

EPIDEMIOLOGIC SURVEY OF CUTANEOUS LEISHMANIASIS: AN EXPERIENCE IN MERIDA, VENEZUELA¹

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Leishmaniasis is common in western and southern portions of Venezuela, where it tends to occur in hot, humid regions below 600 meters. This article describes the results of a survey performed during 1978-1979 in western Venezuela above 600 meters, where leishmaniasis caused by a recently identified parasite (Leishmania garnhami) is common, but where the mucosal lesions often associated with the disease in other areas are virtually unknown.

Introduction

To date, epidemiologic surveys of cutaneous leishmaniasis in Venezuela have addressed themselves to focal outbreaks (1-6). The largest collective effort to delimit the territorial extent of cutaneous leishmaniasis in the country was made by the Venezuelan Health Ministry's Division of Dermatology; organized by Dr. Jacinto Convit, it found the disease in 21 of the country's 23 territorial divisions.

A total of 13,600 cases were treated in 1955-1977, a period when the population grew from 7 to 12 million inhabitants. Despite this, the true incidence and prevalence of the disease in any particular part of Venezuela remains unknown. However, the situation is fairly similar to that in Colombia (7), where the infection (which appears to have been known since the end of the past century) exists in 23 of the country's 32 territorial divisions, and where the estimated prevalence between 1948 and 1955 was on the order of 2,000 cases.

In 1971, when Venezuela's population barely exceeded 10 million inhabitants, the

cumulative total of known cases since 1955 was on the order of 10,900. Many of these occurred within the four states that comprise Venezuela's Andean region, which in 1971 had approximately 1.4 million inhabitants, less than 14 per cent of the national population. The total number of cutaneous leishmaniasis cases recorded in this region during the period in question was 8,000, or 73.4 per cent of the 10,900 cases recorded in the country as a whole (8, 9).

Because of this concentration of cases in the Andean region, we selected that region for a pilot project—an epidemiologic survey of the disease designed to accurately determine the seriousness of the present situation. This survey, conducted during 1978-1979, was made easier by good knowledge of both the Andean region's anthropophilic sandflies (10, 11) and the *Leishmania* species responsible for the disease (12, 13).

Review of Prior Studies

The zoonotic nature of American leishmaniasis has been described by Pifano (14), who systematically defined the foci of the disease and validated allergic and parasitic indexes used as parameters for evaluating changes in those areas. His characterizations (14) have since been used by Herrer and Christensen (15, 16) and by Herrer, Christensen, and Beumer (17) in describing epidemiologic situations in Panama.

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In Venezuela, cutaneous leishmaniasis has traditionally been recognized as a unique clinical entity, with the relative likelihood of mucosal involvement depending partly upon the age of the responsible focus. The first and most complete epidemiologic study of the disease in its own natural environment was made in 1940 by Pifano (1) who, in a later work (14), summarized his epidemiologic experience with leishmaniasis foci in Venezuela's Yaracuy State and classified the foci according to their degrees of development. This classification employed two of the three categories of Pessoa (18), which are as follows:

1) Recent endemic foci. These are characterized by active lesions among the affected population, a high parasite index, a low allergic index, active cases among all age groups, and no cases with mucosal involvement, the latter appearing later.

2) Highly endemic foci. Active lesions and scars are common; 10 to 20 per cent of the population typically shows a positive allergic response, and some 5 to 10 per cent of the inhabitants have mucosal lesions.

3) Old endemic foci. In such foci there are only sporadic active cases; but the allergic index is very high and 15 to 30 per cent of the inhabitants typically have mucosal lesions.

In 1962, Pifano also described the results of Montenegro intradermal tests conducted on inhabitants of the same endemic area where he had observed the disease since 1945 (19). Here he pointed out that 60 per cent of the intradermal tests performed on subjects with old scars yielded positive results, and that 10 per cent of the apparently healthy subjects of the endemic area also yielded positive results. The intradermal test was administered to 1,118 people in the endemic area and elicited a positive response among subjects beginning at 10 years of age, the highest rate of positive response occurring among those 40-55 years old.

Other epidemiologic situations involving more recent endemic foci have been studied by Pons and Londres (2) and by Pons et al.

(20) in Venezuela's northwest region. Both studies dealt with epidemic situations that the authors characterized as hyperendemic. They found that the infection attacked both males and females beginning at age five, and that it was possible to observe a spectrum of community involvement ranging from settlements with a high parasite index and low allergic index to ones with a high allergic index and intermediate parasite index. It is interesting to note that the authors also found infected domestic animals (dogs and donkeys) and, surprisingly, allergic indexes ranging from 9.4 to 21.4 per cent among people without lesions. Overall, in various communities where mucosal lesions were diagnosed, 25 per cent of the population accounted for 42.8 per cent of the positive intradermal reactions.

Elsewhere, Homez and Romero (3) have studied the epidemiologic situation in settlements characterized as having old endemic foci in extreme western Venezuela by administering intradermal tests, and Bonfante et al. (21) have studied recent endemic foci in the northwest region where active cases and individuals with scars were found.

In addition, Pifano et al. (22) have studied two old endemic foci in central Venezuela. The authors detected no mucosal cases but found that intradermal testing of subjects with scars yielded a 40 per cent positive response and that 17 per cent of the healthy subjects tested also responded positively. More recently, Albornoz et al. (23), testing subjects in another locality, obtained positive leishmanin reactions from 60 per cent of these subjects, all of whom exhibited sequelae of previous lesions.

In the Amazonas territory of southern Venezuela, Medina and Vegas (24) examined about 1,000 people in the mid-1960s and found few active cases. However, the rate of positive response to the intradermal test was 72 per cent among subjects over 40 years of age, and various discrete lesions of the nasal mucosa were detected.

Recently, in 1981, Aguilar (6) made a sig-

nificant contribution to knowledge about the epidemiology of cutaneous leishmaniasis in Venezuela. This work describes a 1978 epidemic in a piedmont community of the coastal mountain range that is located in Cojedes State. This community was located in a softwood forest that had been heavily logged. Of the 124 inhabitants, 21 (including a child 3 years of age) had active lesions. The disease was diagnosed parasitologically in 16 subjects, but only 22.6 per cent of the subjects yielded a positive response to the intradermal test. The early appearance of mucosal lesions in four persons was confirmed, and three of these cases were parasitologically confirmed through inoculation of biopsy material into hamsters.

Summarizing the general Venezuelan situation, it can be said that western, central-western, southwestern, and southern Venezuela harbor old endemic foci. These are characterized by low parasite indexes, high allergic indexes, and varying frequencies of mucosal lesions, some of which appear early. In these old endemic foci, as in recent endemic foci, most of the people are attacked regardless of age or sex; domestic animals (dogs and donkeys) are also infected; and a high percentage of apparently healthy individuals give a positive response to the intradermal test. These old endemic foci are located below the altitude of 600 meters in hot and humid regions that are forested or that have been cut over.

In western Venezuela's Mérida State, where the study reported here was conducted, the infection appears widely distributed and very common in rural areas. Valera et al. (25) used direct parasitologic methods and immunoreactions to study 56 cases among subjects of all ages in this state and succeeded in inoculating the parasite into hamsters. A microscopic and electromicroscopic study of the etiologic agent identified it as a new taxonomic form of *Leishmania* that was named *Leishmania garnhami* (12). A unique retractile organelle was seen in this parasite, whose morphology is

stable in the amastigote phase, and this organelle persisted after passages in NNN³ cultures, susceptible laboratory animals (hamsters), and sandfly vectors (26). Some of this parasite's features (including passage in the vector *Lutzomyia townsendi*) are shared with parasites belonging to the *Leishmania mexicana* and *Leishmania braziliensis* complexes (27). The clinical infection in man, as well as infection of domestic animals (dogs and donkeys), is associated with the vector *Lutzomyia townsendi* (Ortiz, 1959), which is commonly found in the region (28).

To ascertain the true prevalence and incidence of this infection in the endemic region, we conducted an epidemiologic survey based on the clinical experience of a dermatologist associated with the study (Valera) whose agreement with available parasitologic and immunodiagnostic research data had already been determined (25). This study employed the Montenegro intradermal test with *Leishmania* antigen (leishmanin), whose specificity is well-known (29). We prepared standardized antigen for use in our assays from a local isolate (JAP/76), using a concentration of 30 µg nitrogen per ml, which is slightly lower than that recommended by Melo et al. (30).

Materials and Methods

The Study Area

The town of Tovar in Mérida State (within Venezuela's Andean region) is the site of the headquarters of the local dermatology service. A check of that service's clinical histories of cutaneous leishmaniasis cases treated between 1974 and 1977 revealed the existence of 87 infected individuals in 33 settlements (*caseríos*) belonging to three Mérida districts. Of these 33 settlements, 30 that had less than 500 inhabitants each were selected and designated as "caseríos with cases." Another 30 settlements

³Novy, Nicolle, and McNeal medium.

without reported cases were then selected in the same general region. Designated "caseríos without cases," they had population sizes and ecologic settings similar to those of the other 30 *caseríos*. Altogether, these 60 rural settlements had roughly 25,000 inhabitants. All 60 were located in the northwest part of Mérida, within an area of about 1,124 square kilometers bounded by 71°30' and 71°50' west longitude, and by 8°15' and 8°40' north latitude (Figure 1).

Selection of Study Settlements

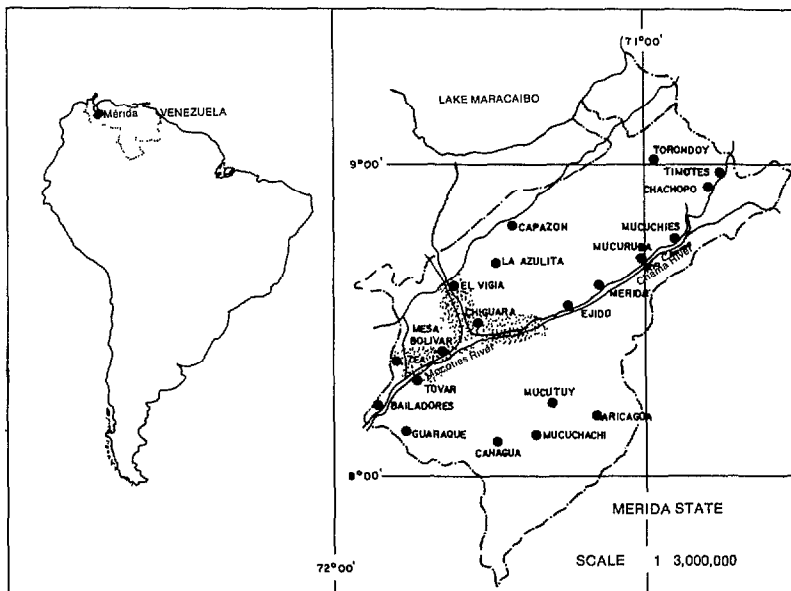
A table of random numbers was used to select settlements from among the group of 60, in order to obtain a study population of

around 2,500 people. In this manner 13 settlements were selected, of which five were in the group with reported cases and eight were in the group without such cases.

Establishment of Ecologic Zones

Three studies have contributed important information about the natural features of the study area. Andressen and Ponte (31) have reported useful climatologic and hydrologic data; Castillo et al. (32) have performed a soil survey in the area, concentrating on the soils of the Mocotíes River Basin; and Sarmiento et al. (33) have described the region's natural vegetation. Using the material offered by these studies, we made a map of the climatic

Figure 1. A map showing the area studied in the state of Mérida, Venezuela. The map at the left shows the location of the state of Mérida; that on the right shows the location of the study area (stippled region) within the state of Mérida, around the confluence of the Chama and Mocotíes rivers.



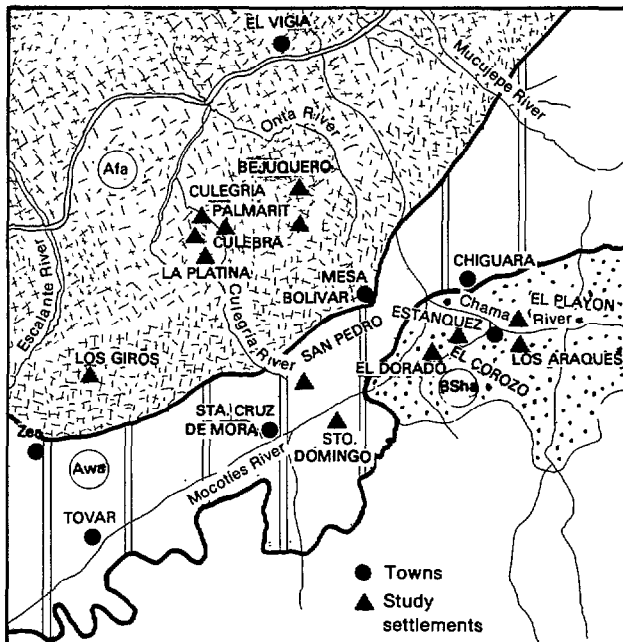
zones prevailing in the study area. This map, based on rainfall levels, separated the study area into three zones—one where the rainfall averages about 600 mm per year, one where it averages around 1,000 mm, and one where it averages between 1,200 and 1,800 mm.

Following Koppen nomenclature (34), these zones have been designated in Figure 2 as having a semi-arid hot climate (BSha), a hot tropical climate (Awa), and a constantly humid tropical climate (Afa). The BSha area shown has considerable drought. The Awa area, where the towns of Tovar and Santa Cruz de Mora are located along the Mocotíes River, has a dry season in the first quarter of the year and average temperatures between 18 and 22°C. The Afa area has year-round rainfall (averaging at least 60 mm in the driest month), with temperatures averaging above 18°C in the coolest month and averaging

above 22°C in the hottest month. Relative humidity averages are between 70 and 80 per cent in the BSha area, between 75 and 90 per cent in the Awa area, and over 80 per cent in the Afa area.

According to Andressen and Ponte (31), there is an inverse linear relationship (with a correlation coefficient of $r = -0.9769$) between average altitude and temperature in the three regions. Using average annual isotherms, we defined two temperatures zones—one “tropical” or “hot” with annual temperatures averaging around 22°C (including the dryer BSha area between 480 and 650 meters above sea level and the Afa area between 80 and 900 meters above sea level) and the other with annual temperatures ranging from 18 to 27°C corresponding to the Mocotíes River Basin (the Awa area, with altitudes ranging from roughly 600 to 1,400 meters).

Figure 2. A map of the study area showing the 13 settlements covered, a few of the major settlements in the region, and the three ecologic zones (Afa, Awa, BSha) used to classify the study settlements.



With regard to natural vegetation, the semi-arid BSha area provides a habitat for certain spring shrubs including cacti (*Lemnaireocereus griseus*, *Cereus hexagonus*, and *Pilocereus lanuginosus*) and for trees not exceeding six meters in height (including the genera *Prosopis* spp. and *Acacia* spp.). The shrub population also includes climbing members of the euphorbia and cactus families (*Croton* sp., *Jatropha* sp., and *Opuntia* spp.). In general, the vegetation within this area is sparse.

According to Sarmiento et al. (33), seasonal mountain forest vegetation predominates along the Mocoties River (the Awa area) between the altitudes of 800 and 1,700 meters, occupying uncultivated portions of the area's riverbanks, terraces, and mountain slopes. This vegetation, growing in areas that have generally been disturbed by man, forms small mosaics between land with permanent crops (such as coffee) and level meadows or sugarcane areas. There are two tree strata—at heights of 12-20 meters and 20-25 meters—in

which *Inga* sp., *Cordia* sp., and *Erythrina* sp. predominate and provide shade for raising coffee. Secondary savannas, at lower altitudes of 550 to 700 meters, consist of grazing-lands dominated by *Panicum maximum*, *Melinis minutiflora*, and *Pennisetum purpureum*.

Of the three climatic zones, the continually humid Afa area descending toward the western piedmont of the Andes has the least-studied flora. Its regular temperature, heavy rainfall, and recent quaternary soils make it suitable for development of mixed tropical rain forest where a wide variety of palms and tall trees belonging to the families Anacardiaceae, Bombacaceae, Burseraceae, Lauraceae, Moraceae, and Sterculiaceae predominate. Human settlements have recently been established in the area's lower reaches, which have been largely deforested.

As Table 1 shows, five of the 13 study settlements containing 854 subjects were located in the portion of the BSha region extending from El Dorado to Los Araques; two settlements

Table 1. The ecologic zones and settlements of the study area showing the population of each settlement, the number of subjects with recent lesions, the number of subjects with scars or old lesions and positive intradermal test results, and the estimated incidence and prevalence of the disease.

Ecologic zone	Settlement	Population	Subjects with active lesions less than one year old	Leishmaniasis incidence (%)	Subjects with positive intradermal test results and scars or lesions active over one year	Leishmaniasis prevalence (%)
Afa	Palmarito	232	2	0.86	31	13
	Bejuquero	196	6	3.06	56	29
	La Platina	32	0	0.00	6	19
	La Culebra	83	7	8.43	26	31
	Los Giros	267	13	4.87	38	14
	Culegria	143	3	2.10	31	22
	Subtotal	953	31	3.25	188	20
Awa	Santo Domingo	397	11	2.77	91	23
	San Pedro	326	9	2.76	69	21
	Subtotal	723	20	2.77	160	22
BSha	El Playón	97	0	0.00	2	2
	Los Araques	292	0	0.00	9	3
	El Dorado	52	1	1.92	9	17
	Estánquez	249	2	0.80	55	22
	El Corozo	164	13	7.93	55	34
	Subtotal	854	16	1.87	130	15
	Total	2,530	67	2.65	478	19

containing 723 subjects (San Pedro and Santo Domingo) were in the Awa region; and six settlements containing 953 subjects, ranging in altitude from 820 meters (Los Giros) to 80 meters (Bejuquero) were in the Afa region.

Epidemiologic Survey

A rough map showing the distribution of all houses or family units was made for each of the 13 study settlements; a schedule for visiting the settlements was drawn up; and the people in the settlements were informed as to the dates of the scheduled visits.

A card was then prepared for each family unit containing the following information: (1) the name of the head of household or family representative, data identifying the residence, and the altitude of the locale; (2) a list of all the household members giving their sex, age, and length of residence in the area; (3) a description of each family's economic activity—i.e., primary production (raising of livestock or crops such as cocoa, coffee, sugar cane, bananas, citrus fruits, etc.) or work as civil servants, traders, or providers of other services; (4) an indication of the distance from the residence to other nearby residences and also to the secondary natural vegetation and to farming areas in the family's possession; (5) a list of the domesticated animals (horses, cattle, pigs, dogs, poultry, etc.) present in the immediate vicinity of the residence.

On the scheduled date a medical dermatologist and an assistant began to interview inhabitants of the settlement in question concerning the existence of ulcers or sequelae of lesions compatible with cutaneous leishmaniasis. The presence of these indications of infection was then confirmed by clinical examination, and the results obtained from all the inhabitants were recorded along with information provided by them concerning the approximate date the infection was noticed and the locality in which it was acquired.

The Montenegro intradermal test was administered to all the inhabitants. The leishma-

nin used in this test was prepared with promastigotes of a local isolate of *Leishmania garnhami* (JAP/76) that had been cultured in Nakamura medium and were in the logarithmic phase of development on the sixth day of being grown at 26°C (12). These promastigotes were suspended in saline solution with 0.5 per cent phenol at a nitrogen concentration equivalent to 30 µg/ml determined by the Kjeldahl method. A tenth of an ml of the suspension of lysed parasites was subsequently injected by the intradermal route into each subject's right forearm, and the same amount of a phenolated solution was injected by the same route into the left forearm. Forty-eight hours later the site of the leishmanin injection was examined for a delayed hypersensitivity response, and the diameter of the induration was measured with a transparent ruler after outlining its perimeter with a marking pencil. Any induration with a diameter of five millimeters or more was considered a positive response.

Whenever possible, subjects with active lesions were transferred to the clinical laboratory for parasitologic confirmation of the diagnosis by isolation of the agent or proliferation of the organism in inoculated male hamsters. All subjects yielding a positive response to the intradermal test were examined for frank or inconspicuous lesions of the oronasal mucosa that could be sequelae of cutaneous leishmaniasis.

Data Analysis

To help estimate the risk of infection, the study subjects were separated into the following four age groups: (a) 0-2 years, including infants and young children who spent most of their time indoors, rarely spent the night outdoors, and rarely visited the areas where adults worked; (b) 3-7 years, including children who occasionally went into areas near their residences at night and who may have served as messengers between one house and another; (c) 8-20 years, including children,

adolescents, and young people who tended to participate actively in productive work according to the family's needs; and (d) over 20 years, including those subjected to cumulative risks as they continued to participate in activities dedicated to commercial production or family maintenance.

The prevalence of the disease was taken to be the percentage of inhabitants who gave a positive response to the intradermal test and who exhibited scars or had borne active lesions for over a year. Similarly, the incidence was taken to be the percentage of inhabitants who bore active lesions less than 12 months old that were compatible with the clinical manifestations of cutaneous leishmaniasis at the time of the clinical examination, whether or not they responded positively to the intradermal test. If the intradermal test was negative, the parasite's presence was confirmed clinically by direct examination of biopsy material taken from the edge of the lesion.

Results

Incidence and Prevalence Data

The incidences and prevalences of cutaneous leishmaniasis that were observed in the 13 study settlements using the above criteria are shown in Table 1. Of the 2,530 people examined, 67 were found to have active cases with lesions less than 12 months old, and 478 were found to yield a positive response to the intradermal test and to have old lesions or scars compatible with sequelae of prior leishmaniasis lesions.

The populations of the various settlements ranged from 32 in the smallest Afa community (La Platina), which had been recently established, to 397 in the largest Awa community (Santo Domingo), which was located on a large coffee plantation and which was over 100 years old.

The highest incidences and prevalences of leishmaniasis were found in two communities (La Culebra and El Corozo) with interme-

diate-sized populations (83 and 164 inhabitants, respectively) situated in the Afa and BSha areas. Most of these communities' family units (57.1 per cent) were engaged in coffee-growing, though many living in level valley areas were occupied with sugar-growing (28.6 per cent) and livestock-raising (17.9 per cent). The two settlements harbored active epidemic foci, the highest incidence being found in La Culebra, where deforestation work had recently been performed.

The lowest incidences and prevalences were found in El Playón and Los Araques in the heart of the BSha region's dry area. Despite the relatively high population of Los Araques (292 inhabitants), only three subjects in this community were found to have scars indicative of prior lesions.

Overall, prevalences in the two Awa settlements were 21 per cent in San Pedro and 23 per cent in Santo Domingo. And in the Afa region, if the community of La Culebra is excepted, the observed prevalences ranged from 13 to 29 per cent. In this regard it appears noteworthy that the leishmaniasis picture in the two Awa settlements was quite similar. Besides having similar prevalences of the disease, both had an apparent incidence slightly below 3 per cent. The fact that most of the families (61 per cent in Santo Domingo and 76 per cent in San Pedro) had members engaged in coffee-growing suggests a possible relationship between leishmaniasis epidemiology and the intensity of coffee cultivation.

Within all the settlements combined, the average incidence of leishmaniasis was 2.7 per cent and the average prevalence was 19 per cent. Three subjects with mucosal lesions were discovered, two of whom had lesions in an advanced stage of development. However, two of the three subjects said they had contracted the infection in distant endemic areas, one of them in Barinas State, in an eastern piedmont area of the Andes Mountains where mucosal lesions were frequent. Therefore, there appeared to be only one subject with locally acquired mucosal lesions in the study popula-

tion, making the apparent incidence of mucosal lesions among the 478 infected subjects 0.21 per cent.

Sex and Age

Of the 2,530 study subjects, 1,414 were males and 1,115 females; and of the 545 cases of leishmaniasis discovered, 286 occurred in males and 259 in females. Statistical analysis of the data showed no significant differences between the two sexes.

With regard to the distribution of cases by age, there is some indication that the percentage of infected individuals in each of the four age groups considered was directly proportional to the median age within that group. Performing a regression analysis with four pairs of values does not yield statistically significant results. However, within each climatic zone (Afa, Awa, and BSha) it is possible to plot the percentage of infected subjects in each age group against the intermediate ages (1, 5, 13.5, and 45 years) of each group. In all three cases, when this relationship was charted it yielded straight-line values with correlation coefficients between 99 and 100 per

cent, suggesting that the percentage of infected individuals in each group tended to be directly proportional to age.

In general, subjects over 20 years old accounted for 60 to 80 per cent of the leishmaniasis cases detected. The medical histories of subjects questioned regarding the age at which they contracted the infection indicated that in the Afa and Awa areas taken together about 40 per cent of the subjects were infected between the ages of 8 and 20 years. The fact that a smaller percentage of people in the BSha area became infected under 20 years of age could relate to immigration and patterns of endemicity prevailing in areas from where new residents had come.

Distribution of Cases within Settlements

Table 2 presents data on the percentage of subjects with positive cases and the percentage of houses with infected subjects. When these two variables are compared for each community by regression analysis, considering the percentage of subjects with cases as the independent variable and the percentage of houses with infected subjects as the dependent vari-

Table 2. Variations in settlements with and without reported cases, by ecologic zone. The values shown are the lowest average for any one settlement and the highest average for any one settlement in the indicated group.

	Ecologic zone					
	Afa		Awa		BSha	
	Settlements without reported cases %	Settlements with reported cases %	Settlements without reported cases %	Settlements with reported cases %	Settlements without reported cases %	Settlements with reported cases %
% of subjects testing positively	12.5-31.3	14.3-28.6	22.9	21.2	2.1-17.3	18.7-25.8
% of houses with infected subjects	47.1-62.5	33.3-71.1	62.5	68.5	5.6-77.8	68.9-91.3
Altitude above sea level (meters)	164-814	486-651	1,015	957	410-508	440-860
Average distance from a residence to forest vegetation (meters)	25-36	20-42	20	20	20	20-24
% of families cultivating:						
Coffee	61.1-94.1	52.6-80.4	61.1	75.9	0.0-28.0	1.4-57.1
Sugar cane	8.3-35.3	10.5-17.7	44.4	24.1	71.4-93.3	28.6-60.7
% of families raising livestock	6.3-36.1	21.1-47.1	0.0	19.0	0.0-71.4	17.9-25.0

able, the result is a straight-line equation with a correlation coefficient of $r = 0.83$. The results suggest it may be possible, in future epidemiologic surveys, to estimate the prevalence of leishmaniasis in a settlement by surveying a random sample of houses in that settlement.

Considering each community's average altitude, average distance between houses, and average distance from houses to natural secondary vegetation as independent variables, and taking the percentage of leishmaniasis cases and the percentage of houses harboring cases as dependent variables, we could find no significant correlations. This suggests that the risk of infection in the study communities was not related to the communities' average altitudes (which ranged from 164 to 1,015 meters above sea level) or to the average distance between homes and natural vegetation (which ranged from 20 to 42 meters).

Prevalences in Communities with and without Reported Cases

Leishmaniasis cases reported to the dermatology services are principally those reported by infected patients. Trying to study the distribution and prevalence of leishmaniasis on the basis of these registered cases results in errors leading to underestimation of the health problem. This is the reason why we preferred to make a blind epidemiologic study—including a group of communities in the study sample from where no cases had been reported for at least four years. Then, as previously noted, communities were selected at random from a group of 60 settlements (30 with reported cases and 30 without) until a sample of at least 2,500 subjects was obtained. This procedure resulted in selection of 13 study communities, five with reported cases and eight without, containing a total population of 2,530 inhabitants. Statistical comparison of the percentages of leishmaniasis cases in each community with reported cases and in each community without indicated that there was no significant difference between the prevalences in the communi-

ties with reported cases and those without such cases.

Prevalences in Similar Communities and Ecologic Zones

An effort was also made to extrapolate the results obtained from the study area (1,124 square kilometers in the state of Mérida) to nearby districts in the same state with similar ecologic zones and a rural population of 104,000 inhabitants. Statistical analysis of the prevalences in each of the 13 communities by ecologic zone (Awa, Afa, or BSha) indicated that there were no significant zone-dependent differences. Specifically, the null hypothesis that average prevalences in settlements within the Awa, Afa, and BSha areas were the same is not denied by an analysis of variance; the estimated value of $F (0.363)$ is not rejected at a level of 10 per cent.

This makes it possible to conclude that the observed prevalences were not attributable to particular geographic or climatologic variations within the study area, and that the findings could be applied to other neighboring areas with the same or similar ecologic zones. If this is true, and if there are no unacknowledged geographic or ecologic barriers, it is very likely that the average prevalences observed (ranging from a low of 17.05 per cent in the BSha area to a high of 22.05 per cent in the Awa area) resemble those in at least six neighboring districts of the state of Mérida.

These six districts, located at altitudes ranging from 200 to 1,400 meters above sea level in the foothills of the Andes Mountains, have a rural population of 104,110 inhabitants, which, if it is distributed by age like the study sample population, would include some 63,500 inhabitants 20 years of age or less. Over the past 24 years, some 39 per cent of this 20 and under group could have been expected to contract leishmaniasis, bringing the total number of cases among the young popu-

lation of working age (24-44 years) to something on the order of 25,000 cases. This suggests that the number of leishmaniasis cases in this age group alone is roughly 10 times higher than the number recorded by the dermatological service in these districts over the past 20 years.

Intradermal Test Results

Leishmanin from *L. garnhami* that had been isolated from a patient in the study area, prepared as previously described, was administered to all 2,530 study subjects. A total of 545 of these subjects bore scars or ulcers that were compatible with leishmaniasis but that had not been parasitologically confirmed. Of the 2,530 subjects, 490 responded positively to this highly specific test (35). Two subjects with recent ulcers and parasitologically confirmed cases (a six month old infant and a man 62 years of age) gave a negative response to the intradermal test.

Of the 2,052 individuals without recent scars or lesions, 12 yielded positive reactions with indurations ranging from 5.0 to 11.0 mm in diameter. One of these 12 subjects, an adult 26 years of age, developed a lesion on the right leg 15 days after receiving the intradermal test injection on the right arm; the etiology of this lesion was parasitologically confirmed.

Discussion and Conclusions

Comparison of the endemicity of cutaneous leishmaniasis in the state of Mérida and in the rest of Venezuela brings out the following differences:

1) Around Mérida, Andean leishmaniasis occurs mainly in areas more than 600 meters above sea level with variable temperatures (18-22°C), although it has been observed in some low tropical areas. In the rest of the country leishmaniasis invariably occurs in tropical regions below 600 meters.

2) The Andean prevalence, measured as the response to intradermal tests with leishmanin, is much greater among older age groups; more than

65 per cent of the population over 20 years of age in endemic areas appears to react positively, while in the rest of the country this percentage is found only in population groups over 40 years of age. This difference, together with the extremely low proportion of cases with actual lesions in the Andean region, indicates that the foci in most of the study communities (11 of 13) were old endemic foci.

3) Mucosal attack is generally found in connection with cutaneous leishmaniasis in most portions of western and northwestern Venezuela. Since the Andean region is situated in the west of the country, it appears as an island where mucosal involvement is almost nonexistent.

4) In the Andean region the percentage of healthy individuals yielding a positive response to the intradermal test is extremely low (0.58 per cent), whereas in the rest of the country this percentage ranges between 10 and 22 per cent. This could be explained by any of the following circumstances: (a) the antigen used in the present study (local isolate JAP/76) could be much more specific or better standardized than the leishmanin used by the other investigators; (b) interpretation of intradermal test results could be subjective (except in the study by Homez and Romero (3), who like ourselves interpreted the appearance of a papule at least 5.0 mm in diameter as a positive response); or (c) *Leishmania garnhami* could be more virulent than the country's other species or subspecies of *Leishmania*, so that, after virtually every transmission to man by sandflies, a patent lesion is produced. (A reinoculation effort by Pifano, which yielded negative results (1), indicates that resistance can be induced without production of an active lesion.)

The endemic leishmaniasis situation in the Venezuelan Andes, specifically in the state of Mérida, bears some similarities to the situation in Peru studied by Herrer (36). Nevertheless, there are also some important differences:

1) The disease known as uta in Peru, whose lesions tend to occur on the face because of the clothing worn in a cold climate, is a benign infection that primarily affects children. The reason for this tendency to afflict children is unknown. In Mérida, Andean leishmaniasis tends to attack older subjects actively engaged in productive labor.

2) Uta is typically found at altitudes from 900 to 3,000 meters above sea level, whereas leishmaniasis of the Venezuelan Andes is found at lower altitudes, generally between 600 and 1,400 meters above sea level.

3) Whereas uta is an infection of the mountain slopes, which have scanty vegetation or desert vegetation and temperatures below 16°C, leishmaniasis of the Venezuelan Andes is commonly found in intramontaine valleys possessing trees and temperatures between 18° and 22°C.

4) Uta affects both children and dogs, with the disease prevalence among children in endemic areas being 20 per cent or more, and with virtually all the dogs in such areas appearing to be infected. We have not specifically studied the infection of animals in the Venezuelan Andes, but in this case the dog and donkey appear to be only sporadically infected.

5) *Leishmania garnhami*, the parasite that produces the endemia in the Venezuelan Andes, possesses a particular organelle in its amastigote form. This organelle, possessed by amastigotes found in the early lesions of human subjects, domestic animals, and experimental animals, is not present in the *Leishmania* parasites responsible for uta. It is also noteworthy, however, that both Venezuelan Andean leishmaniasis and uta are transmitted by sandflies of the *Lutzomyia verrucarum* group, which has a typically Andean habitat.

All of the foregoing helps to define cutaneous leishmaniasis of the Venezuelan Andes as a relatively benign infection affecting the rural population. The prevalence of infection appears to be around 20 per cent in endemic areas, with all age groups and both sexes being involved. Infection is relatively common among members of age groups actively engaged in productive labor. The infection was found to be holoendemic in the study area and to occur in the BSha, Awa, and Afa areas studied, as well as in transitional BSha-Awa and Awa-Afa areas.

With respect to incidence, the underregistration of cases is considerable. On the basis of results obtained to date, it appears that a rural population of 100,000 in Mérida could be expected to have some 2,700 active cases of cutaneous leishmaniasis, a number 55 times greater than the average number of cases recorded annually over the period 1972-1977.

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SUMMARY

Leishmaniasis is well-known in western and southern portions of Venezuela, where it tends to occur in hot, humid regions below 600 meters above sea level and where a significant share of those infected develop mucosal lesions. The disease is also common at higher altitudes in the western state of Mérida, where it is caused by *Leishmania garnhami*, a distinct form of the *Leishmania* parasite. The survey reported here was carried out in order to determine the nature and intensity of leishmaniasis in this latter region.

For that purpose a group of 13 communities with a total of 2,530 residents in the affected area were selected. Basic personal data were gathered on the subjects, each of whom was interviewed, given an

intradermal test for *Leishmania* (delayed reaction), and examined for active lesions or scars compatible with the disease. This procedure detected 67 "active" cases with lesions less than 12 months old and 478 subjects who yielded a positive response to the intradermal test. Only three subjects were found to have mucosal lesions, and two of these had apparently acquired their infections outside the study area. This finding contrasts sharply with the leishmaniasis picture at altitudes below about 600 meters, where mucosal lesions tend to be quite common in old endemic foci of the disease. Observed geographic and climatic variations within the study area did not appear to significantly affect the prevalence of the disease.

In sum, the findings indicate that the leishmaniasis of the Venezuelan Andes caused by *Leishmania garnhami* is a relatively benign infection with a prevalence of about 20 per cent in endemic areas, and with both sexes and all age groups being involved. The infection was found throughout a study area encompassing 1,124 square kilometers, including regions with climates ranging from semi-arid desert to tropical rain forest. However, the disease picture

appears to change at altitudes below about 600 meters. That is, mucosal lesions are a common feature of leishmaniasis in lower portions of western and northwestern Venezuela surrounding the study area. Therefore, the Andean upland, where this particular form of the disease prevails, appears as an island where the disease is common but mucosal lesions are very rare.

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