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THE TRANSMISSION OF SUPRAPYLARIAN *LEISHMANIA* BY THE BITE OF EXPERIMENTALLY INFECTED SAND FLIES (DIPTERA: PSYCHODIDAE)

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Lutzomyia furcata *transmitted* Leishmania chagasi *to a hamster 10 days after being experimentally fed on an infected spleen. An individual female* Psychodopygus carrerai carrerai *that had fed on a hamster lesion caused by* Leishmania mexicana amazonensis *transmitted this parasite 6 days later to another hamster. Transmission electron microscopy of this fly's head revealed a small number of degenerate promastigotes in the foregut, but only a few were attached.*

Key word: sand flies – *Leishmania* – transmission – Brazil

During the past 10 years, a number of workers have recorded the experimental transmission of suprapylarian leishmanias (Lainson & Shaw, 1979) by the bite of sand fly species not thought to play any role in the epizootology of these parasites (Killick-Kendrick, 1979, 1986).

We report here the experimental transmission of *Leishmania* (*L.*) *chagasi* Cunha & Chagas, 1937, by the bite of *Lutzomyia furcata* (Mangabeira, 1941), and *L.* (*L.*) *mexicana amazonensis* by the bite of a sand fly of the genus *Psychodopygus, P. carrerai carrerai* (Barretto, 1946).

L. (L.) chagasi has previously been experimentally transmitted (Lainson et al., 1977, 1984, 1985; Gonçalves et al., 1985; Stephenson & Ward, 1987) only by its natural vector, *L. longipalpis* (Lutz & Neiva, 1912) and, as far as we are aware, there has been no laboratory transmission reported of a suprapylarian *Leishmania* by a *Psychodopygus* species.

MATERIALS AND METHODS

Transmission of *L. m. amazonensis* by *P. c. carrerai* – We collected 149 female sand flies in a Shannon trap (Shannon, 1934) in the high forest of Serra dos Carajás (N2 area, see Ward et al., 1973 for description). Flies were left in the forest overnight (16.05.1985) in a holding cage that contained a hamster infected with *L. m. amazonensis* (IFLA/BR/67/PH8 reference stock). The hamster was anaesthetized and restrained, with only the lesions of the rear feet exposed (see Ryan et al., 1987a&b for procedures). The 85 females that fed were taken to our laboratory in Belém and of those that survived oviposition, nine subsequently attempted to refeed on individual hamsters offered to them, some 6 days after their infective meal. Immediately after the insects had probed and/or fed, the head of each fly was placed in 2.5% glutaraldehyde in 0.07M cacodylate buffer for 1 hour and stored in buffer for 5 weeks until it could be processed further. The head was post fixed in 1% osmium tetroxide for 1 hour, dehydrated in an ethnol series, and treated in an ethanol/Spurr's low viscosity resin mixture before finally being embedded in the same resin. Ultrathin sections were cut on a LKB Ultratome III, mounted on uncoated copper grids, stained for 25 minutes in uranyl acetate and 10 minutes in lead citrate, and examined with a Corinth electron microscope at 60 kV.

Transmission of *L. chagasi* by *L. furcata* – The parasite used was stock HOM/BR/75/L. dPP75 (M.2682) isolated from man in Bahia, Brazil (Lainson et al., 1977). The sand flies were from our colony of *L. furcata* originally reared by Dr. R.D. Ward and, at the time, in its 54th generation. The sand flies (6 days old) were fed on a suspension of heavily infected hamster spleen triturated in defibrinated, heat inactivated rabbit blood (Ward, 1977). The infected flies were refed on hamsters 10 days later, after oviposition, and were offered a saturated sucrose solution throughout their maintainance.

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RESULTS

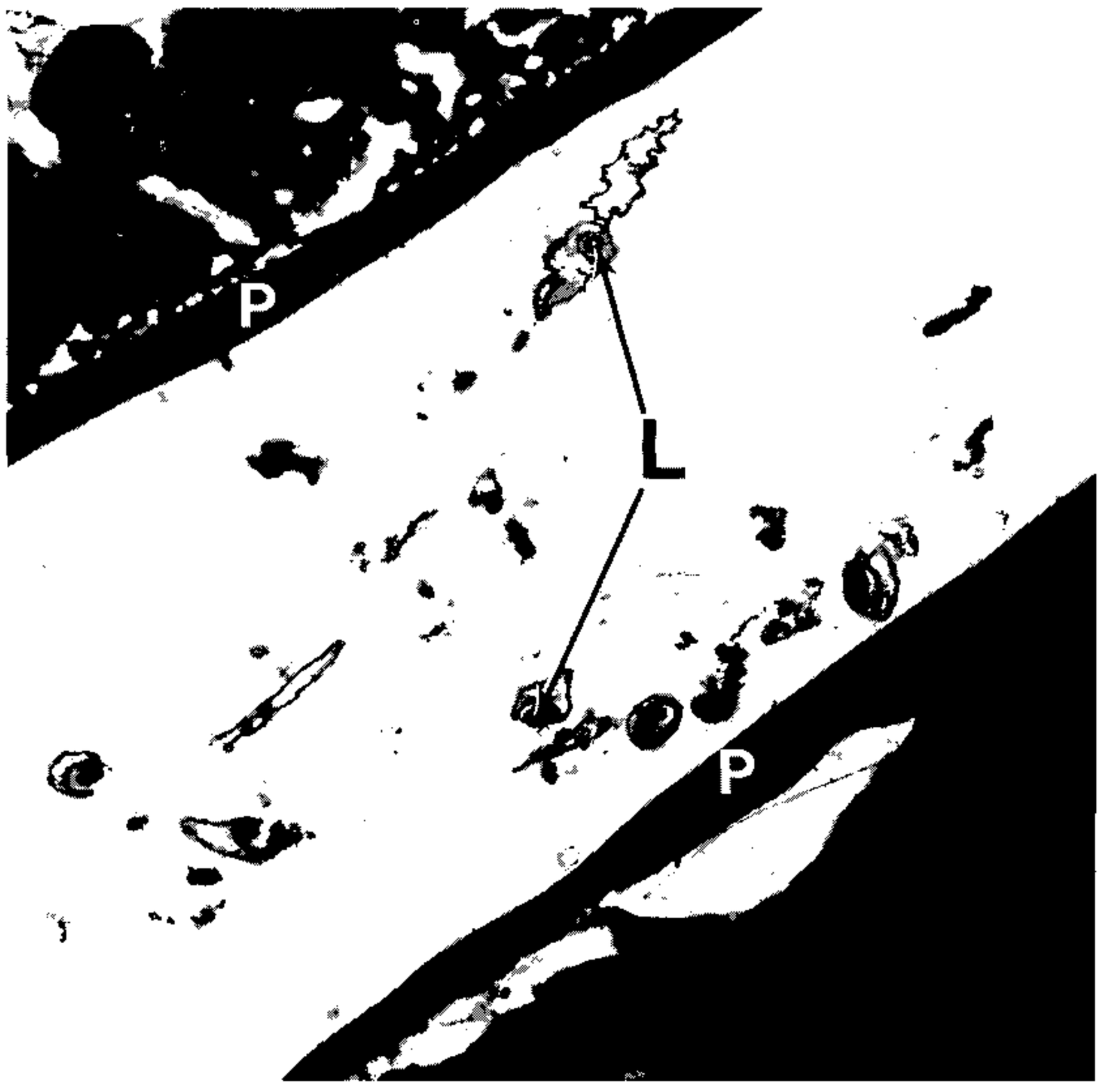
Transmission of *L. m. amazonensis* – Of the 9 flies which had probed or refed, two were uninfected, 2 were not examined and the remaining 5 had well developed infections of the cardia and proventriculus (Richards & Richards, 1971). Four of these infected flies were *P. wellcomei* and the other was *P. c. carrerai.* The latter fly had probed the rear right foot of a hamster for over 5 minutes and had succeeded in obtaining a partial blood meal. The hamster used for the *P. c. carrerai* feed developed a small lesion at the site of the probe which was positive for amastigotes. Electron microscopic examination of the head showed only a light infection of the pharynx and cibarium, and most of these flagellates were unattached and degenerate (fig). No transmissions were obtained by the bite of *P. wellcomei.*

Transmission of *L. chagasi* – Three of the *L. furcata* females that refed on the hamster were dissected, and all were heavily infected in the cardia and proventriculus. The animal appeared sick 8 months later and was killed - the liver and spleen were heavily infected with amastigotes.

DISCUSSION

This the first transmission of *L. chagasi* by a sand fly species which is not the natural vector (Table) and the second record of *L. furcata* transmitting *Leishmania* by bite. As mentioned elsewhere (Ryan et al., 1986[a]), *L. furcata* has, to our knowledge, never been found naturally infected with flagellates, nor do we consider this sand fly of importance in the transmission of *L.* (*L.*) *chagasi* to man as it is not highly anthropophilic (Ryan et al., 1986[b]).

P. c. carrerai has recently been found infected by a *Leishmania* species (Caillard et al., 1986) which is not of the *mexicana* complex. We have not detected flagellates in 549 wild caught *P. c. carrerai* (Ryan et al., 1987[a & b]) but this species remains a suspect as a vector of disease to man because of its marked anthropophilic behaviour (Ward et al., 1973).



Longitudinal section of the head of a female *Psychodopygus carrerai carrerai*, infected with *Leishmania mexicana amazonensis* and fixed immediately post feed (and transmission), X6K, transmission electron micrograph. This shows the cuticular linings of the anterior pharynix (P) and remains of *Leishmania* with flagellar axonemes (L).

TABLE

Experimental transmissions of *Leishmania* species by the bite of sand flies

Leishmania	Sand fly	References
L. b. braziliensis	*P. wellcomei**	Ryan et al., (1987[a])
L. braziliensis complex	*P. s. maripaensis*	Ryan et al., (1987[b])
	*P. s. squamiventris***	
L. chagasi	*L. longipalpis*	Lainson et al. (1977, 1984, 1985); Gonçalves et al. (1985), Stephenson & Ward (1987).
	L. furcata	This paper
L. donovani	*P. argentipes*	Shortt et al. (1931); Napier et al. (1933); Smith et al. (1936, 1940, 1941) Swaminath et al. (1942).
	P. chinensis	Feng & Chung (1941); Ho et al. (1943) Chung et al. (1951); Yuan et al. (1943)
	P. longiductus	Dergacheva & Strelkova (1985)
	P. smirnovi	Dergacheva & Strelkova (1985)
L. garnhami	*L. townsendi*	Scorza et al. (1984)
L. infantum	*P. ariasi*	Rioux et al. (1979)
	P. perniciosus	Pozio et al. (1985)
L. major	*P. papatasi*	Adler & Ber (1941); Kryukova (1941); Killick-Kendrick et al. (1985[a] & [b]); Warburg & Schlein (1986)***
	P. dubosqi	Beach et al. (1984)
L. m. amazonensis	*L. flaviscutellata*	Ward et al. (1977); Ryan et al. (1986[a]) Lainson et al. (1987)
	L. furcata	Ryan et al. (1986[a])
	L. longipalpis	Killick-Kendrick et al. (1977)
	P. c. carrerai	This paper
L. m. mexicana	*L. anthophora*	Endris & Young, in K.-Kendrick (1986)
	L. cruciata	Williams (1966)
	L. diabolica	Lawyer & Young, in K.-Kendrick (1986)
	L. longipalpis	Coelho & Falcão (1962); Coelho et al. (1967[a&b])
	L. renei	Coelho & Falcão (1962); Coelho et al. (1967[a&b])
	L. shannoni	Lawyer & Young, in K.-Kendrick (1986)
	P. spp. +	Strangeways-Dixon & Lainson (1962, 1966)

*This fly was possibly *P. complexus*, which occurs in the study area at proportions of 7 *P. wellcomei*: 1 *P. complexus*.

**This fly was possibly *P. chagasi*, which occurs in the study area at proportions of 30 *P. s. squamiventris*: 1 *P. chagasi*.

***Transmission here refers to the ejection of *Leishmania* during a forced probe of the fly.

\+ The identity of this species is discussed by Ready & Lainson (1982) and Williams (1983).

This table is an unpdate of information given by Killick-Kendrick (1979, 1986).

The transmission of *L. m. amazonensis* by this fly, which had a few degenerating parasites in the pharynx and cibarium, possibly lends support to the "blocked fly" theory of transmission (Shortt & Swaminath, 1928), recently discussed by Jefferies et al. (1986) and Warburg & Schlein (1986). Walters et al. (1987) suggest that the forms in the pharynx and cibarium are ephemeral, continuously generated from parasites at the proventriculus, resulting in so many of the flagellates found there being degenerate. In our observations the degeneration may possibly be attributed to the delay in processing the fly. The difficulty experienced by this fly in obtaining a blood meal in the present experiment supports the suggestion of Killick-Kendrick & Molyneux (1981) that infection with *Leishmania* in some way reduces the ability of

the sand fly to feed: a theory that has found support from other investigators (Beach et al., 1986).

RESUMO

A transmissão de *Leishmania* suprapilária pela picada do flebotomíneo infectado experimentalmente – O protozoário *Leishmania* (*L.*) *chagasi* foi transmitido experimentalmente a um hamster pela picada do flebotomíneo *Lutzomyia furcata*. Os insetos foram infectados através de uma membrana (pele de pinto), utilizando-se formas amastigotas provenientes do baço de um hamster infectado. O baço foi triturado em sangue de coelho. A *L.* (*L.*) *amazonensis* foi transmitida a um hamster pela picada do flebotomíneo *Psychodopygus c. carrerai*, previamente alimentado em lesão de pele de um outro hamster infectado com o parasita. O exame desse flebotomíneo, através de microscópio eletrônico, revelou um número pequeno de flagelados degenerados, livres no lumen do intestino anterior.

Palavras-chave: flebotomíneos – *Leishmania* – transmissão – Brasil

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