J. Med. Entomol. Vol. 16, no. 5: 424–427 23 November 1979

© 1979 by the Bishop Museum

SUSCEPTIBILITY OF SAND FLIES (DIPTERA: PSYCHODIDAE) TO TRYPANOSOMATIDAE FROM TWO-TOED SLOTHS (EDENTATA: BRADYPODIDAE)[1]

By **Howard A. Christensen**[2] **and Aristides Herrer**[3]

Abstract. Xenodiagnostic trials consisted of 1665 laboratory-reared sand flies (*Lutzomyia sanguinaria*, *Lu. gomezi*, and *Lu. trapidoi*) in 114 lots fed on 72 Two-toed Sloths (*Choloepus hoffmanni*). Fifty-eight (80.6%) of the sloths had trypanosomatid infections as follows: *Leishmania braziliensis*, 20 (27.8%); *Endotrypanum schaudinni*, 39 (54.2%); *Trypanosoma rangeli*, 15 (20.8%); and *T. cruzi*, 1 (1.4%), as determined by the biopsy-culture technique. Nineteen animals (26.4%) had multiple infections. Seven (7.7%) of 91 *Lu. sanguinaria*, which fed on 7 animals infected with *Le. braziliensis*, acquired the parasite. A total of 187 (31.1%) of 601 sand flies of 3 species developed flagellates after feeding on 21 animals infected with *E. schaudinni*. Only 1% of 292 flies developed parasites after feeding on 8 sloths with *T. rangeli*.

A biopsy-culture technique was developed by Herrer et al. (1966) for the detection of *Leishmania* and other Trypanosomatidae in natural reservoir hosts. Its effectiveness has been borne out by the large number of isolations of leishmaniae from sylvatic animals in Panama since 1966 (Herrer et al. 1973). Especially productive have been the cultures of skin samples from the hairless areas of the Two-toed Sloth, *Choloepus hoffmanni*, the principal reservoir host of *Le. braziliensis* in Panama (Herrer et al. 1973); infections are completely cryptic in this animal.

As an adjunct to the biopsy-culture technique, we fed a total of 1665 laboratory-reared sand flies of 3 species in 114 lots on 72 newly captured sloths. The present report details the results of these xenodiagnostic feedings and their comparison with the biopsy-culture technique.

MATERIALS AND METHODS

Phlebotomine rearing methods developed by Hertig & Johnson (1961) were used in this study. *Lutzomyia sanguinaria*, *Lu. gomezi* and *Lu. trapidoi*, reared routinely at our laboratory, were selected for xenodiagnostic feeding trials because they are regarded as potential vectors of *Le. braziliensis* in Panama (Christensen & Herrer 1973). The technique of feeding laboratory-reared sand flies on sloths was reported previously (Christensen & Herrer 1972).

The biopsy-culture method was used for screening and identifying trypanosomatid infections in the animals shortly after capture. Cultures of skin biopsies were made routinely from all bare areas of the body including forehead, nose, chin, ears and feet; heart blood also was cultured at this time. All specimens were cultured in modified Senekjie's medium for hemoflagellates (Herrer et al. 1966) at 22–24 °C. Negative cultures were maintained for 30 days before they were discarded. Fresh blood was examined microscopically for intraerythrocytic forms of *Endotrypanum schaudinni* and the motility of other hemoflagellates present. Blood films were fixed in methanol and stained with Giemsa. Autopsies were conducted at the time of death or sacrifice of each animal, and samples of liver, spleen, lungs, bone marrow and blood were cultured routinely.

The presence of intraerythrocytic flagellates was diagnostic for *E. schaudinni*. A pure culture of promastigote flagellates morphologically resembling those of *E. schaudinni* and not infective to hamsters was also diagnostic for this parasite. Promastigotes isolated in culture which produced characteristic lesions in hamsters, from which amastigotes were observed in smears, were identified as *Le. braziliensis*. Cultures containing epimastigotes or trypomastigotes were inoculated intracoelomically into clean *Rhodnius pallescens*. The subsequent transmission of parasites by the bite of these bugs to guinea pigs, as determined by blood smears and reisolation, was diagnostic for *Trypanosoma rangeli*. *Trypanosoma cruzi* was identified by the characteristic morphology of its trypomastigote stage and its infectivity for white mice. Epimastigote flagellates isolated, in culture, were inoculated subcutaneously into white mice. The blood and viscera of all mice were cultured for the presence of parasites.

[1] This work was supported in part by a research grant (AI-01251) from NIAID, NIH, USPHS.

[2] Gorgas Memorial Laboratory, Panama, Repub. of Panama (mailing address: P.O. Box 2016, Balboa Heights, Canal Zone).

[3] Present address: Institutos Nacionales de Salud, Apartado No. 451, Lima, Perú.

TABLE 1. Xenodiagnostic trials on Two-toed Sloths, *Choloepus hoffmanni*, harboring *Leishmania braziliensis*.*

Sloth no.	*Lutzomyia sanguinaria* No. pos./no. fed	% pos.
2944	5/14	36
3217	1/19	5
3221	1/12	8
3225	0/6	0
3234	0/5	0
2999	0/2	0
3215	0/33	0

* Natural infections demonstrated by the biopsy-culture technique.

TABLE 2. Xenodiagnostic trials utilizing 21 Two-toed Sloths, *Choloepus hoffmanni*, infected with *Endotrypanum schaudinni*.

Parameters	Sand flies: *Lu. sanguinaria*	*Lu. gomezi*	*Lu. trapidoi*
No. sloths exposed	20	8	4
No. flies fed	465	109	27
No. flies positive (%)	104 (22)	71* (65)	12 (44)

* Promastigote flagellates obtained in culture from 1 *Lutzomyia gomezi* were inoculated into hamsters and identified as *Leishmania braziliensis*.

RESULTS

A total of 58 (80.6%) of 72 *C. hoffmanni* had single or multiple trypanosomatid infections as follows: *Le. braziliensis*, 20 (27.8%); *E. schaudinni*, 39 (54.2%); *T. rangeli*, 15 (20.8%); *T. cruzi*, 1 (1.4%). Multiple infections of up to 3 trypanosomatid species occurred in 19 (26.4%) of the animals.

Leishmania braziliensis. Of the 72 *C. hoffmanni* used in this investigation, 7 (9.7%) had pure infections of *Le. braziliensis*, as determined by biopsy-culture technique. A total of 91 laboratory-reared *Lutzomyia sanguinaria* fed on these animals (TABLE 1); 7 (7.7%) of the flies were infected. All 5 *Lu. sanguinaria* positive for *Le. braziliensis* after feeding on sloth no. 2944 were dissected 4 days after the blood meal. The hindgut was not seen in 3 of these flies. Hematin residue was present in the midgut of all flies; motile unattached promastigotes were observed within the blood meal bolus of the midgut in 4 of the insects. Motile, attached and free forms were seen in the pylorus (hind triangle) and intestine of the 5th specimen. The single positive fly which fed on sloth no. 3217 was dissected 6 days later. The anterior portion of the pylorus contained a solid mass of attached, ovoid, nonmotile flagellates. The posterior aspect of the pylorus contained many motile free and attached elongated forms. The cardia, midgut, intestine and rectal ampulla showed lesser numbers of motile unattached elongated flagellates. The 1 infected fly which fed on sloth no. 3221 was dissected 7 days later. No trace of blood remained, but a moderate number of motile flagellates were seen attached to the walls of the midgut and intestine.

The biopsy-culture technique isolated only *E. schaudinni* from sloths shown in TABLE 2. However, flagellates acquired by a single *Lutzomyia gomezi* from 1 animal proved infective to hamsters and were subsequently identified as *Le. braziliensis*.

Endotrypanum schaudinni. Twenty-one (29.2%) of the 72 sloths studied had single infections of *E. schaudinni*, by biopsy-culture. Of 601 sand flies of 3 species fed on these animals, 187 acquired the parasite (TABLE 2), an infection rate of 31.1%. One hundred and four (22.4%) of 465 *Lu. sanguinaria* developed infections, as opposed to 71 (65.1%) of 109 *Lu. gomezi*. Twelve (44.4%) of 27 *Lu. trapidoi* became infected. The growth patterns of *E. schaudinni* in *Lu. sanguinaria* and *Lu. gomezi* did not differ significantly. Dissections of 136 infected flies of both species 1–11 days after feeding showed the following parasite distribution: cardia, 14%; midgut, 52%; pylorus, 63%; Malpighian tubules, 19%; hindgut, 60%; and rectal ampulla, 29% (Christensen & Herrer 1976).

The entire intestinal tract of 9 *Lu. trapidoi* was observed 5–7 days after feeding; all had heavy pylorus infections. Seven of the flies also had mod-

TABLE 3. Xenodiagnostic trials on Two-toed Sloths, *Choloepus hoffmanni*, with multiple trypanosomatid infections.

No. sloths	Biopsy-culture isolates	*Lutzomyia* spp.	No. pos./no. fed	% pos.
10	*Le. braziliensis*	*sanguinaria*	41/175	23
	E. schaudinni	*trapidoi*	4/22	18
		gomezi	0/3	0
1	*Le. braziliensis*	*sanguinaria*	0/10	0
	T. rangeli	*trapidoi*	0/1	0
4	*E. schaudinni*	*sanguinaria*	3/46	7
	T. rangeli	*gomezi*	18/28	64
1	*Le. braziliensis*	*sanguinaria*	5/19	26
	E. schaudinni			
	T. rangeli			
1	*Le. braziliensis*	*sanguinaria*	0/11	0
	E. schaudinni			
	T. cruzi			

TABLE 4. Negative xenodiagnostic trials on Two-toed Sloths, *Choloepus hoffmanni,* infected with undetermined flagellates.

SLOTH NO.	BIOPSY-CULTURE ISOLATE	*Lutzomyia* spp.	NO. FLIES FED
2974	Epimastigote	*sanguinaria*	4
3128	*E. schaudinni*	*sanguinaria*	11
	Epimastigote	*trapidoi*	4
3149	*E. schaudinni*	*sanguinaria*	17
	Epimastigote	*gomezi*	8
3296	Promastigote	*sanguinaria*	4

erate hindgut infections, 2 of which extended to the rectal ampulla. Promastigote flagellates were observed in the midgut of 3 specimens. Small clumps of hematin were present in the midgut of 5 females.

Trypanosoma rangeli. Eight (11.1%) of the 72 *C. hoffmanni* had pure infections of *T. rangeli.* Three (1.1%) of 279 *Lu. sanguinaria* fed on these animals became infected. The positive flies were examined at 3, 4 and 6 days after feeding. The entire midgut and intestine of the fly dissected 3 days postprandially contained hematin residue which obscured critical observation, although about 12 motile epimastigotes were seen in the residuum and in the saline dissecting medium. The sand fly dissected 4 days postprandially had unattached motile flagellates in the blood meal bolus of the midgut and the cardia. No blood remnants were noted in the sand fly dissected 6 days after feeding. Four motile unattached flagellates were seen in the midgut of this insect.

Multiple trypanosomatid infections. Positive xenodiagnoses were recorded from 15 of the 17 sloths (TABLE 3). Acquisition rates by the flies ranged from 0 to 64%, with the degree of alimentary tract involvement as follows: cardia, 22%; midgut, 62%; pylorus, 74%; hindgut, 82%; and rectal ampulla, 28%.

Unidentified trypanosomatids. Other trypanosomatids, exclusively or in combination with *E. schaudinni* infections, in 4 sloths failed to develop in 48 sand flies fed on the infected animals (TABLE 4).

Only 1 (0.3%) of 337 sand flies which fed on 14 *C. hoffmanni,* classified as trypanosomatid-free by the biopsy-culture technique, developed a flagellate infection. The parasite was not identified.

DISCUSSION AND CONCLUSION

Johnson & Hertig (1970) were the first to use laboratory-reared sand flies in an attempt to isolate *Leishmania* from sylvatic reservoir hosts. In 7 trials, 317 *Lu. sanguinaria* were fed on 4 Panamanian Woolly Opossums, *Caluromys derbianus.* Nineteen (6.0%) of the flies developed flagellate infections in the gut. In another xenodiagnostic trial by the same workers, 1 of 20 *Lu. gomezi* and 1 of 84 *Lu. sanguinaria* which fed on a Kinkajou, *Potos flavus,* were positive for flagellates. The identification of the trypanosomatids from these animals was not ascertained.

In the present study, 7 (7.7%) of 91 laboratory-reared *Lu. sanguinaria* acquired *Le. braziliensis* from 3 of 7 infected sloths. *Lu. sanguinaria* is 1 of 5 species regarded as potential vectors of *Le. braziliensis* in Panama (Christensen & Herrer 1973). An infection rate of about 8% for a potential vector after feeding on a principal reservoir host seems rather low. However, it should be noted that the parasite is not acquired from the circulatory system, as are most arthropod-borne disease agents, but from limited areas of infected dermis. Phlebotomines must rupture a considerable number of host capillaries to form a hemorrhagic pool to feed. Most individuals are unsuccessful in their initial attempts to ingest blood and randomly probe several areas on the face of a sloth before engorging to repletion. Such activity results in the ingestion of more dermal tissue elements and increases the opportunity for acquisition of leishmanial amastigotes (Christensen & Herrer 1972).

No females of *Lu. gomezi* were available for companion-lot feedings with *Lu. sanguinaria* on the sloths with pure *Leishmania* infections. Johnson & Hertig (1970) found higher overall infection rates in *Lu. gomezi* that in *Lu. sanguinaria,* 55% vs 42%, respectively, which fed on hamsters experimentally infected with several strains of *Leishmania.*

The fact that *Lu. gomezi* acquired *Leishmania* undetected by the biopsy-culture technique does not detract from the usefulness of this technique. Rather, it points out the advantage of using xenodiagnosis as a supportive screening method, and demonstrates the competence of this sand fly vector species in acquiring the parasite from a naturally infected reservoir.

E. schaudinni was the most prevalent sloth trypanosomatid, occurring in 39 (54.2%) of the 72 animals. *Lu. gomezi* appeared to be about 3 times more efficient as a potential vector than *Lu. san-*

guinaria, with infection rates of 65.1% and 22.4%, respectively. The high incidence of this parasite in *C. hoffmanni*, and the relative ease with which phlebotomines acquire the agent from infected animals illustrate the potential error of equating sand fly promastigotes with *Leishmania*. *Leishmania* and *Endotrypanum* flagellates are indistinguishable in the intestinal tract of sand flies. Also, the "posterior station" growth patterns of *Le. braziliensis* and *E. schaudinni* in *Lu. sanguinaria* and *Lu. gomezi* are very similar (Christensen & Herrer 1976).

The majority of multiple infections in sloths was due to *Le. braziliensis* and *E. schaudinni*. Since the infection rates of sand flies from sloths with *E. schaudinni* (187 of 601 or 31.1%) was approximately 4-fold greater than from animals with *Le. braziliensis* (7 of 91 or 7.7%), it is probable that the former parasite made up most of the positive xenodiagnoses from animals with both parasites; triturates of sand flies that fed on these animals were not inoculated into hamsters for confirmation of *Leishmania*. It is likely that multiple infections of *Endotrypanum* and *Leishmania* developed in some of the flies.

The 31.1% infection rate of sand flies fed on sloths with *E. schaudinni* is in sharp contrast to the 1.0% infection rate in the flies fed on animals with *T. rangeli*. Additionally, the occurrence of *T. rangeli* (O. Sousa, pers. commun.) in certain phlebotomine-free Panamanian localities (unpubl. data) rules out the possibility that sand flies play a significant role in the transmission of this trypanosome.

Our xenodiagnostic trials were dependent upon the use of laboratory-reared sand flies, since as many as 16.5% of Panamanian wild-caught females have been reported to be infected with promastigote flagellates (Johnson et al. 1962). Difficulties encountered in the rearing of *Lutzomyia* species have been obvious to all who have attempted to colonize them. The sand fly xenodiagnostic technique, when used as a screening procedure for isolating *Leishmania* from natural reservoirs, is less efficient, more expensive and time-consuming than the biopsy-culture method. However, as an adjunct to the latter method and as an epidemiological investigative tool, it may provide valuable data on vector-reservoir-agent dynamics. It is a method for determining and comparing parasite acquisition rates by vectors from natural reservoirs, and the subsequent developmental patterns of *Leishmania* within the intestinal tract of the sand flies. Such data, when used to supplement basic ecological information on *Leishmania* vectors and reservoirs, are requisites for comprehensive vector competence evaluation.

LITERATURE CITED

Christensen, H. A. & A. Herrer. 1972. Detection of *Leishmania braziliensis* by xenodiagnosis. *Trans. R. Soc. Trop. Med. Hyg.* **66:** 178–79.

1973. Attractiveness of sentinel animals to vectors of leishmaniasis in Panama. *Am. J. Trop. Med. Hyg.* **22:** 578–84.

1976. Neotropical sand flies (Diptera: Psychodidae), invertebrate hosts of *Endotrypanum schaudinni* (Kinetoplastida: Trypanosomatidae). *J. Med. Entomol.* **13:** 299–303.

Herrer, A., H. A. Christensen & R. J. Beumer. 1973. Reservoir hosts of cutaneous leishmaniasis among Panamanian forest mammals. *Am. J. Trop. Med. Hyg.* **22:** 585–91.

Herrer, A., V. E. Thatcher & C. M. Johnson. 1966. Natural infections of *Leishmania* and trypanosomes demonstrated by skin culture. *J. Parasitol.* **52:** 854–57.

Hertig, M. & P. T. Johnson. 1961. The rearing of *Phlebotomus* sandflies (Diptera: Psychodidae). I. Technique. *Ann. Entomol. Soc. Am.* **54:** 753–64.

Johnson, P. T. & M. Hertig. 1970. Behavior of *Leishmania* in Panamanian phlebotomine sandflies fed on infected animals. *Exp. Parasitol.* **27:** 281–300.

Johnson, P. T., E. McConnell & M. Hertig. 1962. Natural and experimental infections of leptomonad flagellates in Panamanian *Phlebotomus* sandflies. *J. Parasitol.* **48:** 158.