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Leishmania braziliensis: Dissemination of Panamanian Strains in Golden Hamsters

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HERRER, A., TELFORD, S. R., JR., AND CHRISTENSEN, H. A. 1979. *Leishmania braziliensis:* Dissemination of Panamanian strains in golden hamsters. *Experimental Parasitology* 48, 359–363. Dissemination of Panamanian strains of *Leishmania braziliensis* was observed in experimentally infected golden hamsters, *Mesocricetus auratus*, during the characterization of 164 strains isolated from patients (67), two species of edentates (88), and dogs (9). A total of 614 hamsters was employed in these studies. Hamsters were inoculated intradermally in the nose with $5-10 \times 10^6$ promastigotes from cultures of strains in their first to third passage *in vitro*. Parasites were recovered by culture from skin samples, viscera, blood and bone marrow. All strains studied disseminated to various areas of the skin and to the ear pinnae. Highest incidence of dissemination occurred in the skin from the tip of the tail, feet, and ears. Positive cultures obtained from liver and spleen were not considered as evidence for metastasis since they may have been due to the transitory presence in the blood of rare parasitized macrophages. Dissemination of various areas of the body was directly proportional to the length of the postinoculation period of the sloth strains.

INDEX DESCRIPTORS: *Leishmania braziliensis;* Hemoflagellate; Protozoa, parasitic; Golden hamster; *Mesocricetus auratus;* Dissemination; Metastasis; Leishmaniasis, cutaneous; Dermatrophy; Cultivation; Sloths; *Choloepus hoffmanni; Bradypus infuscatus.*

INTRODUCTION

In the course of a long-term investigation on the ecology and epidemiology of cutaneous leishmaniasis in the Republic of Panama, many strains of *Leishmania braziliensis* were isolated in culture from patients, dogs, and feral mammals, particularly the two-toed sloth *Choloepus hoffmanni* and the three-toed sloth *Bradypus infuscatus* (Herrer *et al.* 1973a). For characterization, these strains were inoculated routinely into the golden hamster *Mesocricetus auratus*.

Since the 1965 discovery of occult leishmanial infections in the tropical porcupine, *Coendou rothschildi*, (Herrer *et al.* 1966), cryptic cutaneous leishmanial infections have been found in 205 of 230 (89%) sylvatic animals of five mammalian orders in Panama (Herrer and Christensen 1975). Hamsters inoculated with promastigotes from all isolates during the process of leishmanial characterization were examined routinely by skin culture from different parts of the body. In this way it was shown that Panamanian strains of *L. braziliensis* disseminated from the point of inoculation to other areas of the skin in hamsters.

The present paper characterizes the dissemination of 164 Panamanian strains of *L. braziliensis* in 614 hamsters.

MATERIALS AND METHODS

Hamsters. Golden hamsters, 5–7 weeks old, were obtained from the Gorgas Memorial Laboratory colony and from animal dealers in Florida.

Parasite strains. A total of 164 strains of *Leishmania braziliensis* were used, 67 from

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patients, 86 from the two-toed sloth *Choloepus hoffmanni*, 2 from the three-toed sloth *Bradypus infuscatus*, and 9 from dogs. All strains were isolated from natural infections. While human strains were from patients from different parts of Panama, all the infected sloths and dogs were obtained from the central part of the Isthmus.

Inoculation technique. All isolates were grown on modified Senekjie's diphasic medium for hemoflagellates at 22–24 C (Herrer *et al.* 1966); promastigotes were harvested after 8–10 days. Hamsters were inoculated intradermally in the nose with $5-10 \times 10^6$ promastigotes, contained in a volume of 0.05 ml of 0.85% saline solution with 500 units of potassium penicillin and 1 mg of streptomycin/ml. Most of the strains were in their first to third *in vitro* passage. However, a few original isolates also were used as inocula.

Processing of inoculated hamsters. Experimental hamsters were maintained under observation in an animal room at 22–24 C; skin smears from the site of inoculation were made routinely after 22–25 days. Hamsters that died before 1 month postinoculation were not examined, while the remaining animals were followed for periods up to 28 months. At necropsy, skin from feet and tail, samples of ear pinnae, liver, and spleen were triturated in saline plus antibiotics; each sample was inoculated into a set of four tubes of Senekjie's culture medium. Heart blood also was cultured in most cases. Occasionally skin from other parts of the body, as well as bone marrow, was cultured. Cultures were examined microscopically on the 10th, 20th, and 30th days before being discarded.

Skin samples from both ear pinnae were removed and processed as a single sample. Similarly, skin usually was obtained from both hind feet, and at times samples from one or both forefeet also were included. From back and chest regions, single samples of the skin were removed and processed.

Smears from liver and spleen were made from selected animals, stained with Giemsa and examined microscopically for the presence of amastigotes.

Results

The number and source of the strains of *Leishmania braziliensis*, number of hamsters employed, and the periods of observation are presented in Table I.

Table II summarizes recovery of parasites from various sites in the hamster with the strains of human, sloth, and dog origin. Recovery rates were consistently higher in the skin than in the viscera, and the skin of the foot and tail yielded the highest rates. Occasionally, redness and/or moderate swelling of the skin was observed at the tip of the tail and toes, but never in other parts of the skin or ears.

The rate of parasite recovery in relation to the time of infection was determined up to 18 months postinoculation from hamsters inoculated with the sloth strains (Table III); dissemination increased in relation to the postinoculation time.

Microscopic examination of smears made from liver and/or spleen occasionally showed rare amastigotes, usually free and not clearly stained. In no case did we find any indication suggestive of the establishment of the infection in the viscera.

Figure 1 shows that when the golden hamster is inoculated intradermally in the

TABLE I
Strains of *Leishmania braziliensis* Inoculated into Hamsters

Strains of *L. braziliensis*		Hamsters inoculated	
Source	Number	Number	Observed during (months)
Humans	67	176	1–21
Sloths	88	387	1–28
Dogs	9	51	1–9
Totals	164	614	

TABLE II
Recovery of *Leishmania braziliensis* in Culture from Viscera and Skin of Hamsters Infected Experimentally with Different Strains of the Parasite

Strains studied	Viscera				Skin				
	Blood	Bone marrow	Liver	Spleen	Ear pinna	Tail	Foot	Back	Chest
Human	0/64[a]	1/12	28/166	41/169	38/107	30/57	38/49	6/25	6/31
	(0)[b]	(8)	(17)	(24)	(36)	(53)	(78)	(24)	(19)
Sloth	8/86	7/38	57/357	93/357	101/217	92/145	103/138	7/35	9/43
	(9)	(18)	(16)	(26)	(46)	(63)	(75)	(20)	(21)
Dog	0/9	1/7	11/51	20/51	6/35	9/18	3/7	1/7	0/7
	(0)	(14)	(22)	(39)	(17)	(50)	(43)	(14)	(0)
Total	8/159	9/57	96/574	154/577	145/359	131/220	144/194	14/67	15/81
	(5)	(16)	(17)	(27)	(40)	(60)	(74)	(21)	(18)

[a] Number of hamsters with positive culture/total of hamsters examined.
[b] Values in parentheses indicate percentage positive.

nose with virulent cultural forms of Panamanian strains of *L. braziliensis*, the parasites disseminate from the site of inoculation to other parts of the skin, although usually there are no gross indications of the infection.

Discussion

Schnur *et al.* (1973) reported that dissemination patterns in hamsters following intrasplenic inoculation of two Israeli strains of *Leishmania*, *L. tropica*, and an isolate from a gerbil, were very similar but distinct from that of *L. donovani*. We agree with their recommendation that many strains must be examined under standard conditions to determine if such patterns may serve as differential biological characteristics of *Leishmania* species.

In a proposed reclassification of *Leishmania* of the New World by Lainson and Shaw (1972), one of the principal behavioral attributes of parasites of the *L. braziliensis* complex used to distinguish them from

TABLE III
Recovery Rates by Culture of Sloth Strains of *Leishmania braziliensis* from Hamsters in Relation to Postinoculation Time

Material cultured	Recovery of the parasite (months postinoculation)					
	1–6		7–12		13–18	
Heart blood	0/10	(0)[a]	1/39	(2.6)	5/29	(17.2)
Bone marrow	1/5	(20.0)	0/17	(0)	5/15	(33.3)
Ear pinnae	8/26	(30.8)	39/99	(39.4)	47/78	(60.3)
Liver	5/91	(5.5)	16/138	(11.6)	27/107	(25.2)
Spleen	8/92	(8.7)	29/135	(21.5)	41/109	(37.6)
Tail skin	7/18	(38.9)	41/65	(63.1)	35/50	(70.0)
Foot skin	10/15	(66.7)	41/53	(77.4)	40/56	(71.4)
Back skin	0/4	(0)	3/24	(12.5)	5/14	(35.7)
Chest skin	0/4	(0)	2/17	(11.8)	4/13	(30.8)
Totals	39/265		172/587		209/471	
Percentages	14.7		29.3		44.4	

[a] Positive cultures/number of hamster examined (%).

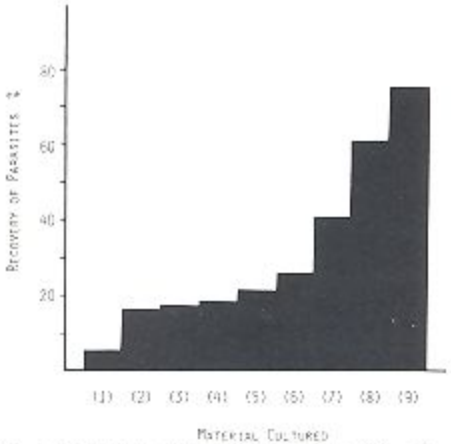

FIG. 1. Recovery rates of 164 strains of *Leishmania braziliensis* from various material cultured from experimentally infected hamsters: (1) heart blood, (2) bone marrow, (3) liver, (4) chest skin, (5) back skin, (6) spleen, (7) ear pinnae, (8) tail skin, (9) foot skin.

parasites from the *L. mexicana* complex was the lack of "metastatic spread" in the skin of hamsters. However, Brazil (1976) reported "metastatic spread of lesions" by a *L. braziliensis braziliensis* strain in 2 of 20 hamsters. The present work documents the fact that Panamanian strains of *L. braziliensis* frequently disseminate to different areas of the skin in the golden hamster. Although we have not observed gross metastatic skin lesions, the capacity of the parasite to spread out from the site of inoculation seems to be a characteristic of Panamanian strains of *L. braziliensis* and, possibly, to nonindigenous strains of this species.

As shown in Table II and illustrated in Fig. 1, the rate of dissemination differs in relationship to certain areas of the skin, and involves the feet, the tip of tail, and ear pinnae the most frequently. Recovery of the parasite by culture from liver, spleen, and bone marrow must be evaluated carefully if we are to understand dissemination to mean the spread of the parasite from the site of inoculation and its establishment elsewhere in the body of the infected animal. Positive cultures from these organs may be due to the accumulation of rare parasitized macrophages found in the blood. Zeledon and Blanco (1963) reported that most amastigotes found in the spleen appear to undergo "degeneration," and do not grow in culture from liver and spleen samples positive on smear and/or sections (Zeledon *et al.* 1969).

It is clear that Panamanian strains of *L. braziliensis* disseminate throughout the skin of hamster, however the infection is cryptic in the majority of cases, and not readily detectable without culturing. In this context, we believe that the dissemination of a Brazilian strain of *L. braziliensis* in 2 of 20 hamsters (Brazil 1976) was not an aberration, but rather characteristic of the species or complex throughout its distribution.

On the other hand, dissemination of Panamanian strains of *L. mexicana* usually results in tumor-like swellings of the extremities (Herrer *et al.* 1973b), in a similar manner to that of isolates from other countries. We have observed also that inoculations of hamsters with *L. mexicana* strains maintained *in vitro* for long periods of time result in the dissemination of the parasite to different areas of the skin but without the production of gross skin lesions. This indicates that the occurrence of tumor-like swellings of the extremities in the golden hamster depends in part on the virulence of the strain inoculated.

The skin biopsy culture technique appears to be the most practical approach to characterize dissemination of cutaneous leishmaniasis among reservoirs and experimental animals.

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