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Enzootic cutaneous leishmaniasis in eastern Panama

I: Investigation of the infection among forest mammals

By A. HERRER, S. R. TELFORD, Jr*. and H. A. CHRISTENSEN

From the Gorgas Memorial Laboratory, Panama City, Panama R. P.

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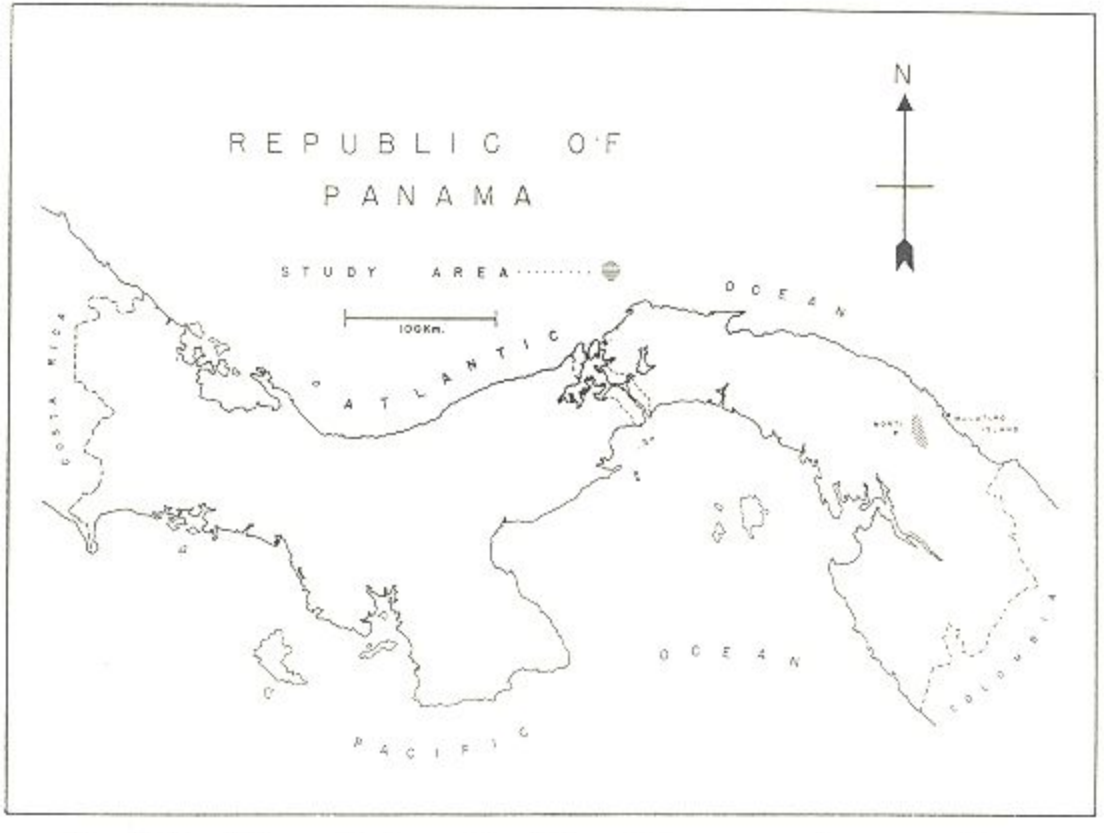
Epidemiological investigations on cutaneous leishmaniasis were carried out in the Sasardi area, San Blas Territory, eastern Panama (see map), between September, 1968 and June, 1969, during which time four trips were made.



Cuna Indians living on Mulatupo Island, just off the coast, are the owners of the region of Sasardi. They sometimes visit the banana plantations or wander around the vicinity of the study area. In addition, since a trail goes westward deeper into the forest in the direc-

*Present address: Department of Natural Sciences, The Florida State Museum, University of Florida, Gainesville, Florida 32601, U.S.A.

tion of Morti, Indians frequently are seen in transit through the Sasardi Camp area. Nevertheless, it seems that most of the forest mammals remain largely undisturbed by humans in the area where our studies were carried out.

Special attention was paid to trapping forest mammals, making ecological observations, and collecting phlebotomine sandflies. In addition, the small group of people remaining in the camp, as well as a few Indians who visited the area, were interviewed and examined for lesions or scars of cutaneous leishmaniasis. The information gathered indicates that cutaneous leishmaniasis is not present among the local human population. However, an American herpetologist who spent some weeks collecting reptiles in the region in February 1967, developed leishmanial lesions on his neck four to five weeks after leaving Sasardi Camp, while a Panamanian workman who was stationed in the Sasardi Camp for some months developed leishmaniasis in March 1967. These are the only examples we have found of human cases of cutaneous leishmaniasis possibly acquired in this area.

A series of three papers will report the results of our studies. This first paper deals with the parasitological aspects of cutaneous leishmaniasis in the forest mammals found there. The second and third papers (Christensen et al., *in press*; Telford et al., *in preparation*) will report the results of entomological and ecological investigations.

MATERIALS AND METHODS

Animals Studied

During the four trips about 480 forest mammals were collected, 386 of which were examined for leishmanial infections. The following animals were studied:

Carnivora	
Bassaricyon gabbii J. A. Allen (olingo)	2
Potos flavus Scheber (kinkajou)	1
Marsupialia	
Didelphis marsupialis L. (common opossum)	5
Marmosa robinsoni Bangs (brown murine opossum)	35
Metachirus nudicaudatus E. Geoffroy St.—Hilaire (brown-masked opossum)	39
Caluromys derbianus Waterhouse (woolly opossum)	3
Primates	
Aotus trivirgatus Humboldt (night monkey)	1
Saguinus geoffroyi Pucheran (marmoset)	23
Rodentia	
Nectomys alfari J. A. Allen (Alfaro's water rat)	1
Oryzomys caliginosus Tomes (rice rat)	18
Oryzomys capito Olfers (rice rat)	39
Tylomys panamensis Gray (white-tailed rat)	11
Agouti paca L. (paca)	2
Dasyprocta punctata Gray (agouti)	3
Diplomys labilis Bangs (arboreal spiny rat)	1
Proechimys semispinosus Tomes (spiny rat)	202

Seven species accounted for 95 per cent. of the animals studied. Predominant animals

were two species of marsupial (the brown murine opossum and the brown-masked opossum), a primate (the marmoset), and four rodents (two species of rice rats, the white-tailed rat, and the spiny rat). Although ecological aspects are discussed in another paper of this series, we may comment that six of the seven predominant mammals were widely distributed throughout the study area and adjacent areas, and the number of specimens obtained in each trip did not vary significantly. The exception was the rice rat, *O. capito*, which was found to be restricted to a small area, and its population decreased markedly. This was apparently due to the intensive trapping.

Processing of the Animals in the Search for Leishmanial Infections

Trapped animals were transported alive by air to the laboratory in Panama city as soon as possible. After their arrival they were examined for the presence of gross skin lesions or scars similar to those of cutaneous leishmaniasis in animals. Skin smears were prepared and examined only in cases with more conspicuous skin alterations. Skin cultures were made from all animals that arrived alive at the laboratory, using a technique previously described (Herrer *et al.*, 1966). Ears, nose and feet were the principal sites from which skin biopsies or necropsies were made in order to obtain material for culturing. Simultaneously, heart blood was also cultured from most of the specimens. Visceral cultures were made from animals that arrived recently dead or that died in the laboratory during processing, as well as from those which were killed in the laboratory. Liver and spleen tissue, either separately or pooled, were frequently cultured, but bone marrow only in a limited number of cases.

A slight modification of the Seneckji's culture medium for leishmania (Herrer *et al.*, 1966) was routinely used both for isolation of the parasite and subsequent *in vitro* maintenance in the laboratory. Original cultures, incubated at air-conditioned room temperature (19–22°C), were microscopically examined for the first time about the tenth day. At least two additional examinations during a period of a month were made for every culture before considering them negative. Cultures positive for haemoflagellates were microscopically examined under phase contrast illumination to ascertain whether or not a promastigote (leptomonad) flagellate was present.

Each strain of promastigote flagellates obtained in culture was inoculated intradermally into the noses of two to six hamsters. Inoculated hamsters were kept under close observation for several weeks to detect the presence of the typical leishmanial swelling at the site of the inoculation, during which time skin smears were made from that site. Those hamsters that showed Leishman-Donovan (L-D) bodies in skin smears were kept alive as long as possible in order to observe the entire course of the infection. Inoculated hamsters with negative skin smears were examined in addition by skin cultures from the nose before being considered as non-infected.

RESULTS

Infected Animals Found and Characteristics of the Infection

The following animals proved to be naturally infected: 14 (36 per cent.) of 39 rice rats, *O. capito*; 8 (4 per cent.) of 202 spiny rats, *P. semispinosus*; 1 (2·8 per cent.) of 35 brown murine opossums, *M. robinsoni*; 1 of 2 pacas, *A. paca*, as well as the single specimen of arboreal spiny rat, *D. labilis*. In four rice rats the infection was demonstrated by skin

smears only; in six rice rats, the brown murine opossum and the arboreal spiny rat both by skin smears and skin cultures; and in the eight spiny rats, four rice rats and the paca, by cultures only.

The infections were localized as follows: in the arboreal spiny rat, the paca and the eight spiny rats, on the ear only; in the brown murine opossum and 12 rice rats, on the tail only; and in two rice rats, both on one ear and the tail.

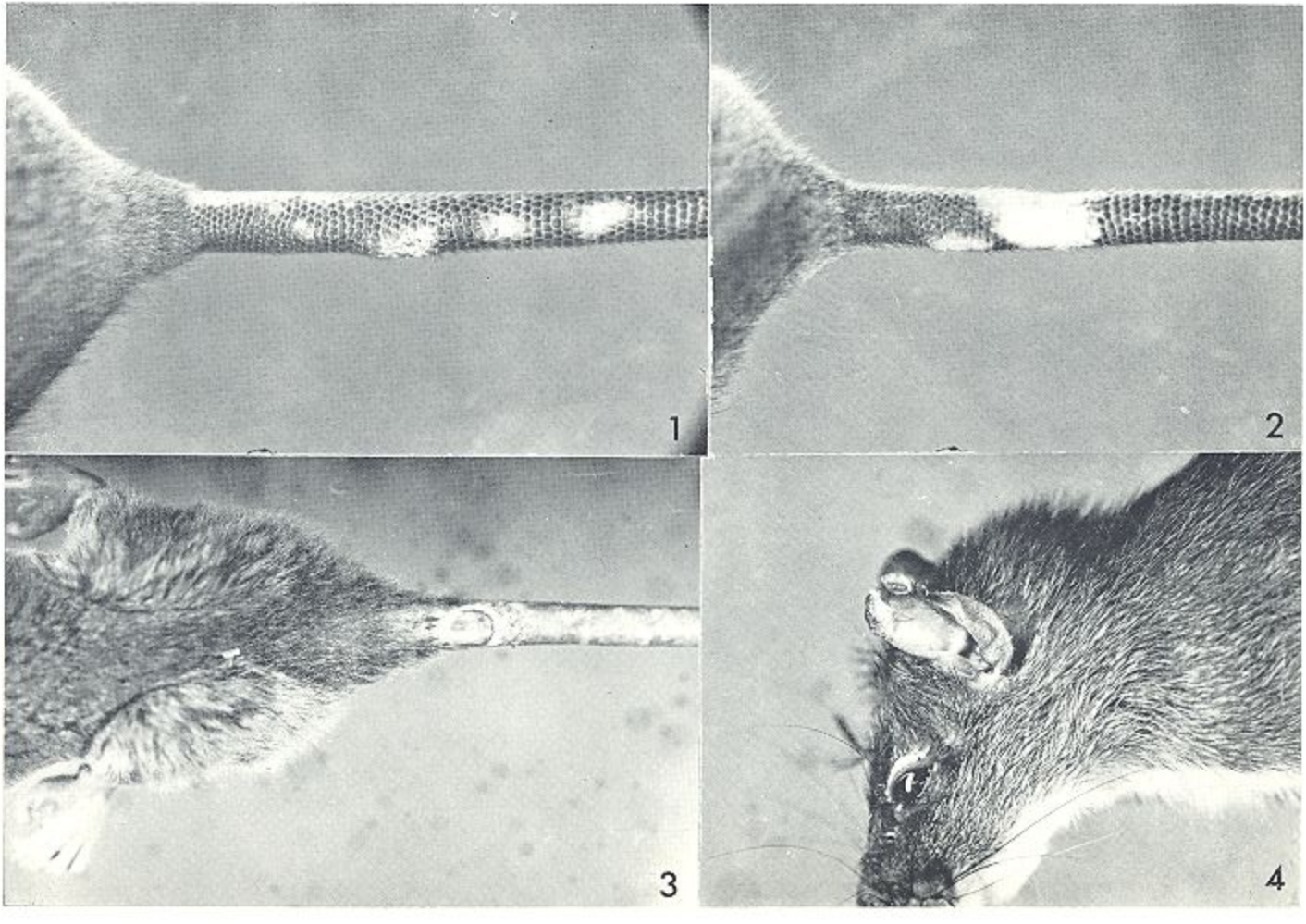
In those animals that showed typical gross skin alterations due to leishmaniasis, the parasite seemed to be restricted to small areas of the skin in all cases, and in general the infection was not intense. Cultures or smears made from the skin some millimetres away from active lesions usually gave negative results. This limited distribution of the parasite was confirmed in a series of four naturally-infected rice rats in which the entire skin of the tail was cultured after being cut into four to six different pieces. Smears from active lesions as a rule contained only moderate numbers of L-D bodies, and those from healing lesions, very few. Although smears from scars sometimes showed rare parasites, especially if the smears were made just from the edge, in every case careful and prolonged examination was required. When tissues from scars were cultured and yielded positive results, usually only some of the inoculated tubes showed growth of the parasite and, in addition, the incubation period was longer than usual.

Gross Skin Alterations

Conspicuous skin lesions or scars due to cutaneous leishmaniasis were found only in the infected rice rats and the brown murine opossum. The single specimen of arboreal spiny rat trapped, which proved to be infected, showed superficial encrusted lesions on the edge of both ears when captured. These lesions almost disappeared after the animal was kept for two weeks in the laboratory. Moreover, only the right ear yielded positive results both by skin smear and pinna culture. In about one-third of the spiny rats different skin alterations were observed as indicated below. The infected paca showed no gross skin alteration at all at the site (ear) from which the parasite was cultured.

Active lesions in the rice rat were usually whitish, somewhat circular in outline, three to seven mm in diameter, and rather prominent (Plate XII, Fig. 1). The surface was rough, dry, and covered by thick scales instead of true crusts. Frequently two or three lesions were found, one near the other, which sometimes became confluent. Exceptionally the lesions were oedematous but never ulcerated. In every case lesions were found only on the dorsal side of the tail, mostly near its base (Plate XII, Fig. 2). From the two rice rats in which parasites were found on the ear also, only one showed a minute and inconspicuous alteration of the skin. Scars on the tail were more typical and similar to those previously described in the same rodent found infected in Brazil (Nery-Guimaraes, 1964; Lainson and Shaw, 1968); they were conspicuously depigmented and frequently the surface was a little depressed below the surrounding normal skin (Fig. 2). The single infected opossum had an ulcerated lesion about seven millimetres in diameter on the base of the tail (Plate XII, Fig. 3). Nevertheless, there was no induration at all around the ulcer, giving the appearance of a recent traumatic lesion. This animal was under observation in the laboratory for 24 days, during which time the lesion remained unchanged in appearance and yielded positive smears on three different occasions.

Spiny rats sometimes showed gross skin alteration due to causes other than leishmani-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asis (Plate XII, Fig. 4). These rodents at times were heavily parasitized by trombiculid mites that produced redness and other skin reactions, especially on the ears and around the eyes. Gross skin alterations were also found, mainly on the ears and tail, that seemed to result from fighting. Four of the eight rats with positive pinna cultures presented depigmentation or redness on the ears, and the other four showed no skin alteration at all. Consequently, it was difficult to associate the presence of skin alterations in this animal with a leishmanial infection.

Search for Dissemination of the Parasite

Absence of metastases. In order to observe the entire course of the infection and the eventual presence of metastases and/or visceral localization, nine naturally-infected rice rats and a single spiny rat were kept under observation in the laboratory for 136–366 days from the date of their capture. During this period these animals were repeatedly examined both by skin smears and skin cultures, except for the spiny rat which was examined only twice, on the 13th and 204th day following its capture. The animals were finally killed, and cultures were made both from the skin and viscera at autopsy.

Four rice rats of this group yielded positive results upon initial examination on days 12, 22, 22 and 35, respectively. Subsequent examinations up to the 246th, 197th, 137th and 366th day, respectively, were negative. In another animal two examinations gave positive results up to the 17th day, followed by three negative on the 42nd, 153rd and 258th day. In the remaining four rice rats the infection was demonstrated to persist up to the 164th, 266th, 267th and 286th day, respectively. A positive skin culture was obtained in the last animal at autopsy (286th day), but two previous examinations on the 18th and 40th day were negative; the other three rice rats yielded repeatedly positive skin smears or skin cultures during the period of study. The single spiny rat studied gave a positive pinna culture at the 13th day, and negative pinna and skin (tail) cultures at autopsy (204th day).

Although the above nine naturally infected rice rats, as well as the single spiny rat mentioned, were carefully observed during the period of time they were under study, none of them showed any clear indication of metastasis to other sites of the skin. Nevertheless, in one rice rat a minute pimple-like formation was discovered on one ear on the 133rd day; L-D bodies were observed the same day. Although part of the pinna was destroyed in making the smear when the lesion was observed for the first time, another smear on the 159th day, as well as the culture at autopsy (266th day), were also positive. The gross skin alteration was so inconspicuous in this case that it was actually discovered by touching instead of looking, so there is the possibility that it was overlooked for some time.

EXPLANATION OF PLATE XII

Natural leishmanial infection among forest mammals in eastern Panama

FIG. 1. Four small active lesions on the tail of the rice rat, *Oryzomys capito*. Lesions are usually located on the dorsal side of the tail, near its base and are not ulcerated.

FIG. 2. Characteristic scar due to leishmaniasis in the rice rat. It is whitish in colour and somewhat depressed below the surrounding normal skin.

FIG. 3. An ulcerated leishmanial lesion at the base of the tail in a brown murine opossum, *Marmosa robinsoni*. There was no induration around the ulcer and it had the appearance of a traumatic lesion. Nevertheless, the parasite was repeatedly demonstrated to be present.

FIG. 4. Encrusted lesion on the ear of a spiny rat, *Proechimys semispinosus*, with both smears and pinna culture negative for *Leishmania*.

Absence of visceral localization. As indicated in the Table, cultures were made from liver and spleen in all infected animals except one rice rat and one spiny rat. In addition, bone marrow was cultured in 13 (53 per cent.) out of the 25 animals, all with negative results. Identical results were obtained when heart blood was cultured from most of these animals.

TABLE

Search for visceral localization of the parasite among the 25 forest mammals with natural leishmanial infection found in Sasardi. Results of the different cultures made.

Animal no.	Sex	Month trapped	Cultures made from: Blood	Ear pinna	Skin*	Liver	Spleen	B. marrow
			Oryzomys capito					
1263	♂♀♀	Sept., 1968	—	C	–**	–	–	
1264	♂♀♀	,, ,,	–	–	+	C	–	
1276	♂♀♀	,, ,,		–	C**	–	–	
1475	♂♂♂	Dec., 1968		+	+	–	–	–
1476	♂♂♀	,, ,,		–	+	–	–	–
1477	♂♂♀	,, ,,		–	–**	–	–	
1483	♂♂♀	,, ,,	–	–	+	–	–	
1486	♂♂♀	,, ,,	–	–	+	–	–	–
1487	♀	,, ,,			+			
1488	♂♂♂	,, ,,		–	–**	–	–	
1540	♂♂♂	,, ,,	–	+	+	–	–	
1543	♂♂♂	,, ,,	–	–	+	–	–	–
1603	♂♂♂	,, ,,	–	–	+	–	–	
1647	♀	March, 1969	–	–	+	–	–	
			Proechimys semispinosus					
1235	♂	Sept., 1968		+		–	–	
1268	♂♂♂	,, ,,	–	+	–	–	–	–
1408	♂♂♂	Nov., 1968	–	+		–	–	–
1516	♂♂♂	Dec., 1968	–	+		–	–	–
1625	♂♂♀	March, 1969	–	+	–	–	–	–
1719	♀♀	,, ,,	–	+	–	–	–	–
1722	♀♀	,, ,,	–	+	–			
1921	♂	June, 1969	–	+	–	–		–
			Diplomys labilis					
1618	♂	March, 1969		+	–	–	–	–
			Marmosa robinsoni					
1905	♂	June, 1969	–	–	+	–	–	–
			Agouti paca					
1963	♀	,, ,,	–	+	–	–	–	–
	Total cultures made		17	24	22	23	22	13
	Positive		0	12	11	0	0	0
	Negative		17	11	10	22	22	13
	Contaminated		0	1	1	1	0	0

* = All skin cultures reported in this table were made from the tail
** = In these cases the infection was demonstrated by skin smear only
C = Contaminated.

Cultures from liver and spleen (usually pooled), bone marrow and heart blood, were also made from most of the animals that yielded negative results in the previous examination for leishmaniasis. Viscera, bone marrow and heart blood cultures were made in 88

47 and 68 per cent., respectively, of all animals processed, including those infected. Although the number of animals examined by these series of cultures was considerable, *Leishmania* was not isolated in any case.

Identification of the Parasite Cultured

Strains of promastigote flagellates cultured from all five species of forest mammals mentioned proved to be *Leishmania*. They infected the golden hamster when inoculated intradermally on the nose, producing typical leishmanial swellings at the site of inoculation and metastatic lesions similar to those due to *L. mexicana* (Garnham, 1962). L-D bodies were extremely abundant, both in the nose swelling as well as in the metastatic lesions. Moreover, hamsters infected with these strains survived a shorter time than those infected with Panamanian strains of *L. braziliensis*. The exact characterization of the strains isolated in Sasardi is currently under way and the results will be reported at a later date.

DISCUSSION

Enzootic Nature of the Infection

Although two persons seem to have acquired cutaneous leishmaniasis in the vicinity of the Sasardi Camp during the last few years, most of the evidence indicates that we are dealing with a real enzootic focus. The Cuna and Choco Indians who visit the nearby banana plantations and occasionally cultivate small patches of the land did not show any indication of the disease. In addition, the involvement of five different species of forest mammals indicates that the infection is well established among the mammalian fauna of that area. Two of the five species found infected showed a great attractiveness to *Lutzomyia olmeca* (Christensen *et al.*, *in press*), a sandfly species that proved to transmit *L. mexicana* to man in Mexico (Biagi *et al.*, 1965). *L. olmeca*, moreover, has been incriminated as the potential vector of cutaneous leishmaniasis among forest rodents in British Honduras (Disney, 1968; Williams, 1970), as has been the closely related *L. flaviscutellata* in Brazil (Lainson and Shaw, 1968). This species of sandfly was found in Sasardi predominantly at ground level, mainly among the forest floor litter and apparently in close ecological association with the forest mammals found infected (Christensen *et al.*, *in press*).

Reservoir Hosts

Due to the small number of specimens processed, the arboreal spiny rat and the paca are not considered further in this discussion. The rice rat, brown murine opossum and spiny rat (14/39, 1/35 and 8/202 infected, respectively) seem to be potential reservoir hosts.

The rice rat, *O. capito*, appears to be the main reservoir host in the area studied. In addition to the high infection rate (36 per cent.) demonstrated in this animal, the infection seems to be a long-lasting one. In four out of nine naturally infected rats maintained in the laboratory for several months, the parasite proved to be present up to the 164th, 266th, 267th and 286th day, respectively, following capture. Taking into consideration that these four specimens were already infected at the time they were trapped, it is obvious that the natural leishmanial infection is long lasting in this species of rice rat.

However, the presence of *O. capito* was limited to a small area, almost restricted to the ecotone formed by an old corn-field and a banana plantation with secondary forest. In addition, it seems that the repeated trapping in the study area decreased its population con-

siderably (Telford *et al.*, *in preparation*). The spotty distribution of this rice rat appears to limit its importance as an effective reservoir host along extensive areas.

Both the spiny rat and the brown murine opossum, with 4 and 2·9 per cent. infection rates, respectively, probably play a less important role as reservoir hosts in the Sasardi area. The reason for their lower infection rate in comparison with that of the rice rat remains to be determined. It may be due either to the nature of the leishmanial infection in these animals or to behaviour patterns on the part of the mammals which would reduce their contact with the insect vector. Nevertheless, in Sasardi the spiny rat appears to be one of the preferred hosts of *L. olmeca*, the sandfly incriminated as a potential vector among forest mammals in this area (Christensen *et al.*, *in press*). A closely-related spiny rat, *P. guayannensis*, has been found infected with a similar strain of *Leishmania* in Brazil (Lainson and Shaw, 1968), as well as three different species of *Marmosa*, two in Trinidad (Tikasingh, 1969) and one in Brazil (Lainson and Shaw, 1969). In both instances other small forest mammals were also involved and *L. flaviscutellata* was found ecologically associated with the infected animals.

Gross Skin Lesions and their Relative Significance

Fifteen (60 per cent.) of the 25 infected animals showed conspicuous and typical leishmanial skin lesions. Such lesions, however, were seen only in the 14 rice rats and the single brown murine opossum. Infections among all eight spiny rats and the paca, on the other hand, presented no gross skin alterations suggestive of leishmanial aetiology at the site from which the parasite was cultured; the single arboreal spiny rat had superficial encrusted lesions on the edge of both ears, but only in one of them was the parasite demonstrated to be present. In brief, 40 per cent. of the infected animals showed no typical gross skin alterations that might indicate the possibility of leishmanial infection in the absence of microscopic examination.

It has been demonstrated recently that leishmanial infections among sloths in Panama, due to *L. braziliensis s. l.*, do not show any skin alteration (Herrer and Telford, 1969). In the same way, a new skin-inhabiting species of *Leishmania* has been found in the porcupine, *Coendou rothschildi*, again in Panama, with complete absence of any skin lesion (Herrer *et al.*, 1966). Moreover, the occurrence of cutaneous leishmaniasis among dogs, with no skin lesions, has also been demonstrated in Peru (Herrer, 1951).

The above findings clearly indicate that cutaneous leishmaniasis in several species of animals, both wild and domestic, may at times show no gross skin alterations. The epidemiological significance of this phenomenon is considerable and merits further investigations.

Behaviour of the Parasite in Mammals with Natural Infection

None of the 25 forest mammals found naturally infected showed any clear indication of metastases, either to other parts of the skin or to internal organs. Since cultures were routinely made from viscera as well as from the skin, the persistently negative results obtained are significant. It seems, therefore, that the parasite is restricted to the site of the insect vector's bite. Moreover, ten infected animals, nine rice rats and one spiny rat, were kept under observation in the laboratory for 136 to 366 days after their capture, during which time all seemed to be in good health. This indicates that the natural infection is rather benign, in marked contrast to experimental infection in the golden hamster.

Comparison of Cutaneous Leishmaniasis among Forest Mammals in Sasardi and the Central Part of the Republic of Panama

With the single exception of the arboreal spiny rat, *D. labilis*, the species of forest mammals found naturally infected in Sasardi live mainly at ground level (*O. capito*, *P. semispinosus*, and *M. robinsoni*) or in burrows (*A. paca*). This suggests an ecological relationship with the sandfly species (probably *L. olmeca*) that transmits the infection in the area studied, which is in marked contrast to the epidemiological picture of cutaneous leishmaniasis among forest mammals in the central part of the Isthmus. In that area, except for two species of spiny rats that live at ground level (*P. semispinosus* and *Hoplomys gymnurus*) in which the parasite was isolated from the heart blood (Annual Report of the Gorgas Memorial Laboratory, 1959), the forest mammals found naturally infected so far are arboreal (Thatcher *et al.*, 1965; Annual Report of Gorgas Memorial Laboratory, 1967 and 1968; Herrer and Telford, 1969). Several species of sandflies associated with these arboreal mammals have been demonstrated to be infected with promastigote flagellates (Johnson *et al.*, 1963), some strains of which proved to be *L. braziliensis* (McConnell, 1963).

The strains or species of *Leishmania* present in each area may also be different. Strains of the parasite isolated in the central part of Panama from different animals have been studied experimentally for years. All these strains appear to be similar and have been described as *L. braziliensis s. l.* Inoculated intradermally on the nose of the golden hamster, they produce a characteristic swelling at the site of inoculation. The parasites disseminate to other parts of the skin only to a moderate degree and after a prolonged time. The strains obtained from the animals that live at ground level in Sasardi however behave in a different way in experimentally-infected hamsters. In addition to the tumour-like swelling of the nose, dissemination of the parasite to different parts of the skin occurs three to four months after inoculation, resembling the pathological picture of *L. mexicana* (Garnham, 1962).

SUMMARY

1. Epidemiological investigations on cutaneous leishmaniasis were undertaken from September 1968 to June 1969 in the Sasardi area, eastern Panama, where an enzootic focus of the infection was discovered among forest mammals. Twenty-five out of 386 animals processed were found to be infected. These included 14 rice rats, eight spiny rats, and one each of the brown murine opossum, the paca and the arboreal spiny rat. Except for the last-named all the species found naturally infected live mainly at ground level.

2. Only the rice rats and the brown murine opossum showed typical gross leishmanial skin lesions, which were usually located at the base of the tail. No such skin alterations were noted in the spiny rats or the paca, although the parasite was cultured from the ears. The single arboreal spiny rat had superficial encrusted lesions at the edge of both ears, but the parasite was demonstrated to be present only in one of them.

3. Nine rice rats and one spiny rat were kept under observation in the laboratory for varying periods of time in order to observe the entire course of the infection. In four of the rice rats the parasite was recovered in culture up to the 164th, 266th, 267th and 286th day, respectively, beyond the date of capture. This indicates that natural leishmanial infection in this rodent is a long-lasting one.

4. The strains of *Leishmania* isolated are currently under study and thus far most of them behave similarly to *L. mexicana* in experimentally-infected hamsters. They produce

a tumour-like swelling at the site of inoculation (the nose), followed months later by remarkable metastatic lesions of the skin at different sites. L-D bodies are extremely abundant both in the nose swelling and in metastatic lesions.

5. The potential role as reservoir hosts of the different mammals found naturally infected in Sasardi is discussed, and a comparison is made of cutaneous leishmaniasis among forest mammals in Sasardi and that which occurs in animals in the central part of the Republic of Panama.

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