

AN EPIDEMIOLOGIC STUDY OF AMERICAN CUTANEOUS LEISHMANIASIS IN MAIPUCO, PERU^{1,2}

Carmen Arzubiaga,³ Jorge Huayanay,³ and Italo Biaggioni³

Little is known about the reservoirs, vectors, transmission patterns, and distribution of American leishmaniasis in the region of the Peruvian Amazon. The work reported here, which involved testing 67 villagers in an area where leishmaniasis cases had been occurring, was performed to help assess the extent of the leishmaniasis problem in that region.

Introduction

Two forms of leishmaniasis occur in Peru. One, known as *uta*, occurs in the western valleys of the Andes and produces a cutaneous lesion of spontaneous remission that rarely causes contiguous mucosal damage. The other, called *espondia*, occurs in the Amazon region and produces cutaneous lesions that in some cases are indistinguishable from those of *uta*. However, it can also produce destructive metastatic mucosal lesions, typically in the nasal septum or the oropharyngeal mucosa (1, 2).

Little is known about the epidemiology of the two forms, particularly in the Amazon region, or about the natural history, distribution, reservoirs, transmission mechanisms, and pathogenic agents of the disease in these areas. Furthermore, an indeterminate number of cases go unreported every year in Peru, largely because of the sociocultural circumstances of the affected populations. Nevertheless, endemic areas are recognized within the country; and the focal behavior of the

disease—involving the reported presence of different clinical forms of leishmaniasis, presumably owing to the existence of several *Leishmania* strains—has created a need to elaborate upon the few studies performed in the affected regions (2, 3, 4, 5).

For some time the value of the Montenegro intradermal test in assessing the prevalence of leishmania infections has been recognized (6), and significant progress has been made with regard to improving both the quality of the antigen and the methods of data analysis employed (7, 8). Nevertheless, some question has remained regarding the test's specificity in ecological settings such as those found in Peru's Amazon region.

Therefore, once an Amazon area that was producing leishmaniasis cases was identified, interest arose in defining the extent of the problem more clearly. The purpose of the work reported here was to determine the prevalence of leishmaniasis infection by means of the Montenegro intradermal test, as a basis for determining the need for later studies. At the same time, the study was designed to evaluate both the intradermal test and the indirect immunofluorescence test as field methods applicable to the particular area and situation involved.

Materials and Methods

The Study Population

The study was conducted in the village of Maipuco, a settlement located in Maipuco

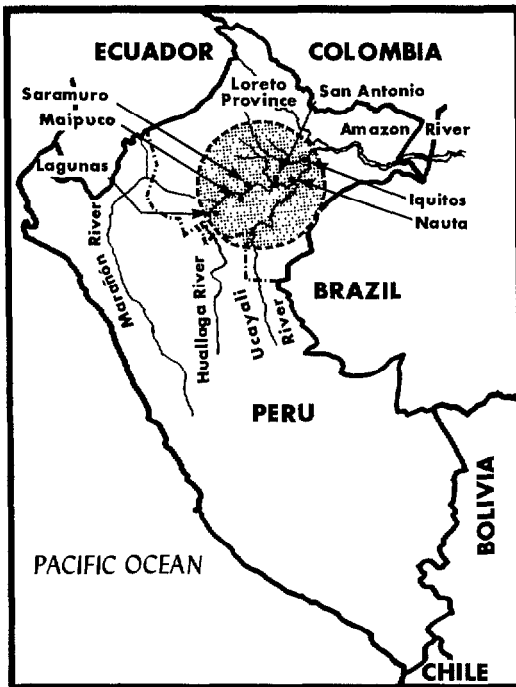
¹Also appearing in Spanish in the *Boletín de la Oficina Sanitaria Panamericana* 96(4), 1984.

²The work reported here was funded in part by the Department of Epidemiology of Peru's Northeast Health Region, Loreto, Peru, and by the United Nations Development Program/World Bank/World Health Organization Special Program of Research and Training in Tropical Diseases, projects SWG 780235 and RSG 780488.

³Research Fellow, Instituto de Medicina Tropical "Alexander von Humboldt, Universidad Peruana Cayetano Heredia, A. P. 5045, Lima, Peru.

District, Loreto Province, Loreto Department, Peru (Figure 1). The village, 240 km southwest of the city of Iquitos on the right bank of the Marañón River, is situated in a low jungle terrain subject to flooding. Its few inhabitants, numbering 380 in all, were distributed by age and sex as shown in Table 1.

Figure 1. A map of Peru showing Iquitos, Lagunas, and the survey village of Maipuco.



As indicated in the table, a survey sample consisting of 67 Maipuco residents was selected. Application of the Kolmogorov-Smirnov test indicated that this sample was representative of the village population at the 95 per cent confidence level (9).

The Intradermal and Indirect Immunofluorescence Tests

Montenegro's intradermal test, exposing the test subject to leishmanin (6), was performed on all 67 members of the survey population. The leishmanin was prepared at the laboratory of the Alexander von Humboldt Institute of Tropical Medicine, using as antigen dead promastigotes of *Leishmania braziliensis* in a saline solution with 0.5 per cent phenol at a protein nitrogen concentration equivalent to 35 µg/mg. This solution (0.1 ml) was inoculated by the intradermal route into the upper inside portion of each subject's left forearm. The subject's response to this inoculation was read at 48 hours by measuring the diameters of the induration at the inoculation site, a measurement of 5 mm or more being taken to indicate a positive response.

To obtain specimens for the indirect immunofluorescence test, blood samples were obtained from the survey subjects and were centrifuged within six hours of their procurement in Maipuco. Sodium azide (0.01 per cent) was added to the resulting serum specimens as a preservative in Iquitos, and the

Table 1. The Maipuco population and survey sample, by age group and sex.

Age group (in years)	Total population				Survey population			
	Males	Females	Total		Males	Females	Total	
			No.	%			No.	%
< 1	25	22	47	(12.4)	3	1	4	(6.0)
1-4	43	37	80	(21.1)	4	4	8	(11.9)
5-14	69	45	114	(30.0)	14	10	24	(35.8)
15-44	54	55	109	(28.7)	15	11	26	(38.8)
45-64	16	12	28	(7.4)	2	3	5	(7.5)
≥ 65	0	2	2	(0.5)	0	0	0	-
Total	207	173	380	(100)	38	29	67	(100)

specimens were transported under refrigeration to Lima. They were then maintained at -70°C until tested for leishmania antibodies by the indirect immunofluorescence method within 15 days of their collection.

Promastigotes of the Yumare strain of *Leishmania braziliensis* isolated by C. F. Pifano in the Department of Yumare, Venezuela, were used as antigen for this test. The general guidelines of the method used by Guimaraes (9, 10) were followed, evaluating dilutions of 1:16, 1:32, 1:64, and 1:128. Serum samples known to contain leishmania antibodies served as positive controls, and a specimen processed only with diluent served as a negative control. Positive results at dilutions of 1:32, 1:64, and 1:128 were to have been considered significant, as the method yields appropriately sensitive and specific results at these concentrations (10).

Results

A total of 18 subjects (26.8 per cent of the 67 tested) yielded positive responses to the Montenegro test. As indicated in Table 2, all were over 15 years of age, and nearly all (17 of the 18) were agricultural workers—including the "sanitarian," who performed the duties of a primary health care worker. None of the 18 exhibited active leishmaniasis at the time the test was performed, but scars clinically compatible with previous leishmaniasis were seen on four of the 18 subjects (22 per cent). No such scars were observed on any of the 49 Montenegro-negative subjects, and none of the 14 Montenegro-positive subjects without dermal scarring had any known history of the disease. The Montenegro-positive subjects included 12 males and six females, making the sex ratio two to one.

Table 2. Data on the 18 subjects responding positively to the Montenegro intradermal test—including each subject's age, sex, occupation, place of infection (if known), presence or absence of cutaneous scarring compatible with leishmaniasis, width of induration resulting from the Montenegro test, and serologic results of the indirect immunofluorescence test.

Subject No.	Age (years)	Sex	Occupation	Place of infection	Presence of scarring	Montenegro test response (width of induration)	Indirect immunofluorescence test responses at all serum dilutions tested ^a
1	39	M	Agricultural worker and sanitarian	Unknown	No	6x5 mm	Negative
2	51	F	Agricultural worker	Maipuco	No	10x11 mm	"
3	36	M	"	Lagunas ^b	No	16x20 mm	"
4	39	M	"	Unknown	No	5x5 mm	"
5	15	M	"	Maipuco	No	8x8 mm	"
6	18	M	"	"	Yes	6x6 mm	Positive, 1:16 only
7	37	F	"	"	Yes	6x6 mm	" " "
8	28	F	Housewife	Lagunas ^b	Yes	5x7 mm	Negative
9	36	M	Agricultural worker	Maipuco	No	13x13 mm	"
10	53	M	"	"	Yes	14x14 mm	Positive, 1:16 only
11	40	F	"	Unknown	No	9x9 mm	Negative
12	49	M	"	"	No	14x15 mm	"
13	19	M	"	Maipuco	No	6x5 mm	Positive, 1:16 only
14	42	M	"	Unknown	No	4x5 mm	Negative
15	38	F	"	Maipuco	No	6x6 mm	Positive, 1:16 only
16	15	M	"	"	No	10x7 mm	" " "
17	16	M	"	"	No	5x5 mm	Negative
18	18	F	"	"	No	5x5 mm	"

^aBecause a positive response at a 1:16 dilution, by itself, was not considered a positive result, no clearly positive results were obtained.

^bLagunas is a town located some 75 kilometers southeast of Maipuco, on the Huallaga River.

In the case of the four subjects with scars, all the scars were found on the lower extremities. The initial lesions involved were said to have lasted anywhere from a month to a year, and the time elapsing between the appearance of the lesion and the survey examination was said to have ranged from one month to 17 years.

It is noteworthy that 58 per cent of the 31 survey subjects above 14 years of age responded positively to the Montenegro test (Table 3), and that 17 of the 25 tested agricultural workers also responded positively. As Tables 4 through 6 indicate, these relation-

Table 5. Relationship between the Montenegro test results and the survey subjects' occupations.^a

Occupation	No. of subjects with indicated Montenegro test response		Total
	Positive	Negative	
Agricultural worker	17 ^b	8	24
Other	1	41	43
Total	18	49	67

^aSignificant at $p < 0.01$.

^bIncluding the one agricultural worker also employed as a sanitarian.

Table 3. Responses to the Montenegro test of all the survey subjects categorized by sex, age group, and occupation.

Age group (in years)	Montenegro test response:												Total	
	Positive						Negative							
	Agricultural workers		Occupied at home		Other occupation		Agricultural workers		Occupied at home		Other occupation			
	M ^a	F ^a	M	F	M	F	M	F	M	F	M	F		
< 1	-	-	-	-	-	-	-	3	1	-	-	-	-	4
1-4	-	-	-	-	-	-	-	4	4	-	-	-	-	8
5-14	-	-	-	-	-	-	2	-	12	10	-	-	-	24
15-44	10 ^b	4	-	1	-	-	5	1	-	5	-	-	-	26
45-64	2	1	-	-	-	-	-	-	-	2	-	-	-	5
≥ 65	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Total	12	5	0	1	0	0	7	1	19	22	0	0	-	67

^aM = males, F = females.

^bOne of these 10 also worked as a sanitarian.

Table 4. Relationship between the Montenegro test results and the survey subjects' age.^a

Age (in years)	No. of subjects with indicated Montenegro test response		Total
	Positive	Negative	
< 15	0	36	36
≥ 15	18	13	31
Total	18	49	67

^aSignificant at $p < 0.01$.

Table 6. Relationship between the Montenegro test results and the presence of scarring compatible with cutaneous leishmaniasis.^a

	No. of subjects with indicated Montenegro test response		Total
	Positive	Negative	
Subjects with scarring	4	0	4
Subjects without scarring	14	49	63
Total	18	49	67

^aSignificant at $p < 0.01$.

ships as well as the relationship between the presence of leishmaniasis-compatible scarring and the Montenegro test results were found to be statistically significant. No statistical significance was found between the Montenegro and indirect immunofluorescence test results using the contingency coefficient⁴ (12), the results of the latter test being generally negative.

Discussion

The value of the intradermal test for determining exposure to leishmaniasis is well established. Many years ago, Wagner demonstrated the existence of delayed hypersensitivity to leishmaniasis among guinea pigs previously inoculated with inactive forms of the infective agent (6). However, Montenegro was the first to use this cutaneous test for the diagnosis of human leishmaniasis, which is why the test bears his name (6). Later, in 1940, Pessoa and Pestana applied the intradermal reaction for clinical epidemiologic purposes, and many subsequent observations have since verified the test's great sensitivity and worth (13).

It is also generally recognized that the intradermal reaction remains positive for years, even for life (14), following clinical remission of the disease. The test thus permits retrospective diagnosis of leishmania infections and hence a fairly precise determination of the prevalence of the disease.

The leishmanin used in the present survey has been shown by work not yet published to be highly sensitive and specific, demonstrating the latter quality by inducing only one false positive response in a survey population of 300 apparently healthy schoolchildren (15).

In general, the findings obtained (a 27 per cent positive response in village residents with few scars or dermal lesions characteristic of leishmaniasis) are comparable to those reported by other studies of American leish-

maniasis (13, 16) and are similar to those obtained by Herrer in a highland jungle area (Tambopata, Puno, Peru) where it was found that 26 per cent of 1,613 residents studied were Montenegro-positive, of whom 21 per cent had no history of dermal lesions (4).

As various authors have suggested, the presence of reactors without any history or evidence of lesions may be explained by the occurrence of subclinical infections (4, 14, 17). In other countries, cases of positive intradermal reactions have been described that resulted from exposure to animal leishmanias (18, 19), but such reactions have not been demonstrated in our area. Cross-reactions with Chagas' disease (*Trypanosoma cruzi*) antibodies have also been described (20); however, this matter is somewhat controversial, and in any event no cases of Chagas' disease have been reported in the Maipuco area.

The fact that positive responses occurred only in people over 14 years old, most of whom were males and farmers, indicates that most exposure to the insect vector occurred far from the village—in the course of farming work or while hunting in river gorges. The absence of positive reactors among those under 14 suggests that transmission of the disease within the household is improbable, and that the leishmaniasis vector and/or the natural hosts of the disease are not present in the village proper. (There is no proof that people, apart from serving as accidental hosts, can become reservoirs of the disease agent.)

The agents presumed responsible for American leishmaniasis in Peru are *Leishmania braziliensis braziliensis* and *L. braziliensis guyanensis* on the eastern slopes of the Andes and in the Amazon region; and *L. peruviana* on the western slopes of the Andes (2). Small feral mammals are considered possible hosts, but relatively little is known about the vectors and reservoirs of these agents in the Amazon region. Elsewhere, on the western slopes of the Andes, recent studies by Herrer have recovered leishmanias from triturates of *Lutzomyia peruensis*, and it has therefore been postulated that this sandfly species could be a natural vec-

⁴Employed to estimate the degree of association between two nonparametric variables.

tor in that region (21). In discussing this vector, Herrer describes it as having habits that keep it outside of settled areas, a behavior pattern consistent with what was found in our study.

Regarding the immunofluorescence test, Oddo and Cascio demonstrated in 1963 that sera from patients with Mediterranean visceral leishmaniasis could be shown to react with cultures of *Leishmania donovani* by the indirect immunofluorescence method (6). The value of this technique for diagnosing kala-azar (the disease caused by *L. donovani*) was demonstrated a year later by Duxbury, who also reported a cross-reaction with sera from patients with American leishmaniasis (22, 23). Finally, Camargo (24) showed that when such sera were treated with a heterologous antigen, then the cross-reactions could be eliminated, and sera monospecific against *L. braziliensis* (principal agent of American leishmaniasis) could be obtained.

Experience using the indirect immuno-

fluorescence test, especially in Brazil, has shown it to be particularly useful in diagnosing and monitoring patients with active American leishmaniasis, with the test results being positive in over 90 per cent of the cases (6). The antibody titers obtained are not correlated with the degree of parasitism in the lesions or with the intensity of the Montenegro reaction (25, 26, 27), although some authors have found sera from patients with delayed forms of the disease and mucosal lesions to yield higher antibody titers than sera from patients with recent cutaneous lesions (27).

It has also been reported that the antibody titers obtained tend to decrease or disappear after treatment (25), so that unlike the Montenegro test the indirect immunofluorescence test is negative during the inactive (cicatrical) phase of the disease. These findings are consistent with those of the present study, in which no active disease cases were observed and the indirect immunofluorescence test detected no clearly significant antibody titers.

ACKNOWLEDGMENTS

The authors wish to thank Drs. Hugo Lumbreras and Humberto Guerra for reviewing the manuscript and offering suggestions, and Dr. Jorge Sibina, Director of Peru's Northeast Health Region, and Dr. Eduardo García, Head of the Department of Epidemiology, for their assistance.

SUMMARY

The study reported here tested residents at a Peruvian Amazon village in an area where American leishmaniasis cases were occurring. To help assess the extent of the problem, as well as the specificity of the Montenegro intradermal test in the Peruvian Amazon, 67 residents at the lowland jungle village of Maypuco in Loreto Department were selected. Each member of this representative population sample was given the Montenegro test, and each provided a blood specimen for later indirect immunofluorescence testing.

The immunofluorescence test results were negative, but 18 of the 67 subjects (26.8 per cent) responded positively to the Montenegro test. Four of the 18 positive subjects exhibited scars compatible with prior leishmaniasis. None of the other subjects

were found to have such scars, nor were any subjects found to have active leishmaniasis lesions.

Because all 18 Montenegro-positive subjects were above 14 years of age and most were men engaged in farmwork, it would appear that most exposure to the insect vector occurred outside the village proper, and that transmission of the disease within the home was unlikely.

Also, unlike the Montenegro test, the indirect immunofluorescence test tends to become negative once the active phase of the disease has passed. That circumstance is compatible with the reported survey findings, which detected no active cases and no clearly positive immunofluorescence test responses.

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