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HOSTS OF SANDFLY VECTORS OF *LEISHMANIA BRAZILIENSIS GUYANENSIS* IN THE CENTRAL AMAZON OF BRAZIL*

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Abstract. The blood meals of 2,569 phlebotomine sandflies from areas endemic for cutaneous leishmaniasis in the central Amazon of Brazil were tested by the microcapillary precipitin method to determine their vertebrate hosts. The two-toed sloth, *Choloepus didactylus*, was the predominant host of two incriminated vectors of *Leishmania braziliensis guyanensis* in the region, *Lutzomyia umbratilis* and *Lu. anduzei* (64.0% and 63.6%, respectively). The *Lu. "shannoni"* group, a complex of several species in which females are indistinguishable, also fed predominantly on sloths (73.0%). Species comprising the *Lu. "shannoni"* group have not been implicated as vectors of leishmaniasis; however, their feeding patterns in the study area illustrate their potential involvement in the transmission of the parasites to two-toed sloths, which are the principal reservoir hosts of *L. braziliensis* in Panama. Rodents, and particularly porcupines, were the second most frequently fed-on mammal by *Lu. umbratilis* (11.6%) and the *Lu. "shannoni"* group (8.5%).

Lutzomyia anduzei (Rozeboom, 1942) was first reported to be a possible vector of cutaneous leishmaniasis by Floch and Abonnenc in French Guyana,[1] based on epidemiological data. Wijers and Linger, working in Surinam, provided more substantive evidence of this sandfly's involvement in the transmission of leishmaniasis in their report,[2] in that 12 of 1,209 *Lu. anduzei* collected in an endemic area were infected with promastigote flagellates. The flagellates from two of the flies were inoculated into the skin on the back of a hamster; however, parasites were not demonstrated on histological examination after 1 month. It is probable that some of the sandflies reported by these investigators[1,2] were *Lu. umbratilis* rather than *Lu. anduzei*, since Ward and Killick-Kendrick discovered that *Lu. anduzei* in Pará State, Brazil, was actually a complex comprised of several closely related taxa,[3] one of which was later described by Ward and Fraiha as a new species, *Lu. umbratilis*.[4] Promastigotes found by Lainson et al.[5] in 4 of 55 *Lu. umbratilis* (reported as *Lu. anduzei*) from northern Brazil produced nodular lesions at the site of inoculation in hamsters, and amastigotes were demonstrable on stained smears from the nodules. Arias and Freitas isolated leishmanial promastigotes (infective to hamsters) from three *Lu. anduzei* and 12 *Lu. umbratilis* in the Manaus area of central Brazil.[6,7]

The subject of the present paper concerns the results of precipitin tests on the blood meals of 2,574 sandflies, collected from tree trunks during the latter study, to delineate their vertebrate hosts. The object was to determine the potential reservoirs of leishmaniasis by the elucidation of the feeding profiles of incriminated vectors.

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MATERIALS AND METHODS

Study sites

The study sites comprised four terra firma forest localities along the Torquato Tapajos Highway (AM-010), an area known to be endemic for *Leishmania braziliensis guyanensis*, the etiological agent for "pian-bois." These areas were: 1) Cidade Nova, 2) Colonia Santo Antonio, 3) Ducke Forest Reserve, and 4) CEPLAC Field Station.

Cidade Nova and Colonia Santo Antonio are two residential areas about 5 km from the city of Manaus, 2 km before the entrance to the airport. Cidade Nova is a recently deforested area where the government is constructing 15,000 low-cost houses. There have been several clinical cases of cutaneous leishmaniasis from this area during the time of this study. The entrance to Colonia Santo Antonio is only several hundred meters down the highway, and is an area which has been colonized for many years. Ducke Forest Reserve is a biological reserve 26 km northeast of the city of Manaus on the AM-010 highway; this area has been the site of many sandfly studies and has been characterized by Penny and Arias.[8] CEPLAC Field Station is 30 km northeast of Manaus, 4 km from the Ducke Forest Reserve. *Leishmania b. guyanensis* has been isolated from naturally infected *Lu. umbratilis* and *Lu. anduzei* collected in the CEPLAC Field Station and the Ducke Forest Reserve.

Sandfly collections

All collections were from sandfly resting sites on large trees. Only blood-engorged females were collected, and these were placed individually in cotton-stoppered glass tubes.

Sandflies were dissected in a drop of physiological saline and the blood meals were transferred to numbered sections of No. 1 Whatmann filter paper. All samples were refrigerated and subsequently wrapped in aluminum foil and mailed to Gorgas Memorial Laboratory in Panama where they were analyzed.

Processing of blood meals

Filter papers containing sandfly blood-meal smears were placed in 12 × 75-mm test tubes containing 0.3 ml of 0.85% buffered saline; the material was then refrigerated overnight at 5°C. The next day each blood meal was gently expressed from the filter paper with a wooden applicator stick. The filter paper was removed from each tube and the eluate tested with a Hemastix to determine if the material contained sufficient blood to process by the microcapillary precipitin test. The antigens in the eluate were then screened by class, order, and family-specific antisera. Preparation of the antisera has been reported previously.[9]

TABLE 1

Vertebrate hosts, as determined by the microcapillary precipitin test, of Lutzomyia umbratilis *from Manaus, Brazil*

Hosts	No. blood meals	% of total
Mammalia (unidentified)	56	5.7
Perissodactyla (unidentified)	13	1.3
Edentata		
Bradypodidae (sloths)	624	64.0
Unidentified	31	3.2
Myrmecophagidae (anteaters)	19	2.0
Rodentia		
Erethizontidae (porcupines)	113	11.6
Unidentified	70	7.2
Heteromyidae (spiny pocket mice)	1	0.1
Muridae (roof rats)	1	0.1
Primates		
Unidentified	8	0.8
Cebidae (monkeys)	6	0.6
Lagomorpha		
Leporidae (rabbits)	15	1.5
Marsupialia		
Didelphidae (opossums)	11	1.1
Carnivora		
Procyonidae (coatis, raccoons, etc.)	4	0.4
Unidentified	2	0.2
Artiodactyla		
Suidae (pigs)	1	0.1
Total	975	100

RESULTS

A total of 2,569 blood meals from the following sandfly species was tested by the precipitin method: *Lu. umbratilis, Lu. "shannoni"* group, *Lu. anduzei, Lu. tuberculata* and *Lu. furcata*. The vertebrate hosts of 1,955 (76.1%) of these sandflies were identified, in the majority of cases to the family level of specificity.

Lutzomyia umbratilis, the principal vector species in the study areas, was the most prevalent sandfly collected from the tree trunks. Edentates were the predominant hosts of *Lu. umbratilis*; sloths comprised 64.0% of all feedings (Table 1). Of the 624 females which had fed on sloths, 207 tested with *Choloepus* genus-specific antisera were positive indicating that the majority, if not all, of sloth feedings were derived from *C. didactylus*, the two-toed sloth. A single double feeding on Bradypodidae and Cebidae also was detected.

TABLE 2

Vertebrate hosts, as determined by the microcapillary precipitin test, of Lutzomyia "shannoni" *group from Manaus, Brazil*

Hosts	No. blood meals	% of total
Aves (unidentified)	1	0.1
Mammalia (unidentified)	74	7.8
Perissodactyla (unidentified)	3	0.3
Edentata		
Bradypodidae (sloths)	694	73.0
Unidentified	19	2.0
Myrmecophagidae (anteaters)	17	1.8
Rodentia		
Erethizontidae (porcupines)	81	8.5
Unidentified	8	0.8
Dasyproctidae (agoutis)	2	0.2
Carnivora		
Procyonidae (coatis, raccoons, etc.)	21	2.2
Unidentified	4	0.4
Felidae (cats)	1	0.1
Primates		
Cebidae (monkeys)	12	1.3
Unidentified	2	0.2
Callithricidae (marmosets)	1	0.1
Lagomorpha		
Leporidae (rabbits)	8	0.8
Marsupialia		
Didelphidae (opossums)	3	0.3
Total	951	100

Blood-engorged females of the *Lu. "shannoni"* group may have included *Lu. shannoni, Lu. dendrophila,* and *Lu. abonnenci* in the collection since females are indistinguishable. This group represented the second most common taxa encountered in the study. Although species of this group are not considered to be of importance as *Leishmania* vectors with respect to clinical cases in the Manaus region,[7] their feeding habits are very similar to that of *Lu. umbratilis* in that sloths accounted for 73.0% and porcupines 8.5% of the 951 hosts identified (Table 2). Similarly, all 291 of the 694 Bradypodidae tested with *Choloepus*-specific antisera were positive. In addition to the single feedings, one specimen had fed on members of three families, Bradypodidae, Didelphidae and Dasypodidae.

Twenty-two *Lu. anduzei,* another proven *Leishmania* vector species in the region,[7] had fed on the following hosts: Bradypodidae 14 (63.6%) Erethizontidae three (13.6%), Myrmecophagidae two (9.1%), and one each (4.6%) on Cebidae, an unidentified edentate and an unidentified mammal. The blood meals of five *Lu. tuberculata* were identified as from two unidentified mammals, and one each from Bradypodidae, Didelphidae, and Leporidae. Two *Lu. furcata* blood meals tested were from unidentified rodents.

It should be noted that the small amount of blood and/or blood meals older than about 24 hours in specimens tested precluded the identification of some hosts below the class or order level of specificity. Antisera titers decrease towards the generic level due to necessary absorption with sera from cross-reacting hosts.

DISCUSSION

Precipitin tests, performed by Dr. P. F. L. Boreham, on *Lu. umbratilis* collected in north Brazil from widely separate trees and reported by Lainson and Shaw[10] showed that 35 females from one batch had fed on the following animals: primates 20 (51.3%), rodents 11 (28%), and anteaters 8 (18%). Analysis of 36 additional blood meals of *Lu. umbratilis* from three adjacent trees showed that 35 had fed on primates and one on a porcupine. Lainson and Shaw emphasized the fact that samples of sandfly vectors from highly localized habitats can be misleading in attempts to elucidate the vertebrate source of their leishmaniasis, if the sandfly species concerned is an opportunistic feeder.[10] These authors agreed, however, that the results of blood-meal tests on a given sandfly species collected from numerous widely dispersed areas, over a long period must eventually afford some indication of the most likely reservoir(s) of *Leishmania,* especially if they are related to the detection of the parasite in dissected sandflies from the same area at the same time. Inasmuch as our study involved numerous collecting sites over an area extending for 30 km during the period from June 1978 to October 1980, and since during the study *L. b. guyanensis* was isolated from *Lu. umbratilis* and *Lu. anduzei,* the preponderant number of feedings by *Lu. umbratilis* on *C. didactylus* incriminates this edentate as a natural reservoir host of *L. b. guyanensis* in the terra firma forest of the Manaus area. Our findings, and the recent report by Lainson et al.[11] of isolating *L. b. guyanensis* from *C. didactylus* captured in Jari, Pará State, Brazil, implicate two-toed sloths as one of the principal reservoirs of the disease in the north-

ern Amazon region. Another species of two-toed sloth, *Choloepus hoffmanni,* previously had been incriminated as the principal reservoir of *L. braziliensis* in the Republic of Panama.[12–16] Two-toed sloths should be considered as potential reservoirs of parasites comprising the *L. braziliensis* complex throughout the distribution of these edentates from Nicaragua to central Brazil.

The limited but pertinent data presented on the feeding habits of the second *L. braziliensis* vector, *Lu. anduzei,* further emphasizes the significant role of *C. didactylus* in the epidemiology of the disease in the Brazilian endemic foci studied.

Although none of the 128 promastigote flagellates isolated from the *Lu. "shannoni"* group by Arias and Freitas infected hamsters,[7] the high frequency of sloth feedings by these taxa illustrates their potential involvement in the transmission cycle of leishmaniasis. If such involvement is established in this group in the future by isolating *Leishmania* parasites, the most important role of this complex undoubtedly would be the transmission between sloths, with a very minor role in transmission to man because of their rather minimal anthropophagic habits.[17]

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