings (Reithinger and Davis 1999). Existence of these interactions in urban conditions, and local transmission, is increasingly important (Desjeux 2002). Understanding urban environmental changes and phlebotomine vectors is necessary to design appropriate disease prevention and control strategies (Campbell-Lendrum et al. 2001).

Cutaneous leishmaniasis in the New World is traditionally considered as a sylvatic disease, with people becoming infected when they visit forested areas (WHO 1991). Rapidly changing environmental conditions in many tropical regions have an enormous influence on vector populations and consequently on disease transmission.

Trujillo (48,000 inhabitants), a city in the Venezuelan Andes, had incidences for cutaneous leishmaniasis between 1994 and 1997 of 10.2, 9.1, 9.4, and 5.1 cases per thousand, respectively (Scorza et al. 1999). In this study, we examined spatial and temporal abundance and diversity of phlebotomine sand flies. Leishmaniasis has been known for almost 60 years in this area (Tálamo 1946).

We stratified 86 localities according to transmission persistence. This was estimated as the maximum number of consecutive months with cases, as a mode for identifying places with active transmission. We related these locations to altitude, number of sand flies captured within a 200-m radius of human dwellings, parous proportion, and number of inhabitants and persistence of leishmania transmission.

MATERIALS AND METHODS

Study area: Collection sites included 6 urban sectors in Trujillo City in northwestern Venezuela (9°22′24″N, 70°26′08″W), in the Andean ecoregion, with altitudes from 600 to 1,010 m.

Criteria used for selection of these localities were cutaneous leishmaniasis transmission detected as new cases among children under 12 years old and incidences per thousand from 1994 to 1997, information from previous surveys of sand fly vectors (Mogollón et al. 1977, Márquez and Scorza 1984, Rojas and Scorza 1986, Scorza 1989), differences in ecological features such as vegetation type and height, and presence of risk factors for *Leishmania (Viannia) braziliensis* (Viannia) (Guevara et al. 1993).

Mean annual rainfall of 800 mm was confirmed by using data from a meteorological station located at the Centro "José W. Torrealba," Núcleo Universitario "Rafael Rangel," Trujillo. Two rainy seasons occurred, from April to May and from August to September (75% of the rainfall) for the study period (1995–2000). Vegetation corresponds to premontane dry forest (bs-PM) in the Holdridge classification (Ewel et al. 1979).

We assumed that each locality was relatively iso-

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Table 1. Geographical distribution of cutaneous leishmaniasis cases with prevalence and transmission in urban localities (2000).

Station	Urban locality[1]	Altitudinal range[2]	No. inhabitants	No. cases	Prevalence (%)	Transmission
La Barranca	9/21	600–690	2,373	76	3.20	Medium
Villita	6/7	720–800	1,943	66	3.39	Medium
Numa Quevedo	14/25	760–800	15,278	259	1.69	Lower
San Jacinto	14/18	810–825	13,460	681	5.05	High
Carmona/La Hacienda	11/15	830–1,010	4,123	246	5.96	High
Total	54/86	600–1,010	37,177	1,328	3.57	Medium

[1] Locality with cases/total localities in area.
[2] Meters above sea level.

lated, because the distance between any pair of these is >1 km and is beyond the sand fly dispersal range (Young and Arias 1992).

Sampling methods: Entomological surveys were made before and after rainy seasons for 5 years. Collections were made at 2-wk intervals by using 2 Shannon light traps set 0.5 m above the ground and 100 m from houses. Traps were operated from 1800 to 2000 h. Trap locations were constant during the study. Sand flies were collected by aspirator (Maroli et al. 1997) and transported to the laboratory under constant conditions of temperature (21°C) and relative humidity (75%).

Sand flies were dissected to assess the parous condition (Márquez and Scorza 1984). Criteria for identification followed those of Young and Duncan (1994).

Epidemiological data: Leishmaniasis cases were diagnosed and treated in the Centro "José W. Torrealba," Núcleo Universitario "Rafael Rangel," Universidad de Los Andes. A longitudinal study of leishmaniasis incidence was made for relating with rainfall regime. The maximum number of consecutive months in a locality with leishmania cases was calculated from monthly leishmaniasis incidence data by using cumulative data from 1982 to 1999.

The monthly relationship of sand fly abundance and cutaneous leishmaniasis incidence was calculated by linear regression, as the independent variable (sand fly abundance) and dependent variable (number of new cases/locality/year).

Data entry and analysis: An altitude and spatial location were entered by using data from a global positioning system Garmin™ II tracker (Magellan Systems Corporation, San Dimas, CA). Sand fly species composition, abundance, β diversity, and parity also were entered. To test the hypothesis that *Lutzomyia youngi* (Feliciangeli & Murillo) was the most abundant species (as previous surveys suggested), a test for equality between 2 proportions from the same group with mutually exclusive categories was conducted. For each locality, the numbers of individuals (by species) were expressed as a proportion of the total number collected (all species). These were compared in a pairwise fashion by using a test for proportions: % of ♀♀ parous/total dissected = proportion of ♀♀ parous and % ♀♀/total capture × station = proportion of species A, B, and so on. To test the similarity, richness and β diversity among stations from the 6 localities were calculated by the Sørensen similarity index (SSI), which was calculated as:

$$Cn = \frac{2jn}{(an + bn)} = \frac{\sum \text{lower abundant common species}}{\sum \text{species in locality a} + \text{species in locality b}}.$$

This index will be equal to 1 when both localities share exactly the same species and number of individuals per species, and will be equal to 0 if no common species occur. This index combines richness and abundance, and is dependent on abundance (Southwood 1978).

The Czekanowski similarity index (CSI) was calculated as:

$$Cs = \frac{2j}{(a + b)} = [2(\text{no. species common in locality a} + \text{locality b})]/(\text{no. species in locality a} + \text{no. species in locality b}).$$

This index was used to compare species richness between localities and years and is independent of the number of individuals captured per species.

Cross-correlation analyses were applied to abundance and precipitation time series for female *L. youngi* and *L. ovallesi* (Ortiz). The data for sand fly abundance results of all surveys were converted to the number of female sand flies per locality per year.

RESULTS

Cutaneous leishmaniasis was widely distributed (in 54 of 86 localities), with prevalence rates declining in the following regions: Carmona-La Hacienda, San Jacinto, Villita, La Barranca, and Numa Quevedo (Table 1). After 5 years and about 160 h of sand fly collections in the 6 stations, sand fly species richness differed by station. We identified

Table 2. Surveillance of sand fly (*Lutzomyia*) populations in urban conditions in Trujillo, Venezuela.

Station	*L. youngi*		*L. ovallesi*		*L. shannoni*		*L. lichyi*		*L. scorzai*	
	C[1]	P[2]	C	P	C	P	C	P	C	P
Carmona	1,872	4,100	1,420	280	2	0	23	3	745	35
Hacienda	6,200	1,700	1,380	150	1	0	2	0	293	49
Numa Quevedo	500	300	100	30	0	0	1	1	60	10
La Barranca	1,000	420	250	93	1	1	7	3	120	13
San Jacinto	12,100	3,410	4,200	1,920	2	0	0	0	232	50
Villita	25,870	6,670	7,690	2,755	7	2	18	6	190	10
Total	64,390	16,600	15,040	5,228	13	3	51	13	1,640	167
Parous proportion	0.0312		0.0420		0.0279		0.0308		0.0474	

[1] C, total capture by corrected values: no. of captured sand flies by no. species/capture effort (no. traps × no. capture × no. of month × no. of night traps).

[2] P, total parous.

12 species among 102,962 sand flies captured (Table 2).

Distribution of species according to abundance and diversity

The captured sand fly species ranked according to presence in the place of collection as common, occasional, or rare. *Lutzomyia youngi, L. ovallesi, L. scorzai* (Ortiz), *L. gomezi* (Nitzulescu), *L. lichyi* (Floch & Abonnenc), and *L. shannoni* (Dyar) were common and occurred at all localities every year (Table 2).

Lutzomyia walkeri (Newstead), *L. migonei* (França), *L. erwindonaldoi* (Ortiz), and *L. hermandezi* (Ortiz) were occasional, and present only in 4 stations (Hacienda, Carmona, San Jacinto, and Villita) at least on 1 occasion. Other species such as *L. trinidadensis* and *Lutzomyia* sp. were rare and were not caught in all years in the same locality (Numa Quevedo, Hacienda, and Carmona). *Lutzomyia youngi* was the most abundant species collected in all localities (62.5%) and predominated over all other collected species regardless of the station or locality. *Lutzomyia ovallesi* was the next predominant species (14.6%). In general, the abundance of these species showed low variation among years, but high variation between localities.

During 1995, the most similar communities, based upon the β diversity index, were Carmona, La Hacienda, and Villita (CSI = 0.9). The most diverse communities were La Barranca and Numa Quevedo (CSI = 0.65). For the years 1996 and 1997, all localities were uniform (CSI = 0.5). The statistical analysis for 1998 and 1999 showed a significant variation ($P < 0.05$) in species richness (number of species) between years within each locality. The SSI also was used to compare diversity between years within each locality, and ranged from 0.60 at Carmona to 0.84 at La Hacienda.

Sand fly communities showed annual variation in species richness and diversity, but such variations were affected more by changes in abundance than changes in species composition. *Lutzomyia ovallesi, L. lichyi,* and *L. scorzai* had the highest coefficient of variation between the years 1998 and 1999 (63% and 23%, respectively).

The number of specimens of *L. youngi, L. ovallesi,* and *L. scorzai* were significantly higher after rainfall periods and lower before rain periods (Fig. 1). Linear regression analyses of total sand fly abundance on precipitation were not significant for precipitation during the 5-year period ($r = 0.39$, $P < 0.005$). Analysis of sand fly abundance after and before precipitation showed a high correlation coefficient for *L. youngi* ($r = 0.714$, $P < 0.0001$) and *L. ovallesi* ($r = 0.743$). Cross-correlation analyses between the percentage of sand flies and cutaneous leishmaniasis cases were significant ($r = 0.4655$, SE = 0.118).

Distribution of species according to incidence and persistence of leishmaniasis

Considerable numbers of parous female *L. youngi* and *L. ovallesi* in all localities were correlated with incidence of leishmaniasis cases by locality. Sand fly species have an associated distribution in all localities and altitude did not greatly influence their urban distribution (Table 2). Three of 12 species (*L. youngi, L. ovallesi,* and *L. scorzai*) occurred simultaneously at all stations, and the proportions of parous female sand flies from total samples collected were 3.1%, 4.2%, and 4.7%, respectively (Table 2).

Anthropophilic sand fly species such as *L. youngi, L. ovallesi, L. shannoni, L. lichyi,* and *L. scorzai* may occur in different habitats, including indoors. In this study we caught these species in peridomestic habitats associated with humans and domestic animals.

DISCUSSION

Trujillo City has long experienced leishmaniasis cases. Most recently (Scorza 1985, Scorza and Rojas 2002) cases often have been acquired in or near homes, as evidenced by the high frequency of women and children or family groups infected with the disease, (Rojas and Scorza 1986, Morales and

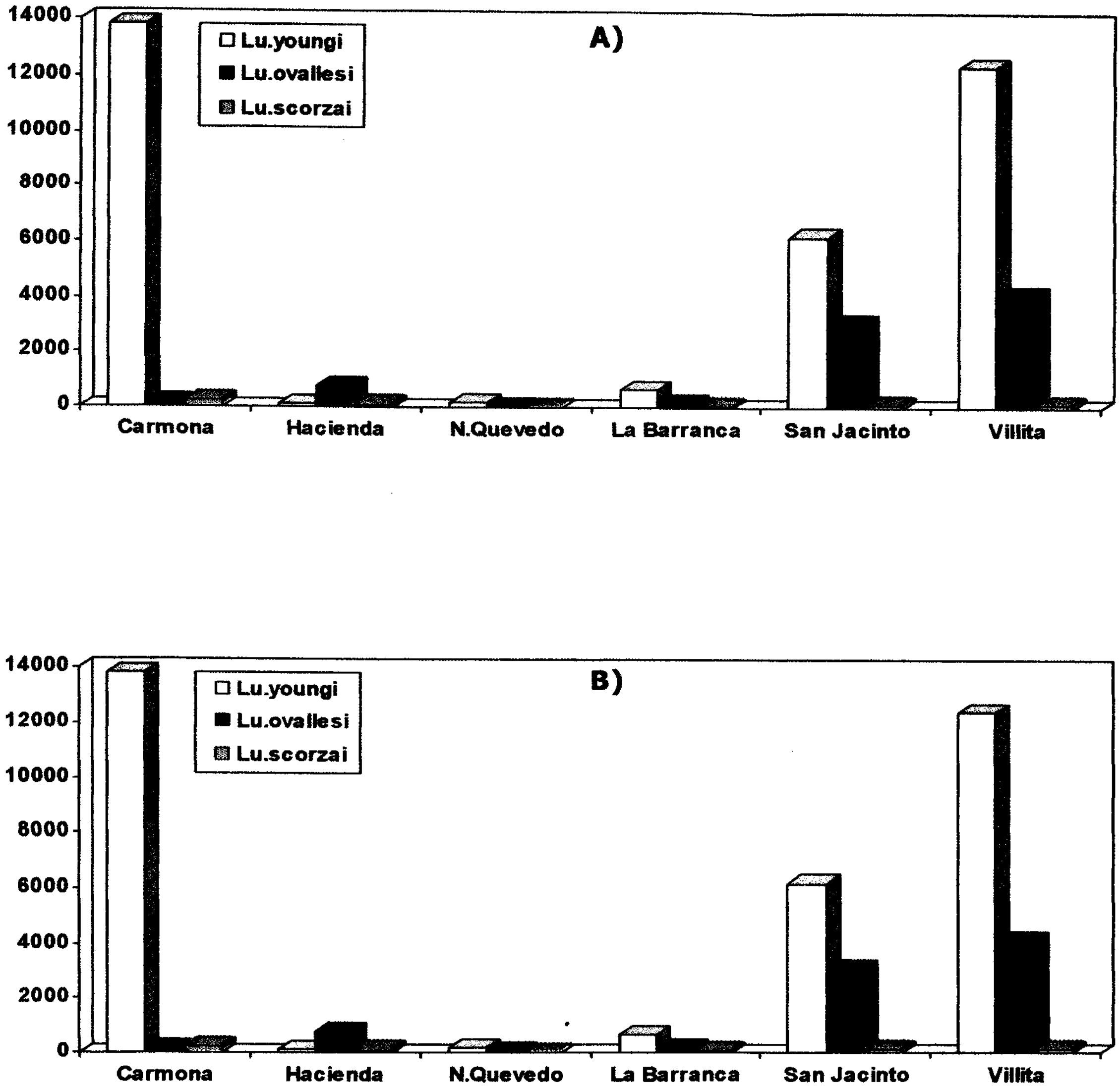

Fig. 1. Sand fly frequency in 6 studied sectors from Trujillo, Venezuela (1995–2000). (A) Before the rainy season (precipitation: $\bar{x}$ = 58.6 mm, SD = 6.4). (B) After the rainy season (precipitation: $\bar{x}$ = 67.9 mm, SD = 4.9).

Rojas 2000). The sand fly fauna identified from the Trujillo area (Mogollón et al. 1977, Scorza 1989) that are naturally infected with *Leishmania* sp. also suggests a domestic or peridomestic transmission cycle (Scorza and Rojas 2002).

In this paper, we report the capture of 5 anthropophilic sand fly species in a peridomestic area, probably because of the proximity of the collection sites to vegetation suitable for resting. *Lutzomyia youngi* is a very common species of the Verrucarum Group in the Andean region (Jiménez et al. 2000, Agudelo et al. 2002) and may be active in houses (Scorza et al. 1984, Scorza and Rojas 1989). The high population density and the large proportion of parous females, coupled with observations on the altitudinal distribution for this sand fly species under new urban conditions, suggest a strong vectorial capacity. *Lutzomyia ovallesi* also has been associated with transmission in a domestic cycle (Feliciangeli and Rabinovich 1998, Gómez et al. 1998), and both species are associated with marsupials as primary reservoir of *L.* (*V.*) *braziliensis* in Carmona and Hacienda localities (Scorza et al. 1986). These are important in understanding the ecoepidemiology of urban leishmaniasis.

Lutzomyia youngi and *L. ovallesi* also have been reported in other, similar epidemiological settings in Venezuela (Feliciangeli and Rabinovich 1998, Bonfante and Barroeta 2002, González et al. 2002), Colombia (Travi et al. 1988, Bejarano et al. 2002), and other researchers from Brazil (Gómes et al. 1986, Tolezano 1994, Valente et al. 2002,) formulated a hypothesis for the existence of novel independent transmission cycles. Further investigations confirming this new epidemiological situation are required.

Twelve sand fly species are present in this urban focus of cutaneous leishmaniasis. These species apparently are adapted to urban conditions, and control strategies for them are necessary in domestic habitats. In the present study, collections were carried out in peridomestic environments associated with habitats where abundant vectors could be increased mainly by human activities, with limited influence by climatic factors.

The phlebotomine fauna of Trujillo City, Venezuela, has not exhibited major, epidemiologically significant changes at a microgeographical or spatial level. *Lutzomyia ovallesi* and *L. youngi* are naturally infected with *Leishmania (V.) braziliensis* (Scorza and Rojas 2002) and show abundance related with foci of high or persistent transmission.

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