PHLEBOTOMINE SAND FLY (DIPTERA: PSYCHODIDAE) SEASONAL DISTRIBUTION AND INFECTION RATES IN A DEFINED FOCUS OF LEISHMANIA TROPICA

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Abstract. A two-year study was conducted of phlebotomine sand fly fauna in a defined focus of Leishmania tropica. A total of 17,947 sand flies representing 10 species were collected from the location. Phlebotomus guggisbergi, a vector of L. tropica in Kenya, was the most prevalent species through the entire period, representing about 80% of the total catch. There was marked seasonal fluctuation in the populations of the three most common species, with highest population levels reached in December and lowest levels reached in July and August. Leishmania-like infections were encountered in 489 P. guggisbergi. No flagellate infections were observed in any other species of sand fly. Although infected P. guggisbergi were collected during each month of the year, the percent parous infected flies was highest (27.5%) during the November through January time period. These data show that the greatest risk of transmission to humans at this focus occurs during December, when the vector is prevalent and infections are common.

It has been more than 10 years since the first report of autochthonous human cases of cutaneous leishmaniasis (CL) due to Leishmania tropica in Kenya. Subsequent surveys focused on additional cases in indigenous Kenyans. This led to the discovery of a defined focus of the disease in Muruku Sublocation, Laikipia District, and the determination that Phlebotomus guggisbergi is a vector. Further studies have led to the discovery of additional foci of L. tropica-induced CL in central Kenya and the Rift Valley. More recent determination of a new species of Phlebotomus further complicates the ecology of the disease in the newer foci. We report here on the results of a two-year survey of the sand fly fauna at the well-defined, first reported focus of CL in Kenya.

MATERIALS AND METHODS

Description of the study area. The study site, as first described by Lawyer and others, encompassed a 1-km gorge at the head of which was a shallow semi-circular cave with a 100 m-wide entrance. The site was located in the Muruku Sublocation, Laikipia District in Kenya. A shallow pond at the base of the cave, which was present for about 10 or 11 months of the year, was used as a watering hole for domestic animals. The cave showed extensive use by rodents as well (Figures 1 and 2).

Sand fly collection and examination. Five light trap locations were selected along the mouth of the cave, in areas with abundant crevices and chambers. Four trap locations were selected in the gorge floor below the cave, at distances of approximately 40, 55, 70, and 100 m from the mouth of the cave. Additional trap sites were selected in the environs of a homestead of two confirmed CL cases on the rim of the gorge, 500 m from the cave (designated homestead #3). The homestead consisted of two buildings, an adjacent pen for goats and sheep, and a pen for 5–8 cattle.

We performed one-week surveys at monthly intervals from January 1989 through March 1991. Sand flies were captured using solid-state army miniature light traps (John W. Hock Co., Gainesville, FL) and castor oil-soaked papers (21 × 29.5 cm). Traps were set in the afternoon, just before sunset and collected in the morning, just after sunrise. Light traps were set in two of the five cave positions and each of the four gorge positions each night. Five light traps were placed in and around the buildings at homestead #3. In the cave, 25 papers were placed in rock crevices and potential animal dens each night. An additional 25 papers were placed in and around homestead buildings, animal pens, and rock crevices and animal burrows along the escarpment below the homestead. Captured sand flies were processed as described by Young and others, which included removing sand flies from the light traps or papers, washing them in a 2% detergent solution, followed by rinsing in normal saline, and then placing the flies in a cryotube with dimethyl sulfoxide and slowly freezing in liquid nitrogen. The frozen material was transported from the field back to the laboratory in Nairobi. In the laboratory, frozen flies were thawed, rinsed in sterile saline, disinfected, dissected, and identified. The sand flies were identified using classical morphologic characteristics. Characterization of parity was also performed, since when compared with infection status, it would an indication of the prevalence of Leishmania-infected hosts. Parasites from infected sand flies were cultured and samples were cryopreserved. The parasites were characterized by cellulose-acetate electrophoresis. Additionally, a number of isolates were analyzed by in situ hybridization of kinetoplast DNA. The blood meals of freshly blood-fed sand flies were removed and dried for later host source determination by ELISA, using methods described elsewhere.

RESULTS

A total of 17,947 sand flies representing 10 species were collected from the Muruku focus over the two-year period of January 1989 to March 1991 (Table 1). Phlebotomus guggisbergi was the most prevalent species, comprising approximately 78% of the total collection. Approximately 89% of the P. guggisbergi were collected from the cave sites. Phlebotomus guggisbergi, Sergentomyia africana, and S. bedfordi were the only species present in sufficient numbers to allow analysis of seasonal distribution (Figures 3 and 4). Four species, S. affinis, S. clydei, S. schwetzii, and S. unifor-
mis, were represented by a single specimen each. Light traps provided the most effective method of collecting sand flies at this location; however, there were sex-related differences in capture success between light traps and paper traps (Table 1). Leishmania-like infections were found in 489 P. guggisbergi, the only species found infected. Approximately 90% of these were from the cave sites. Of 327 isolates characterized to date, all have proven to be L. tropica s.l. Although infected P. guggisbergi were found throughout the year, the percent infected was generally highest each year during the November to December time frame, when up to 27.5% of the parous flies were infected with promastigotes (Figure 3). The greatest percent positive parous flies in a single collection, 74.6% (47 of 63), was from a light trap in the cave in April 1990 (Figure 5). The blood meals of 11 freshly fed flies were examined for host determination by ELISA. The hosts could be determined for only three flies, which had been collected by light trap in the goat pen. As expected, the host was goat.

DISCUSSION

Phlebotomus guggisbergi was the predominant species of phlebotomine sand fly in the study area, and the only member of its genus present. As a vector of L. tropica in Kenya, it was the main focus of the study. Sergentomyia spp. feed primarily on reptiles, but there are reports of species feeding on mammals, including humans. They probably represent no more than an occasional minor nuisance to local residents.

The population dynamics of the sand flies in Muruku Sublocation were greatly affected by seasonal variations in temperature and precipitation. In Kenya, the months of June–August exhibit the coolest nighttime temperatures, often dip-
ping as low as 5°C on the Laikipia Plateau, particularly in July. This is also the time of year for the most rainfall (long rains) in the Laikipia plateau region. The cave-dwelling habits of *P. guggisbergi* probably protect it from rainfall. Whereas some species of Kenyan *Phlebotomus* may experience post-rainy season increases in population (Lawyer PG, unpublished data), for *P. guggisbergi* the cool temperature may serve to inhibit reproduction or delay development and thus keep its population low during June–August. The combination of warmer weather and the short rains, which occurs during the period of late October–early December, may provide favorable conditions for the rise in population levels. The presence of infected flies throughout the year suggests the continual presence of the reservoir host. However, the increased infection rates during certain times of the year suggest an increased availability of the animal host of *Leishmania* at those times. Elsewhere, *L. tropica* is thought to be anthropoponic, with humans serving as the reservoir. The paucity of human infection during times of increased vector infections lends doubt to this theory for Kenya. In our study site, the extremely high infection rate in parous flies from the cave indicates that a reservoir is either common or the almost exclusive source of the blood meals. Although work has yet to be done on the daily activity cycle of *P. guggisbergi*, we have noted while setting traps that flies start coming out of the recesses of the cave only after sunset, with the concomitant decrease in ambient temperature. We noted their presence again in the mornings, but only within 30–45 min after sunrise. Domestic animals frequent the cave area, particularly the waterhole at its base, generally between 10:00 AM and 6:00 PM, and thus are not present in close proximity when the flies are active. Although tests have shown that goats and sheep are preferred hosts of *P. guggisbergi*, we do not find increased evidence of infection from those flies captured in the environs of homestead #3, where goats and sheep are readily available. We believe that the search for the reservoir should be directed to a sylvatic host more intimately associated with the cave. The discovery of rock hyraxes (*Procavia johnstoni*) infected with *L. tropica* elsewhere in Kenya should be followed up in the Muruku

**Table 1**

<table>
<thead>
<tr>
<th>Species</th>
<th>Collection method</th>
<th>Cave M/F</th>
<th>Valley M/F</th>
<th>Homestead #3 M/F</th>
<th>Other M/F</th>
<th>Total M/F</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. guggisbergi</em></td>
<td>LT</td>
<td>3,490/8,176</td>
<td>82/618</td>
<td>133/327</td>
<td>1/2</td>
<td>3,706/9,123</td>
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<tr>
<td></td>
<td>PT</td>
<td>698/127</td>
<td>N/A</td>
<td>361/50</td>
<td>8/10</td>
<td>1,067/187</td>
</tr>
<tr>
<td><em>S. africana</em></td>
<td>LT</td>
<td>106/262</td>
<td>119/251</td>
<td>20/114</td>
<td>1/13</td>
<td>246/640</td>
</tr>
<tr>
<td></td>
<td>PT</td>
<td>7/12</td>
<td>N/A</td>
<td>28/53</td>
<td>287/221</td>
<td>322/286</td>
</tr>
<tr>
<td><em>S. antennata</em></td>
<td>LT</td>
<td>0/0</td>
<td>1/0</td>
<td>2/9</td>
<td>0/0</td>
<td>3/9</td>
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<tr>
<td></td>
<td>PT</td>
<td>0/0</td>
<td>N/A</td>
<td>0/4</td>
<td>0/0</td>
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</tr>
<tr>
<td><em>S. bedfordi</em></td>
<td>LT</td>
<td>38/160</td>
<td>70/240</td>
<td>98/972</td>
<td>31/175</td>
<td>237/1,547</td>
</tr>
<tr>
<td></td>
<td>PT</td>
<td>17/57</td>
<td>N/A</td>
<td>64/310</td>
<td>15/86</td>
<td>96/453</td>
</tr>
<tr>
<td><em>S. ingrami</em></td>
<td>LT</td>
<td>3/2</td>
<td>6/3</td>
<td>0/0</td>
<td>0/0</td>
<td>9/5</td>
</tr>
<tr>
<td></td>
<td>PT</td>
<td>0/0</td>
<td>N/A</td>
<td>0/0</td>
<td>0/1</td>
<td>0/1</td>
</tr>
<tr>
<td><em>S. squamipleuris</em></td>
<td>LT</td>
<td>0/1</td>
<td>0/2</td>
<td>0/0</td>
<td>0/0</td>
<td>0/3</td>
</tr>
<tr>
<td></td>
<td>PT</td>
<td>0/0</td>
<td>N/A</td>
<td>0/0</td>
<td>0/0</td>
<td>0/0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>LT</td>
<td>3,637/8,601</td>
<td>278/1,114</td>
<td>253/1,422</td>
<td>33/190</td>
<td>4,200/11,327</td>
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<tr>
<td></td>
<td>PT</td>
<td>722/196</td>
<td>N/A</td>
<td>453/417</td>
<td>310/318</td>
<td>1,485/931</td>
</tr>
</tbody>
</table>

* M/F = males/females; N/A = not available, no paper traps were set in Valley sites.
area where a different rock hyrax species (*Procavia capensis*) occurs.

The overlap of high vector population levels and high infection rates shows that there is a window of greatest risk of infection to area residents. Educating the nearby residents of this risk might serve to reduce human disease. In the future, disease control efforts should be directed to reduce this risk through aggressive vector and/or reservoir control programs.

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FIGURE 5. Parity rate of *Phlebotomus guggisbergi* collected at the cave site in the Muruku Sublocation in Kenya from February 1989 through March 1991.

REFERENCES