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MODIFICATION OF SAND FLY BITING BEHAVIOR BY *LEISHMANIA* LEADS TO INCREASED PARASITE TRANSMISSION

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Abstract

To attempt rodent-sand fly-rodent transmission of *Leishmania major,* laboratory-reared *Phlebotomus doboscqi* were fed on *L. major*-infected mice and then refed on uninfected mice 21 days later. Flies which refed either probed 1–2 times and took a full blood meal in less than 10 minutes or probed 3 or more times and took little or no blood during a period of 15 minutes or more. When dissected, 7 of 8 flies which experienced difficulty in obtaining a blood meal had flagellates in their cibaria, an observation supporting the hypothesis that parasites in this part of the alimentary canal modify normal blood feeding behavior. None of the infected females which probed 1–2 times had similar anterior station infections. Infected sand flies transmitted *L. major* to uninfected mice and a single fly, transferred from 1 mouse to the next while repeatedly attempting to take blood, infected 5 mice.

During a year-long survey in Baringo District, Kenya, we collected 9,182 female sand flies. Only 2 of the 278 *P. duboscqi* captured during this collection were infected with *L. major;* however, 18 of the 789 small rodents from this area were infected with *L. major.* Parasite interference with normal blood feeding may explain how a relatively small population of *P. duboscqi,* only a few of which are infected with *L. major,* can amplify parasite transmission thereby maintaining a disproportionately large reservoir in local rodents.

Beach et al.[1] reported transmission of *Leishmania major* by bite of a naturally-infected *Phlebotomus duboscqi* sand fly, captured in Baringo District, Kenya. During this incident the infected fly probed repeatedly, transmitting parasites at 11 different biting sites, but was unable to take blood. The number of infective bites delivered by this female was greater than that predicted based on the 1–2 bites delivered by uninfected females in the course of obtaining a blood meal (Beach, personal communication). Laboratory studies[2–7] have also described how *Leishmania* infections interfered with sand fly blood feeding and, in some cases, elicited a greater than expected number of bites by females attempting to take blood. It has been suggested that such changes in sand fly biting behavior are of adaptive value to the parasite because they promote transmission.

The transmission experiments discussed in this paper were carried out using laboratory-reared *Phlebotomus duboscqi* infected with *Leishmania major.* We characterized the blood feeding behavior of infected females and then dissected them to determine if flagellates were present in the cibarium, an experiment predicated on the hypothesis that such infections interfere with cibarial blood meal sensing receptors, thereby changing normal blood feeding behavior.[7,8]

The existence of a mechanism to increase the number of infective bites delivered by a female sand fly may explain the low *Leishmania major* infection rate of *Phlebotomus duboscqi* in an area with sizeable *L. major* rodent reservoir.

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MATERIALS AND METHODS

Sand flies

Phlebotomus duboscqi females were taken from the first and second generations of a laboratory colony begun in June 1983. The flies were reared according to previously described procedures.[9] Because they took multiple blood meals, females were fed 3–4 times on *L. major*-infected BALB/c mice and then refed on uninfected mice 21 days after first taking blood. Unless otherwise stated, 4 or 5 randomly selected females were used in each transmission attempt. Infected, as well as uninfected females were held in individual cages at 95% rh, 27°C and had access to a small slice of apple which was changed daily. All blood feeding was done in the early morning when ambient temperatures were lowest and light intensity diminished.

After attempting transmission, infected flies were dissected in saline to determine presence and location of parasites in the alimentary canal. The head was severed from the body and the anterior midgut removed and examined for promastigotes. Finally, the pharynx, cibarium, and mouth parts were dissected, intact, from the head and examined for parasites.

Probing behavior was quantified by recording the number of probes, that is the number of times the proboscis was raised and lowered with subsequent insertion of the feeding stylets. We also recorded the time between the first and last attempt to take blood or until successful completion of the blood meal. The volume of blood imbibed was determined by comparison of the visible blood meal taken by infected sand flies with that taken by paired uninfected controls.

Leishmanial strains

Nairobi *Leishmania* Bank isolate 144, a strain of *L. major* isolated from a wild-caught *Phlebotomus duboscqi* and characterized by cellulose acetate electrophoresis,[1] was used in this study. The spleen from a mouse already infected with 144 was hom1ogenized in 1 ml of saline, and 0.1 ml of the resulting amastigote suspension was inoculated subcutaneously into the noses of 5 mice. Approximately 45 days later, sand flies were allowed to feed around the lesions which developed at the site of inoculation.

Mice

The BALB/c mice used in transmission attempts were killed 60 days after being bitten on the nose by infected *Phlebotomus duboscqi.* To verify infection, tissue from the nose and spleen of each animal was cultured

TABLE 1

P. duboscqi *infected with* L. major: *Biting behavior, blood meal volume and duration of biting period compared with the presence of parasites in the sand fly's cibarium*

Biting behavior, blood meal volume, and biting period duration	Parasite in cibarium	
	Yes	No
Flies probing 1–2 ×; taking full blood meal in 10 min	0/16	16/16[1] 10/10[2]
Flies probing ≥ 3 ×; taking small or no blood meal in 15–20 min	7/8	1/8[3]

[1] When dissected 10 females with midgut infections only; 6 females with no apparent infection.
[2] Uninfected controls.
[3] Heavy midgut infection, no parasites observed in anterior station.

animal was cultured in Schneider's Drosophila Medium (GIBCO) supplemented with 20% heat-inactivated fetal bovine serum,[10,11] 250 µg/ml 5-fluorocytosine (SCH-20FBS). The cultures were incubated at 25°C and monitored for promastigotes over a 14-day period.

Field work

Field studies were carried out near the town of Marigat, Baringo District, Rift Valley Province, Kenya (0°30'N lat., 36°E long.). Sand flies were collected daily during the period between February 1983 and January 1984 inclusive using CDC light traps and hand aspirators. All flies were dissected, examined for parasites, and mounted for taxonomic identification. Flagellates from infected flies were inoculated into SCH-20FBS, sent to our laboratory in Nairobi and characterized using the technique of cellulose acetate electrophoresis.[12]

RESULTS

The effect of Leishmania major *on* Phlebotomus duboscqi *biting behavior*

Twenty-four *Phlebotomus duboscqi* were used in a transmission experiment 21 days after first feeding on an infected mouse. The biting behavior, amount of blood imbibed and duration of the biting period were compared with the presence of parasites in cibarium of each sand fly (Table 1). When dissected, 7 of 8 flies which probed 3 or more times and took little or no blood over a period of 15–20 minutes had parasites in their cibaria. Of the remaining 16 females which probed 1–2 times and took a full blood meal within 10 minutes, 10 had midgut infections which had not spread to the anterior station (here defined as the part of the alimentary canal located in the head of the insect and including the pharynx, cibarium, and the remainder of the buccal cavity), and 6 were uninfected.

Transmission of Leishmania major

Upon postmortem examination, 2 of the 5 mice bitten by potentially infective *Phlebotomus duboscqi* in the preceding experiment were culture positive for *Leishmania.*

Three *Leishmania major*-infected *Phlebotomus duboscqi,* observed to probe repeatedly without engorging, were subsequently used in a multiple transmission experiment 25 days after their first infective blood meal. Each fly was exposed to a different series of 5 BALB/c mice. When a fly had probed 1–2 times on the first mouse in the series it was transferred to the-next until all 5 mice had been bitten or until the fly showed no further interest in attempting to take blood. Upon dissection, all 3 flies were found to have heavy midgut infections extending forward into the anterior station. One of these flies transmitted *L. major* to all 5 mice it fed upon; a second infected 3 of 5 mice and the last fly bit and infected only 1 mouse. Three control females, uninfected but otherwise identical in age and prior blood feeding experience to experimental flies, probed only on the first mouse, took a full blood meal and thereafter showed no inclination to feed on mice 2–5 in their respective series.

Leishmania major *infections in natural populations of* Phlebotomus duboscqi

Sand flies were collected by light trap and aspiration over a 12-month period in Baringo District, Kenya. These collections yielded a total of 9,182 female sand flies of which 278 were *Phlebotomus duboscqi*. Two of the *P. duboscqi* females were infected with *Leishmania major* (Table 2).

DISCUSSION

Experimental and natural *Leishmania* infections interfere with sand fly blood feeding. Increased biting, a corollary of this interference, is important because it amplifies the transmission potential of an infective sand fly and therefore the contribution made by such an insect to the spread of leishmaniasis. Parasite interference with cibarial sensilla controlling engorgement is thought to be a cause of obstructed blood feeding behavior in *Leishmania*-infected sand flies.[7,8] The presence of parasites in the cibaria of 7 of the 8 sand flies in our study, which displayed excessive biting and had difficulty taking blood, provides only circumstantial support for this hypothesis. However, because certain *L. major*-infected *Phlebotomus duboscqi* probe repeatedly and fail to engorge, this parasite-sand fly combination may be of value in conducting a more rigorous investigation of the "sensilla" hypothesis.

TABLE 2

Female sand flies caught in Baringo District, Kenya during the period February 1983–January 1984 inclusive: total and number infected

	Collection method			
	Aspiration		Light trap	
Species	Total	Positive[1]	Total	Positive
P. duboscqi	52	2[2]	226	0
P. martini	35	0	302	0
P. rodhaini	1	0	1	0
S.[3] *antennatus*	1,182	9	232	1
S. bedfordi	3,613	10	849	1
S. schwetzi	368	7	756	11
S. africanus	220	1	72	0
S. clydei	114	2	689	5
S. squamipleuris	7	0	435	3
S. adleri	7	0	16	0
S. granger	1	0	1	0
Total	5,603	31	3,579	21

[1] Positive for flagellates upon dissection.
[2] Flagellates identified as *L. major* by cellulose acetate electrophoresis.
[3] *Sergenfomyia.*

An alternative explanation of how *Leishmania* interfere with sand fly blood feeding involves mechanical blockage of the pharynx or esophagus by large numbers of parasites, some of which are flushed forward as the flow of blood, unable to pass the parasite block, moves back through the mouth parts into the wound.[13] According to this hypothesis, "blocked" sand flies would also have difficulty engorging on blood; however, 10 of our experimental flies that took full blood meals after probing once or twice were found to have heavy infections in their anterior midguts. Thus, in our work, large numbers of parasites localized between the head and the abdominal midgut failed to interfere with normal blood feeding. Whether flies with heavy infections in their anterior midguts transmit parasites when they feed is not known, but the biting behavior of such insects is not the same as that observed in flies with parasites in the cibarium as well as the anterior midgut.

The tsetse fly, *Glossina m. morsitans,* infected with *Trypanosoma (T.) brucei* probes more frequently than uninfected controls,[14] a behavior change attributed to parasite interference with mechanoreceptors monitoring blood flow rate in the proboscis. However, this explanation has been recently questioned.[15] A similar increase in probing behavior occurs in *G. m. morsitans* infected with *T. (N.) congolense*.[16] In addition, changes in blood feeding behavior are known to occur in mosquitoes infected with malaria and arboviruses[17,18] and in plague-infected fleas.[19] The strategy of eliciting increased probing or other transmission-favoring events in hematophagous insects appears to have been invoked during evolution of several vector-pathogen relationships.

The natural infection rate in *Phlebotomus duboscqi* from Baringo District, 0.7%, is low when compared with promastigote infection rates for other species of sand flies,[20] which range from 0.2% to 10.5%. Based on our collection methods, 6 of the 11 species of sand flies in Baringo are more abundant than *P. duboscqi,* suggesting that the population size of this species is relatively small. The prevalence of *L. major* in the Baringo rodent population, where 18 of 789 animals, representing 15 species in 6 genera, were infected with *L. major*,[21] may have been due to the occurrence of a greater than expected number of infective bites by *P. duboscqi* with its potential for increasing the amount of transmission attributable to a small population of vector sand flies.

Despite attempts at standardization, we observed variation in infectivity, localization of *Leishmania major* in the alimentary canal and biting behavior of our experimentally-infected flies. However, we did experience some success employing colonized *Phlebotomus duboscqi* in experimental transmission studies. Two

possible explanations for this success are suggested. Other colonized sand flies suffer high mortality rates during oviposition.[8] Due to this oviposition-associated death, most females infected for transmission work die while laying eggs, prior to seeking additional blood and transmitting parasites. However, 75% of our *P. duboscqi* females survived oviposition and thereafter readily took as many as 4 additional blood meals. This allowed us to avoid the somewhat artificial treatment of denying females an oviposition site in order to keep them alive until attempting transmission, or of infecting flies by membrane feeding them on suspensions of promastigotes in media that did not stimulate oogenesis—2 methods from other studies[8,22] for circumventing oviposition. In addition, the prolonged survival of adult *P. duboscqi,* which averaged 25 days in this study, favors transmission by affording more time for *L. major* infections to develop. Finally, the use of NLB 144, only recently isolated from a sand fly and passaged only once in mice, reduces the possibility of loss of infectivity to the insect as a result of continual passage in laboratory culture or animals, a problem known to occur with certain rodent malarias.[23]

This study demonstrates that *Leishmania* interfere with normal blood feeding, eliciting a greater than expected number of infective bites/ sand fly. It is suggested that such changes favor the spread of *Leishmania* in Kenya where populations of vector sand flies have relatively low densities and infection rates.

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REFERENCES

1. Beach, R., Kiilu, G., Hendricks, L., Oster, C., and Leeuwenberg, J., 1984. *Leishmania major* in Kenya: Transmission to a human by bite of a naturally infected *Phlebotomus duboscqi* sand fly. *Trans. R. Soc. Trop. Med. Hyg.* (In press.)
2. Adler, S., and Ber, M., 1941. The transmission of *Leishmania* by bite of *Phlebotomus papatasii. Indian T. Med. Res., 29:* 803–809.
3. Chung, H. L., Feng, L. C., and Feng, S. L., 1951. Observations concerning the successful transmission of kala-azar in North China by bites of naturally infected *Phlebotomus chinensis. Peking Nat. Hist. Bull., 19:* 302–326.
4. Strangways-Dixon, J., and Lainson, R., 1966. The epidemiology of dermal leishmaniasis in British Honduras Part III. The transmission of *Leishmania mexicana* to man by *Phlebotomus pessoanus,* with observations on the development of the parasite in different species of *Phlebotomus. Trans. R. Soc. Trop. Med. Hyg., 60:* 192–207.
5. Williams, P., 1966. Experimental transmission of *Leishmania mexicana* by *Lutzomyia cruciata. Ann. Trop. Med. Parasitol. 60:* 608–609.
6. Molyneux, D. H., Lewis, D. J., and Killick-Kendrick, R., 1977. Aspects of microecology of Leishmania. Ecologie des Leishmanioses, *Colloques Internationaux du CNRS No. 239:* 31–40.
7. Killick-Kendrick, R., Leaney, A. J., Ready, P. D., and Molyneux, D. H., 1977. *Leishmania* in phlebotomid sand fly, IV: The transmission of *Leishmania mexicana amazonensis* to hamsters by the bite of experimentally infected *Lutzomyia fongipalpis. Proc. R. Soc. B., 196:* 105–115.
8. Killick-Kendrick, R., 1979. Biology of *Leishmania* in phlebotomid sand flies. Pages 395–460 *in* W. H. R. Lumsden and D. A. Evans, eds., *Biology of the Kinetoplastida.* Academic Press, London and New York.
9. Beach, R., Young, D. G., and Mutinga, M. J., 1983. New phlebotomine sand fly colonies: rearing *Phlebotomus martini, Sergentomyia schwetzi* and *Sergentomyia africana* (Diptera: Psychodidae). *J. Med. Entomol., 20:* 579–584.
10. Childs, G. E., Foster, K. A., and McRobert, K. J., 1978. Insect cell culture media for cultivation of New World *Leishmania. Int. J. Parasitol., 8:* 225–258.
11. Hendricks, L. D., Wood, D. E., and Hajduk, M. E., 1978. Haemoflagellates: commercially available liquid media for rapid cultivation. *Parasitology, 76:* 309–316.
12. Kreutzer, R. D., and Christensen, H. A., 1980. Characterization of *Leishmania* spp. by isozyme electrophoresis. *Am. J. Trop. Med. Hyg. 29:* 199–208.
13. Shortt, H. E., and Swaminath, C. S., 1928. The method of feeding of *Phlebotomus argentipes* with relation to its bearing on the transmission of kala-azar. *Indian J. Med. Res., 15:* 827–836.
14. Molyneux, D. H. and Jenni, L., 1981. Mechanoreceptors, feeding behavior and trypanosome transmission in *Glossinai Trans. R. Soc. Trop. Med. Hyg., 75:* 160–163.

15. Moloo, S. J., 1983. Feeding behavior of *Gfossina morsitans morsitans* infected with *Trypanosoma vivax, T. congolense* or *T. brucei. Parasitology, 86:* 51–56.
16. Roberts, L. W., 1981. Probing by *Glossina morsitans morsitans* and transmission of *Trypanosoma (nannomonas) congolense. Am. J. Trop. Med. Hyg., 30:* 948–951.
17. Rossignol, P.A., Ribeiro, J.M. C., Spielman, A., 1984. Increased intradermal probing time in sporozoite-infected mosquitoes. *Am. J. Trop Med. Hyg., 33:* 17–20.
18. Grimstad, P.R., Ross, Q. E., and Craig, G. B., Jr., 1980. *Aedes triseriatus* (Diptera: Culicidae) and La Crosse virus, II: Modification of mosquito feeding behavior by virus infection. *J. Med. Entomol. 17:* 1–7.
19. Wheeler, C. M., and Douglas, J. R., 194 I. Transmission studies of sylvatic plague. *Proc. Soc. Exp. Biol. Med., 47:* 65–66.
20. Williams, P., and De Vasconcellos Coelho, M., 1978. Taxonomy and transmission of *Leishmania. Adv. Parasitol., 16:* 1–42.
21. Githure, J. I., Beach, R. F., and Lightner, L. K., 1983. The isolation of *Leishmania major* from rodents in Baringo District, Kenya. *Trans. R. Soc. Trop. Med. Hyg.* (In press.)
22. Beach, R., 1983. Localization of *Leishmania donovani* in experimentally infected sand flies: an indicator of vectorial competence, Pages 158–162 *in* P. M. Tukei and A. R. Njogu, eds., *Current Public Health Research in the Tropics,* Proc. 4th Annual Medical Scientific Conference. Kenya Medical Research Institute, Kenya Trypanosomiasis Research Institute, Nairobi, Kenya.
23. Carter, R., and Diggs, C. L., 1977. Plasmodia of rodents. Pages 359–465 *in* J. P. Kreier, ed., *Parasitic Protozoa, Vol. Ill.* Academic Press. New York.