Monthly variation in natural infection of the sandfly Lutzomyia ayacunchensis with Leishmania mexicana in an endemic focus in the Ecuadorian Andes

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In order to collect information on the role of Lutzomyia ayacunchensis in the transmission of leishmaniasis in a newly discovered Andean endemic focus in Ecuador, a longitudinal field study was carried out over 13 months. Monthly dissections were made of a minimum of 200 anthropophilic sandflies, collected at night during the month. A total of 2600 flies was separated from a small number of Lu. osornoi, another anthropophilic species in the area, and dissected; 95 (3-65%) were naturally infected with Leishmania mexicana promastigotes. The parasites were always located in the sandfly midgut. The current study revealed a marked monthly variation in both natural infections with Leishmania and in biting activity of sandflies in the endemic area, demonstrating a high transmission rate during the period from the early rainy season to the early or mid dry season (February to July).

The current study site at Paute, Department of Azuay, Ecuador was confirmed for the first time as an area endemic for a new type of leishmaniasis in the Andes (Hashiguchi et al., 1987). The disease is clinically very similar to the Peruvian uta, but the causative agents and vectors are completely different. The former have recently been identified as Leishmania mexicana and Le. major-like by zymodeme, serodeme and schizodeme analysis (Hashiguchi et al., 1991) and the latter as a newly-described species, Lutzomyia ayacunchensis Caceres & Galati, 1988 (Takaoka et al., 1990). This species (Lu. ayacunchensis) belongs to the Lu. vexator species group, of which Lu. peruensis has already been incriminated as a vector of Le. peruviana in the Peruvian Andes (Herrera, 1982). The female of Lu. ayacunchensis is very similar to that of Lu. peruensis, and can be separated only by slight differences in the shape of the spermatheca (Takaoka et al., 1990). Following a survey of this area two species of sandfly, Lu. ayacunchensis and Lu. osornoi were found; the former is considered to be the more important species as a vector of leishmaniasis. A preliminary epidemiological survey suggested that transmission of leishmaniasis in the area occurred during the rainy season. In order to demonstrate this, we carried out a longitudinal sampling study of the natural
infection rates of sandflies with *Leishmania* promastigotes at different times throughout the year.

**MATERIALS AND METHODS**

**The Study Site**
The chosen study site was Mount Cenaculo, adjacent to Canton Paute, a small town in the Department of Azuay (Fig.). The study continued over 13 months at the same collecting site, which was a 4 m² area among rocky slopes with alpine vegetation consisting of grasses, low shrubs and *Agave*, at 2500 m above sea level. Many cases of human leishmaniasis were discovered by our epidemiological survey (Hashiguchi et al., 1991) in houses lining a road along the base of Mount Cenaculo. Ecuador has only two seasons, a long dry summer from May
TABLE

Monthly variation in natural infections of the sandfly Lutzomyia ayacuchensis with Leishmania mexicana in an endemic focus, Paute, Department of Azuay, Ecuador

<table>
<thead>
<tr>
<th>Date</th>
<th>Time in hours required to collect 200 flies*</th>
<th>Average density (fl/h)†</th>
<th>No. of +ve flies (%)</th>
<th>Average Temperature (°C)</th>
<th>Average Humidity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sep 88</td>
<td>9</td>
<td>22.2</td>
<td>3 (1.5)</td>
<td>15</td>
<td>50</td>
</tr>
<tr>
<td>Oct 88</td>
<td>8</td>
<td>25.0</td>
<td>2 (1.0)</td>
<td>15</td>
<td>50</td>
</tr>
<tr>
<td>Nov 88</td>
<td>10</td>
<td>20.0</td>
<td>2 (1.0)</td>
<td>17</td>
<td>50</td>
</tr>
<tr>
<td>Dec 88</td>
<td>22</td>
<td>9.0</td>
<td>0 (0.0)</td>
<td>16</td>
<td>65</td>
</tr>
<tr>
<td>Jan 89</td>
<td>20</td>
<td>10.0</td>
<td>0 (0.0)</td>
<td>17</td>
<td>60</td>
</tr>
<tr>
<td>Feb 89</td>
<td>1</td>
<td>200.0</td>
<td>8 (4.0)</td>
<td>18</td>
<td>75</td>
</tr>
<tr>
<td>Mar 89</td>
<td>1</td>
<td>200.0</td>
<td>15 (7.5)</td>
<td>18</td>
<td>80</td>
</tr>
<tr>
<td>Apr 89</td>
<td>0.5</td>
<td>400.0</td>
<td>14 (7.0)</td>
<td>17</td>
<td>80</td>
</tr>
<tr>
<td>May 89</td>
<td>0.4</td>
<td>500.0</td>
<td>15 (7.5)</td>
<td>17</td>
<td>75</td>
</tr>
<tr>
<td>Jun 89</td>
<td>0.8</td>
<td>250.0</td>
<td>12 (6.0)</td>
<td>16</td>
<td>60</td>
</tr>
<tr>
<td>Jul 89</td>
<td>1</td>
<td>200.0</td>
<td>13 (6.5)</td>
<td>16</td>
<td>60</td>
</tr>
<tr>
<td>Aug 89</td>
<td>6</td>
<td>33.3</td>
<td>7 (3.5)</td>
<td>16</td>
<td>60</td>
</tr>
<tr>
<td>Sep 89</td>
<td>7</td>
<td>28.5</td>
<td>4 (2.0)</td>
<td>16</td>
<td>55</td>
</tr>
</tbody>
</table>

*In each month, 200 flies were collected and dissected.
†Fly density per hour in each collection.

to December and a short rainy winter from January to April. We therefore decided to start our study in the second half of summer, and to continue through the winter and the first half of the summer of the next year. With this schedule it was possible to collect data on the dry season both before and after the rainy season.

Sandfly Collection

The aspirators were glass tubes, 8 mm in diameter, connected by rubber tubing to plastic carrying bottles each capable of holding 50 sandflies. Each fly collection started at 18.30 hours. The collection time varied from several minutes to four hours per day, depending on the fly density. In this study our main purpose was to obtain enough sandflies for dissection, i.e. a minimum of 200 Lu. ayacuchensis per month, irrespective of the total collection time required. The same procedure was employed for all collections and involved two persons, the collector and the human 'bait'. The collector aspirated the flies immediately after they alighted on the bare legs of the human bait volunteer. Sandfly carrying bottles containing living individuals were always kept inside insulated boxes until they could be dissected.

Fly Identification and Dissection

After initially separating Lu. ayacuchensis from Lu. osornoi by size, the females were dissected according to the procedure described previously (Hashiguchi et al., 1985) under aseptic conditions. After the flies were dissected in a drop of sterile saline, their guts were isolated and examined for Leishmania promastigotes. The remaining parts of the flies served for species identification; the spermathecae, individual sperm ducts and flagellomeres were examined to confirm identifications of Lu. ayacuchensis.

Parasite Isolation and Identification

Infected guts were aspirated by syringe in sterile saline, and inoculated into the nose of golden hamsters. Amastigotes were recovered from the animals about two months later, and then inoculated into culture medium (Hashiguchi et al., 1985) for future characterization. The isolate
(WHO designation code: IAYA/EC/89/PA11) from these flies was identified as *Le. mexicana* by molecular techniques such as reactivity patterns with specific monoclonal antibodies, isoenzyme electrophoresis, and restriction endonuclease fragment patterns of kinetoplast DNA (Hashiguchi *et al.*, 1991).

**RESULTS**

A total of 2600 female *Lu. ayacuchensis* were dissected during the study; 95 (3.65%) were infected with *Le. mexicana* promastigotes.

As shown in the Table, there were higher natural infection rates during the period of six months from February to July. This period corresponded to the time when sandfly densities were high compared with other months. There was a notable variation in the time required for collection of about 200 flies in each month. The total time required for the collection of the monthly catch was used to estimate an average fly density in flies per hour (f/h).

At the beginning of the study, during September and November 1988, a minimum of three nights, with approximately three hours each of collection, was necessary each month. During December 1988 and January 1989, i.e. the end of the dry season and the beginning of the rainy season, sandfly catches took six nights with three to four hours per night. The rainy season started on the second week of January 1989. During the rainy months of February, March and April 1989 a sudden increase in fly density was recorded. During these months only 30–60 minutes on a single night was required to collect 200 sandflies. Both the fly density and the natural infection rate were high, at 200 and 400 f/h and 4–0 and 7–5%, respectively. In May, June and July 1989, at the end of the rainy season and the beginning of the dry season, again a single night was sufficient to collect the monthly quota of 200 sandflies; and in August and September 1989, the middle of the dry season, two and four nights respectively were required to obtain the monthly quota.

*Leishmania* promastigotes were always observed in the midgut of sandflies, but exceptionally a few parasites (only three to five) were observed in the hind triangle. Most of this hindgut localization of promastigotes, however, seemed to be caused by cover-glass pressure during microscopical observation.

**DISCUSSION**

The study revealed a marked monthly variation in both the natural infections with *Leishmania* and the biting activity of sandflies in the Paute endemic area, suggesting a high transmission intensity during the period from the beginning of the rainy season to the beginning or middle of the dry season. To date there have been several studies on seasonal variation in natural infections and in sandfly density. Certain sandfly species of the genus *Lutzomyia* are known to reach maximum numbers during the rainy season (Chaniotis *et al.*, 1971), while others peak in the dry season (Shaw and Lainson, 1972). No such study, however, is available for the Andean endemic area of leishmaniasis. The present findings differ from those of previous workers, which is probably a reflection of the different ecological conditions encountered in the Andean plateau, as follows:

(a) The natural infection rate gradually increased from December and January (0–0%) to February (4–0%), reaching a peak between March and July (6–0–7–5%). These data indicate that high natural infections of sandflies are present during the principal three months of the rainy season and the first four months of the dry season.

(b) The sandfly density also increased in February 1989 and reached a peak (500 f/h) in May, when the rainy season was practically over. Thereafter, sandfly density gradually decreased during June and July to 200–250 f/h and reached a markedly low rate (33 f/h) in August.

(c) Comparison of the natural infection rates and fly densities showed the following relationship. Both values increased in February, but decreased separately; a relatively high level of fly density lasted from March to June, while sandfly natural infection was maintained at a high level until August. The natural infection rate reached a peak in March, two months
before the fly density did, and this high level of infection continued until July, two months after the highest fly density had been reached.

Promastigotes were found in the midgut of L. ayacuchensis females, suggesting that they belonged to Le. mexicana of the Le. mexicana complex. In Peru, where another type of the disease (uta) is prevalent in the Andes, the promastigotes in the vector sandfly L. verrucarum were concentrated in the hindgut, demonstrating the characteristic of Le. peruviana of the Le. braziliensis complex (Lianson et al., 1979). Such behavioural differences between the Leishmania from the Ecuadorian and Peruvian Andes are very important, and interesting from the epidemiological and clinical points of view.

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REFERENCES


