

RESERVOIR HOSTS OF CUTANEOUS LEISHMANIASIS AMONG PANAMANIAN FOREST MAMMALS

ARISTIDES HERRER, HOWARD A. CHRISTENSEN, AND RONALD J. BEUMER

Gorgas Memorial Laboratory, Apartado 6991, Panama 3, Republic of Panama

Abstract. Principal results obtained during a 7-year period (1965-1972) of investigations on natural cutaneous leishmaniasis among Panamanian forest mammals are reported. A total of 2,947 feral mammals, belonging to 6 different orders and 42 genera, were studied. Leishmanial infections were demonstrated in 192 (6.5%) of these animals. Infected animals belong to 5 of the 6 orders investigated. Cutaneous leishmaniasis among Panamanian forest mammals was found to be produced by three different species of *Leishmania*: *L. braziliensis*, *L. mexicana* and *L. hertigi*. Most of the animals in which the infection was demonstrated are considered incidental hosts. Principal hosts are: the two-toed sloth, *Choloepus hoffmanni*, for *L. braziliensis*; the rice rat, *Oryzomys capito*, for *L. mexicana*; and the tropical porcupine, *Coendou rothschildi*, for *L. hertigi*. The infection rate of the two-toed sloth varies considerably in different localities studied, the high incidence of infection among the porcupine was relatively stable, and the rice rat has been found infected in a single locality in eastern Panama.

To our knowledge, the spiny rat, *Proechimys semispinosus*, was the first forest mammal found naturally infected in the New World with a species of *Leishmania* similar to those which produce cutaneous infections in humans. This important finding was reported by workers at the Gorgas Memorial Laboratory in Panama.¹ The parasite, isolated from the heartblood, proved to be infective to a volunteer and to the golden hamster, *Mesoericetus auratus*. This discovery had a profound effect on the investigative approaches concerned with the reservoir hosts of cutaneous leishmaniasis in tropical forested areas of the New World. Starting at this time emphasis shifted from domestic animals to forest mammals as potential reservoir hosts, in contrast to the previous half century of investigations.

Since the demonstration of natural leishmaniasis in the spiny rat, a considerable number of forest animals from several mammalian orders have been found infected in different neotropical areas.²⁻¹¹ Both *L. braziliensis* and *L. mexicana*, species that infect humans in the New World, have been found in these animals. In addition, a host-specific species, *Leishmania hertigi*, was found in the tropical porcupine, *Coendou rothschildi*.¹²

The present paper reports results of studies on cutaneous leishmaniasis in Panamanian forest mammals from April 1965 through March 1972.

Previous publications have partially reported information contained in this paper.

MATERIALS AND METHODS

Processing of the Animals Studied

Most of the animals studied were trapped or caught alive, but some specimens were shot. About 90% of the animals were obtained by our own personnel, and the rest were bought from local animal dealers or received as gifts.

All animals were carefully observed for gross skin alterations which might suggest a leishmanial infection. Skin smears were made in those cases with suspicious skin alterations, and were examined after being fixed in methyl alcohol and stained with Giemsa stain. As soon as possible after the animals were brought to the laboratory, cultures were made from different bare areas of the skin, mainly ears, nose and feet. After November 1967 blood was also routinely cultured. The technique used in culturing skin samples has been described previously.¹³ Animals which gave positive promastigote cultures were kept alive as long as possible at the laboratory to observe the course of the infection. Skin cultures were made periodically from these animals. Visceral cultures, mainly from liver and spleen, were made at autopsy of animals that died or were killed. Smears from viscera, as well as cultures from blood clots, were made regularly only from the two-toed sloth, *Choloepus hoffmanni*. A slightly

TABLE 1

Panamanian forest mammals examined in the search for natural leishmanial infections during 1965-1972, and results obtained

Common name	Scientific name	Number			Species of <i>Leishmania</i> involved
		Examined	Infected	Percent	
MARSUPIALIA		527	1	0.2	
Wooly opossum	<i>Caluromys derbianus</i>	75	0	0.0	
Brown murine opossum	<i>Marmosa robinsoni</i>	64	1	1.6	<i>L. mexicana</i>
Four-eyed opossum	<i>Philander opossum</i>	99	0	0.0	
Brown-masked opossum	<i>Metachirus nudicaudatus</i>	55	0	0.0	
Common opossum	<i>Didelphis marsupialis</i>	232	0	0.0	
Water opossum	<i>Chironectes minimus</i>	2	0	0.0	
PRIMATES		169	2	1.2	
Night monkey	<i>Aotus trivirgatus</i>	64	1	1.6	<i>L. braziliensis</i>
Howler monkey	<i>Alouatta villosa</i>	3	0	0.0	
Capuchin	<i>Cebus capucinus</i>	10	0	0.0	
Spider monkey	<i>Ateles fusciceps</i>	5	0	0.0	
Marmoset	<i>Saguinus Geoffroyi</i>	87	1	1.2	<i>L. braziliensis</i>
EDENTATA		605	69	11.4	
Giant ant-eater	<i>Myrmecophaga tridactyla</i>	1	0	0.0	
Lesser ant-eater	<i>Tamandua tetradactyla</i>	16	0	0.0	
Silky ant-eater	<i>Cyclopes didactylus</i>	18	0	0.0	
Three-toed sloth	<i>Bradypus infuscatus</i>	163	2	1.2	<i>L. braziliensis</i>
Two-toed sloth	<i>Choloepus hoffmanni</i>	396	67	16.9	<i>L. braziliensis</i>
Nine-banded armadillo	<i>Dasybus novemcinctus</i>	11	0	0.0	
LAGOMORPHIA		6	0	0.0	
Forest rabbit	<i>Sylvilagus braziliensis</i>	6	0	0.0	
RODENTIA		1,504	116	7.7	
Tropical red squirrel	<i>Sciurus granatensis</i>	29	0	0.0	
Variegated squirrel	<i>Sciurus variegatoides</i>	6	0	0.0	
Spiny pocket mouse	<i>Liomys adspersus</i>	44	0	0.0	
Spiny pocket mouse	<i>Heteromys desmarestianus</i>	25	0	0.0	
Rice rat	<i>Oryzomys alfaroi</i>	1	0	0.0	
Rice rat	<i>Oryzomys caliginosus</i>	26	0	0.0	
Rice rat	<i>Oryzomys capito</i>	97	14	14.4	<i>L. mexicana</i>
Rice rat	<i>Oryzomys albigularis</i>	2	0	0.0	
Rice rat	<i>Oryzomys</i> sp.	13	0	0.0	
Alfaro's water rat	<i>Nectomys alfari</i>	3	0	0.0	
White-tailed rat	<i>Tylomys panamensis</i>	140	0	0.0	
White-footed mouse	<i>Peromyscus nudipes</i>	1	0	0.0	
Cane rat	<i>Zygodontomys microtinus</i>	30	0	0.0	
Cotton rat	<i>Sigmodon hispidus</i>	45	0	0.0	
Roof rat	<i>Rattus rattus</i>	76	0	0.0	
Porcupine	<i>Coendou rothschildi</i>	104	92	88.5	<i>L. hertigi</i>
Paca	<i>Agouti paca</i>	8	1	12.5	<i>L. mexicana</i>
Agouti	<i>Dasyprocta punctata</i>	19	0	0.0	
Spiny rat	<i>Proechimys semispinosus</i>	766	8	1.0	<i>L. mexicana</i>
Spiny rat	<i>Hoplomys gymnurus</i>	30	0	0.0	
Arboreal spiny rat	<i>Diplomys labilis</i>	39	1	2.6	Not yet definitely characterized
CARNIVORA		136	4	2.9	
Raccoon	<i>Procyon cancrivorus</i>	6	0	0.0	
Coati	<i>Nasua nasua</i>	8	1	12.5	<i>L. braziliensis</i>
Kinkajou	<i>Potos flavus</i>	107	2	1.9	<i>L. braziliensis</i>
Olingo	<i>Bassaricyon gabbii</i>	9	1	11.1	<i>L. braziliensis</i>
Tayra	<i>Eira barbara</i>	1	0	0.0	
Grison	<i>Galictis allamandi</i>	2	0	0.0	
Skunk	<i>Conepatus semistriatus</i>	1	0	0.0	
Jaguarundi	<i>Felis yagouaroundi</i>	2	0	0.0	
Totals		2,947	192	6.5	

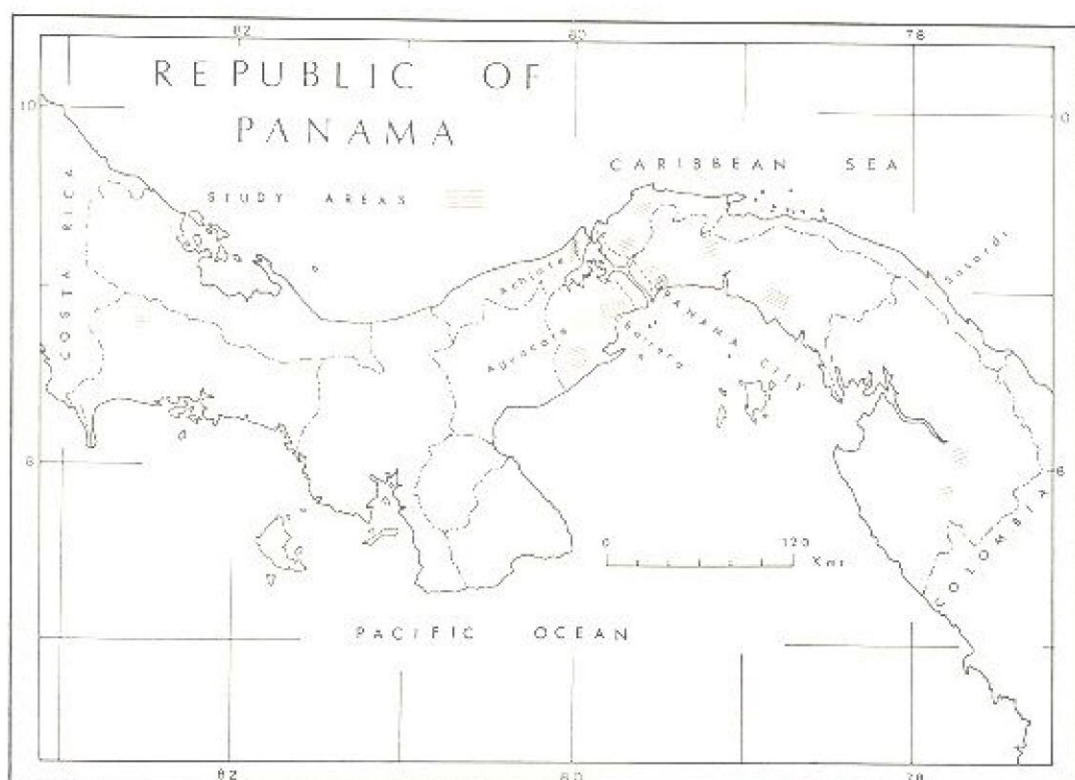


FIGURE 1. Map of the Republic of Panama showing the study areas where the animals studied were collected.

modified diphasic Senekji's culture medium for leishmaniae was used routinely.

In addition to the above diagnostic techniques, starting in 1971 the xenodiagnostic technique also has been used in the two-toed sloth.²⁴

Identification of *Leishmania* Species

Leishmanial strains isolated in cultures were identified by studying their infectivity for the golden hamster, as well as the course of the infection they produced in this rodent. Four- to 7-week-old hamsters were inoculated intradermally in the nose with 5 to 10×10^6 promastigotes from cultures of strains recently isolated; volume of the inoculum was about 0.05 ml. Inoculated animals were maintained alive as long as possible.

RESULTS

Forest Mammals Found Infected

A total of 2,947 feral mammals belonging to 6 orders and 42 genera was studied, and leish-

manial infections were demonstrated in 5 orders and 13 genera. Table 1 shows the species of forest mammals studied and those found infected, as well as the species of *Leishmania* involved in each case.

About 85% of the animals studied were collected in central Panama; only 15% and less than 1% of the total were obtained from eastern and western Panama (Fig. 1), respectively.

Species of *Leishmania* Found

Three dermatropic species of *Leishmania* were found infecting Panamanian forest mammals. They are: *L. braziliensis*, *L. mexicana*, and *L. hertigi*, which may be differentiated readily from one another by using the golden hamster as an experimental host.

L. hertigi remains at the site of inoculation only transiently and does not produce any swelling or other gross reaction. *L. braziliensis* produces a long-lasting infection, with a swelling of moderate proportion at the site of inoculation, which de-

TABLE 2

Comparative prevalence of the leishmanial infection between the two-toed sloth, *Choloepus hoffmanni*, and the porcupine *Coendou rothschildi*, in three representative areas of Central Panama

Study area	Two-toed sloth, <i>C. hoffmanni</i>			Porcupine, <i>C. rothschildi</i>		
	Number studied	Infected with <i>L. braziliensis</i>	Infection rate (%)	Number studied	Infected with <i>L. hertigi</i>	Infection rate (%)
Achiote	35	13	37.1	16	14	87.5
Chorrera	43	2	4.7	15	15	100.0
Aguacate	119	31	26.1	44	37	84.1
Totals	197	46	23.4	75	66	88.0

creases with time. Metastases to other parts of the skin occur in some cases, but they are usually inconspicuous and take more than 5 months to appear. The intensity of the parasitism is moderate, both in the swelling at the site of inoculation and in metastatic lesions. *L. mexicana* produces a tumor-like swelling at the site of the inoculation, which usually persists until the death of the animal. Metastases to other parts of the skin occur more frequently and in a shorter period of time. Metastatic lesions achieve tremendous size and contain enormous numbers of parasites.

Geographic Distribution of the Three Leishmanial Species

Geographic distribution patterns of the three species of *Leishmania* show significant differences. *L. hertigi* was present practically everywhere its vertebrate host was trapped and studied, and the infection rate did not show any marked difference from one region to another. Infected porcupines were obtained both from the Atlantic and Pacific sides of central Panama as well as from the eastern side of the Isthmus (Darien Province). In addition, porcupines collected in primary forest, secondary vegetation and cultivated areas showed similar infection rates. *L. mexicana* so far appears to be restricted to the eastern side of Panama, around the Sasaki area. *L. braziliensis* is associated mainly with the two-toed sloth in central Panama. Infected sloths are found both from the Pacific and Atlantic sides of this region.

Comparative Prevalence of Leishmanial Infections

It is possible to estimate the comparative prevalence of leishmanial infections for certain

representative areas from the information we have gathered. These estimations will be limited to the infections due to *L. braziliensis* and *L. hertigi*.

The infection rate of *L. braziliensis* in the two-toed sloth and that of *L. hertigi* in the porcupine may be compared in three areas of the Province of Panama from which adequate numbers of animals were obtained. These are: Achiote, El Aguacate and La Chorrera (Fig. 1). The first of these is located on the Atlantic side and the other two on the Pacific side of the Isthmus. Both two-toed sloths and porcupines from these three localities have been studied intensively during at least 3 consecutive years and results obtained are condensed in Table 2.

Persistence of High Prevalence Rate of *Leishmania braziliensis* and *L. hertigi* Infections

A natural leishmanial infection in a two-toed sloth was first demonstrated in February 1968. Thereafter, infection rates determined on the basis of calendar years were as indicated in Table 3.

Figures contained in Table 3 represent the overall prevalence rate for all areas studied.

TABLE 3

Prevalence rate of *Leishmania braziliensis* infection in the two-toed sloth, *Choloepus hoffmanni*

Data	Year			
	1958	1969	1970	1971
Specimens processed	162	88	67	50
Number found infected	24	9	19	13
Infection rate (%)	14.8	10.2	28.4	26.0

TABLE 4

Persistence of a high Leishmania hertigi infection rate in the porcupine, Coendou rothschildi

Data	Year						
	1965	1966	1967	1968	1969	1970	1971
Specimens processed	8	7	43	22	6	13	5
Number found infected	6	7	36	20	6	13	4
Infection rate (%)	75.0	100.0	83.7	90.9	100.0	100.0	80.0

Prevalence for different localities varies considerably, from areas where no leishmaniasis has been demonstrated to others with very high prevalence rates.

The porcupine was found infected with *L. hertigi* for the first time in April 1965. Since then, it has shown persistently high infection rates throughout the whole study period, as is indicated in Table 4.

DISCUSSION

Cutaneous Leishmaniasis Among Forest Mammals in Panama

Forest mammals reported here with natural cutaneous leishmaniasis belong to 13 different genera (Table 1). The spiny rats, *Proechimys semispinosus* and *Hoplomys gymnurus*, and the kinkajou, *Potos flavus*, have been reported previously as hosts of *L. braziliensis* in Panama.^{1,15} Our findings now raise to 14 the total number of forest mammalian species found with natural leishmanial infections in this country. This is by far the largest number of feral hosts ever found in any single country of the New World.



FIGURE 2. The two-toed sloth, *Choloepus hoffmanni*, main reservoir host of *Leishmania braziliensis* in central Panama. The infection rate in this edentate varies considerably with the locality.

Three different dermatropic species of *Leishmania* have been found. This fact, together with the number of mammalian species in which natural leishmanial infection has been demonstrated, indicates that cutaneous leishmaniasis is widely distributed among Panamanian forest mammals. In fact, the infection was demonstrated in 5 of the 6 mammalian orders of feral animals studied (Table 1). Lagomorpha is the single order in which natural leishmaniasis was not found; however, only 6 specimens of this order were processed.

It is expected that natural cutaneous leishmaniasis among the mammalian fauna of other neotropical forested areas may occur in a similar way to that found in Panama.

Principal Reservoir Host for Each Leishmanial Species

Since several mammalian species have been found with natural leishmaniasis, it is necessary to assess the importance of each one as reservoir hosts. In assigning a role as important reservoir host the following two conditions seem necessary: 1) infected specimens should be found repeatedly; and 2) the mammalian species involved must be common. The low infection rates of most of the mammalian species found infected (Table 1) implicate them as incidental hosts only. This may be due either to low host susceptibility, or to lack of a close ecological association with the insect vector.

Table 1 shows that only 3 forest mammals fulfill the prerequisites mentioned above for being considered important reservoir hosts. They are the two-toed sloth, *Choloepus hoffmanni* (Fig. 2), for *L. braziliensis*; the tropical porcupine, *Coendou rothschildi* (Fig. 3), for *L. hertigi*; and the rice rat, *Oryzomys capito* (Fig. 4), for *L. mexicana*.

Each of the above reservoir hosts shows certain characteristics: 1) the two-toed sloth persistently



FIGURE 3. The tropical porcupine, *Coendou rothschildi*, host of *Leishmania hertigi*, a recently described parasite. This porcupine is the only mammalian species so far found infected with *L. hertigi*, and always shows a high infection rate.



FIGURE 4. The rice rat, *Oryzomys capito*, principal reservoir host of *Leishmania mexicana* in Panama. Although this rat is found in different regions of the Panamanian territory, the infection with *L. mexicana* so far seems restricted to the area around Sasardi, eastern part of the Isthmus.

has been found infected for the last 4 years in central Panama, and has shown infection rates as high as 50% in Las Tablitas, Achioté area. No other forest mammal in Panama shows a similar persistence in the infection by *L. braziliensis*, which emphasizes the importance of this edentate as the main reservoir host of this species of *Leishmania*; 2) the tropical porcupine is the single forest mammalian species, among the 47 species studied comprising almost 3,000 specimens, found naturally infected with *L. hertigi*, suggesting that this species of *Leishmania* is host-specific. This rodent has been found infected in all the study areas where it was obtained, and always showed a very high infection rate; and 3) the rice rat *O. capito* has been found infected with *L. mexicana* only in a locality of the Sasardi area, eastern Panama, where an infection rate of 36% was demonstrated.¹⁹ Although this rice rat is also present in central Panama, where 48 specimens have been studied, no infections due to *L. mexicana* were found. The restriction of *L. mexicana* to a small focus in Sasardi may be due to the close ecological association maintained there between the reservoir host and the insect vector, *Lutzomyia olmeca*.¹⁶

The possible role of incidentally infected forest mammals in maintaining leishmaniasis in nature remains to be determined.

Difference in the Prevalence Rate of Porcupine and Sloth Leishmaniasis

Table 2 shows a clear difference in the prevalence of leishmaniasis between the porcupine and

the two-toed sloth in three study areas of central Panama. The infection rate in the porcupine is always much higher and does not vary as markedly from one study area to another, in contrast to the prevalence rate found in the sloth leishmaniasis.

The high infection rate (100%) of porcupine leishmaniasis in La Chorrera study area is particularly interesting. Primary forest has been destroyed here for many years and the land has been used mainly for agricultural purposes and to graze cattle. Secondary forest areas are rare. The current situation concerning the vectors in this study area is unknown.

Sloth leishmaniasis showed its highest prevalence rate in the Achioté study area. In a locality of this area, Las Tablitas, 8 of 16 (50%) of sloths examined proved to be infected. This study site is found partially in the Canal Zone, where primary forest is present and phlebotomine sandflies are abundant.

Nature of the Infection

The course of the infection, the skin reaction due to leishmaniasis and the sites (skin, blood, viscera) from which the parasite was cultured varied considerably in relation to the species of *Leishmania* and the vertebrate hosts involved. These aspects will be considered in future publications.

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