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

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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 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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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*.

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*.

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

<table>
<thead>
<tr>
<th>Collection area</th>
<th>Sand fly species</th>
<th>No. examined</th>
<th>No. positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parque das Laranjeiras</td>
<td><em>Lu. o. nociva</em></td>
<td>2,374</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><em>Lu. flaviscutellata</em></td>
<td>456</td>
<td>0</td>
</tr>
<tr>
<td>Acariquara</td>
<td><em>Lu. o. nociva</em></td>
<td>1,586</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td><em>Lu. flaviscutellata</em></td>
<td>623</td>
<td>6</td>
</tr>
</tbody>
</table>

### 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.

<table>
<thead>
<tr>
<th>Isolate designator</th>
<th>Patient’s sex</th>
<th>Patient’s age</th>
<th>Date of isolate</th>
<th>No. of patient’s lesions</th>
<th>Probable area of transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td>IM-360</td>
<td>M</td>
<td>39</td>
<td>6 February 1981</td>
<td>29</td>
<td>BR-174, KM. 32</td>
</tr>
<tr>
<td>IM-644</td>
<td>M</td>
<td>23</td>
<td>26 April 1982</td>
<td>1</td>
<td>BR-174, ZF-2</td>
</tr>
<tr>
<td>IM-745</td>
<td>M</td>
<td>23</td>
<td>13 October 1982</td>
<td>1</td>
<td>Vivenda Verde</td>
</tr>
<tr>
<td>IM-1865</td>
<td>M</td>
<td>11 November 1983</td>
<td>4</td>
<td>BR-174, ZF-3</td>
<td></td>
</tr>
<tr>
<td>IM-1963</td>
<td>M</td>
<td>38</td>
<td>3 March 1984</td>
<td>1</td>
<td>BR-174, KM. 60</td>
</tr>
</tbody>
</table>

* 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).

<table>
<thead>
<tr>
<th>Animal species</th>
<th>No. examined</th>
<th>No. positive</th>
<th>% positive</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Proechimys guyannensis</em></td>
<td>14</td>
<td>7</td>
<td>50</td>
</tr>
<tr>
<td><em>Marmosa cinerea</em></td>
<td>7</td>
<td>2</td>
<td>29</td>
</tr>
<tr>
<td><em>Dideiphis marsupialis</em></td>
<td>128&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Others</td>
<td>70</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mostly periurban captures.

*The other 243 patients were infected with *Le. b. guyannensis*. 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.