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ESTIMATION OF THE MINIMUM NUMBER OF *LEISHMANIA MAJOR* AMASTIGOTES REQUIRED FOR INFECTING *PHLEBOTOMUS DUBOSCQI* (DIPTERA: *PSYCHODIDAE*)

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ESTIMATION OF THE MINIMUM NUMBER OF *LEISHMANIA MAJOR* AMASTIGOTES REQUIRED FOR INFECTING *PHLEBOTOMUS DUBOSCQI* (DIPTERA: *PSYCHODIDAE*)

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ABSTRACT

Background. Leishmaniasis is a vector-borne disease in which *Leishmania* parasites are transmitted by the bite of phlebotomine sand flies. Amastigotes are ingested by the sand fly vector with a blood meal taken from an infected host. This is followed by their differentiation into metacyclic promastigotes which are selectively released and permitted to migrate interiorly so as to make them available for transmission by bite. However, the actual number of amastigotes ingested by the sand fly in the blood meal is not known.

Objective: To investigate the minimum number of *Leishmania major* amastigotes required to cause an infection in *Phlebotomus duboscqi* following an infective blood meal.

Design: A laboratory based study.

Setting: Centre for Biotechnology Research and Development, Kenya Medical Research institute, Nairobi.

Results: Dissection of all fed sand flies at six days post-infective blood meal revealed that blood containing one amastigote per 0.3 μ l in a total volume of 0.5ml was able to cause an infection in the sand flies, but very few sand flies got infected (7.6% and 9.6% respectively). Concentrations of ten amastigotes per 0.3 μ l in 0.5ml gave infection rates of 35.4% and 26.3% respectively, suggesting that even when the concentration of amastigotes in a bloodmeal was high, not all sand flies feeding on it were able to pick up the parasites.

Conclusions: These observations suggests that one amastigote is sufficient to cause an infection to a sand fly and as a result of multiplication in the gut and the existence of mechanisms that increase the number of infective bites delivered by a female sand fly they are able to sustain the transmission of leishmaniasis in an area.

INTRODUCTION

The aetiological agents for the human parasitic disease leishmaniasis, protozoan of the genus *leishmania*, are obligate parasites of mammalian macrophages (1). Upon transmission to the human body by the bite of

infected sand flies, the parasites invade the macrophage cells by receptor-mediated process and take shelter inside phagolysosomes (2). *Leishmaniae* have been shown to be acidophilic organisms (3) and to be resistant to the cytolytic factors of macrophages (4, 5). Without being killed inside the macrophage

phagolysosome, the parasite cell is transformed into non-motile ovoid form called the amastigote and multiplies inside the phagolysosome (2).

While the life cycle of *Leishmania* parasites within their Phlebotomine vectors varies depending on the parasite/vector pairs involved, some general conclusions can be made regarding the development of suprapylarian species within their invertebrate hosts (6–8). Amastigotes are ingested by the sand fly vector with a blood meal taken from an infected host. This is followed by their differentiation into metacyclic promastigotes which are selectively released and permitted to migrate interiorly so as to make them available for transmission by bite.

Although infections to sand flies have been documented both in the laboratory and in the field, the actual number of amastigotes ingested by the sand fly during a blood meal is not known. It is however known that parasitaemia differs among patients (9) and it is possible that patients with few circulating parasites and not showing any symptoms such as hepatosplenomegally could serve as a source of parasites to sand flies. In the present study, we sought to estimate the minimum number of amastigotes capable of causing an infection in sand flies using *L. major* the aetiologic agent of 'Old World' cutaneous leishmaniasis (OWCL) in its natural vector *Phlebotomus duboscqi* Neveu-Lemaire (Diptera: *Psychodidae*). To the best of our knowledge, similar work has not been done elsewhere.

MATERIALS AND METHODS

Parasites. Isolation, cultivation and purification of *L. major* amastigotes from infected BALB/c footpads was carried out as previously described (10). Considering that a sand fly imbibes 0.3–0.5µl blood (11), an infective inoculum consisting of either one

or ten amastigotes per 0.3 or 0.5µl of blood was tested to determine whether or not it could lead to infection of all, or at least over 50% of *P. duboscqi* sand flies.

Sand fly feeding: Sand flies used during the experiments were obtained from an established colony at the Kenya Medical Research Institute (KEMRI) and reared as previously described (12). In this experiment, female *P. duboscqi* were divided into four groups of 120 each. One group was membrane-fed on defibrinated rabbit blood containing one amastigote/0.3µl and the other group on blood containing ten amastigotes/ 0.3µl in 0.5ml total volume of blood. The remaining two groups of *P. duboscqi* were fed on blood containing one amastigote/0.5µl or ten amastigotes per 0.5µl respectively. Sand flies were left to feed *ad libitum* for one hour. Engorged females were separated from unfed ones after 12 hours. They were maintained on sterile sugar solution for six days. On day six, all surviving sand flies were dissected and examined for the presence or absence of parasites and their forms (9, 13).

RESULTS

Sand fly infection rates: Dissection of all fed sand flies at six days post-infective blood meal revealed that blood containing one amastigote in 0.30µl and in 0.5µl was able to cause an infection in the sand fly, but very few sand flies get infected (7.6% and 9.6% respectively). Concentrations of ten amastigotes in 0.3µl and in 0.5µl gave infection rates of 35.4% and 26.3% respectively suggesting that even when the concentration of amastigotes in a blood meal was high, not all sand flies feeding on it were able to pick up the parasites. Results of the dissections are summarised in Table 1.

Table 1

A summary of results of dissections of P. duboscqi fed on different numbers of L. major amastigotes

No. of amastigotes in blood	No. of sand flies used	No. of sand flies engorged	No. of sand flies engorged & infected
1 / 0.3µl in 0.5ml	120	52	4
10 / 0.3µl in 0.5ml	120	48	17
1 / 0.5µl in 0.5ml	120	62	6
10/0.5µl in 0.5ml	120	57	15

DISCUSSION

In this study, it was shown that blood containing one amastigote in 0.3µl or 0.5µl of blood were able to cause an infection in sand flies but with a very low infection rate (2.08 and 8.1%). Increasing amastigote numbers tenfold in the same volume of blood led to increased rate of infection in sand flies. Failure of any potentially infective blood meal to infect sand flies feeding on it can be attributed to the fact that amastigotes are never uniformly distributed in blood. Low infectivity rates in *P. duboscqi* have also been observed in the field and under laboratory conditions (14). These workers were able to show that sand fly collections over a 12 month period in Baringo District, Kenya, two of the 278 *P. duboscqi* females collected were infected with *L. major*. Furthermore, a single sand fly transferred from one mouse to the next while repeatedly attempting to take blood, infected five mice under laboratory conditions. In a field-based study to find the vector for visceral leishmaniasis caused by *L. donovani*, it was shown that only a few *P. martini* are usually infected, indicating that despite high endemicity in Baringo District, only a small number of sand flies transmit the disease (15). Similar results have also been observed in Machakos District (16). These results together with our observations suggest that the infection rates in sand flies under laboratory or field situations are generally low. Thus the existence of a mechanism to increase the number of infective bites delivered by a female sand fly may explain the low *L. major* infection rates of *P. duboscqi* in the laboratory or in the field (14). In cases where patients from the field are found to be antibody positive through the Leishmanin skin test without detectable parasites, it may be necessary to carry out xenodiagnosis using laboratory-bred sand flies to pick up parasites as an alternative to splenic aspiration.

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