PUBLIC HEALTH

Sand Flies Fauna Involved in the Transmission of Cutaneous Leishmaniasis in Afro-Colombian and Amerindian Communities of Choco, Pacific Coast of Colombia

PATRICIA DUQUE, IVAN D. VÉLEZ, MARCELA MORALES AND DIANA SIERRA

Programa de Estudio y Control de Enfermedades Tropicales PECET-Universidad de Antioquia, A.A. 1226, Medellín, Colombia

Neotropical Entomology 33(2):255-264 (2004)

Fauna Flebotomínea Involucrada en la Transmisión de Leishmaniasis Cutánea en las Comunidades Negra e Indígena de la Costa Pacífica del Choco, Colombia

RESÚMEN - Se llevo a cabo un estudio prospectivo sobre diversidad de fauna flebotomínea en la Costa Pacifica colombiana y su relación con la epidemiología de la leishmaniosis, donde se capturó un total de 5.365 ejemplares en trampas de luz, madrigueras de mamíferos, en reposo y en cebo humano. Se colectó un total de 42 especies de flebotomíneos pertenecientes a los géneros *Lutzomyia, Brumptomyia* y *Warileya* e igualmente se encontraron especies altamente antropofilicas e incriminadas en la capacidad vectorial como *Lutzomyia gomezi* (Nitzulescu), *Lu. hartmanni* (Fairchild & Hertig) y *Lu. trapidoi* (Fairchild & Hertig). Además se encontró que *Leishmania panamensis* es la especie de parásito que está afectando a la población en general siendo la prevalencia de infección de la enfermedad un 57%, para los indígenas, mientras en la población negra sólo el 26% ha tenido contacto con el parásito. En los indígenas no hay diferencias en la positividad para la prueba de Montenegro entre los sexos: 57% de los hombres y 58.5% de las mujeres son positivas. Mientras que en la comunidad negra 39% de los hombres son positivos para esta prueba, frente a 10% de las mujeres, reflejando esto las diferencias en el comportamiento de las dos etnias frente al bosque. Estos resultados confirman que la transmisión de *L. panamensis* en el área de estudio está restringida a las áreas rurales y ligada a la actividad de las personas y a la ubicación con respecto al bosque de las viviendas.

PALABRAS CLAVE: *Lutzomyia*, Emberá, *Leishmania panamensis*

ABSTRACT - A prospective study was conducted on the diversity of phlebotomine in the pacific coast of Colombia and its relationship with the eco-epidemiology of leishmaniasis. A total of 5,365 phlebotomines were collected on light traps, in human baits also standing still on walls, trees and mammalian caves. In total, 42 phlebotomine species were identified belonging to the genera *Lutzomyia, Brumptomyia* and *Warileya.* Among these, the highly anthropophilic vector species *Lutzomyia gomezi* (Nitzulescu), *Lu. hartmanni* (Fairchild & Hertig) and *Lu. trapidoi* (Fairchild & Hertig). *Leishmania panamensis* was the main parasite species affecting the population, with a prevalence of 57% in Indians and 26% in black people. In the Indian community no difference was found in the skin test (Montenegro) between sexes, being positive in 57% of indian males and 58.5% in indian females. In contrast, 39% of black males and 10% black females were found to be positive in the skin test. These results confirm that the transmission of *L. panamensis* in the studied area is restricted to the rural areas and linked to people activities and the location of their homes.

KEY WORDS: *Lutzomyia*, Emberá, *Leishmania panamensis*

The Pacific coast of the Colombian department of Chocó is one of the most biodiverse areas in the world. Classified ecologically as very humid tropical forest (Holdridge 1996), it has 4000-8000 mm of rainfall each year, a mean temperature of 24ºC and 90% relative humidity. It is also one of the regions with the highest prevalence of tegumentary leishmaniasis in Colombia. The region is populated principally by Afro-Colombians who generally live on the coast in houses in open areas and by Amerindians of the Emberá community who live near the headwaters of rivers, either in *palafíticas* dwellings within the forest or small communities in clearings.

The spatial distribution of leishmaniasis is not homogenous

but occurs in foci whose limits are determined by the spatial distribution and relative density of the vectors, phlebotomine sand flies of the genus *Lutzomyia* (Rioux 1996). The presence of the vectors is in turn determined by ecological factors such as vegetation, altitude, geomorphology, and climate. Previous studies of sand flies on the Pacific Coast of Colombia have been carried out in the departments of Chocó, Nariño and Valle (Young 1987, Loyola *et al*. 1988, Travi *et al*. 1988).

The objective of this study was to determine the eco-epidemiological risk of infection by *Leishmania* spp. in Emberá Indians and compare it with that of the Afro-Colombian population living both on the coast and the River Valle basin. Comparisons were made of the prevalence of infection and disease due to *Leishmania* spp., as well as spatial distribution, abundance and relationship with human habitations of sand fly vector species.

Materials and Methods

Description of the Study Area. The study was carried out in the municipality of Bahía Solano, including the El Valle settlement, the Utria National Park and Cabo Corrientes located on the coast; the Valle River Basin, the Emberá Indian community consisting of the villages of El Brazo, Boroboro and Posamanza as well as Santa María de Condoto in the Serranía del Baudó mountain range, approximately 30 km from the coast; and Cerro Mutatá, an area of primary forest at 485 masl (meters about sea level), in the Serranía de Baudó. A total of 120 Afro-Colombians live in the Valle River Basin, their livelihood based on agriculture and cattle-raising.

The Emberá communities of the Valle Arriba reserve have a total population of approximately 320. On the reserve primary forest occurs relatively close to the dwellings with areas of crops and high undergrowth surrounding many houses.

Study of the Human Population. An active search was made for cases of tegumentary leishmaniasis in both the Afro-Colombian and Emberá populations. The clinical histories of people with lesions suspected of being due to leishmaniasis were noted and parasitological diagnostic tests (smears test and culture of the lesion) performed on these individuals.

Epidemiological Survey by Montenegro Skin Test. After explaining the risks and potential benefits involved, the Montenegro intradermal skin test, that consist in the intradermal application of 0.1 ml of *Leishmania* antigen (leishmanina), was applied to consenting members of the Afro-Colombian and Emberá populations to determine the prevalence of *Leishmania* infection in each community. These included schoolchildren resident in the urban zone of the El Valle settlement, 104 Afro-Colombians of the River Valle Basin and 253 Indians of Valle Arriba. Results were read after 48h, employing the ballpoint pen technique recommended by the World Health Organisation (WHO 1990). All indurations ≥ 5 mm in diameter were considered to indicate a positive result.

Sand Fly Sampling Survey. The study of the sand fly fauna was carried out along a transect from the Coast to Santa María de Condoto (150 masl) and including the urban area and outskirts of El Valle, Valle River Basin, Valle Arriba Indian reserves and Santa María de Condoto and the upper Cerro Mutatá at 485 masl (Fig. 1).

The sampling methods used were: *CDC light traps* installed from 18:00h - 06:00h simultaneously in intra, peri and extradomiciliary areas and also in the undergrowth and crowns of trees at approximately 15 m above ground level; The captures were also made on protected human bait and diurnal resting sites (tree trunks and animal burrows), these traps (three per night), were placed weekly during five days in differents locations in the dry season and in the rain season; the specimens were stored in 70% alcohol and transported to the laboratory for clearing, mounting and identification using the taxonomic keys of Young & Duncan (1994).

To analyse results and reduce variability due to the distribution of the insects themselves and the influence of different environmental factors (Bidlinmayer 1969), numbers of sand flies captured in light traps and on human bait collections were converted to Williams' geometric mean (Mw) using the following formula:

$$Mw = (\text{Antilog. ML})\text{-l ML} = \text{Log}\,(X+l)/n$$

where ML = Represents the logarithmic mean of the collection per trap, n = No. sampling days multiplied by no. traps; and X = No. sand flies captured per trap-night.

Results

Active Search for Cases. Samples were taken from all patients who presented cutaneous lesions compatible with leishmaniasis. The disease was confirmed parasitologically in nine patients (four Indians and five Afro-Colombians) aged 5-68. Lesions of the *ulcer franca* type predominated (68%), mostly located on the legs or arms (84%). All the patients had already undergone empirical or homeopathic treatments with plants or caustic substances.

In three of the confirmed cases it was possible to isolate and identify the strains involved. All samples were cultivated in NNN medium, positives cultures were exposed to NNN modificated to enhance parasites numbers; then the parasite were identified with the monoclonal antibodies technique (Diane Mc Mahon Pratt, Yale University) that identifies *Leishmania* (*Viannia*) *panamensis.*

Patients with confirmed diagnoses of leishmaniasis received treatment with Glucantime at a dose of 20 mg Sb^{5} 1k/d/20 days. Post-therapeutic clinical evaluation confirmed scarring of all the lesions and no relapses were observed during one year of observation.

Prevalence of Infection by *Leishmania* spp. All 161 Afro-Colombian children aged 4-15 in El Valle were Mn-ve. Results of this test according to age group and sex for the other two populations are presented in Table 1. In the Valle River Basin 66% of males aged over 15 were Mn+ve. There were no differences between the sexes in the Emberá reservation with regard to skin test positivity but a high number of very young children were Mn+ve.

Entomological Survey. In all 5365 phlebotomines belonging to 42 species were collected (67.1% females), including 37

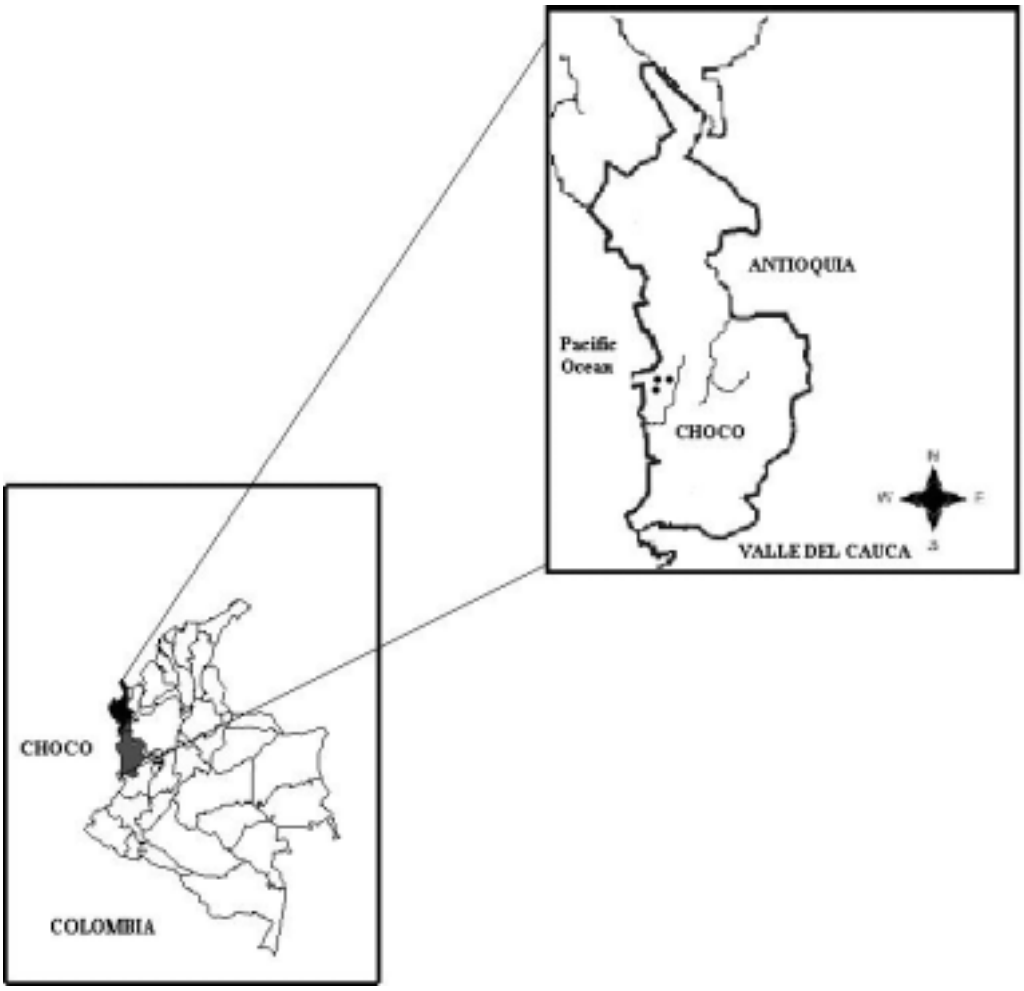

Figure. 1. Outline map of Colombia, showing the location of sampling sites.

species of the genus *Lutzomyia* (three species identifications to be confirmed), three of *Brumptomyia* and two of *Warileya.* Four species are recognised vectors of *Leishmania*, i.e., *Lutzomyia hartmanni* (Fairchild & Hertig), *Lu. trapidoi* (Fairchild & Hertig), *Lu. gomezi* (Nitzulescu) and *Lu. panamensis* (Shannon). The species collected with CDC light traps and human bait are listed in Table 2.

*Light Trap Samples.*The total of 1,464 sand flies were collected, with females predominating in 77% of the samples. This method sampled the greater number of phlebotomine species (33) although *Lu. hartmanni*, *Lu. trapidoi* and *Lu. gomezi* were the most abundant.

Human Bait Collections. The total of 1,510 sand flies were collected in 270 man-h (1,481 females and only 29 males) belonging to 15 *Lutzomyia* and two *Warileya* species. The most abundant anthropophilic species were *Lu. gomezi* (39.5%), *Lu. hartmanni* (21.0%) and *Lu. trapidoi* (20.5%). Collections were also made from diurnal resting sites. A total of 2,352 sand flies was obtained from animal burrows, the most abundant species being *Lu. reburra* (Fairchild & Hertig), *Lu. triramula* (Fairchild & Hertig) and *Lu. carpenteri* (Fairchild & Hertig). Further 59 specimens were collected resting on trees,

Table 1. Percentage of individuals giving a positive response to the Montenegro skin test, sorted by age and sex in the communities of the River Valle Basin (Afro-Colombian) and Valle Arriba Reservation (Emberá Indian), Choco, Colombia.

Age group	Males		Females		Overall	
	Afro-Colombian (n = 56)	Emberá (n = 123)	Afro-Colombian (n = 48)	Emberá (n = 130)	Afro-Colombian (n = 104)	Emberá (n = 253)
0-9	6.6	17.0	0	36.5	3.9	26.6
10-14	16.7	50.0	0	61.5	9.3	55.2
15-44	61.0	91.0	12.5	78.8	38.4	84.8
44+	72.7	85.7	25.0	100	49.1	94.4
Overall	39.0	57.7	10.4	58.1	25.9	57.3

Table 2. Number and relative abundance (%) of phlebotomine sand fly species captured according to sampling method on the Pacific Coast of Choco, Colombia.

Species	CDC Light trap		Human bait		Animal burrows		Trees		Total
	No.	%	No.	%	No	%	No.	%	
Lutzomyia reburra	32	2.2	1	0.06	1,395	59.3			1428
Lu. hartmanni	509	34.8	313	21.0	2	0.08	1	1.6	825
Lu. gomezi	152	10.4	589	39.5			4	6.7	745
Lu. trapidoi	357	24.4	306	20.5	10	0.4	1	1.6	674
Lu. triramula	7	0.4			546	23.2			553
Lu. panamensis (Shannon)	77	5.3	89	6.0					166
Lu. olmeca bicolor	142	9.7	2	0.1					144
Lu. sanguinaria (Fairchild & Hertig)	16	1.1	117	7.8	2	0.08			135
Lu. carpenteri	9	0.7			124	5.3			133
Lu. carrerai thula Young	32	2.2	62	4.2					94
Lu. aragaoi (Costa Lima)	12	0.8			76	3.2	2	3.4	90
Lu. camposi (Rodríguez)					77	3.3			77
Lu. trinidadensis (Newstead)	51	3.5	2	0.13					53
Lu. saulensis (Floch & Abonnenc)	10	0.7			33	1.4			43
Lu. isovespertilionis	3	0.2					29	49	32
Lu. aclydifera	3	0.2					21	35	24
Lu. sordelli (Shannon & Del Ponte)	21	1.4							21
Lu. barrettoi sp.	1	0.06			13	0.55			14
Lu. ayrozai (Barreto & Coutinho)	2	0.06	3	0.19					5
Lu. carrerai sp.	5	0.3							5
Lu. shannoni (Dyar)	3	0.2	1	0.06			1	1.6	5
Lu. guyanensis (Floch & Abonnenc)	3	0.2	1	0.06					4
Lu. barrettoi mayuscula Young					3	0.12			3
Lu. bifoliata Osorno- Mesa, Morales, Osorno & Hoyos	3	0.2							3
Lu. caprina Osorno- Mesa, Morales & Osorno			4		1	0.04			1
Lu. (micropygomyia) sp. Barreto	1	0.06							1
Lu. ovallesi (Ortiz)	1	0.06							1
Lu. (Psathyromyia) sp. Barreto	1	0.06							1
Lu. triacantha (Mangabeira)			1	0.06					1
Lu. recurva Young	1	0.06							1
Lu. rorotaensis (Floch & Abonnenc)	1	0.06							1
Lu. yuilli	1	0.06							1
Lutzomyia sp_1	3	0.2	14	0.9					17
Lutzomyia sp_2	1	0.06							1
Lu. flaviscutellata (Mangabeira)	2	0.06							2
Lu. ubiquitalis (Mangabeira)			4	0.2					4
Lutzomyia sp_3	1	0.06							1
Brumptomyia galindoi (Fairchild & Hertig)	1	0.06			25	1.1			26
B. leopoldoi (Rodríguez)					24	1.0			24
Brumptomyia sp. Fran a & Parrot					1	0.04			1
Warileya rotundipennis Fairchild & Hertig			4	0.2					4
W. nigrosacculus Fairchild & Hertig			1	0.06					1
Total	1,464	0	1,510	0	2,332	0	59	0	5,365

the most abundant being *Lu. gomezi*, *Lu. isovespertilionis* (Fairchild & Hertig) and *Lu. aclydifera* (Fairchild & Hertig).

The distributions spatial of sand fly species captured in the different subregions from the Coast to the Serranía del Baudó (0-100m) and Mutatá (485m) are presented in Table 3. *Lu. hartmanni, Lu. trapidoi* and *Lu. olmeca bicolor* Fairchild & Theodor, were the most widely distributed species and were found from 0-485 m, while *Lu. gomezi* was only found from the coast to the reservation and *Lu. panamensis* was present in all the subregions at elevations below 100 m, including the Serranía del Baudó.

The mean densities of *Lutzomyia* species captured with light traps in the canopy and forest understorey undergrowth are shown in Table 4. Seventeen simultaneous

Table 3. Spatial distribution of sand fly species captured on the Pacific Coast of Choco, Colombia.

Sand fly species	Elevation zone (masl)				
	Coast (0)	River (0 –100)	Reserve (100)	Baudo (100 – 200)	Mutata (485)
Lutzomyia aclydifera	X				X
Lu. aragaoi	X		X	X	
Lu. ayrozai	X		X		
Lu. barrettoi mayuscula			X	X	
Lu. barrettoi sp.			X		
Lu. bifoliata		X		X	
Lu. camposi				X	
Lu. caprina				X	
Lu. carpenteri	X		X	X	
Lu. carrerai thula	X		X	X	X
Lu. carrerai sp.	X			X	
Lu. flaviscutellata					X
Lu. gomezi	X	X	X		
Lu. guyanensis	X			X	
Lu. hartmanni	X	X	X	X	X
Lu. isovespertilonis	X		X		X
Lu. olmeca bicolor	X	X	X	X	X
Lu. ovallesi				X	
Lu. panamensis	X	X	X	X	
Lu. reburra	X		X	X	
Lu. recurva			X		
Lu. rorotaensis			X		
Lu. sanguinaria	X	X	X	X	
Lu. saulensis	X		X		
Lu. shannoni	X	X			
Lu. sordelli	X		X	X	
Lu. trapidoi	X	X	X	X	X
Lu. triacantha				X	
Lu. trinidadensis		X	X		X
Lu. triramula	X	X	X	X	
Lu. ubiquitalis			X		
Lu. yuilli		X			
Lutzomyia sp_1					X
Lutzomyia sp_2			X	X	
Lutzomyia sp_3					X
Lu. (*Micropygomyia*) sp[1]					
Lu. (*Psathyromyia*) sp.	X				
Brumptomyia galindoi	X			X	
B. leopoldoi			X	X	
Brumptomyia sp.			X		
Warileya nigrosaculus			X		
W. rotundipennis				X	

samples were collected in the crowns of the trees at approximately 15 m above ground level and in the understorey at 0.5 m. Fourteen sand fly species were captured in the former, *Lu. trapidoi* being the most abundant. Thirteen species were collected in the understorey, *Lu. hartmanni* being most numerous.

Relative Density of Vector Species According to Vegetation Type. The relative densities of the vector species captured with light traps among the different types of vegetation indicated that *Lu. hartmanni* was the most abundant species in primary forest, except in Santa María de Condoto where *Lu. panamensis* predominated. In the areas of high undergrowth and crops, *Lu. hartmanni* had a similar density to that of *Lu. trapidoi*, these species being more abundant than all others. In pastureland *Lu. gomezi* predominated. The relative density of *Lu. trapidoi* presented few variations among the different types of vegetation.

Table 4. Mean density (Xw/flies/trap-night) of sand flies in canopy and understorey.

Species	Elevation (masl)	
	< 0.5	6 – 15
Lutzomyia aragaoi	0.096	0.039
Lu. carpenteri	0.122	0.039
Lu. carrerai thula	0.334	0.360
Lu. carrerai sp.	-	0.174
Lu. gomezi	-	0.039
Lu. guyanensis	-	0.122
Lu. hartmanni	0.757	0.039
Lu. olmeca bicolor	0.414	0.039
Lu. panamensis	0.122	0.080
Lu. reburra	0.200	0.363
Lu. recurva	0.059	-
Lu. sanguinaria	0.211	0.212
Lu. shannoni	-	0.039
Lu. sordelli	0.059	-
Lu. trapidoi	1.158	1.700
Lu. trinidadensis	0.059	-
Lu. triramula	0.059	0.039

Relative Abundance of Phlebotomines with Respect to Human Dwellings. The collections were make with CDC light traps and human bait, intra, peri and extra domicile in the Afro-Colombian and natives populations as following:

Afro-Colombian Populations. At the seaside of Utria and settlement Valle, phlebotomines were collected during ten nights. The phlebotomines´s density in the intradomicile and peridomicile was almost nule (Fig. 2). This result is similar to Montenegro skin test, which was 100% negative for the very young children of settlement Valle. In the forest *Lu hartmanni* was the most abundant species.

In the Valle River Basin the collections were made during seven nights. In the intra and peridomicile *Lu. gomezi*, was the most predominant meanwhile in the forest the specie most predominant was *Lu. hartmanni*; *Lu. gomezi* was the less found (Fig. 2). As showed by the Montenegro skin test, this population is infected mainly in the forest, the 7% of very young children and the 13% of women less than 44 years old has been in contact with the parasite.

Emberá Indian Population. In the Emberá Indian community of Valle Arriba collections were made during 26 nights where *Lu. gomezi* y *Lu trapidoi* were found most frequently in the intradomicile. In contrast, *Lu. trapidoi* y *Lu. hartmanni* were found in the peridomicile, and *Lu. hartmanni* was the most abundant specie in the forest. Regarding to the mean density of flies/hour/men comparing to domicile we can see that in the native resguard *Lu. gomezi* is predominant in the intradomicile, *Lu trapidoi* and *Lu gomezi* in the peridomicile and *Lu hartmanni* and *Lu. trapidoi* in the extradomicile.

Diurnal Human Bait of Phlebotomines. Sampling on human bait during daylight was attempted on several occasions in all the subregions. Sand flies were captured very sporadically, except on one afternoon after a tree had been cut down, when 80 examples of *Lu. trapidoi* were caught in 1 man-hour.

Discussion

Cutaneous leishmaniasis was shown endemic in rural areas of all the life zones of the Pacific coast. There was however no evidence of transmission in the urban area of the El Valle settlement.

In the interior of the dwellings the most frequent and abundant species were all known vectors of *Leishmania*, i.e., *Lu. hartmanni*, *Lu. trapidoi*, *Lu. gomezi* and *Lu. panamensis*. Another suspected vector (*Lu. olmeca bicolor*) was most abundant in forest. In the peridomicile *Lu. trapidoi* predominated, with a density similar to that observed in the forest; in the latter habitat *Lu. hartmanni* was clearly more abundant predominated over the other species.

In this field study in the Valle River Basin, where dwellings are surrounded principally by small pastures, crops and scrub and the forest is sparse, the predominance of *Lu. gomezi* is very clear both indoors and in the peridomicile. The density of this species was much lower inside houses.

The Emberá communities consist of two types of dwelling: the *escaladas* in the centre of the settlements, free of surrounding vegetation; and those within the forest surrounded by crops. The mean densities of sand flies captured in the two types of dwelling of the Emberá reserve are shown in Table 5. Density was clearly higher in the peripheral *tambos* surrounded by vegetation. The mean densities of vector species in dwellings surrounded by vegetation and clearings are shown in Table 6.

The principal *Leishmania* species in the region was confirmed as *Leishmania* (V) *panamensis* and clearly patients are responding adequately to treatment with pentavalent antimony derivatives at the doses recommended by the Colombian Ministry of Health 1994.

As shown by the results of the intradermal Montenegro reaction, Emberá children are in contact with *Leishmania* from a very early age. About 20% of the children under four years old are Mn+ve. It may be postulated that people become infected in or around houses at night, when the phlebotomines present their greatest biting activity and all members of the family are most exposed to their bites. The felling of trees constitutes an additional risk since this may result in contact with *Lu. trapidoi* during the day. The *tambos* located on the periphery of the villages and thus closest to the forest present greater intradomicile vector activity thus inhabitants have a greater risk of infection with the parasite.

In contrast among the Afro-Colombian community of the River Valle males over 15 years old were Mn+ve, suggesting that infection occurs in the extradomicilary environment or the forest where men may pass several days clearing trees for pasture, planting crops. They are also exposed to sand fly bites when they go out hunting at night.

Prevalence of *Leishmania* infection is different for the two races. Among the Emberá there is a high prevalence (57%) of infection, while only 26% of Afro-Colombians show signs of contact with the parasite. Among the Indians there was no significant difference in the prevalence of Mn+ve individuals between the sexes, with 57.0% of men and 58.5% of women positive. By contrast in the Afro-Colombian community 39% of men were positive vs. 10% of women.

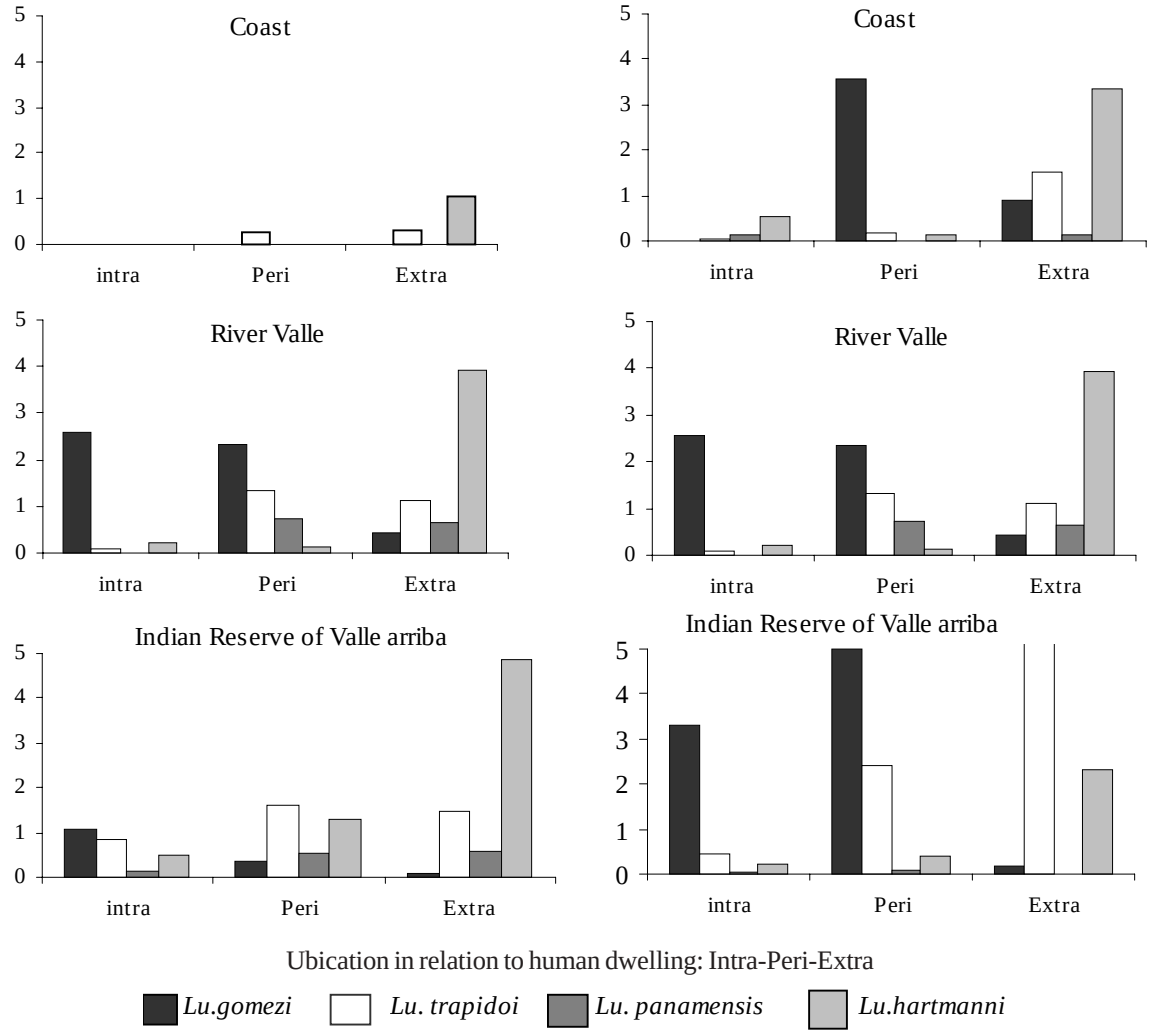

Figure 2. a) Mean density of flies/ CDC Trap/ night in relation to human dwellings; b) Mean density of flies/hour/man in relation to human dwellings.

Table 5. Mean density of insects/CDC trap-night inside *tambos* in the centre and periphery of Emberá settlements Choco, Colombia (no. samples in parentheses).

Valle Arriba reserve

Species	Intradomicile	
	Centre (16)	Periphery (10)
Lu. gomezi	0.139	4.478
Lu. hartmanni	0.250	0.966
Lu. panamensis	0.139	0.072
Lu. trapidoi	0.220	2.546

Valle River

Species	Pasture and vegetation (7)	Intradomicile (7)
Lu. gomezi	2.567	0.414
Lu. hartmanni	0.219	3.930
Lu. panamensis	0.000	0.626
Lu. trapidoi	0.104	1.213

These results reflect differences in the behaviour of the two ethnic groups regarding the forest. The Afro-Colombian communities build their houses at some distance from the forest and only the men and youths work there. Children remain in school or at home helping the women with their domestic chores.

As mentioned previously, Emberá men and women work in the forest accompanied by all their children. The other subregion where a Montenegro test survey was carried out was at sea level, in the urban zone of El Valle, a relatively deforested area with forest and cropland at some distance from the villages and no trees within the limits of these communities. The results of sand fly sampling and the skin test survey demostrated that there was no intradomiciliary biting activity and no children of school age were Mn+ve.

In Tumaco (department of Nariño), where environmental conditions are similar, Weigle (1986) found that transmission of *Leishmania (Viannia) braziliensis* occurred in rural areas including primary forest, secondary forest and mangroves. They frequently diagnosed infection in children under five

Table 6. Mean density (x_w flies/man) of sand flies captured on protected human bait inside dwellings surrounded by vegetation vs in clearings in the Valle Arriba reserve (VA) and Santa María de Condoto (SMC).

Species	Clearings		Surrounded by vegetation	
	VA	SMC	VA	SMC
Lu. gomezi	0.57	0	4.06	0
Lu. hartmanni	0.37	0.30	0.26	0.06
Lu. panamensis	0.09	0.03	0	0
Lu. trapidoi	0.55	0	0.25	0
No. trap-nights	14	6	17	5

years old, leading them to suspect that transmission occurred in or around houses. Later Travi *et al*. (1988) found children under four infected although they did not detect any intradomiciliary biting activity of *Lutzomyia*, leading them to suggest that *Leishmania* transmission occurred in the peridomicile.

On the other hand when results were analysed no difference was observed between Mn+ve prevalences of children living in *tambos* at the centres of villages and those living on the periphery (data not presented). This may be explained by the fact that Emberá families only remain in villages for six months of the year, spending the rest of their time living on their farms in *tambos* surrounded by vegetation.

These results confirm that transmission of *Le. panamensis* in the study area is restricted to rural areas, linked to human activities and the position of human dwellings with respect to the forest.

A variety of capture methods should be used to determine species diversity (Alexander 2000). However light trapping was the most effective method used in the present study, allowing 76% of all species present to be sampled, including all the anthropophilic ones.

The relative abundance of man-biting species were similar in samples collected with light traps and human bait. However collections from diurnal resting sites revealed the presence of a further six species.

Lu. gomezi was principally associated with highly intervened areas, such as the Valle River Basin where pastures predominate. Its density diminished with increasing forest cover until it became difficult to find in primary forest. This contrasts with the pattern of distribution of *Lu. hartmanni*. Previous researchers have noted a similar tendency in Colombia Porter & Defollart (1981), Venezuela Feliciangeli (1987) and Panama. Fairchild and Hertig cited by Porter (1986) suggested that *Lu. gomezi* is a semi-domestic species adapted to deforested areas, where it feeds on a great diversity of mammals, being particularly attracted to pigs. In the *tambos* and most houses of the Valle River Basin, where people live in close proximity to pigs, the density of this species is very high. In Montebello (Antioquia) it was found inside houses on coffee plantations. Samples on human bait also showed this species to be highly endophilic Vélez *et al.* (1991).

In epidemic outbreaks of cutaneous leishmaniasis, *Lu. gomezi* was the most abundant anthropophilic species and also was incriminated as the vector due to its endophilic biting habits (Velez *et al.* 1987, 1991). In cropland and forest the predominant vector species were *Lu. trapidoi* and *Lu. hartmanni*.

In Bajo Calima (Buenaventura, department of Valle del Cauca) *Lu. trapidoi* represented 94% of all man-biting sand flies and was incriminated as the vector of *Le. panamensis* (Christensen *et al.* 1983, Travi *et al.* 1988) We found *Lu trapidoi* to be a species that tolerated deforestation and was as frequent in forest as in intervened areas. By contrast other authors have described this species as occurring in mature forest, for example in Bajo Calima where it represented 90% of specimens captured (Christensen *et al.* 1983, Travi *et al.* 1988). Porter (1981) reported that it was rarely captured in pastureland.

Sand flies were particularly abundant in mammal burrows, examples being found in 15/40 holes examined. Results of captures in resting sites suggest that armadillo burrows are the preferred feeding and resting sites of *Lu. reburra*: more than 90% of the females captured were gravid and had blood meals. Sampling from resting sites within the forest, except for the burrows, was not very successful. One of the species encountered in tree holes was *Lu. isovespertilionis*, which has been associated with the roosting sites of bats, on which it feeds (Memmott 1992).

It appears that the risk of *Leishmania* infection occurs throughout the study area except in the urban area of El Valle. Four sand fly species with recognised vectorial capacity are present, their distribution governed by the vegetation types present in each habitat. Intradomiciliar and peridomiciliar contact with vectors occur at night when dwellings are close to the forest and during the day within the forest, associated with tree felling. The element that determines the greatest risk of infection in the different habitat types must be the density of infected mammalian reservoirs of *Leishmania*.

Some of the species collected in this study that are important for transmission have been studied concerning distribution, density, antrophofilia and intradomiciliary activity. *Lu. trapidoi* that is highly antrophofilic, with peridomiciliary and intradomiciliary habits, has been found naturally infected in areas of the Colombian´s Pacific Coast and Magdalena Medio (Travi *et al*. 1988, Young *et al*. 1987); its vectorial capacity was demostrated in the laboratory for experimental infection (Jaramillo *et al*. 1994). *Lu. hartmanni* is antrophofilic with wide distribution in the Colombian´s Pacific Coast (Barreto *et al.* 1989) and Andean region with intradomiciliary and peridomiciliary habits. It was reported by Montoya-Lerma & Baena (1995) in high densities in Gorgona Island and Colombian Pacific Coast. *Lu. gomezi* has shown a wide distribution in the Colombian´s Andean and Pacific Coast. It is highly antropophilic and has also been reported in the intradomicile and peridomicile (Travi *et al.* 1988,Vélez 1990, Martínez *et al.* 1995). It appears that this specie is alternating the transmission with *Lu. trapidoi.*

The almost total absence of vegetation for a radius of approximately 200 m around human settlements acts as a barrier that makes it difficult for sand flies to reach the houses, as was observed in Santa María de Condoto and El Valle.

Marked differences were observed between the two communities with regard to the epidemiological risk of infection. For Afro-Colombians the microfocus of transmission was the forest, whereas for the Emberá it was the *tambo* and the peridomicile.

Acknowledgments

This study received financial support from Colciencias (project no. 1115-04-018-92), the Instituto Francés de Estudio Andinos (IFEA) and the University of Antioquia. We are indebted to Dr. Bruce Alexander for their critical reviews of this manuscript prior to submission. We thank the research staff of the Programa de Estudio y Control de Enfermedades Tropicales (PECET) and members of the Afro-Colombian and Indian communities who collaborated with the study.

Literature Cited

Alexander, B. 2000. Sampling methods for phlebotomine sand flies (Diptera: Psychodidae) Med. Vet. Entomol. 14: 1-13.

Barreto, P., J. Montoya, Y. Solarte, C. Jaramillo, M. Barreto, A. Sánchez & J.L. Freyre. 1989. Notas sobre algunos flebótomos *Lutzomyia* (Diptera: Psychodidae) en tres focos de leishmaniasis cutánea en el Occidente de Colombia. Colombia Méd. 20: 53-59.

Bidlíngmayer, W.L. 1969. The use of logarithms in analyzing trap collections. Mosq. News 33: 535-538.

Christensen, H.A., G.B. Fairchild, A. Herrera, K.M. Johnson, D.G. Young & A.M. Vasquez. 1983. The ecology of cutaneous leishmaniasis in the republic of Panama. J. Med. Entomol. 20: 463-484.

Feliciangeli, D. 1987. Ecology of sandflies (Diptera: Psychodidae) in a restricted focus of cutaneous leishmaniosis in Northern Venezuela II. Species composition in relation to habitat catching methods and hour of catching. Mem. Inst. Oswaldo Cruz 82: 125-131.

Holdridge, L. R. 1966. The life zone system. Adasonia: l99-203.

Jaramillo, C., B. Travi & J. Montoya. 1994. Vector competence of some Neotropical sandflies for the *Leishmania* (*Viannia*) *braziliensis* complex. Med. Vet. Entomol. 8: 1-7.

Loyola, E., A. Alzate, A. Sanchez & A. Gonzalez. 1988. Epidemiology of natural focus of *Leishmania braziliensis* in the pacific Lowlands of Colombia. I.V. Observations on sandflies. Trans. Roy. Soc.Trop. Med. Hyg. 82: 408

Memmott, J. 1992. Patterns of sandflies dístribution in tropical forest: a causal hipotesis. Med. Vet. Entomol. 6: 188-194.

Martínez, G.S. Uribe & I. Velez. 1995. Behavior and intradwelling-vertical-distribution of *Lutzomyia gomezi* Nitzulescu 1931 in Montebello, Antioquia, Colombia. Bol. Dir. Malariol. San. Amb. 35: 197-204.

Montoya-Lerma, J. & M.L. Baena. 1995. Man-biting sand flies (Diptera: Psychodidae) from Gorgona, an island off of the Pacific Coast of Colombia. Bibl. J.J. Triana (Bogotá) 11: 73-78.

Ministerio de Salud. Terapeutica: En leishmaniosis: Plan Nacional de Control Santafé de Bogotá D.C. Trazo Ltda; 197-201.

Organización Mundial de la Salud. 1990. Lucha contra la leishmaniasis. Serie de informes técnicos No. 793. Ginebra, p. 177.

Porter, C.H. & D.G. Young. 1986. TA new species of phlebotomine sandfly (Diptera: Psychodidae) from Guatemala. J. Med. Entomol. 23: 236-243.

Porter, C.H. & G.R. De Foliart. 1981. The man biting activity of phlebotomine sandflies (Diptera: Psychodidae) in a tropical wet forest enviroment in Colombia. Arq. Zool. S. Paulo 30: 8l-l58.

Rioux, J.A., G. Lanotte, F. Peter, J. Dereure, O. Alkalay, F. Pratlong, I.D. Vélez, N.B. Fikri, R. Maazoun, M. Denial, J.M. Jarri, A. Zahaf, R.W. Ashford, M. Cadi-Soussi, R. Killick-Kendrick, N. Benmansour, G. Moreno, J. Perieres, E. Guilvard, M. Zribi, M-F. Kennou, R. Knechtli & E. Serres. 1986. Les leishmanioses cutanées du bassin mediterraneen occidental. p. 365-395. In Leishmania. Taxonomie et phylogenese Applications Eco-épidémiologiques, Int Coll CNRS/INSERM/OMS (2-6 July 1984), Institut Méditer-ranéen d'Etudes Epidémiologiques et Ecologiques, Montpellier, p. 365-395.

Travi, B.L., J. Montoya, Y. Solarte, L. Lozano & C. Jaramillo. 1988. Leishmaniasis in Colombia. I. Studies on the phlebotomine fauna associated with endemic foci in the Pacific Coast region. Am. J Trop. Med. Hyg. 39: 261-266.

Vélez, I.D. 1990. Leishmaniasis cutánea en Montebello, Antioquia: Determinación del riesgo epidemiológico de contraer la enfermedad. Actas XI Congreso Colombiano de Medicina Interna. Cali, p. 99-105.

Vélez, I.D., M. Wolff, R. Valderrama, J.P. Escobar & L. Osorio. 1991. Community and environmental risk factors associated with cutaneous leishmaniasis in Montebello, Antioquia, Colombia, p. 261-274. In Leishmaniasis control strategies, IDRC, México.

Vélez, I.D., S. Ospina, G. Henao, P. Le Pape, M. Correa, M. Wolf & L. Jaramillo. 1987. Epidemiología de la leishmaniasis cutánea en San Roque, Antioquia. Bol. Epid. Antioquia 12: 354-359.

Weigle K.A., N.G. Saravia, M. De Davalos, L.H. Moreno & A. Dalessandro. 1886. *Leishmania braziliensis* from the Pacific Coast region of Colombia: Foci of transmission clinical spectrum and isoenzyme phenotypes. Am. J. Trop. Med. Hyg. 59: 49-52.

Young, D.G., A. Morales, R.D. Kreutzer, J.B. Alexander, A. Corredor, R.B. Tesh, C. Ferro & C. de Rodríguez. 1987. Isolation of *Leishmania braziliensis* (Kinetoplastida: Trypanosomatidae) from cryopreserved Colombian sandflies (Diptera: Psychodidae). J. Med. Entomol. 24: 587-589.

Young, D.G. & M. Duncan. 1994. Guide to the identification and geographic distribution of *Lutzomyia* sandflies in Mexico, the West Indies, Central and South America (Diptera: Psychodidae), Associated Publishers, Gainesville, Florida, 881p.

Received 23/12/02. Accepted 29/01/04.