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Leishmaniasis in Bolivia. I. *Lutzomyia longipalpis* (Lutz & Neiva, 1912) as the vector of visceral leishmaniasis in Los Yungas

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Abstract

A relatively high leishmanial infection rate was found in the phlebotomine sandfly *Lutzomyia longipalpis* collected from three villages of the Los Yungas region (Department of La Paz, Bolivia). 2,578 female sandflies were dissected. In three houses surveyed in Santa Barbara promastigote infection rates of *Lu. longipalpis* were 4·2, 2·2 and 3·2% respectively. Anatomical localization of the infection in the insect, and biochemical characterization of the strains indicate that the parasite belongs to the *Leishmania donovani* complex. The geographical area and the biotopes of *Lu. longipalpis* are discussed in relation to the vector-parasite relationship.

Introduction

Epidemiological studies on kala-azar in neo-tropical regions have been carried out mainly in the north-east and north of Brazil, where the sandfly *Lutzomyia longipalpis* is considered to be the vector of the disease (DEANE, 1956; DEANE & DEANE, 1964). However, since the pioneer works of CHAGAS *et al.* (1937, 1938), the presumption that this sandfly plays a leading role in the transmission of kala-azar has remained based only on indirect evidence (DEANE & DEANE, 1954b; LAINSON *et al.*, 1977a, LAINSON & SHAW, 1979; LAINSON *et al.*, 1983). This evidence is: (i) the predominance of *Lu. longipalpis* in most foci of human and canine visceral leishmaniasis, (ii) the same distribution of the insect and the human disease throughout Latin America, (iii) the distribution around human habitations and endophily, (iv) the attraction to man, (v) the experimental infection obtained from infected patients, dogs and foxes, (vi) the experimental transmission of the parasite from hamster to hamster by the bite of infected *Lu. longipalpis*, and (vii) the elimination of the disease after systematic spraying of houses with insecticides.

Only a few studies of the natural infection rate in *Lu. longipalpis* have been made to date (DEANE & DEANE, 1954a; RYAN *et al.*, 1984; LAINSON *et al.*, 1985) and biochemical characterization of the strains has not been completed but it seems that the *L. donovani* complex may be implicated in spontaneous infections.

DEANE & DEANE (1954a) studied a focus of visceral leishmaniasis in the suburbs of Sobral (Ceara), Brazil, situated at an altitude varying from 100 to 800 metres. At the end of December, just before the rainy season, they found two of 141 *Lu. longipalpis* dissected to be infected (1·4%). The infection was heavy with a supra-pylarian localization, similar to infection by *L. chagasi* s.l. (LAINSON *et al.*, 1977b). A third specimen was found among 876 additional dissected *Lu. longipalpis*, giving an infection rate of 0·3% (1017 sandflies examined).

During an epidemic outbreak, LOPES (1956) found in Jacobina (Bahia) eight of 209 (3·8%) *Lu. longipalpis* infected with promastigotes: four of the infected specimens came from chicken coops, three from pig-sties and one from a tree trunk sheltering hens. In the same city, SHERLOCK & PESSOA (1966) and SHERLOCK & GUITTON (1969) found one *Lu. longipalpis* infected of 91 samples examined.

RYAN *et al.* (1984) have recently recorded the presence of heavy suprapylarian promastigote infections in 8 of 1,500 (0·5%) *Lu. longipalpis* on the island of Marajó, Pará.

In 1984, LAINSON *et al.* (1985) in the focus of Santarém, Pará State, found 35 (7·14%) of 491 *Lu. longipalpis* dissected to be infected, and the parasite was transmitted to hamsters by the bites of infected wild flies.

From October 1982, epidemiological studies on the vector of visceral leishmaniasis in Bolivia have been carried out. In the Los Yungas area (Department of La Paz), both canine and human visceral leishmaniasis were described recently (ANGLES *et al.*, 1982; DESJEUX *et al.*, 1983), confirming a suspicion that kala-azar may occur in this region (GATTI *et al.*, 1939). VELASCO (1973) surveyed the phlebotomine sandfly fauna during the cool and dry winter season (from June to August); he noted, for the first time, the presence of *Lu. longipalpis* and found it very common and widespread (present in 65% of collections).

These observations led us to investigate the rate of natural infection among the population of *Lu. longipalpis* in this area.

Materials and Methods

Area studied

Our area of investigation (see Fig.) is located in the village of Santa Barbara, near the village of Chijchipa, where a second case of kala-azar was described in November 1982 (Urjel & Desjeux, 1982, personal communication). Santa Barbara is situated at the foot of a valley overlooked to the south-east by the village of Coroico, the county-town of Nor-Yungas Province.

The Yungas constitute a very peculiar geographical terrain. They are steep-sided valleys in the foothills of the Eastern Andean Cordillera. Santa Barbara, at an altitude of 950 m, is overlooked by the mountains of Uchumachi and Kusilluni, rising to a height of 2500 metres. The valley of Coroico River is directed north-east. The climate is subtropical with an average yearly temperature of 18°C, and a mean annual rainfall of 1200mm. Some of the inhabitants are black and live under poor conditions, cultivating coffee, coca plants and citrus trees. Steep slopes going down from Coroico are covered with wet subtropical forest. Coffee

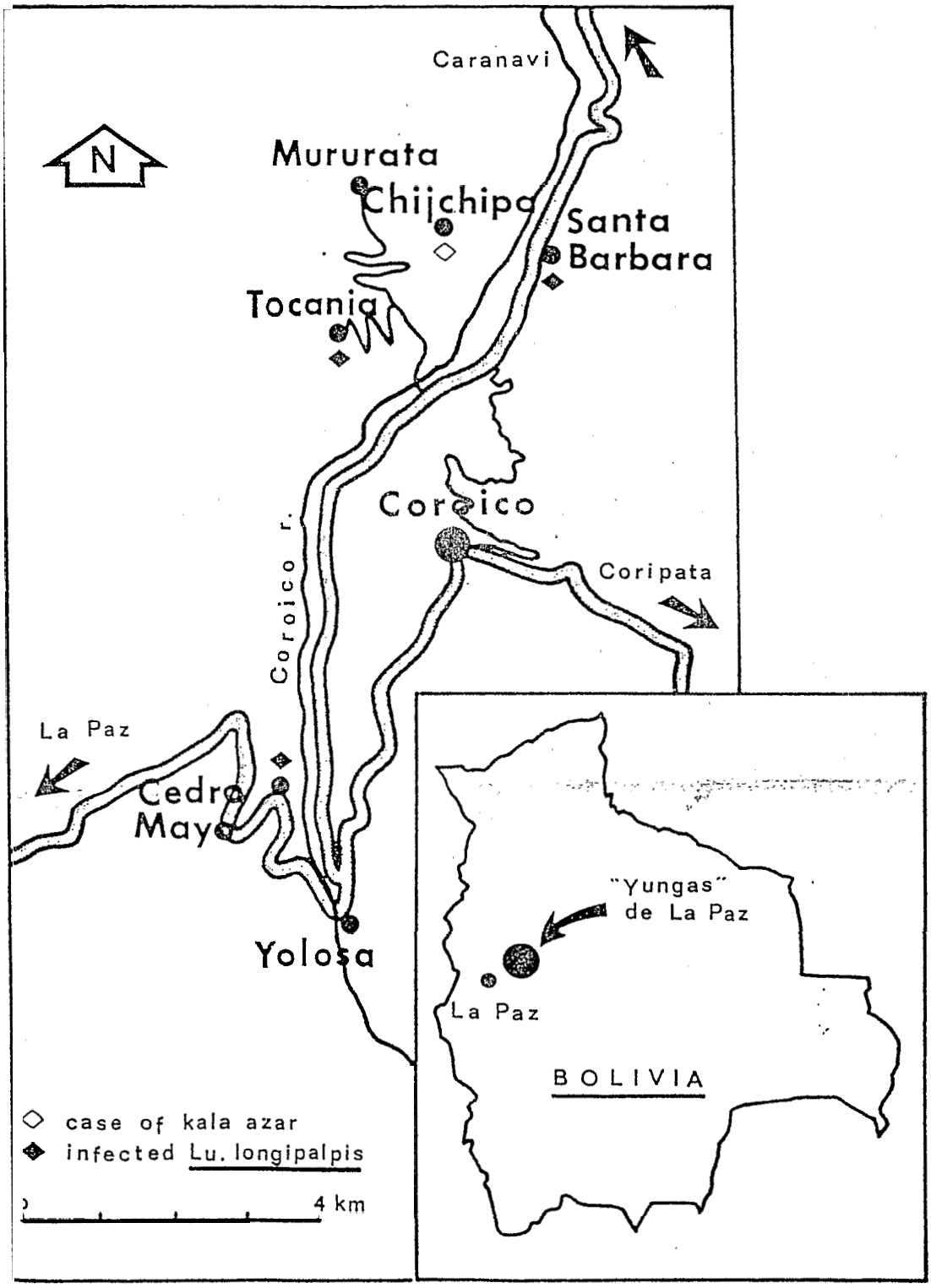

Fig. Map showing the area surveyed.

plantations and cultivated plots surround the villages and are situated at the bottom of the valleys. This area is less populated and much more humid than that of Coripata/Khala-Khala, where canine visceral leishmaniasis was described (ANGLES *et al.*, 1982).

Three villages were surveyed: Santa Barbara (950 m), with houses scattered all along the Coroico River; Tocania (1320 m), on the north-western slope near Chijchipa; and Cedro Mayo (1370 m), situated on a slope, 9 km from Santa Barbara.

Sandfly collections

Phlebotomine sandflies were collected in October and November 1982, at the beginning of the rainy season. Collections, using human bait, were made between 19 and 24 hours, around isolated houses.

Isolation of Leishmania *from sandflies*

For all sandflies collected, species were identified by the examination of genitalia. Females were dissected; when found infected by promastigotes, the intestinal tract was picked up from the slide and carefully disrupted in 0·5 ml of sterile 9‰ NaCl. The suspension was thrown into modified NNN rabbit blood agar base (DECKER-JACKSON & HONIBERG, 1978), with a (40% v/v, overlay/base) liquid overlay of PBS.

The tubes were examined every week and discarded if negative after one month. The isolations were routinely passaged.

Isoenzyme characterization of the stocks

Multiplication of the stocks was obtained dividing the contents of a single tube between larger tubes; cultures were routinely harvested, centrifuged at 2500 rpm for 10 min and washed (three times); pellets were stored at −80°C until use. Just before electrophoresis, an equal volume of hypotonic enzyme stabilizer was added. Lysis of the parasites was achieved through freeze-thawing procedures repeated three times.

The following enzymic systems were used for characterization:

Malate dehydrogenase E.C.1.1.1.37 (MDH)
Malate dehydrogenase (oxaloacetate decarboxylating) $NADP^+$ E.C.1.1.1.40 (ME)
Isocitrate dehydrogenase E.C.1.1.1.42 (ICD)
6 Phosphogluconate dehydrogenase E.C.1.1.1.44 (6PGDH)
Glucose 6 phosphate dehydrogenase E.C.1.1.1.49 (G6PDH)
Phosphoglucomutase E.C.2.7.5.1. (PGM)
Mannose phosphate isomerase E.C.5.3.1.8. (MPI)
Glucose phosphate isomerase E.C.5.3.1.9. (GPI)
Glutamate dehydrogenase E.C.1.4.1.2. (GDHNAD and GDHNADP$^+$)
Aconitate hydrolase E.C.4.2.1.3. (ACON)
Peptidase E.C.3.4.11.11. (PEP)

Five reference strains were used:

ITMAP 263 *L. infantum* (man)—Morocco, 1967.
M 2682 *L. chagasi* s.l. (man)—Bahia State, Brazil, 1974
M 2904 *L. braziliensis braziliensis* (man)—Pará State, Brazil, 1975.
PH 8 *L. mexicana amazonensis* (*Lu. flaviscutellata*)—Pará State, Brazil, 1967
M 5088 *L. hertigi deanei* (*Coendou prehensilis*)—Pará State, Brazil, 1978.

The electrophoresis were carried out on cellulose acetate plates (Helena Laboratories) running at 200 volts for 30 min. All cell buffers were used with 20% sucrose, their preparation being adapted from LANHAM *et al.* (1981) and as previously described (TIBAYRENC & DESJEUX, 1983).

Results

Infection rate of Lu. longipalpis

Lu. longipalpis (see Table) represented 96% of all the phlebotomine sandflies captured. 2578 females were dissected. Promastigote infection rates of *Lu. longipalpis* were, 4·2, 2·2 and 3·2% respectively in three houses surveyed in Santa Barbara; in the fourth, a nearby poultry farm with walls covered with a coating containing residual insecticide, no infected sandflies were found.

Two *Lu. longipalpis* of 240 (0·8%) were found infected in a house located 300 metres below the village of Tocania. Very similar results were obtained in Cedro Mayo: two specimens out of 187 were infected (1%).

In every infected sandfly, the parasitism was heavy, with distension of the upper digestive track (proventriculus and cardia). The localization of the promastigotes in the suprapyloric region together with their large size, suggest that the parasite belongs to the *L. donovani* complex (LAINSON *et al.*, 1977b).

Isolation of Leishmania *from* Lu. longipalpis *specimens*

Five strains were isolated from infected *Lu. longipalpis* females, and then subcultured weekly. Semi-

Table I—Results of catches and dissections of *L. longipalpis* achieved around human habitations in the focus of visceral leishmaniasis in Los Yungas (Coroico village), Department of La Paz. October/December 1982. Dwellings (1) (3) (5) and (6) had dogs.

Village	Santa Barbara				Tocania	Cedro Mayo
Altitude	950 m				1320 m	1370 m
Situation	Bottom of valley				North-west slope of valley	As Tocania but 9 km upstream
	(1)	(2)	(3)	(4)	(5)	(6)
Dwelling	Dom Angel family	Adriana family	Nicolas family	Poultry farm	Sabala family	Pinedo family
No. of *L. longipalpis* dissected	1910	45	91	105	240	187
No. of *L. longipalpis* infected (+)	81	1	3	—	2	2
Rate of infection	4·2%	2·2%	3·2%	—	0·8%	1·0%

massive cultures were obtained, giving sufficient, material for enzyme electrophoresis.

Isoenzyme characterization of the stocks

The electrophoretic result for the five Bolivian stocks studied showed, for 12 enzymes, a consistent enzymic profile, similar to that of ITMAP-263 (*L. infantum*) and M-2682 (*L. chagasi* s.l.); they were easily distinguished from *L. m. amazonensis*, *L. b. braziliensis* and *L. h. deanei*. We conclude that the five strains of *Leishmania* isolated from *Lu. longipalpis* specimens belong to the *L. donovani* complex.

Further details of these enzyme similarities between *Lu. longipalpis* Bolivian stocks, one stock from a human Bolivian visceral case, and visceral *Leishmania* reference stocks from Brazil and Morocco, will be published elsewhere (DESJEUX *et al.*, in press), discussing their significance and taxonomic consequences.

Discussion

The Yungas region of Bolivia is well known as a focus of mucocutaneous leishmaniasis or espundia (DESJEUX, 1974, 1976; DESJEUX *et al.*, 1974), which affects a large proportion of the inhabitants. The discovery in 1982 of two cases of visceral leishmaniasis in the same area, associated with widespread canine visceral leishmaniasis, together with the finding of numerous specimens of *Lu. longipalpis* with a relatively high infection rate, provokes evidence that in this region transmission cycles of both visceral and mucocutaneous leishmaniasis co-exist.

The geography is favourable and this area, with its mild climate but with an Amazonian influence, presents an extraordinary diversity of biotopes. However, the visceral leishmaniasis focus studied shows the general characteristics observed in other neotropical foci, i.e., (i) villages with scattered houses, (ii) poor agriculture on small plots of land, with secondary vegetation, (iii) part of the population being black (negro), as in the foci of Venezuela (PIFANO *et al.*, 1976) or Brazil (VIANNA MARTINS *et al.*, 1956; SHERLOCK & PESSOA, 1966); (iv) location in warm foothills in which DEANE & DEANE (1964) had noted (as *bosqueirao* or *pe da serra*) that *Lu. longipalpis* infection rates are higher in the valleys than on the slopes; and (v) in our three foci, peri-domestic phlebotomine sandflies represent a considerable inconvenience to the inhabitants, with *Lu. longipalpis* predominant. We have observed that the populations of this species are particularly aggressive in the spring (September to December), as observed by ARJONA (1971) in Colombia.

However, in spite of the fact that most features in our foci are similar to others in South America, two peculiarities may be noted in this focus—the altitude level of 1100-1600 metres, and the co-existence of both visceral and mucocutaneous leishmaniases, a fact rarely mentioned in the literature so far.

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