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Experimental transmissions of *Leishmania mexicana amazonesnis* Lainson & Shaw, between hamsters by the bite of *Lutzomyia flaviscutellata* (Mangabeira)

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Lutzomyia flaviscutellata (Diptera: Psychodidae) remains the only proved vector of *Leishmania mexicana amazonensis* in the neotropics. Thus, in North Brazil, 45 out of 7,322 flies of this species were found naturally infected and the parasite was isolated from 15 of these sandflies following inoculation of the flagellates into the skin of hamsters (LAINSON & SHAW, 1968; WARD *et al.*, 1973). A parasite closely resembling *L. m. amazonensis* has also been isolated from naturally infected *L. flaviscutellata* in Trinidad (TIKASINGH, 1975). We would like to report, here, on the experimental transmission of *L. m. amazonensis* by the bite of laboratory-bred *L. flaviscutellata*, thus completing our chain of evidence incriminating this insect as the major vector in nature.

Strain M2269, isolated in 1973 from a single-lesion infection of a man from Magalhães Barata, Pará State, was used in the present work. It had been maintained in the laboratory by serial passage in hamsters. To infect the sandflies we used a hamster with large leishmanial histiocytomata on the nose and hind feet. Between the 8th and 10th November, 1976, the animal was anaesthetized on three occasions with Nembutal (Abbott) and placed in a cage with about 60 female *L. flaviscutellata:* these flies were from the ninth laboratory generation of our colony. A total of 25 flies fed, and after oviposition they were given a daily opportunity to feed again on anaesthetized, uninfected hamsters, which were laid on their backs in the cage. The number of flies which fed between one and four times is shown in Table 1.

As our main aim was to transmit the parasite, only five flies that had consistently refused to feed again or were obviously weak were dissected. One of these had fed four times and was uninfected; whilst another which had fed again on the 14th November was dissected four days later and had promastigotes throughout the mid- and fore-gut, with a cluster of parasites radiating from the posterior rim of the pharynx (eight to ten-day-old infection). The mid-gut forms were long, thin, highly active parasites, whilst those apparently attached to the pharynx were shorter and more rounded. No parasites were seen inside the pharynx, cibarium or proboscis. A similar infection, 13 to 15 days old, was observed in a fly that had fed three times. Of two further flies, that had refused to re-feed, one had an eight to ten-day-old infection with flagellates confined to the mid-gut; the other was a 16 to 18-day-old infection which was similar to those seen in the re-fed flies mentioned above, except for the added presence of promastigotes in the crop. Thus, four out of five of the dissected flies were found to be infected.

The six hamsters, on which flies had been re-fed, were kept in separate cages and were first examined on the 30th November, which was four to 17 days after the possibility of transmissions. There was no evidence of infection, but when re-examined ten days later four of the hamsters had small, red swellings on their feet, and Giemsa-stained smears revealed abundant amastigotes. Metastatic spread of the parasites from one foot to another is very unlikely at such an early stage of infection (23 to 27 days), and it is most probable that nine transmissions took place, rather than four. It is impossible to say, however, whether these transmissions were by the bites of separate flies or by the interrupted feeding of single flies.

The present results show that *L. flaviscutellata* can transmit *L. m. amazonensis* only three to five days after the infective feed. It is interesting that STRANGWAYS-DIXON & LAINSON (1962) transmitted *L. m. mexicana* Biagi in just under four days after the sandfly was infected.

Table I – The number of *L. flaviscutellata* fed and transmissions of *L. m. amazonensis*

Date	Number of flies fed				Approximate no. of days since infective feed	Transmission
	1st feed	2nd feed	3rd feed	4th feed		
8.11.76	11				–	—
9.11.76	14				–	—
10.11.76	1				–	—
13.11.76		3			3–5	+ve lesions on 3 feet
14.11.76		5			4–6	+ve lesion on 1 foot
16.11.76		3			6–8	+ve lesions on 3 feet
18.11.76			3		8–10	+ve lesions on 2 feet
22.11.76					12–14	—ve
26.11.76					16–18	—ve

It remains to be seen if this very short developmental period before transmission by the sandfly is a characteristic of the *L. mexicana* sub-species.

Transmission of *L. m. amazonensis* by *L. flaviscutellata* was also achieved during a third feed, with an eight to 10-day-old infection, and the presence and location of abundant parasites in the fly dissected after 16 to 18 days suggests that even older infections may be transmitted. Further investigations are now necessary to determine if a single fly can repeatedly transmit at each re-feed.

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