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SHORT REPORT: EPIDEMIOLOGIC STUDIES ON CUTANEOUS LEISHMANIASIS IN EASTERN PANAMA

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Abstract. The Panamanian Ministry of Health, through the Interamerican Development Bank, contracted the Gorgas Memorial Laboratory to conduct epidemiologic studies on leishmaniasis and malaria in eastern Panama from July 1984 through June 1985. Preliminary results of the biomedical and entomologic teams investigating the epidemiology of cutaneous leishmaniasis in the eastern part of the country are presented in this short report. The principal findings of the study revealed 1) a large disparity in the incidence and prevalence of the disease among the five communities investigated; 2) the appearance of self-cures without the benefit of effective treatment; 3) a relatively high percentage of subclinical cases; and 4) determination of the sandfly vector species for each community. Also reported here is a case of a double infection with two distinct species of *Leishmania*, *L. mexicana* and *L. amazonensis*, in a single individual.

The present study investigated epidemiologic parameters of cutaneous leishmaniasis among five communities in eastern Panama in which week-long surveys were conducted. This study was reviewed and approved by the Panamanian Ministry of Health. Four of these study sites (San Miguel, Torti, Santa Fe, and Meteti) are adjacent to the Interamerican Highway (Figure 1). The fifth community (Boca de Sabalo) is located in the extreme southeastern part of the country, and was accessed by boat via an extensive river system (Figure 1). Although the primitive working conditions in the communities precluded isolation and characterization of the parasite, it was assumed that all of the cases of leishmaniasis encountered were due to *Leishmania panamensis* since it has been the predominant species identified from human cases in Panama. However, several months after the termination of the project, one of the investigators (JLP) developed a lesion from which a biopsy specimen was obtained and found to be caused by *L. amazonensis*.[1] The locality in which the infection occurred could not be determined since this investigator traveled extensively throughout Panama during the study period.

In 1977, several U.S. soldiers conducting jungle exercises near the Caribbean entrance to the Panama Canal developed leishmaniasis identified by personnel at Gorgas Memorial Laboratory (GML) as caused by *L. amazonensis*.[1,2] In October 1986, the first indigenous case of infection with *L. mexicana* in Panama was isolated from one of two lesions from a young mestizo individual involved in hunting and agriculture in the northern and eastern part of the country.[1] Eight subsequent clones of parasites from each lesion revealed a double infection with *L. mexicana* and *L. amazonensis*. A new species, *L. colombiensis*, was isolated from four sand flies and a sloth in Panama, and from two humans and a sand fly in Colombia.[1] With regard to the remaining two species indigenous to Panama, *L. hertigi* appears to be host-specific for the porcupine *Coendou rothschildi*, and *L. aristidesi* is known only from a single focus in a coastal forest in eastern Panama from rodents and a single marsupial and has never been isolated from humans.[3,5] Identification of *Leishmania* species at the GML has been based upon *in vitro* characterization, pathologic manifestations in the golden hamster, and cellulose acetate isoenzyme electrophoesis.[1,6]

Racial diversity among the five communities in the present study ranged from a predominance of a mixture of Indians (Chocoe or Cuna), Hispanics, Blacks, or Caucasians, classified as mestizos, in San Miguel to a population composed mostly of Indians in Boca de Sabalo. Our biomedical team examined and provided minimal treatment for a variety of ailments to 933 patients, each of whom were given intradermal leishmanin skin tests (Montenegro). However, results of the skin tests were obtained for only 317 individuals who returned after 48 hr to have their clinical history of cutaneous leishmaniasis assessed. The annual incidence (individuals with lesions or scars acquired within the past year) and prevalence (those exhibiting lesions or scars) ranged from a high of 27.0% and 77.3% in San Miguel to a low of 1.6% and 3.8% in Boca de Sabalo, respectively (Table 1). The Panamanian Ministry of Health requested that we begin our investigation on leishmaniasis in the community of San Miguel due to a reported outbreak of the disease during the initial preparation of our study. We noted that only 15% of the population was native to the area, while 49% and 31% of the people had immigrated from the western Province of Los Santos and Panama City, respectively. Leishmaniasis has rarely been reported from these localities. Although we did not record the racial complexity of the people of San Miguel, the 1980 Panamanian census listed a population of 278 with no indigena (Indians). The vast majority of the people were of mixed decent; Indians were not encountered in this community. We were interested in learning if there were differences among the races in the occurrence of subclinical cases, which may indicate some measure of increased resistance to leishmaniasis among the different groups. Although limited in scope, the results appear to indicate no significant differences, which we believe is important.

The true nature of subclinical cases is not clearly understood. The presence of asymptomatic cases of infection with *L. mexicana* has been reported.[7] Hoogstraal and Heyneman noted that "The leishmanin skin test is not a specific response and may reflect transitory nonclinical infections with *Leishmania* not normally found in man."[8] *Leishmania hertigi* was reported to have a prevalence rate of 88% among porcupines in Panama, and microcapillary precipitin tests performed at the GML showed that 107 (100%) of 1,065 blood meals of *Lutzomyia ylephiletor*, a principal vector of

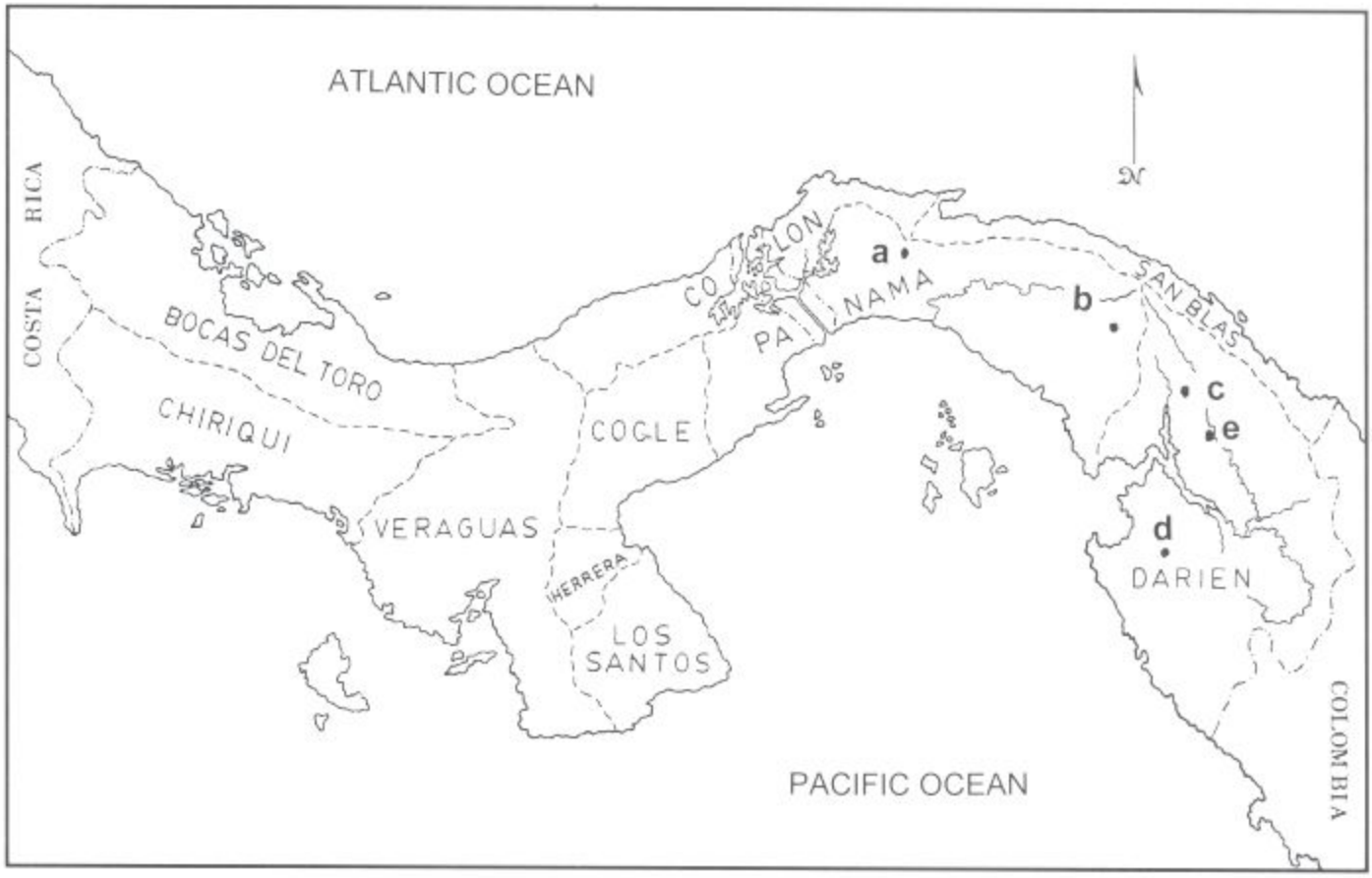

FIGURE 1. Map showing location of communities adjacent to the Interamerican Highway in eastern Panama where epidemiologic studies on cutaneous leishmaniasis were conducted. a = San Miguel; b = Torti; c = Santa Fe; d = Boca de Sabalo; e = Meteti.

L. panamensis among humans in this country, had fed on porcupines.[4,9] Tests in humans to determine if experimental inoculation with *L. hertigi* parasites would stimulate an immune response detectable by the leishmanin skin test have not been conducted. However, the natural transmission of *L. hertigi* to humans as a plausible rationale for subclinical leishmanial infections in Panama appears to be a far less likely hypothesis than may be explained by the innate immune competence differences among individuals and variations in the virulent and invasive properties of different strains of *L. panamensis*. It has yet to be determined if subclinical cases of Panamanian cutaneous leishmaniasis confer any degree of protection against future episodes of the disease.

Leishmania panamensis previously had been considered to be a progressive, nonhealing, chronic disease requiring a course of antimonial injections to effect a cure. Many of the more than 1,000 cases diagnosed at the GML clinic required several courses of treatment and recrudescence was not an uncommon occurrence. A few cases were known to persist for more than 10 years (Johnson CM, Christensen HA, Vasquez AM, unpublished data). Of 72 cases exhibiting scars resembling leishmaniasis in our study, 64% had been treated with antimonial injections by physicians, 30% had lesions that cleared after self-treatment with a variety of supposedly ineffective medications, including Vaseline® (Chesebrough Ponds, Inc., Greenwich, CT), honey, tobacco, mud, cold cream, and Clorox® (The Clorox Co., Oakland, CA), and 6% had lesions that cleared without any treatment. Fourteen of the 22 self-treated cases and two of the four untreated cases had skin tests done; all tested positive.

The skin test antigen used in this study was prepared from *L. panamensis* promastigotes isolated from a 23-year-old woman from Bocas del Toro Province with a two-month history of the disease. Promastigotes were grown in modified Senekjie's culture medium overlaid with 0.85% saline as described by Herrer and others,[9] harvested after eight days, rinsed three times in normal saline, and centrifuged at 3,000 rpm for 30 min following each rinse. The sediment was suspended in a 1:10,000 dilution of merthiolated, modified, sterile Coca's solution (0.5 g of NaCl and 0.4 g of $NaHCO_3$ in 100 ml of distilled water). The organisms were counted with a hemocytometer, and sufficient merthiolated alkaline solution added to obtain a final concentration of 10×10^7 promastigotes/ml, constituting a final stock solution maintained at 2–8°C. Sterility checks for viable promastigotes, bacteria, and fungi were made using Senekjie's, thioglycollate, and Sabouraud's media, respectively. Sensitivity and specificity were determined by intradermal inoculation of 0.1 ml of working dilution (stock solution diluted 1:10 in merthiolated alkaline solution to a final concentration of 1×10^6 promastigotes/ml) into the forearm of the source patient and additional cases. An induration > 5 mm in diameter was recorded as positive.

TABLE 1
Leishmaniasis infections in communities of eastern Panama

Community Race	Prevalence* No. positive/no. tested (%)	Annual incidence† No. positive/no. tested (%)	Subclinical‡ No. positive/no. tested (%)
San Miguel	129/167 (77.3)	45/167 (27.0)	4/59 (6.8)
Mestizos	Undetermined	Undetermined	Undetermined
Blacks	Undetermined	Undetermined	Undetermined
Indians	Undetermined	Undetermined	Undetermined
Not recorded	129/167 (77.3)	45/167 (27.0)	4/59 (6.8)
Torti	26/184 (14.1)	11/184 (6.0)	7/81 (8.6)
Mestizos	23/139 (16.6)	11/139 (7.9)	7/76 (9.2)
Blacks	0/3 (0.0)	0/3 (0.0)	0/3 (0.0)
Indians	0/1 (0.0)	0/1 (0.0)	0/1 (0.0)
Not recorded	3/41 (7.3)	0/41 (0.0)	0/0 (0.0)
Santa Fe	22/133 (16.5)	12/133 (9.0)	10/61 (16.4)
Mestizos	20/106 (18.9)	12/106 (11.3)	10/47 (21.3)
Blacks	2/20 (10.0)	0/20 (0.0)	0/12 (0.0)
Indians	1/7 (14.2)	0/7 (0.0)	1/1 (100)
Meteti	27/265 (10.2)	3/265 (1.1)	8/89 (9.0)
Mestizos	27/254 (10.6)	3/254 (1.2)	8/88 (9.1)
Blacks	0/3 (0.0)	0/3 (0.0)	0/0 (0.0)
Indians	0/2 (0.0)	0/2 (0.0)	0/0 (0.0)
Not recorded	0/6 (0.0)	0/6 (0.0)	0/0 (0.0)
Boca de Sabalo	7/184 (3.8)	3/184 (1.6)	6/27 (22.2)
Mestizos	0/24 (0.0)	0/24 (0.0)	0/4 (0.0)
Blacks	4/38 (10.5)	1/38 (2.6)	3/8 (37.5)
Indians	3/122 (2.5)	2/122 (1.6)	3/15 (20.0)
Total	211/933 (22.6)	74/933 (7.9)	35/317 (11.0)

* % of lesions or scars among the people examined.
† % of active lesions or scars acquired within the year prior to the study.
‡ % of positive skin test (Montenegro) results without evidence of lesions or scars.

The entomologic team of this project collected and identified 6,810 phlebotomines, which included 30 of the 74 species indigenous to Panama.[10] Collection methods included the use of human bait, Centers for Disease Control light traps, Shannon traps, and battery-operated aspirators in tree buttress resting sites. Population densities of the four sand fly species implicated as vectors of *L. panamensis* in Panama (*Lu. panamensis, Lu. ylephitelor, Lu. gomezi,* and *Lu. trapidoi*) varied greatly among the different communities. In San Miguel, *Lu. ylephiletor* comprised 629 (78.0%) of 806 sand flies collected, and should be considered the principal vector at this locality. *Lutzomyia gomezi* comprised 21 (7.4%) of 285, 162 (18.8%) of 863, and 297 (22.9%) of 1,299 sand flies collected in Boca de Sabalo, Santa Fe, and Meteti, respectively, and represents an important vector species in these communities. *Lutzomyia panamensis* was the dominant vector species in Torti, comprising 1,025 (28.8%) of 3,557 flies collected, although *Lu. trapidoi* was also a common vector species, accounting for 365 (10.3%) of 3,557 sand flies.

This project has contributed to a better understanding of the epidemiology of cutaneous leishmaniasis in rural communities adjacent to the Interamerican Highway in eastern Panama.

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