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NATURAL INFECTIONS WITH PROMASTIGOTES IN MAN-BITING SPECIES OF SAND FLIES IN LEISHMANIASIS-ENDEMIC AREAS OF ECUADOR

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Abstract. In order to determine the vectors of leishmaniasis in Ecuador, 1,054 man-biting sand flies from the Department of Cañar were dissected and examined for promastigotes. There were 2 man-biting species, *Lu. trapidoi* and *Lu. hartmanni* in this endemic area of the disease. The infection rates were 7.7% in the former and 3.9% in the latter species, demonstrating the different rates in various localities and altitudes of the study areas. There was an association between infection rates and the time of day, suggesting some connection with biting activity of sand fly species. In collections using human bait at 7 study areas in 5 Departments, 6 man-biting species were recognized, indicating different dominant species in each area. It was assumed that the dominant species would play an important role as the principal vector of leishmaniasis in each endemic area. As to species determination of the present *Leishmania* promastigotes, suffice it to say that the parasites are *Leishmania* sp., presumably *L. braziliensis* s.l., until the isolates have been typed.

American cutaneous or mucocutaneous leishmaniasis, caused by *Leishmania braziliensis* sensu lato, is endemic in most provinces of Ecuador, where it is a considerable public health problem. Little information, however, has been available on epidemiological features of the disease, such as morbidity of inhabitants, vectors and reservoir hosts in each endemic area.

Although Rodriguez made taxonomical works of the sand fly, recording 17 species, some of these species have been estimated to be potential vectors of leishmaniasis in Ecuador without the evidence of natural infection.[1–4]

For a better understanding of the epidemiology of leishmaniasis in this country, we initiated investigations on the infection of human residents, reservoir hosts (wild mammals) and sand flies in endemic areas. The present paper deals with the dissection of sand flies to detect natural infection with *Leishmania* promastigotes, and of collections of man-biting sand flies in different endemic areas using human bait.

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MATERIALS AND METHODS

Study areas

All the collection sites were located on the Pacific coastal slope on the cordillera of the Andes, ranging from 100 m to 2,000 m above sea level (Fig. 1).

In a preliminary survey of man-biting species and natural infections of sand flies with promastigotes, the collections were carried out at 7 study areas (Nos. 1–4 and 7–9 in Fig. 1). Each site is known to be situated in an endemic area of leishmaniasis.[5]

In order to perform a detailed investigation of natural infection, 6 localities with different altitudes, 350 m above sea level, 600 m, 950 m, 1,200 m, 1,500 m and 2,000 m, were selected as study areas in the Department of Cañar. The areas are in the southeast of Ecuador, 2°30′S latitude and 79°10′W longitude, distributed in 3 villages of the Department—Ocaña, Javin and Suscal. In the first 2 villages, large areas of dense and humid primary forests were reclaimed by new settlers to cultivate sugarcane, maize, coffee and cacao along the main asphalt road from Gua-

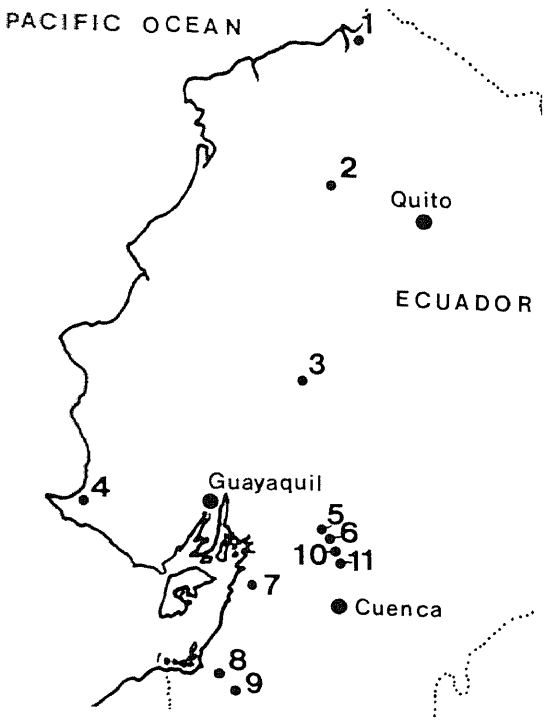

FIGURE 1. Outline map of the Republic of Ecuador, South America, showing study areas. **1.** Tachina (100 m above sea level), Department of Esmeraldas; **2.** Quininde (200 m), Department of Pichincha; **3.** Quevedo (La Mana) (400 m), Department of Los Rios; **4.** Olón (150 m), Department of Guayas; **5.** Ocaña (350–600 m), Department of Cañar; **6.** Ocaña (950 m), Department of Cañar; **7.** Naranjal (1,000 m), Department of Guayas; **8.** Portovelo (500 m), Department of El Oro; **9.** Zaruma (1,100 m), Department of El Oro; **10.** Javin (1,200–1,500 m), Department of Cañar; **11.** Suscal (2,000 m), Department of Cañar.

yaquil to Cuenca. The last village, Suscal, is located at around 2,000 m, showing a characteristic alpine flora of the Andes.

Sand fly collections

All the collection sites were located close to human residences in each station. Two persons engaged in each fly catch; one served as human bait and the other, collector. The individual serving as bait sat with his shirt off and trousers rolled up to the knee; the collector aspirated the sand flies using a collecting tube as they alighted on the exposed skin. In examinations of natural infections, most of the flies were captured between 1900 and 2400 hours. Hourly examinations of natural infections at Ocaña (350 m), however, were carried out from dusk through dawn.

Sand fly dissections

Although sand flies caught were usually dissected within 3 days after collection, a certain portion of the flies were reared up to 5 or 6 days, in order to observe the migration of promastigotes therein. All the dissections were made in fresh materials, by employing the method of Johnson et al.[6] with a slight modification. The internal organs detected were covered by 18 × 18-mm glass cover slips and then examined microscopically at ×400 and ×1,000 magnifications. All the fly identifications were made at the time of dissection by observing the spermathecea and cibarial armatures, except fixed specimens in alcohol from the area, Naranjal, in Table 1.

Promastigote inoculation into hamsters

For the purpose of species differentiation between *L. braziliensis* s.l. and *L. mexicana* s.l., we made observations on the characteristic development of lesions in hamsters, by inoculating promastigotes from wild-caught sand flies. The flagellates harvested from naturally-infected flies were inoculated into the nose and base of tail of 18 Syrian hamsters, *Mesocricetus auratus*. The flagellates from each individual fly were inoculated into 8 hamsters. Four developed nodules and 1 a shallow ulcer in *Lutzomyia trapidoi*, and 4 (2 developed nodules) in *Lu. hartmanni*. Moreover, 3 animals (1 developed nodule) received materials pooled from 3 each of *trapidoi* and 3 (all negative for lesions) from 2 each of *hartmanni* in the case of a small parasite burden in a fly. Thus, almost all of the present promastigotes were utilized for experimental animal inoculations, though a portion of the parasites served for morphological studies.

RESULTS

Man-biting species and natural infections of sand flies at different endemic areas

As a preliminary survey, man-biting sand fly species and natural infections of the flies with promastigotes were examined at different endemic areas of leishmaniasis. In total, the fly collections revealed the following 6 species: *Lu.*

TABLE 1

Man-biting sand fly species and natural infections with promastigotes in flies collected at 7 different endemic areas

Locality* (meters)	Sand fly species	No. flies collected	Abundance** in %	No. positive*** (%)
1. Tachina	*gomezi*	81	98.8	0
(100)	*panamensis*	1	1.2	0
2. Quininde	*panamensis*	19	55.0	0
(200)	*trapidoi*	13	38.2	0
	gomezi	1	2.9	0
	hartmanni	1	2.9	0
3. Quevedo	*trapidoi*	42	75.0	1 (2.4)
(400)	*shannoni*	7	12.5	0
	hartmanni	7	12.5	0
4. Olón	*gomezi*	62	95.4	0
(150)	*shannoni*	3	4.6	0
7. Naranjal	*hartmanni*	27	42.9	not dissected
(1,000)	*serrana*	22	34.9	not dissected
	trapidoi	14	22.2	not dissected
8. Portovelo	*gomezi*	42	84.0	0
(500)	*hartmanni*	7	14.0	0
	serrana	1	2.0	0
9. Zaruma	*hartmanni*	51	79.7	0
(1,100)	*gomezi*	9	14.1	0
	serrana	4	6.2	0

* Each locality number corresponds with that in Figure 1; figures in parentheses show meters above sea level.
** Percent of each species per total numbers collected in each locality.
*** Number or percent of positive flies with promastigotes among the sand flies collected and dissected.

gomezi, trapidoi, hartmanni, shannoni, serrana and *panamensis* (Table 1). Dominant species varied with localities and their altitudes; for example, in Tachina at an altitude of 100 m above sea level, the dominant species was *Lu. gomezi* whose abundance was 98.8%, whereas in Zaruma (1,100 m) it was *Lu. hartmanni* (79.9%). It was found that *Lu. gomezi, trapidoi* and *panamensis* were rather dominant at low land less than 500 m, while *hartmanni* and *serrana,* were dominant at high land in Ecuador.

By dissecting a total of 288 sand flies, except 63 flies from Naranjal, 1 (0.003%) was found to be positive for *Leishmania* promastigotes. In this preliminary fly dissection, thus, only 1 locality, Quevedo, harbored 1 positive *Lu. trapidoi.*

Natural infections of sand flies with promastigotes at Cañar

Sand fly infections with promastigotes showed a considerably high rate at Ocaña, Department of Cañar. For this reason, we selected this area as our main study site. In the Department, furthermore, 33 (11.9%) out of 278 examinees were found to be positive for active and/or cured leishmanial lesions in our epidemiological survey, the results of which will be published elsewhere.

Although fly collections were made at 6 sites distributed in 3 villages, no flies were found at the last village. Only 2 man-biting species were found in this Department: *Lu. trapidoi* and *hartmanni.* A total of 1,054 female *Lu. trapidoi* and *hartmanni* were examined for natural infections with promastigotes (Table 2). As a whole, positive flies were observed in 38 (7.7%) of the 491 *Lu. trapidoi* and 22 (3.9%) of the 563 *Lu. hartmanni.* In *Lu. trapidoi,* natural infections were only recognized at Ocaña; no fly was positive at Javin. On the other hand, *Lu. hartmanni* was positive for the parasite at Ocaña and Javin, though a relatively low rate of infection was found in the latter village. This species, moreover, had a wide range of vertical distribution in the present endemic area.

The promastigotes found in *Lu. trapidoi* and *hartmanni* revealed indistinguishable morphology from *Leishmania.* When the parasites were inoculated into hamsters, they took from 1 to 3 months to produce a small nodule or shallow

TABLE 2

Natural infections of sand flies with promastigotes in 4 locations of Ocaña, Department of Cañar, Ecuador

Locality* (meters)	Total no. sand flies caught	Leishmanial infections in sand flies					
		Lu. trapidoi			*Lu. hartmanni*		
		No. examined	No. positive	%	No. examined	No. positive	%
5. Ocaña (350–600)	658	470	38	8.1	188	11	5.9
6. Ocaña (950)	180	20	0	0.0	160	6	3.8
10. Javin (1,200–1,500)	216	1	0	0.0	215	5	2.3
11. Suscal (2,000)	0	0	0	0	0	0	0
Total (%)	1,054	491	38	7.7	563	22	3.9

* Each locality number corresponds with that in Figure 1; figures in parentheses show meters above sea level.

ulcer at the site of inoculation, and the amastigotes were scanty in smear specimens taken from the lesions.

Hourly examinations on natural infections at Ocaña (350 m)

From the viewpoint of transmission of leishmaniasis, it is interesting to examine the infection in connection with the biting activity of vector sand flies. For this purpose, sand flies were captured from dusk through dawn, and then they were dissected. *Lu. trapidoi* showed a high infection rate between 1800 and 2400 when the flies actively attacked their human bait (Table 3); thereafter, only 1 fly (1/102) was positive for promastigotes. The other species, *Lu. hartmanni,* on the other hand, attacked the bait evenly from dusk through dawn with a slightly higher frequency between 0200 and 0400. In this species, infections were found at every examination, except the period between 0400 and 0600. The infection rate ranged from 3.4% to 11.6% between 1800–0400.

Location of promastigotes in the sand fly gut

Most of the promastigotes attached to the epithelium of the sand fly gut, and the hind triangle was the main area infected with the parasites. They were round to oval in shape and small in size (Fig. 2), and showed rosette formation. At the outer edge of the rosette, a few of spindle-shaped to long, thin and active promastigotes with long flagella were observed and some of them were found swimming in the lumen. Table 4 shows the presence of promastigotes in the organs of *Lu. trapidoi* and *hartmanni* dissected within 3 days of collection. In the table, the result of observations on 10 randomly selected positive individuals of both species is shown. Although the flagellates were mainly found in the hind tri-

TABLE 3

Natural infections of sand flies with promastigotes in sand flies collected from dusk through dawn at 350 m above sea level, Ocaña, Department of Cañar, Ecuador

Time of day	Total no. sand fly caught	Leishmanial infections in sand flies			
		Lu. trapidoi		*Lu. hartmanni*	
		No. examined	No. positive (%)	No. examined	No. positive (%)
1800	148	111	10 (9.0)	37	3 (8.1)
2000	102	76	8 (10.5)	26	1 (3.8)
2200	112	77	6 (7.8)	35	2 (5.7)
2400	97	39	0 (0.0)	58	2 (3.4)
0200	82	39	0 (0.0)	43	5 (11.6)
0400–0600	54	24	1 (4.2)	30	0 (0.0)

angle, some were also observed in the hindgut, rectal ampula, stomach and Malpighian tubules in both sand fly species. The result indicated that the parasites were exclusively located in the posterior parts of the fly gut, and were not beyond cardia or the esophagus, during the early dissections (within 3 days) after collection.

To know the influence of time elapsed after blood meal, we dissected sand flies 5 or 6 days after collection, rearing them at ambient temperature (25–27°C) in the laboratory. Of the 53 fully engorged flies, a total of 8 (2/8 *trapidoi* and 6/45 *hartmanni*) were found to be positive for the parasites (Table 5). In this case, too, the hind triangle was the main infection site. It was noticeable to mention, however, that there were considerable numbers of actively moving and spindle-shaped promastigotes found in the stomach and Malpighian tubules of sand flies. This finding suggested that the parasites were moving forward into the anterior parts of the gut with the passage of time.

There were no differences in the location and the density of flagellates in the gut between the fed and the unfed fly groups. But, most of the sand flies which had taken a bloodmeal harbored fully developed eggs at dissection on day 5 or 6 post-feeding.

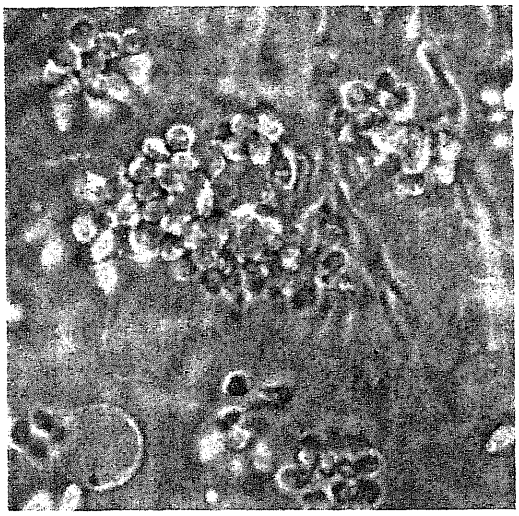

FIGURE 2. Promastigotes attached to the epithelium of hind triangle of *Lu. trapidoi.* ×1,000.

DISCUSSION

In the current study, 2 species of sand flies, *Lu. trapidoi* and *Lu. hartmanni,* were, for the first time, found to be infected with promastigotes of *Leishmania* in Ecuador. The parasite was considered to belong to *L. braziliensis* s.l., based mainly on the location and form in the sand fly, showing a predominant hindgut infection, and on the indolent development of lesions in Syrian hamsters without metastasis.[7] In this paper, however, suffice it to say that the present parasites are *Leishmania* sp., probably *L. braziliensis* s.l., until the isolates have been typed.

Lu. trapidoi, has already been incriminated as a principal vector of leishmaniasis in Panama.[6] According to Lainson and Shaw, 10 sand fly species, viz., *Lu. intermedia, pessoai, umbratilis, trapidoi, ylephiletor, gomezi, olmeca olmeca, flaviscutellata, wellcomei* and *panamensis,* were incriminated as the principal vectors of leishmaniasis in Central and South American countries, while *Lu. verrucarum, peruensis, olmeca bicolor* and *longipalpis* have been highly suspected as vectors.[8] On the other hand, our second species, *Lu. hartmanni* has never been recorded in the list of vectors of leishmaniasis in any part of the New World.

On the examinations of flies in the Department of Cañar, *Lu. trapidoi* showed a slightly higher rate of infection with promastigotes, as compared to *Lu. hartmanni.* This gap might be caused by the difference in distribution patterns of these 2 species. The former species was dominant in low land, whereas the latter was dominant in high land where the intensity of transmission would be weak, because of a relatively low density of sand fly population.

When Panamanian sand flies, *Lu. sanguinaria* and *gomezi* were fed on hamsters infected with strains believed to be *L. braziliensis,* the infections were characterized by growth of promastigotes in the hindgut, especially the hind triangle, with or without growth in the midgut.[9] In their study, over 90% of the flies had attached flagellates in the hindgut, and almost half of these were found only in the hind triangle. On the other hand, *L. mexicana* strains caused midgut infections alone in both above species of sand flies, confining only to the midgut in over 70% of the flies, without even free flagellates in the hindgut. These findings in sand flies have long been believed to be useful information for differentiating *L. braziliensis* s.l. from *L. mexicana* s.l. In the present early dissection (within 3 days) after fly

TABLE 4

Presence of promastigotes in the internal organs of sand flies, Lu. trapidoi *and* Lu. hartmanni, *within 3 days after collection; 10 of each were randomly selected from individuals positive for parasites*

Sand fly species	No.	Internal organs*				
		Stomach	Hind triangle	Hindgut	Rectal ampulla	Malp. tubules
Lu. trapidoi	1	−**	+	−	−	−
	2	−	+	−	−	−
	3	−	+	−	−	−
	4	−	+++	−	−	−
	5	−	+++	+	+	−
	6	−	−	+	−	−
	7	−	+++	++	+	−
	8	−	++	+	+	−
	9	+	−	−	−	+
	10	−	+	−	−	+
Lu. hartmanni	1	−	+++	−	−	−
	2	−	+	−	−	−
	3	−	+	−	−	−
	4	−	−	−	+	−
	5	−	+	−	−	−
	6	−	+	−	−	−
	7	−	+++	+	+	−
	8	−	+++	++	+	+
	9	−	+++	−	−	−
	10	−	+++	−	−	−

* Each organ was identified according to the description by Johnson et al.;[6] no parasite was observed in buccal cavity, pharynx, esophagus and cardia.

** −, no parasites; +, less than 20; ++, 21–50; +++, more than 51.

collection, most (85.0%) of the promastigotes observed were located in the hind triangle, though a few parasites were also found in other posterior parts of the gut. It was, however, rare to find promastigotes in the anterior parts of the gut such as the stomach. When the flies were kept at ambient temperatures for 5 or 6 days after the bloodmeal, the promastigotes tended to show a forward migration, particularly into the stomach.

By examining natural infections of sand flies collected from dusk through dawn, we tried to compare the relationship between infection rate and blood seeking time of the flies. The blood seeking behavior of sand flies would be influenced by the condition of individual flies, such as parous or nulliparous, and also by species difference of the fly itself. It is reasonable to assume that the infected flies in the present study have been attempting to take a second or perhaps a third or fourth bloodmeal, feeding at least once

TABLE 5

Presence of promatigotes in the internal organs of sand flies dissected 5 or 6 days post-feeding

Sand fly species	No.	Internal organs*				
		Stomach	Hind triangle	Hindgut	Rectal ampulla	Malp. tubules
Lu. trapidoi	1	−**	++	+	−	−
	2	+	+++	++	+	+++
Lu. hartmanni	1	−	+++	+	−	−
	2	++	++	+	−	++
	3	−	+++	−	−	−
	4	−	++	+	+	−
	5	++	+++	+	++	−
	6	+	+++	++	+	+

* Each organ was identified according to the description by Johnson et al.;[6] no parasite was observed in buccal cavity, pharynx, esophagus and cardia.

** −, no parasites; +, less than 20; ++, 21–50; +++, more than 51.

on infected hosts, as noted by Johnson et al.[6] *Lu. trapidoi* revealed a high infection rate between 1800 and 2400, while *Lu. hartmanni* were positive for promastigotes between 1800 and 0400 with a peak rate of infection between 0200 and 0400. This difference may be caused by the different biting activities of the 2 species. It was found that a large number of the former species attacked human bait in early dusk, but such a remarkable fluctuation in numbers has not been observed in the latter. It is known that infection rates of sand flies with *Leishmania* promastigotes are affected by method of collection. At Cerro Campana, Panama, 8.0% of *Lu. ylephiletor* collected from human bait were infected, while at the same time and place and only a small distance away, sand flies collected when attracted to light had an infection rate of only 3.2%.[6] Thus, various factors affect the infection rates of sand flies with *Leishmania* promastigotes.

In sand fly collections at 7 different localities endemic for leishmaniasis, 6 man-biting species were captured from human bait. Of these 6, 4 sand fly species, *Lu. gomezi, trapidoi, shannoni* and *panamensis,* were found to be naturally infected with strains believed to be *L. braziliensis* in Panama.[6]

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