STUDIES ON THE INFLUENCE OF THE PRESENCE OF DOMESTIC ANIMALS ON INCREASING THE TRANSMISSION PROBABILITIES OF LEISHMANIASIS

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ABSTRACT

In the endemic regions of leishmaniasis in tropical countries, a great majority of poor residents of rural areas, keep a variety of domestic animals for socioeconomic and other reasons. The vector sandflies feed several times on different animals. It was found that feeding on certain healthy domestic animals may potentially increase the transmission powers of parasite Leishmania carrying vectors. This conclusion is based on the observation that in areas where these animals were kept, sandflies were more numerous, and carried a larger parasite load. The occurrence of such a phenomenon provides a selective advantage to the transmission of Leishmania among vertebrates. These results may enable the health authorities to adopt policies concerning the presence of domestic animals in endemic areas which may result in significant reduction in the numbers of Leishmania cases in the human beings in these areas.

KEY WORDS: Blood-feeding, domestic animals, Leishmania, sandflies, transmission probabilities, vector

INTRODUCTION

Humans and animals get the sleeping sickness infection by the bite of female sandfly vector infected with Leishmania Ross, 1903, a parasitic protozoan. Sandflies feed sev-

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eral times during their life span on many different animal species (Ribeiro et al., 1986, Morrison et al., 1993). Feeding patterns of sand flies diverge with locality and date of collection. *Lutzomyia longipalpis* Lutz and Neiva, 1912 were found to feed predominantly on bovine, but feedings were also recorded on pigs, equines, humans, dogs, opossums, birds, and reptiles (Morrison et al., 1993).

Moreover, the proximity of humans to dogs and poultries appears to increase the risk of *Leishmania* infection considerably (Aguilar et al., 1984, Alexander et al., 2002, Moreira et al., 2003). They become infected by ingestion of blood from infected people or animals. Although an important scourge of mankind, the transmission dynamics of the disease has not been fully elucidated yet.

In this study, in an endemic area of Brazil, a combination of laboratory and field work was applied to understand the role of domestic animals in the vector competence of sandflies in transmitting *Leishmania* parasites. The results are presented and discussed in this paper.

**Area and Climate**

This study was conducted in a village situated in an endemic region of a rural area of Brazil (District of Corte de Pedra, South-east of Bahia, Brazil - 13° 32' LatS, 39° 25' LonE). The climate is hot and humid with mean annual temperature of 25° C with average maximum temperature of 34°C and an average minimum temperature of 16°C. The area's average rainfall is of 1,100 mm per year. There are remaining areas of tropical rain forests and growing crops of cocoa, banana and cassava.

**Materials and Methods**

The sandflies were collected during the night time, using improved battery operated CDC light traps (Sudia and Chamberlain, 1962). These light traps were placed around the houses and in the vicinities where the animals were tethered. The sandflies were captured in high numbers (two to three thousands per night) during all seasons over two years (February 2002 to October 2004).

The predominant sandfly species was *Lutzomyia intermedia* Lutz and Neiva, 1912 (99%), a vector of *Leishmania braziliensis* Vianna, 1991. Other species were *Lu. ayrozai* Barreto and Coutinho, 1940 and *Lu. yuilli* Young and Porter, 1972 and *Lu. whitmani* Antúnez and Coutinho, 1939 accounting for only 1.0%. Captured sandflies were kept in a thermal container with controlled humidity and temperature (26°C and 80% humidity) for 3 to 4 hours until brought to the laboratory.

These sandflies were infected with parasite *Leishmania* through an artificial membrane feeder in the laboratory. Mouse blood mixed with *Leishmania braziliensis* amastigotes (1.106 per mL) isolated from footpad lesions.
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of experimentally infected hamsters (*Mesocricetus auratus* Waterhouse, 1839) was used as stated by Nieves and Pimenta (2000). Blood-engorged female sandflies were kept in a cage and ad libitum on a sugar diet till the fourth day after the infection. This period is necessary for the blood meal to be completely digested in the gut. During this time, parasite *Leishmania*’s lifecycle is completed in the lumen of the alimentary tract of the vector. A control group of 200 sandflies was separated while the rest was utilized in different experiments.

These sandflies were anaesthetized on ice and the species were identified. Sandflies, *Lu. intermedia* were dissected for the presence of parasites under an optical microscope. The infection rate (percentage of infected *Lu. intermedia* per group) was noted, and the parasite density (number of parasites per fly) and proportion of metacyclic parasites (percentage of the differentiated parasite form infective for vertebrates per fly) were quantified. These are standard methods to estimate the establishment of *Leishmania* species in a sandfly since it provide an evidence that the sandfly is a competent vector transmitting the parasite to a vertebrate host as explained by Sacks and Kamhawi (2001).

**Observations**

A series of five similar experiments were carried out in the endemic region during dis-

Table 1: The infection patterns of infected sandflies according to the source of the second ingested blood meal.

<table>
<thead>
<tr>
<th>Blood Source (domestic animals)</th>
<th>Infected Sandflies (%)</th>
<th>Parasites/fly (Number)</th>
<th>Metacyclics (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse (<em>Mus musculus</em>) Control¹</td>
<td>44</td>
<td>1840</td>
<td>15</td>
</tr>
<tr>
<td>Mouse (<em>Mus musculus</em>)</td>
<td>47</td>
<td>1507</td>
<td>17</td>
</tr>
<tr>
<td>Human (<em>Homo sapiens</em>)</td>
<td>64</td>
<td>6743</td>
<td>12</td>
</tr>
<tr>
<td>Donkey (<em>Equus asinus</em>)</td>
<td>87</td>
<td>54771</td>
<td>13</td>
</tr>
<tr>
<td>Cattle (<em>Bos taurus</em>)</td>
<td>50</td>
<td>5000</td>
<td>16</td>
</tr>
<tr>
<td>Horse (<em>Equus caballus</em>)</td>
<td>58</td>
<td>51771</td>
<td>14</td>
</tr>
<tr>
<td>Sheep (<em>Ovis aries</em>)</td>
<td>30</td>
<td>51371</td>
<td>15</td>
</tr>
<tr>
<td>Pig (<em>Sus scrofa</em>)</td>
<td>27</td>
<td>2257</td>
<td>15</td>
</tr>
<tr>
<td>Dog (<em>Canis familiaris</em>)</td>
<td>50</td>
<td>914</td>
<td>13</td>
</tr>
<tr>
<td>Chicken (<em>Gallus domesticus</em>)</td>
<td>90</td>
<td>1889</td>
<td>16</td>
</tr>
</tbody>
</table>

¹=Control group of sandflies received only the first infective blood meal.
tinct seasons of the year. It was seen that 44% of the field-captured sandflies become experimentally infected, presenting an average of 6.6x104 parasites per sandfly with 15% of them recognized as differentiated metacyclic forms (control group in the table: 1). After characterization of the *Lu. intermedia* experimental infection pattern, the infected sandflies were separated into 9 groups of 200 per cage.

Each group of sandflies was allowed to take a second non-infective meal from a blood sample drawn from humans and animals such as mouse (*Mus musculus* Linnaeus, 1758), cattle (*Bos taurus* Linnaeus, 1758), horse (*Equus caballus* Linnaeus, 1758), dog (*Canis familiaris* Linnaeus, 1758), pig (*Sus scrofa* Linnaeus, 1758), sheep (*Ovies aries* Linnaeus, 1758), poultries (*Gallus domesticus* Linnaeus, 1758) and donkey (*Equus asinus* Linnaeus, 1758). Each blood meal consisted of a mixture of blood from at least three donors from the same animal species (with no apparent cutaneous leishmaniasis) living in the village.

The blood-engorged sandflies were again fed only with sugar ad libitum for four days, until the new non-infective blood meal had been completely digested. Afterwards, the groups of sandflies were dissected and analysed again for infection using the same parameters described above. These procedures assisted in understanding the possible role (or possible interference) of non-infective blood meals from domestic animals on the infection process in infected vectors (table: 1).

It was observed that the pattern of sandfly infection changed according to the blood sources. There was a twofold increase in the infection rate among sandflies that were refed on blood of poultries (90%) and donkey (87.5%). Approximately double the number of sandflies had infection detectable under the optical microscope in comparison to the control group (sandflies which received only the first infective blood meal but not the second normal blood meal of the animals). The infection rates increased moderately in infected sandflies that were refed on blood from human (64%), horse (58.5%), dog (50%) and cattle (50%).

On the other hand, the infection rates decreased in the sandflies that were refed on the blood from sheep (30.5%) and pig (27%). The infection rate was about the same in the sandflies refed on blood from mouse (47%). In the controls, the initial infection rate was constant. The type of blood ingested by the infected sandflies also affected the density of parasites. The sandflies that refed on blood from human, cattle, donkey and horse had double or triple the numbers of parasites inside their gut. Sandflies refed on the other host groups, had a small increase or even decrease in the intensity of parasites after the second blood feed.

The proportion of metacyclics per fly did not alter significantly after the second nor-
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Lu. intermedia sandfly seems to have a marked opportunist blood feeding habit, since they can bite avidly any vertebrate host. In this study, it was found that refeeding the infected sandflies on the blood of some domestic animals enhances their ability to transmit Leishmania. Therefore, in endemic areas where these animals are found and provide a source of blood meal for sandflies, the number of infected sandflies with more parasites may always be higher. This fact may provide a selective advantage to the vectorial competence of sandflies by encouraging in transmitting Leishmania to vertebrates.

Based on these facts, it seems that infected sandfly vectors that refeed on the blood of healthy domestic animals enhance the transmission competence of leishmaniasis, depending on the domestic animal species. This pattern is similar to findings of other study with American sandfly Lu. migonei França 1920 using L. braziliensis (Nieves and Pimenta, 2000). The authors found that refeeding infected sandflies in experimental animals affected the development of Le. braziliensis and Le. amazonensis within the sandfly in different ways, influencing both parasite density and morphological changes, by increasing the number of metacyclics in some sandflies.

It is interesting to note that in our experiments with domestic animals, the proportion of metacyclics per fly remained almost
the same in any of the groups after the second normal blood meal. Since the sandflies appear to take the same amount of blood independently of the animal source, it seems that the influence of the blood meal in the transmission of *Leishmania* infection is altered principally by the components of the ingested blood, affecting consequently, the insect's metabolic process.

In conclusion, the influence of the association between human and domestic animals on the transmission of leishmaniasis is still poorly understood. The presence of dogs and poultries around humans settlements in the endemic areas of leishmaniasis has been considered a high risk for human infection in epidemiological studies (Aguilar et al., 1984, Alexander et al., 2002, Moreira et al., 2003). However, this experimental study indicates that by raising some species of domestic animals, even those which are not reservoirs, may potentially influence disease transmission in endemic areas of leishmaniasis. This result may enable health authorities to adopt policies concerning poor communities who raise domestic animals, to significantly reduce the number of human cases in such areas. This study also opens a new frontier in vector biology, which requires more studies on the role of domestic animals in the transmission of not only leishmaniasis but also of other vector borne diseases such as malaria, filariasis and dengue.

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