OBSERVATIONS ON THE PARASITE *LEISHMANIA MEXICANA AMAZONENSIS* AND ITS NATURAL INFECTION OF THE SAND FLY *LUTZOMYIA OLMECA NOCIVA*[1]

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INTRODUCTION

Lutzomyia olmeca nociva Young and Arias is a phlebotomine sand fly of the medically important subgenus *Nyssomyia,* morphologically distinct from the closely related *Lu. flaviscutellata* (Mangabeira). These two species are sympatric in the Manaus area of Brazil. *Lu. flaviscutellata* is known to be the principal vector of *Leishmania mexicana amazonensis*[6] Lainson and Shaw in the lower Amazon Basin *(1, 2),* where it is thought likely to be the only vector of this parasite *(3, 4).*

Because of the close relation of *Lu. o. nociva* to *Lu. flaviscutellata*—as well as to *Lu. o. olmeca* (Vargas and Díaz Nájera), the vector of *Le. mexicana mexicana* Biagi—we felt that a search for leishmanial parasites in *Lu. o. nociva* was justified. In 1984 we initiated a directed search for vectors of *Le. m. amazonensis* in the Manaus area, where this parasite had not yet been isolated from sand flies, but where isolates from man and sylvatic mammals had routinely been obtained *(5, 6).*

MATERIALS AND METHODS

As Figure 1 indicates, we used two periurban collection sites near Manaus that are within seven kilometers of the city center. The older Parque das Laranjeiras site has been described previously *(5).* The new site to which traps were moved, in the Acariquara

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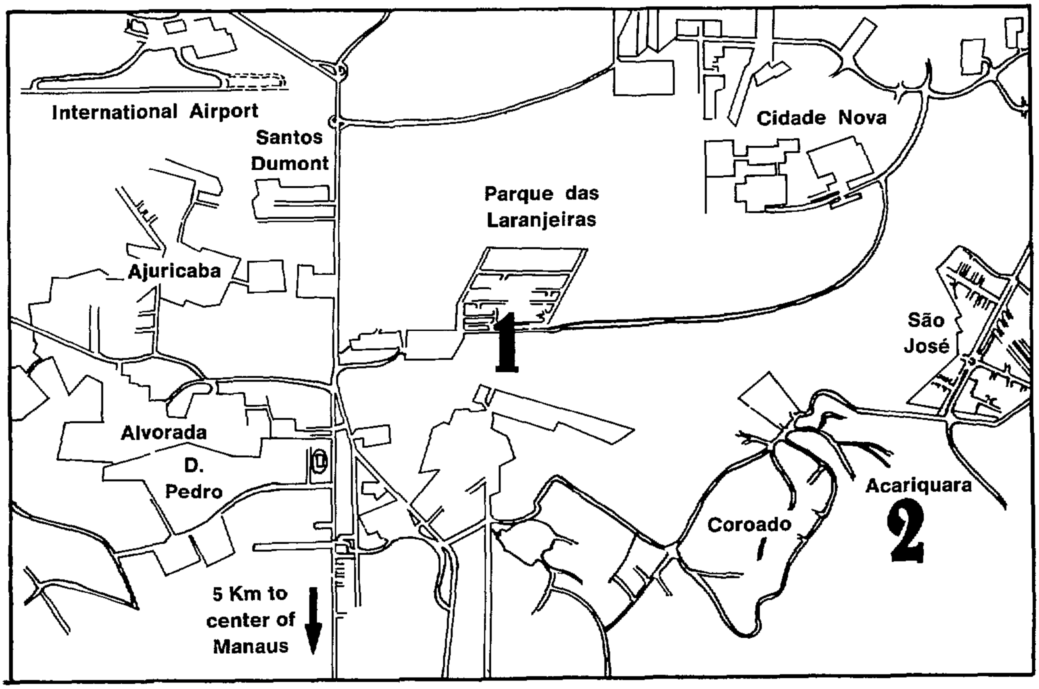
[6] Recently elevated to specific rank as *Leishmania amazonensis.* See R. Lainson and J. J. Shaw; Evolution, Classification, and Geographical Distribution; in: W. Peters and R. Killick-Kendrick (eds.); *The Leishmanias in Biology and Medicine,* vol. 1; Academic Press, London (in press).

FIGURE 1. A map of the outskirts of Manaus showing the two collection sites.



residential area, is very similar to the Parque das Laranjeiras site.

Sand flies at these sites were captured with simplified Disney traps and box traps as described by Ready et al. *(7)* and Ward *(8)*, respectively. Captured flies were taken to the laboratory during the early morning hours. Dissection commenced immediately and continued until 3 p.m. or until the supply of specimens was exhausted. The dissection techniques used have been reported elsewhere *(9)*.

The guts of parasite-positive sand flies[7] were teased apart in saline. This material was then inoculated into biphasic blood agar, and in most cases was also inoculated subdermally into the noses of golden hamsters. Identification of the parasite as *Le. m. amazonensis* was based on observation of rapid growth in culture, rapid formation of large lesions with abundant parasites at the site of inoculation in the hamsters, and the appearance under the microscope of Giesma-stained tissue smears.

In addition, isolates obtained from two humans, three *Lu. o. nociva*, and four *Lu. flaviscutellata* were characterized as *Le. m. amazonensis* by monoclonal antibody techniques *(10)*.

[7] All those in which flagellate infections of the gut were observed upon dissection. No parasites other than *Le. m. amazonensis* (isolated and confirmed in hamsters) were detected in this sample.

Results

Table 1 shows the *Le. m. amazonensis* infections found in female sand flies captured at the two collection sites. While the Parque das Laranjeiras site yielded no positive results, four *Lu. o. nociva* and six *Lu. flaviscutellata* from the Acariquara site were found to be infected with *Le. m. amazonensis*.

TABLE 1. Results obtained by testing 5,039 sand flies captured in the Manaus area for infection with *Le. m. amazonensis*.

Collection area	Sand fly species	No. examined	No. positive
Parque das Laranjeiras	*Lu. o. nociva*	2,374	0
	Lu. flaviscutellata	456	0
Acariquara	*Lu. o. nociva*	1,586	4
	Lu. flaviscutellata	623	6

Tables 2 and 3 show relevant data obtained for human subjects and animals living in areas around Manaus. Of 248 patients with leishmaniasis who were examined over a six-year period ending in December 1984 and from whom *Leishmania* was isolated and identified, only five (2%) had cases of infection with *Le. m. amazonensis*.[8] Data on these five individuals are listed in Table 2. Regarding animals, wild specimens of various species were captured north of the Amazon River within a 50 km radius of the city of Manaus between 1979 and 1984 and were tested for *Le. m. amazonensis*. The percentages yielding *Le. m. amazonensis* isolates are shown in Table 3.

TABLE 2. Human leishmaniasis cases in the Manaus area from which *Le. m. amazonensis* was isolated in 1981–1984. The areas of transmission listed in the last column are shown in Figure 2.

Isolate designator	Patient's sex	Patient's age	Date of isolate	No. of patient's lesions	Probable area of transmission[a]
IM-360	M	39	6 February 1981	29	BR-174, KM. 32
IM-644	M	23	26 April 1982	1	BR-174, ZF-2
IM-745	M	23	13 October 1982	1	Vivenda Verde
IM-1865	M	?	11 November 1983	4	BR-174, ZF-3
IM-1963	M	38	3 March 1984	1	BR-174, KM. 60

[a] The areas cited are those where the patients said they believed they were exposed to sand flies. See Figure 2.

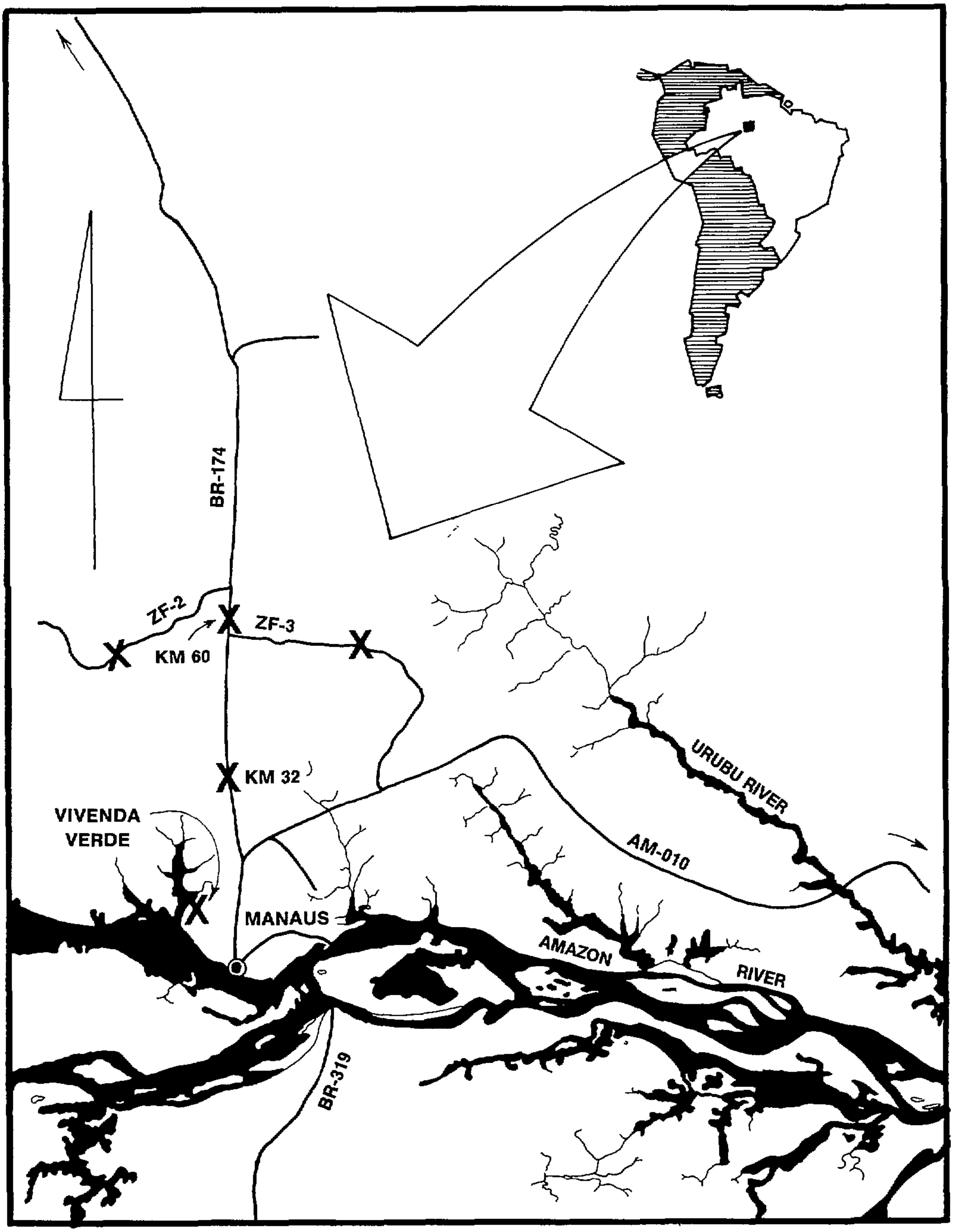
TABLE 3. Wild animals captured in the Manaus area from which *Le. m. amazonensis* was isolated. Some of these data were previously reported in Arias et al. *(6)*.

Animal species	No. examined	No. positive	% positive
Proechimys guyannensis	14	7	50
Marmosa cinerea	7	2	29
Didelphis marsupialis	128[a]	4	3
Others	70	0	0

[a] Mostly periurban captures.

[8] The other 243 patients were infected with *Le. b. guyanensis*. These 248 patients were referred to our laboratory for parasitologic diagnosis of skin lesions. Leishmaniasis was confirmed by the authors by observation of amastigotes in Giemsa-stained smears of tissue samples from the edge of the lesions. *Le. m. amazonensis* infections were diagnosed by isolation, culture in blood agar, and hamster inoculation.

FIGURE 2. A map of the area around Manaus showing where the five human cases of *Le. m. amazonensis* infection appear to have been contracted.



Discussion and Conclusions

The presence of *Lu. o. nociva* and *Lu. flaviscutellata* naturally infected with *Le. m. amazonensis* in the same area further emphasizes the complexity of *Leishmania* transmission cycles in the New World. Other leishmanias are known to be transmitted by more than one vector. For example, the known vectors of *Le. braziliensis guyanensis* Floch are *Lu. umbratilis* Ward and Fraiha *(11-13)*, *Lu. anduzei* (Roseboom) *(11, 12)*, and *Lu. whitmani* (Antunes and Coutinho) *(14)*. The known vectors of *Le. b. braziliensis* Vianna are *Lu. pessoai* (Coutinho and Barretto), *Lu. intermedia* (Lutz and Neiva) *(15)*, and *Psychodopygus wellcomei* Fraiha, Shaw, and Lainson *(16)*. And the known vectors of *Le. braziliensis panamensis* Lainson and Shaw are *Lu. trapidoi* (Fairchild and Hertig), *Lu. ylephiletor* (Fairchild and Hertig), *Lu. gomezi* (Nitzulescu) *(17)*, and *Psychodopygus panamensis* (Shannon) *(18)*.

Our finding that moving the trapping site to a nearby area of apparently similar forest resulted in the capture of infected phlebotomines recalls an observation by Lainson and Shaw *(1)*, who captured *Lu. flaviscutellata* infected with *Le. m. amazonensis* 300 meters from a site where all sand flies of this species had been uninfected. Whether or not these results indicate the existence of discrete foci of transmission is still unclear.

The low prevalence of human leishmaniasis due to *Le. m. amazonensis* in the Manaus area is in harmony with the low infection rates found in *Lu. flaviscutellata* and *Lu. o. nociva.*

Although the infection rate in *Lu. flaviscutellata* was over five times as high as in *Lu. o. nociva,* the number of infected sand flies caught in the traps was approximately equivalent for the two species. Moreover, out of 207 specimens of these species taken on human bait *(19)*, *Lu. o. nociva* specimens outnumbered those of *Lu. flaviscutellata (20)*. This suggests that *Lu. o. nociva* may be at least as important as *Lu. flaviscutellata* as a vector of *Le. m. amazonensis* to man in the Manaus area, where the former species was among the five most commonly collected on human bait *(19, 20)*.

Lu. flaviscutellata has been convincingly implicated as the principal and probably sole vector of *Le. m. amazonensis* in the lower Amazon Valley *(4)*. This is the first time that *Lu. o. nociva* has been implicated as a possible vector of *Le. m. amazonensis,* and also the first report of *Lu. flaviscutellata* being infected with this parasite outside the state of Pará.

Summary

In 1984 the authors began a search for vectors of the leishmania parasite *Leishmania m. amazonensis* in the Brazilian Amazon city of Manaus by capturing sand flies at two periurban collection sites and seeking to isolate the parasite from them. One collection site yielded no positive specimens, but the other yielded 10 sand flies infected with *Le. m. amazonensis*.

Six of the positive specimens belonged to the sand fly species *Lutzomyia flaviscutellata,* and four belonged to the species *Lu. olmeca nociva.* This is the first time that the latter species has been implicated as a possible vector of

Le. m. amazonensis and the first report of *Lu. flaviscutellata* being infected with *Le. m. amazonensis* outside of Pará State.

The capture of infected flies at one site but not at another similar nearby site is reminiscent of previous results reported by Lainson and Shaw *(1)*. Whether or not these results indicate the existence of discrete transmission foci is still unclear.

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United States Recognizes Chilean Eradication of Foot-And-Mouth Disease

The United States Department of Agriculture has recognized Chile as being free of foot-and-mouth disease, an act that opens the way for export of Chilean cattle and sheep to the United States and eventually to other countries as well. Chile thus becomes the first South American country acknowledged by U.S. authorities to be free of the disease.

Chile undertook a long campaign to rid itself of foot-and-mouth disease in the late 1960s, a campaign initially supported by the Inter-American Development Bank. Apparent eradication in 1979 was followed by a setback in 1984, when a new local outbreak occurred. However, that outbreak was successfully contained, and Chile has maintained close control over its seaports, airports, and frontier areas since that time.

Vigilance is needed to maintain this situation, because the disease still exists elsewhere in South America and the responsible virus is easily imported with inadvertently contaminated products. However, the cost of keeping the disease out is expected to decline as time passes; meanwhile, Chile is realizing an added benefit by applying the institutional structure developed against this ailment to combat other animal diseases.

Source: Inter-American Development Bank, *IDB News in Brief*, March 1987, p. 12.