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AMONG DOGS IN PANAMA

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NATURAL CUTANEOUS LEISHMANIASIS AMONG DOGS IN PANAMA

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Abstract. A search for cutaneous leishmaniasis among dogs was conducted in several forest settlements of central Panama from 1968 to 1973. A total of 11 (3.3%) of 333 animals examined was found infected and, in 9 of these, parasites were isolated in culture and characterized as *Leishmania braziliensis*. Infected dogs occurred in three settlements, one of which was free of human leishmaniasis. In the latter case, infections were acquired in the nearby forest during hunting excursions. Ulcerated skin lesions primarily on the lower aspect of ears, or depigmentation and inflammation of the nostrils were manifested, with persistence as long as 45 months. The parasites did not disseminate from the lesion to viscera or other areas of the skin. The dog may serve as an incidental reservoir host of human leishmaniasis and/or a liaison of the infection between the jungle and forest settlements in the Republic of Panama.

Dogs have received attention as potential reservoir hosts of cutaneous leishmaniasis in the New World for many years. The first occurrence of the disease was reported from Brazil in 1912.¹ This finding was confirmed the following year, during one of the first epidemiological investigations on cutaneous leishmaniasis in the Neotropical region.² Thereafter, several additional cases were recorded from Brazil,³ Argentina,⁴⁻⁶ Peru,⁷ and Venezuela;⁸⁻⁹ a single case was seen in Panama, in 1943.¹⁰

In America, the dog has been experimentally infected with cultures of several strains of dermatotropic species or subspecies of *Leishmania*,¹¹⁻¹⁵ as well as with infective material from man and animals.¹⁴⁻¹⁶ An infection was also produced with triturates of phlebotomine sandflies (*Lutzomyia intermedius*), the flies having fed 3 days previously on a patient.¹⁷

The dog is a high risk animal because of its susceptibility to infection and jungle foraging activities. Nevertheless, the extent of its importance as an incidental reservoir host and/or liaison of leishmanial infection in the Neotropical forests has not been determined.

We began an intensive search for natural leishmaniasis in the dog in 1968, as part of a long-term ecological and epidemiological investigation conducted in Panama by the Gorgas Memorial Laboratory. This paper reports our findings to June 1973.

MATERIALS AND METHODS

Our studies were carried out in several villages and forest settlements of central Panama. Dogs were examined for the presence of gross skin alterations (ulcerations, depigmentation, scaly areas) especially on the nose, ears and feet. The owners were questioned about the age of each dog, as well as previous evidence of skin lesions and other information indicative of possible leishmanial infection. Skin smears were taken in the field from every dog showing gross skin alterations. A limited series of skin smears and cultures were made from normal skin of animals in Majecito Arriba. All preparations were examined after fixing in methanol and staining with Giemsa.

Dogs found positive by skin smear were transferred to the laboratory for further study. There, they were maintained under close observation and subjected to periodic skin smears as well as blood and skin cultures. Necropsy was performed on those animals killed or dead from other causes. Viscera (mainly spleen, liver and bone marrow) and skin were cultured; the cultures were made from the leishmanial lesions and several normal areas. Modified Senekjic's culture medium¹⁸ for hemoflagellates and in certain instances Noguchi's semisolid culture medium for leptospira, were used to isolate the parasite.

Parasite strains were characterized during the initial three transfers in vitro by inoculation of a 0.05 ml suspension, containing 5 to 10 × 10⁶ of 7- to 9-day old promastigotes, intradermally in the nose of hamsters. Subsequently, the animals

TABLE 1

Survey of dogs for natural leishmanial infections in forested areas of central Panama

Locality	Year	Number		% infect.
		Exam.	Infect.	
Western side of the Canal Zone				
El Aguacate	1968	20	0	0.0
	1969	42	1	2.4
	1970	44	0	0.0
	1973	37	1	2.7
Subtotal		143	2	1.3
Caimito	1970	35	0	0.0
La Valdosa	1970	28	0	0.0
Cacao	1970	23	0	0.0
Eastern side of the Canal Zone				
Majecito Arriba	1971	55	5	9.1
	1972	25	2	8.0
Subtotal		80	7	8.8
Loma de Mercurio	1973	15	2	13.3
Others	1966-1973	9	0	0.0
Grand total		333	11	3.3

were observed at weekly intervals for approximately 2 months, noting the type of swelling at the site of inoculation and the time of its appearance. From the 3rd month, the hamsters were particularly examined for the presence of gross metastatic skin lesions. The intensity of parasitism that resulted was determined by skin smears. At necropsy, cultures were made from liver and spleen, and in some cases, from the ear pinna and tail skin. Methods evaluating these criteria have been published previously.¹⁹

RESULTS

Prevalence of the infection

Eleven (3.3%) of 333 dogs were infected (Table 1). All positives were adults and were found in three localities where the forest environment was undergoing alteration at the time of our visits. Seven were hunting dogs, which apparently acquired the infection deep in the forest apart from the owner's dwelling. Among the remaining 4 cases, 2 occurred in a new small settlement where an epidemic outbreak took place 2 or 3 years previously, while 2 were from a village that had been in existence for several decades and where the human disease persisted endemically.

TABLE 2

Results of skin smears and skin cultures from dogs

	Condition of the skin examined		
	Normal	Suspicious alteration ¹	Conspicuous lesion ²
Smears			
No. dogs examined	19	54	11
No. infected (%)	0 (0)	0 (0)	8 (73)
Cultures			
No. dogs examined	18	4	10
No. infected (%)	0 (0)	0 (0)	9 (90)

¹ Possible traumatic lesions and/or reactions to the biting of certain arthropods, mainly ticks.

² Ulcerated lesions showing some induration at periphery, concomitant with characteristic depigmentation and/or inflammation of the nostrils.

Dogs were subjected to special examination according to the condition of the skin and the results obtained with each of the two study methods (Table 2). Related to such observations, it is noted that in Majecito Arriba and surrounding areas in the Bayano region owners frequently amputate dogs' ears when this organ is affected by chronic ulcerated lesions (Fig. 3). According to the description of such lesions by the local people and in view of their resistance to ordinary treatment, it seems very likely they are of leishmanial origin.

Nine strains were established in culture. Five were easily isolated and maintained in vitro for numerous transfers in Senekjic's medium. The isolation of the other four strains and their maintenance in vitro during the 2nd to 3rd transfers were only possible with Noguchi's semisolid medium for leptospira.

The following additional cultures were made from infected dogs received at the laboratory: 49 samples of viscera, mainly spleen, liver and bone marrow, from 7 dogs; 97 normal skin samples, the majority from feet, ears, nose, chin, and rump, from 8 animals; and 17 blood samples from 8 dogs. All the above gave negative results. Smears and culture from normal skin of dogs in Majecito Arriba also were negative (Table 2).

Lesions in the dog

Each of the 11 dogs in which leishmaniasis was demonstrated showed conspicuous skin alteration. Seven had ulcers either on the nose, ears, or both (Figs. 2-3), reaching the nasal mucosa in two.

Three others had typical depigmentation of the nostrils (Fig. 1), and a fourth showed a polyp-like formation accompanied by slight depigmentation of one nostril.

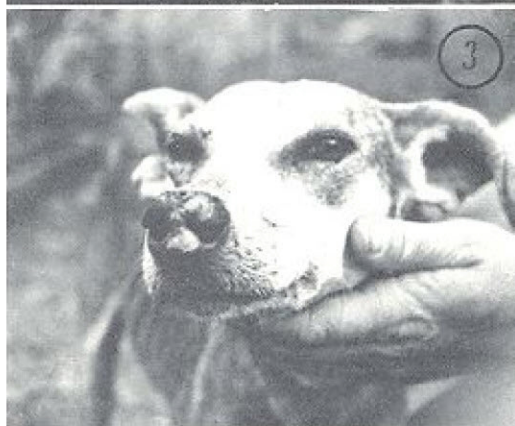
In five dogs the infection persisted throughout observation periods of 3, 5, 6, 38 and 45 months, and in two others examined for 15 and 18 months, the lesions healed at the 7th and 11th month, respectively; cultures from the scars did not reveal the presence of parasites. Among the remaining infected dogs, one escaped a few days after being brought to the laboratory and three were not studied on a regular basis.

The lesion on the dog with the 45-month-old infection grew larger with time (Fig. 1), and at necropsy the infection was seen to involve the nasal mucosa. The second dog with a long-lasting infection (38 months) developed histoplasmosis after 3 years. This animal originally had two ulcerated leishmanial lesions, one on the left nostril and the other on the lower aspect of the right ear. Upon being killed, due to the histoplasmosis, the lesion on the nostril was active, had continued to increase in size, and provided a positive culture for *Leishmania braziliensis*; the other lesion had healed and the culture from the scar was negative.

Parasite levels were very low in all the cases, regardless of the type of lesion involved. Skin smears from the lesions usually required prolonged and careful microscopic examination to find the organism.

Characterization of the parasite

Strains isolated in culture from nine dogs were characterized in a total of 91 hamsters, 72 of which survived long enough to determine the results of the inoculations. Infections were



FIGURES 1 to 3. 1. Inflammation and nostril depigmentation of a dog with natural cutaneous leishmaniasis. This animal was observed in the laboratory for 45 months, during which time the lesion enlarged; the picture was taken after 15 months. At necropsy *Leishmania braziliensis* was isolated from the skin and nasal mucosa. 2. A 13-mm diameter skin lesion (arrow) of leishmanial etiology on the lower aspect of one ear in the 5th month of observation (a tick was removed from the smaller lesion seen in the picture). At the time this case was discovered, leish-

manial lesions were apparent on both ears. However, 6.5 months later, when the animal was killed, only one lesion remained active. 3. An ulcerated lesion on the dorsal and frontal aspects of the nose, reaching to the mucosa. The right ear also showed a leishmanial lesion, although the greater part of this organ had been amputated by the dog's owner in an attempt to cure the animal.

demonstrated in 70 (97.2%) of these hamsters. Swelling at the site of inoculation was moderate and recognizable at 15 to 20 days postinoculation. No gross metastatic lesions were observed, although the parasites persisted in the nose tissue throughout observation (up to 19 months). These characteristics permitted us to identify the strains as *L. braziliensis*. The parasite was recovered in culture from viscera, especially the spleen, more frequently than is generally possible with human and sloth strains. Sometimes positive cultures also were obtained from ear and/or tail skin, although no gross indication was noted.

DISCUSSION

The susceptibility of the dog to cutaneous leishmanial infection in Panama appears to be similar to that in other Neotropical areas. No cases have been reported from this country despite the intensive ecological investigations during recent years.

The search for natural infections among dogs involves certain difficulties and hazards, particularly in the jungle. Gross indications of the infection vary from inconspicuous skin depigmentation to large ulcerated lesions. This complicates differentiation of skin lesions due to other etiologies. In addition, the parasitism normally is so light that microscopic examination of skin smears from active leishmanial lesions frequently fails to demonstrate leishmaniae. As a result of these circumstances, natural leishmanial infections among dogs have been overlooked in Panama.

The occurrence of natural canine leishmaniasis was demonstrated in El Aguacate, Majecito Arriba, and Loma de Mercurio, forest settlements which are distinct. El Aguacate is a village established about 75 years ago, in which much of the forest flora and fauna still persist, enabling endemic persistence of human cutaneous leishmaniasis. Majecito Arriba, colonized much more recently, has been utilized for grazing cattle. The original vegetation has been cleared and grass planted, resulting in a rapid disappearance of leishmaniasis. It was in this community that hunting dogs foraged in the nearby forest and became infected.²⁰ Loma de Mercurio, the third study site, is a recent, small and isolated settlement. In 3 years the whole area had been cleared for farming. During that time an intensive epidemic of cutaneous leishmaniasis occurred among

the members of five families.²¹ Two dogs with active lesions in this settlement apparently acquired the infections during the epidemic. Similar situations probably occur elsewhere. Pons and Londres reported an excellent example of the involvement of domestic animals during an epidemic outbreak of human cutaneous leishmaniasis in Venezuela.⁹

Ecological and epidemiological considerations support the identification of the nine isolated strains as *L. braziliensis*. On several occasions humans as well as dogs with active leishmanial infections of similar evolution were found in homesteads, suggesting the possibility that both infections came from the same source and were acquired simultaneously.^{20, 21} Results of recent studies in central Panama indicated that dogs are attractive to *Lutzomyia panamensis* and *Lu. sanguinaria*, 2 of the 5 phlebotomine sandfly species that have been incriminated as vectors of *L. braziliensis* in this country.²²

From the onset, the forest is cut and burned by small groups of settlers colonizing the jungle. The extent of sandfly persistence in forest communities depends mainly on the degree to which the area has been cleared and on the subsequent use of the land. We are still able to obtain sandflies in Quebrada Bonita, a locality along the Trans-Isthmian Highway, although the region has been occupied for many years and human leishmaniasis has disappeared completely. The sudden and violent change in ecological conditions compels the local mammalian fauna to abandon the area. Among these mammals are the reservoir hosts of the sole human leishmanial parasite thus far found in Panama, *L. braziliensis*. Despite the persistence of sandflies, the incidence of infection diminishes until the disease disappears. As a result of the occurrence of sandfly vectors in such a forest settlement, there remains a possibility of occasional leishmanial infections among humans. The rarity of isolated cases among persons living in areas free of leishmaniasis for many years illustrates this situation. It seems likely that the dog does serve as a carrier of the infection in these instances, i.e., as a liaison between the old settlements and nearby endemic jungle localities.

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