LEISHMANIASIS CONTROL STRATEGIES

A CRITICAL EVALUATION OF

IDRC-SUPPORTED RESEARCH

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Esta serie incluye ponencias de reuniones, informes internos y documentos técnicos que pueden posteriormente conformar la base de una publicación formal. El informe recibe distribución limitada entre una audiencia altamente especializada.

Leishmaniasis control strategies

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Leishmaniasis control strategies: A critical evaluation of IDRC-supported research

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Edited by
Pandu Wijeyaratne, Tracey Goodman
and Carlos Espinal

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Tegumentary Leishmania Infection and Disease in Colombia: Evaluation of Incidence and Risk Factors

K.A. Weigle[1] and N.G. Saravia[2]

Background and Theoretical Framework

American tegumentary leishmaniasis (ATL) caused by the species complex Leishmania (viannia) braziliensis is endemic throughout much of Central and South America (1). Although the manifestations are only rarely fatal, mucosal lesions cause devastating mutilations and cutaneous lesions are chronic burdens, occassionally disfiguring (2). Due to the complex life cycles of this vector-bourne zoonosis control of transmission has not been possible (3). Even case management, as a means for control of the disease burden is hampered by inefficient diagnostic methods (4,5) and costly, difficult to administer, potentially toxic chemotherapy (6,7). Initial progress towards development of vaccines to prevent ATL or supplement case management has been encouraging (8-11). However our limited understanding of the protective nature of the naturally acquired immune response to L. viannia in humans hinders the development of effective vaccine strategies. (12, 13). Development, evaluation and adaptation of control programs for ATL must consider the underlying pathogensis of this illness, which continues to be elucidated.

Observations from this project and works of others (14,15,16), including animal models, have enabled the construction of a theoretical framework for the natural history of L. braziliensis infection for residents of an endemic area, where repeated exposure to Leishmania laden sandflies may occur (Figure 1). Although not all stages of this framework are yet measurable, this framework is critical to planning future studies and control measures. Three concepts are central to this framework: 1. Exogenous reinfection is a frequent cause of recurrent lesions in endemic settings. 2. A substantial number of infections are not clinically manifest initially. 3. Latent infection is a common outcome of primary infection.

Newborns or new residents are considered to be naive hosts who have no history of contact, infection, or sensitization with Leishmania. Naive hosts, depending on their occupation, behaviors and the ecological setting of their residence may or may not be bitten by infected sandflies, represented by the transition (1) to the parasite contact stage. Dependent on host natural resistance, parasite dose, and parasite pathogenic capacity, following a parasite contact, the exposed host may either become infected or remain uninfected. Those infected either progress initially to clinically apparent lesions

[1] Department of Epidemiology, University of North Carolina, NC, USA.

[2] Fundacion CIDEIM, Cali, Colombia.

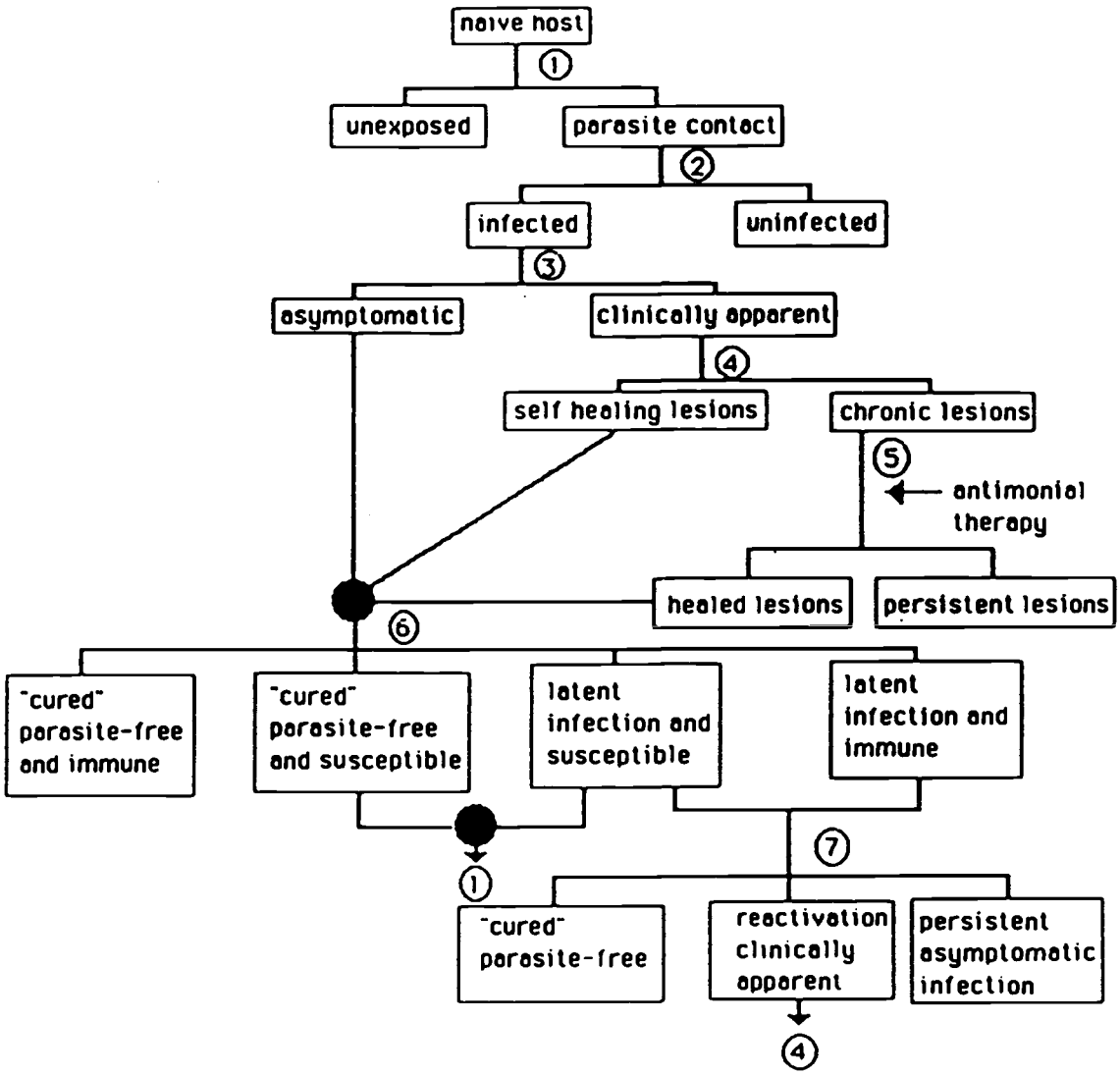

Figure 1. Theoretical Framework, Natural History of Leishmania braziliensis infection diagrams the most common pathways experienced by residents of an endemica area. Each box represents a potential stage in clinical progression. Transitions between stages are indicated by solid vertical lines, (stems), and are identified by encircled numbers. Nodes (●) indicate where branches may lead to similar outcomes. Arrows pointing to encircled numbers indicate that the clinical course is repeated beginning at the numbered transition.

or remain asymptomatic. Those suffering clinically apparent infections often self heal within two to eight weeks while others continue to manifest chronic lesions. The stage of chronic lesions is actually more complex than depicted. Initial lesions often heal in parallel with the presentation of satellite lesions or nearby superficial nodules, presumably infected lymph nodes. These nodules may either self heal or necrose and become verrugous nodules, or ulcers. Over time, this cycle may repeat itself. Less frequently new lesions develop distant from the primary lesions, presumably through hematogenous metastasis. Even though some lesions self heal, the host is not able to control Leishmania replication or manifestations. Furthermore, a lesion may repeatedly activate, heal and reactivate, while other lesions are continually active in the same host. Clearly there is great variety of clinical expression during the stage that is termed chronic lesions, the common feature being the continued presence of at least one active lesion. In most subjects all lesions will eventually become quiescent or healed lesions, this transition being speeded by anti-leishmanial therapy such as pentavalent antimonials. The term "healed" simply indicates that complete reepithelialization, (scarring), has occurred in all lesions and no signs of inflammation are present, the term "cured" being reserved for the absence of parasites with multiplicative potential. Rarely, persistent lesions are resistant to both host defenses and antimonial therapy and never heal.

Once all manifestations of infection are quiescent, the host is considered to have progressed to one of four possible states with respect to harboring parasites and Leishmania-specific acquired immunity (transition 6). Hosts that have completely eliminated parasites are designated as "cured" whereas hosts harboring clinically quiescent Leishmania are termed latently infected. Hosts that have acquired a Leishmania specific immune response that protects them from subsequent exogenous Leishmania reinfection are operationally defined as immune. On the other hand, hosts whose acquired immune response to Leishmanias is not protective against subsequent exogenous reinfection are defined as remaining susceptible. In reality a gradient of relative protective acquired immunity is more likely than two exclusive states. The combination of these two characteristics, parasite persistence (latency) and protective capacity of the acquired immune response, define the four possible states: Cured, immune; Cured, susceptible; Latently infected, susceptible; and Latently infected, immune. These hypothetical states will determine the potential for new lesions developing through either reinfection (re-enter cycle as "naive", transition 1) or reactivation (transition 7). Only those who have reached the cured, immune state would be expected to never experience lesions due to either reinfection or reactivation.

This framework raises numerous questions, the answers to which have direct implications for selection of control strategies for a given endemic setting. A dynamic balance between the transition states exsist such that at any point in time in a given setting persons will be distributed among these possible states. For instance, in a new settlement of a zoonotic focus most persons will be experiencing transitions 1, 2 and 3. Clearly interventions aimed at decreasing infective-vector-human contact will be more cost-effective than case management. On the other hand, several years after short lived

epidemics such as that observed in Dagua, Colombia, (17) all individuals will have progressed to either chronic lesions or one of the "cured" or latent states. In such a setting neither immunization nor vector control will be as appropriate as case management. Carefully conducted longitudinal studies can estimate the rates of transition between these states and the risk factors that impact upon the probability of a particular transition. From such an understanding tools for rapid community assessments can be developed. Estimation of the incidence of clinically apparent leishmaniasis in previously naive hosts is required to predict the potential impact of primary prevention strategies such as vector control or immunization. To date the few studies that have provided incidence rates for leishmaniasis have not excluded previously infected individuals (18,19,20) or have relied on passive reporting (18). In some settings passive reporting may underestimate the incidence by many fold (19,20).

Other questions raised by this framework include: What proportion of infections are subclinical? and, What are the determinants of whether clinically apparent disease develops? In endemic areas of frequent, low level exposure to Leishmania strains it appears that subclinical infection is more frequent (20,21). Subclinical infection in humans by Leishmania of low virulence may naturally elicit protective immune responses, a phenomenon that could be replicated to advantage for vaccine development (22). The extent to which naturally acquired immune response protects against subsequent reinfection may influence the likelihood that artificially induced immunity will confer lasting protection.

Our studies and those of others have attempted to measure rates of transitions such as disease incidence rates or recurrence rates. Such parameter estimates provide an overall sense of the likelihood of such events, but may mask important sources of variations in disease manifestations. By addressing risk factors for events such as infection, clinical disease, or recurrence biological determinants for such events can be uncovered. For instance the observation that recurrences were more frequent in residents of endemic areas focused our attention upon the hypothesis of exogenous reinfection (6). Risk factors assessments can also point toward specific intervention such as modifiable risk factors, or indicate the subpopulations toward which intervention would be most efficiently directed (19,20).

The objectives of this project were as follows:

1. To determine the incidence of leishmanial infection and disease in a defined rural population residing in the Inguapi Area of Tumaco, Colombia (Figure 2).

2. Identify and measure risk factors for acquiring leishmanial infection and dermal disease in this population cohort.

3. Determine and compare the recurrence rate of tegumentary leishmaniasis in patients who have been previously diagnosed and treated in Phase I with that of individuals whose leishmanial lesions healed spontaneously, or whose primary Leishmania infection was subclinical.

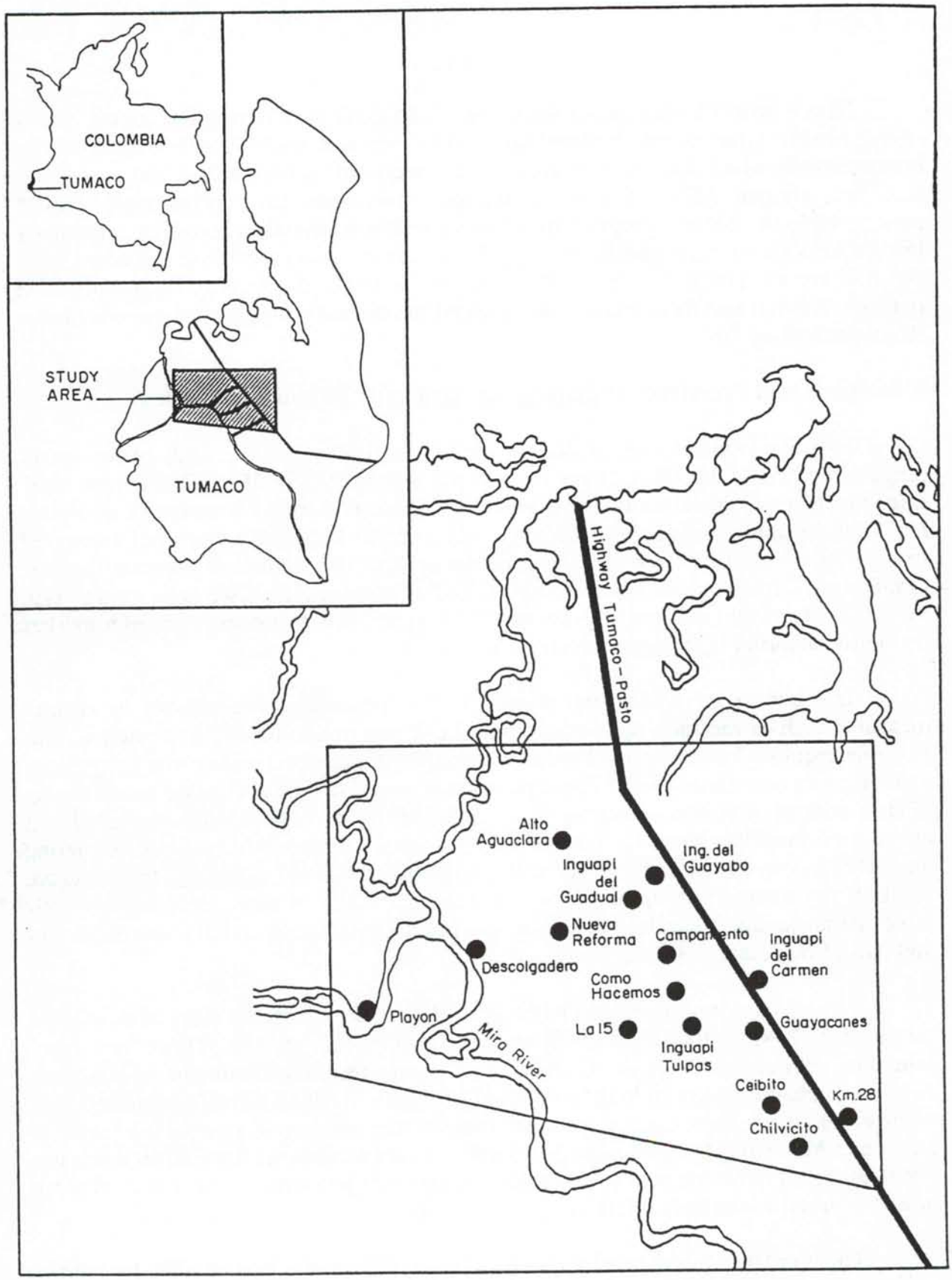

Figure 2. Insert: Location of the municipality of Tumaco within Colombia, and location of the study area within the municipality of Tumaco. Lower map: 15 villages and settlements in relation to the major road (solid black) and rivers and estuaries (double lines).

Three unanticipated issues were raised and dealt with during the course of this study. First because no standardized skin test antigen was available either nationally or internationally when this study began we were required to standardize and evaluate a skin test antigen (23). Secondly, passive surveillance for leishmaniasis proved unacceptable therefore a program of active surveillance with corresponding community involvement and an appropriate strategy for diagnostic evaluations was instituted after the first survey. Lastly a careful dissection of the molecular biology of the infecting parasite of initial and recurrent lesions revealed the diversity of pathways responsible for recurrent lesions (16).

I. Incidence and Prevalence of Leishmania baziliensis Infection and Disease

Previous studies of the incidence of ATL indicates that the rates of new cases ranges from 2.3-15.5/1000 annually in endemic settings (15,18 19). These "new case" rates are helpful indicators of the burden of disease requiring treatment in one year. However, because cases resulting from newly infected indivdiuals were not separated from recurrences, these "new case" rates do not indicate the number of preventable cases of infection. Analogous to epidemiological studies of tuberculosis, we have utilized skin test positivity as an indicator of previous infection and skin test conversion as a marker for newly acquired leishmania infection.

The burden of leishmanial disease that is potentially preventable by control measures such as vaccines, and vector control (4), was measured as the incidence rates of newly acquired infections and lesions in a defined population residing in a Leishmania endemic area over three years. These rates are required for the evaluation and planning of such control programs. Because the mobility of the study population exceeded that anticipated, incidence rates were estimated by incidence density, which allows for varying lengths of follow-up times. However in long established foci of Leishmania transmission, much of the disease burden and source of subsequent illness may reside in previously infected individuals. Therefore the amount of prevalent infection and illness is important and was also measured in this study.

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Methods

Study Area: The study area consisted of fifteen contiguous villages settlements in the rural sector of the municipality Tumaco, Department of Nariño, Colombia (Figure 2). No cases of Chagas disease or visceral leishmaniasis have ever been diagnosed in the study area. All previous Leishmania isolates from the municipality of Tumaco belonged to the L. braziliensis species complex, being identified as either L. b. panamensis or L. b. braziliensis by isoenzyme phenotype. The study area is 27 km^2, between 10-30m above sea level, with 232 days of rainfall and an average of 2,600 mm rainfall annually. The mean annual temperature is 25.9°C. Ecologically the area is classified as humid tropical rain forest, although presently the villages represent a spectrum of micro-ecological conditions depending on the extent of human intervention and deforestation. The residences are constructed primarily on wooden platforms with thatch roofs, and thatch or wooden walls. The primary occupation is subsistence farming supplemented by cash crops of cacao and plantain. The cultivated fields are usually located distant from dwellings although smaller adjacent vegetable gardens are common. Agricultural workers must travel through humid forest to access their fields. The predominant anthropophilic vectors in the study area are Lutzomyia trapidoi and Lu. gomezi, infected with promastigotes at a rate of 0.1% and 0.2% respectively. Insecticide spraying is provided by the Colombian Malaria Erradication Service (SEM) and 93.9% of households in the study area reported spraying in the preceding 12 months.

Study design: A prospective cohort study was carried out, the reference population being the entire population of the 15 locales. Detailed maps of all residences within the study area were constructed, each occupied dwelling was designated by village number and household number. Acquisition of infection was assessed by leishmanin skin testing during the three surveys spaced approximately one year apart. Acquisition of leishmanial lesions was assessed during the surveys and through three years of surveillance, from the first survey until December 1, 1989.

First survey: The study procedures were explained in full. Written informed consent was obtained from all participants; parents or guardians provided consent from minors. The names, sequentially assigned numbers, age, gender, relation to head of household, and date of interview of each household were recorded. For each resident an Individual Questionnaire regarding socio-demographic behavioral and occupational risk factors was completed. A complete exam of the skin and the upper respiratory

mucosa was performed and a leishmanin skin test applied, and read 48-54 hours later (23). In addition, the head of the household, defined as the individual providing the greatest economic support within the household, was queried as to the size, location, and ecological conditions of the family's landholdings, use of insecticide, and recognition of vectors. The construction and ecological environment of the residence was recorded after direct observation.

Second survey: Approximately one year later, changes in family composition were noted. In addition, a Transfer Form was completed for each individual whose location had changed since the first survey, noting the dates of departures arrivals, birth, and the village and household of both prior and new place of residence. All participants were examined for leishmanial lesions and LST reactivity was determined for all prior residents who had not reacted to the LST during the first survey, and for new residents.

Third survey: One year after the second survey, transfers were again noted, and physical exams repeated for both new residents and residents remaining from the prior surveys. All present residents were skin tested, regardless of their reactivity to prior skin tests.

Leishmaniasis surveillance: Upon completion of the first survey, the head of household was provided an inscription card listing all surveyed household members and briefly explaining the leishmaniasis surveillance, diagnostic, and treatment program. Families were enrolled in a leishmaniasis surveillance program consisting of heightened community awareness and recognition of leishmanial lesions. In each locale, one or two health promoters were taught to recognize, record, and refer such cases to the survey team. In addition, a member of the survey team visited each village twice monthly to review the case finding records with the promoter and evaluate persons with skin lesions.

Physical exams: Participants were provided either gowns or loose shorts after disrobing. Scarred lesions and active lesions were recorded as described in Table 1. The initial classification of active lesions served to guide diagnostic evaluations described in the following section.

Diagnosis of leishmaniasis: Due to the intensive surveillance and early evaluation of lesions, we intentionally evaluated many more lesions than were likely to be caused by Leishmania. In an attempt to diagnose all lesions caused by Leishmania, we evaluated lesions ranging from characteristic to atypical. Many of the latter were self-healing and/or bacterial in origin. Individuals who were LST reactive, presented with typical lesions, or presented with non-purulent lesions were evaluated diagnostically. Individuals with purulent lesions were treated with systemic antibiotics and local debridement. Diagnostic evaluations were performed for suspect or atypical lesions that did not heal by two of four weeks, respectively. Diagnostic evaluation consisted of dermal scraping, histopathology and culture of aspirate with the addition of the inoculation of biopsy material into hamster in selected circumstances as previously described (4). The LST was applied at the time of diagnosis. Using this protocol, it is possible that suspect or atypical, spontaneously healing lesions went undetected, but unlikely that clinically important lesions were missed.

Table 1: Criteria for Active and Healed Skin Lesions
Initial Clinical Classification

Active lesions criteria	Healed lesions criteria
1. No history of trauma	1. No history of trauma
2. During over 2 weeks	2. Duration over 2 weeks
3. Round or ovale ulcer	3. Round or ovale scar
4. Ulcer with raised borders	4. Smooth surfaced scar
5. Smooth nodule	5. Depressed scar
6. Satellite lesions	
6. Scar hypo or hyper pigmented	
7. Localized adenopathy	

Number of Criteria	Classification
>5	Typical
3 or 4	Suspect
<3	Atypical

Number of Criteria	Classification
>5	Typical
3 or 4	Suspect
<3	Atypical

Diagnostic categories, active lesions

P = Parasitological, Leishmania isolated or visualized
C = Clinical - lab supported meeting for of the following
1. Clinical classification either typical or suspect
2. Histopathology compatible with leishmaniasis; granuloma or mononuclear,
histiocytic chronic inflamatory infiltrate
3. LST reactive
4. Other diagnoses excluded (Sporotrichosis, Cancer, Leprosy)
5. Response to antimonial therapy

IM = Immunologically supported - meet two criteria
1. Clinical category either typical or suspect
2. LST converted at time of diagnoses

L = Likely diagnosis, does criteria for P,C or IM diagnostic categories, clinical
classification is typical or suspect and meets at least one of the diagnostic criteria #2-5 of the clinical-lab diagnostic category.

The definitions of the four diagnostic categories employed are displayed in Table 1. Although all four of these diagnostic categories are at least as strict as those currently utilized in epidemiological studies of leishmaniasis (15, 21), they were devised to represent increasing levels of certainty regarding a leishmanial etiology from likely, immunological, clinical-lab to parasitological categories. Cases which fit any of the four categories were considered as diagnosed leishmaniasis.

Outcome definitions: A prevalent case of leishmaniasis was defined as the presence of active lesions of diagnosed leishmaniasis at the time of the first survey in which an individual participated. A prevalent infection was defined as LST reactivity at the time of a participant's first survey. An incident infection was defined as skin test conversion, from LST non-reactive to LST reactive. An incident case of leishmaniasis was defined as lacking active skin lesions during their first survey who later developed active lesions diagnosed as leishmaniasis. Incident cases were further subdivided based on prior LST reactivity clinical history and prior skin lesions. An "Incident lesion and Infection" (I.I.) was defined as previously LST non-reactive, lacking a scar during prior surveys and lacking a typical scar and clinical history of leishmaniasis. An "Incident lesion, but Pre-existing infection" (I.P.) was defined as being LST reactive prior to lesion onset but lacking a prior characteristic scar or history of leishmaniasis. When new lesions presented in someone with a prior history or scar typical of leishmaniasis who was known to be LST reactive prior to onset of the new lesions, the case was defined as "Recurrent lesion, Pre-existing infection" (R.P.). New skin lesions presenting in individuals with pre-existing typical scars and/or history, who were skin test negative prior to the onset of the new lesions were defined as "recurrent lesion, new infection" (R.N.). These four definitions were operational and do not necessarily imply a particular sequence of pathogenesis.

Person time calculations: Time at risk was calculated differently for infection rates. Persons who remained LST non-reactive were considered to be at risk for infection during the time interval between two or three non-reactive skin tests. New residents were considered to be at risk for infection after their first negative LST with the exception of newborns who were assumed to be risk from day of birth. Persons who moved from one locale to another were considered to have contributed person time to their first locale until the date of the move. Persons whose skin test converted were assumed to be at risk for infection from the date of their first negative LST until the date of skin test conversion, which was estimated as the midpoint between the negative and positive skin test.

With the exception of newborns, individuals were considered to be at risk for leishmaniasis from the date of their first physical exam until the date of the first censoring event. Censoring events included: 1. Onset of new skin lesions diagnosed as leishmaniasis; 2. Departure from study area; 3. Refusal or absent for yearly physical exam; 4. End of surveillance program on 12/01/89. Infants born during the study who were enrolled and examined were considered to be at risk from day of birth until the first censoring event. When departure date was unknown it was estimated to be 6 months after the preceding survey in which the individual participated.

Results

Study population: In total 2858, 2714, and 2252 individuals participated in the first, second, and third surveys respectively. Between the first and second survey 742 residents left the area. During the second survey 598 new participants, including 110 newborns were enrolled. Between the second and third surveys 589 residents left the area. During the third survey 127 new participants, 104 of whom were newborns were enrolled. By the third survey 1771 (62%) of the original participants remained in the study area.

Nearly all residents listed on family registers participated in the skin exam and LST (Table 2) only 202 declined participation. Most of the non-participants were absent during time of visits to household. Villages with lower participation were located along waterways and highways and had a greater degree of absenteeism. Participation rates were greater for children and women who are less frequently absent from the home. Because the LST requires two appropriately timed contacts, data on LST results are available for fewer persons than were interviews and physical exams. Of those interviewed in the first survey, 98.7% (2822/2888) also underwent the physical exam; whereas LST results are available for 92.9% (2656/2888). The rates of participation were nearly identical for the three surveys.

Cumulative Prevalence of Infection: Villages varied considerably with respect to the percent of residents previously infected, based on the prevalence of LST reactivity during the first survey (Table 2). The villages with lowest prevalence are located along highway and river borders where the dense population has virtually eliminated forest vegetation. The highest prevalence was observed in dispersed settlements, Campamento, and La Quince, in which most households are surrounded by tropical forest. The rank of villages with respect to prevalence remained constant during the three surveys. Cumulative prevalence of infection increased with age, reaching a plateau at 30 years of age. Prior infection was three times greater in males than females.

Incidence of infection: The incidence rate of infection was 6.6/100 person-years at risk overall and greater in the first year. In general, villages with a greater prevalence also were found to have higher incidence rates ($p=<0.1$). One exception to this correlation is the village La Quince which was recently settled, thus explaining the lack of cumulative prevalence of infection but the high acquisition at infection. The greatest rates of infection were in those between 30 and 60 years of age. Incidence was greater for males than females, RR=1.7 (8.5/4.9).

Leishmaniasis cases: More than half of the diagnosed cases of leishmaniasis demonstrated in active prior lesions at time of enrollment (Table 3). Of the prevalent cases, three were actually recurrences of cases diagnosed during Phase I. L.b. braziliensis was isolated in only two prevalent case, whereas all remaining isolates from both prevalent and incident case were characterized as L.b. panamensis. The majority of

Table 2: Participation and prevalence of leishmanin skin test (LST) reactivity during the first survey by village.

Village	Population n	Interviewed n	Interviewed %[1]	Skin tested n	Skin tested %[2]	LST reactive n	LST reactive %[3]
Inguapí del Guadual	370	357	96.5	346	96.9	40	11.6
Inguapí del Guayabo	86	86	100.0	82	95.3	7	8.5
Nueva Reforma	255	242	94.5	228	94.2	41	18.0
Campamento	33	33	100.0	33	100.0	5	15.2
Como Hacemos	14	14	100.0	10	71.4	2	20.0
La Quince	58	58	100.0	57	98.3	2	3.5
Inguapí las Tulpas	132	132	100.0	127	96.2	15	11.8
Alto Aguaclara	217	186	85.7	134	72.0	18	13.4
Descolgadero	330	286	86.7	272	95.1	4	1.5
Playón	307	225	73.3	198	88.0	1	0.5
Guayacanes	320	295	92.2	254	86.1	16	6.3
Ceibito	200	200	100.0	193	96.5	28	14.5
Km 28	335	286	85.4	273	95.5	28	10.3
Chilvicito	92	89	96.7	88	98.9	8	9.1
Inguapí del Carmen	405	369	91.1	361	97.8	31	8.6
Total	3,154	2,858	90.6	2,656	92.9	246	9.3

1 = % of population interviewed
2 = % of those interviewed whose LST was applied and read
3 = Chi^2 = 76.9, d.f.= 12, P=<0.0001

Table 3: DESCRIPTION OF DIAGNOSED CASES OF TEGUMENTARY LEISHMANIASIS

	Prevalent Cases	Incident Cases
Diagnostic Category		
Parasitologic	35	19
Clinical - lab	5	2
Immunologic	0	9
Typical	3	4
TOTAL	43	34
Parasite identification		
L. b. braziliensis	2	0
L. b. panamensis	23	14
TOTAL	25	14
Classification incident cases		
Incident lesion and infection (II)		24
Incident lesion, pre-existing infection (IP)		1
Recurrent lesion, pre-existing infection (RP)		6
Recurrent lesion, new infection (RN)		3
TOTAL		34

incident cases lacked prior lesions and were LST non-reactive at enrollment. Four of the six cases who demonstrated both prior healed lesions and LST reactivity at enrollment were actually recurrences of cases originally diagnosed during Phase I.

Distribution of cases over time: During the diagnostic evaluation of individuals presenting with new lesions, the duration of the presenting lesion was obtained. Based on the date of the diagnostic evaluation and lesion duration, the date of onset was estimated. Seasonality was not apparent in the pattern of lesion onset (Figure 3). Many of the non-diagnosed cases appeared during the largest cluster of cases 5/88-11/88 indicating that our diagnostic categories probably excluded several cases of leishmaniasis.

Incidence of leishmaniasis: Overall the incidence of diagnosed cases or leishmanias who presented with new lesions either during surveillance or yearly surveys was 4.6 cases per 1000 person years at risk, Table 4. There was a large variation by locale in the incidence rate, however, the highest overall incidence rate observed was 20 per 1000 person years. Hence, the study area should be considered to be of low to moderate endemicity. The incidence of illness was correlated with the rate of infection of each villace. (correlation coef.=0.69, p=0.004) this would indicate a relatively constant rate of pathogenecity across villages. Overall, for every 100 newly infected individuals 9.25 were diagnosed as having leishmaniasis during the first two study years. The incidence rate of leishmaniasis was relatively constant year to year. The highest incidence of leishmaniasis was observed in the ten to twenty-nine years old age group and in males (Table 5).

Relationship between pre-existing LST reactivity, and scars to risk of leishmaniasis: Risk of illness was greatest in persons who were previously infected (LST reactive) and had a typical scar at time of enrollment (Table 6). Prior to enrollment these 213 individuals had evidently suffered a clinically apparent infection. Then during the three years of surveillance six of these individuals developed a recurrence. Even for initially LST non-reactive individuals, the presence of a typical scar was associated with a three fold increase in risk. However, for persons lacking a typical scar, pre-existing LST reactivity did not confer an increased risk of leishmaniasis. These individuals had already demonstrated their ability to confront Leishmania infection without manifesting skin lesions and demonstrated an illness rate similar to that of persons without clinical immunological evidence of prior contact with Leishmania. The largest fraction of new cases of leishmaniasis developed in previously naive hosts (attributable risks = 70.6%)

Discussion

In the study area Leishmania infection was much more common than disease both at enrollment and during the longitudinal follow-up period. It is uncertain as yet whether the low pathogenecity observed was due to parasite characteristics, parasite dose or host characteristics. In other settings we and others have observed a larger fraction of the clinical manifestations in LST reactive individual. However, most prior studies were

Figure 3. Frequency of leishmaniasis by time of onset of lesions and diagnostic criteria.
I. Only parasitologically diagnosed cases
II. All disgnosed cases (categories, P, C, IM and L)
III. All evaluated cases

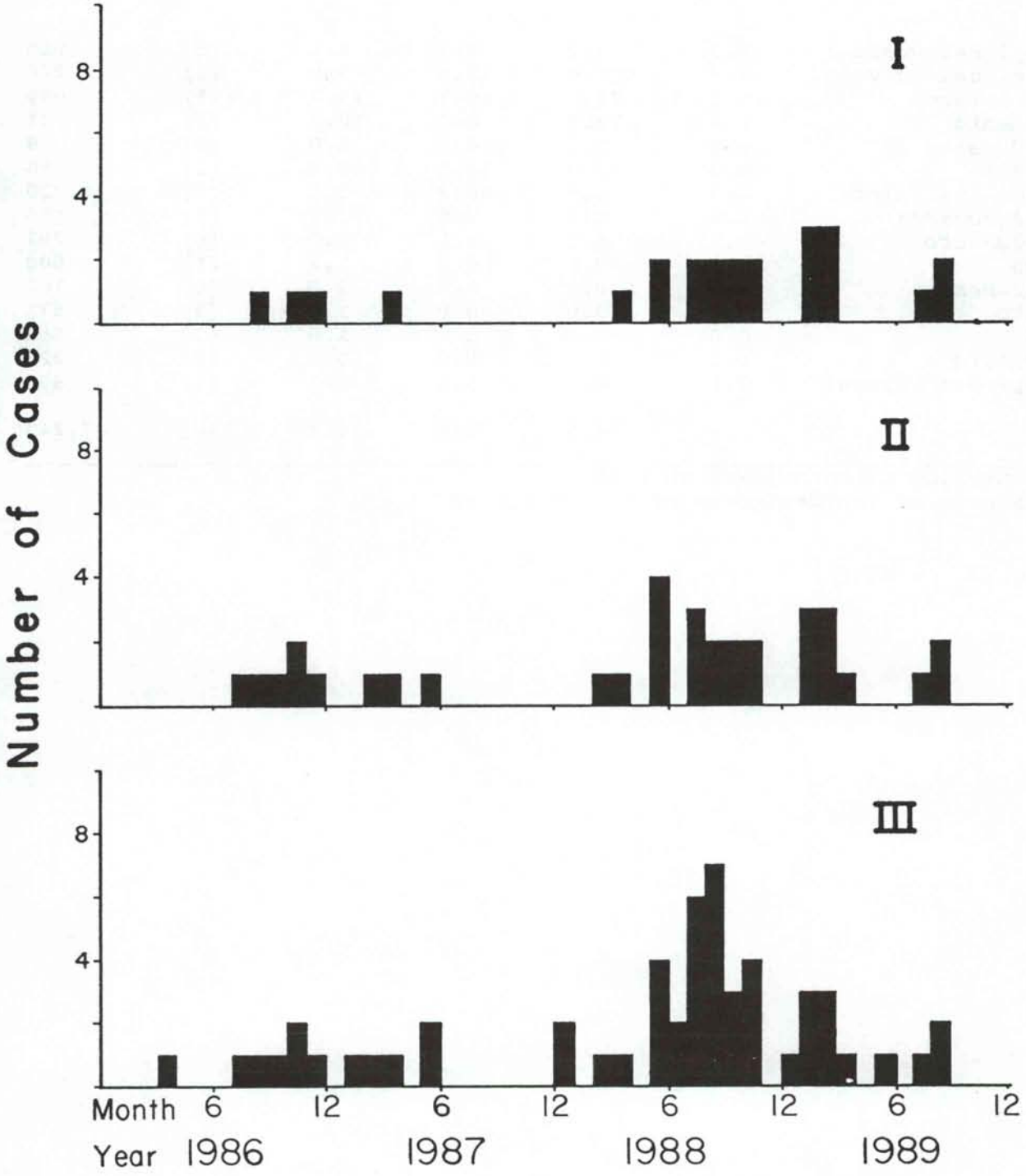


Table 4: INCIDENCE RATE OF TEGUMENTARY LEISHMANIASIS BY VILLAGE OF RESIDENCE

	Year of Observation			Total		
	1st	2nd	3rd		Cases	Person
	Rate*	Rate	Rate	Rate	(N)**	Years
Inguapi del Guadual	15.2	3.2	6.1	8.3	(8)	965
Inguapi del Guayabo	0.0	0.0	15.6	5.0	(1)	200
Nueva Reforma	9.2	21.6	16.1	15.7	(11)	699
Campamento	0.0	38.5	0.0	12.3	(1)	81
Como Hacemos	0.0	0.0	0.0	0.0	(0)	9
La Quince	0.0	37.0	34.5	20.4	(2)	98
Inguapi las Tulpas	0.0	0.0	10.2	3.1	(1)	320
Alto Aguaclara	0.0	0.0	7.0	2.2	(1)	456
Descolgadero	3.7	8.2	4.1	5.3	(4)	757
Playon	5.3	0.0	0.0	1.7	(1)	600
Guayacanes	0.0	0.0	0.0	0.0	(0)	707
Ceibito	0.0	9.6	0.0	3.5	(2)	571
Km 28	0.0	0.0	5.2	1.5	(1)	662
Chilvicito	0.0	0.0	0.0	0.0	(0)	226
Inguapi del Carmen	0.0	0.0	5.0	1.1	(1)	892
TOTAL	3.6	4.8	5.8	4.7	(34)	7,243

*Rate per 100 persons-years at risk
** = Number of incident case of leishmaniasis

Table 5: INCIDENCE RATE OF TEGUMENTARY LEISHMANIASIS BY AGE AND GENDER

	Year of observation			Total		
Age Group	1ST	2ND	3RD		Cases	Person
(Years)	Rate*	Rate	Rate	Rate	(N)**	Years
0 - 9	0.0	1.1	3.3	1.4	(4)	2782
10 - 29	5.9	9.8	10.7	8.6	(20)	2320
> 30	5.3	4.2	4.5	4.7	(10)	2141
TOTAL	3.6	4.8	5.8	4.7	(34)	7243
Gender:						
Female	0.8	4.1	2.7	2.5	(9)	3603
Male	6.2	5.6	9.0	6.9	(25)	3640

*Rate per 1000 person years at risk
** = Number of incident cases of leishmaniasis

Table 6: INCIDENCE OF LEISHMANIASIS BY INITIAL IMMUNE AND CLINICAL STATUS

Status	Cases	Person Years	Rate*	Relative Risk	Attributable Risk
Scar Present**					
LST Positive	6 (RP)***	213	28.2	7.13	17.6%
LST Negative	3 (RN)	240	12.5	3.16	8.8%
Scar Absent:					
LST Positive	1 (IP)	394	2.5	0.642	2.9%
LST Negative	24 (II)	6074	3.9	1.00	70.6%

* = Rate per 1000 person years at risk
** = Typical scar present
***= Case category

cross-sectional and thus unable to consider the dynamic and varied natural history of tegumentary leishmaniasis (19,21). In this study area a considerable fraction of incident cases of leishmaniasis (as many as 26%) may not be preventable by measures that attempt to reduce infections, because these individuals are already infected. Furthermore, the number of prevalent active cases exceeded the number of incident cases observed over three years, illustrating that for this chronic, recurrent infection, control programs must address both the infections and the cases that have accumulated over the preceding decades. In more highly endemic settings where up to 80% of the population are already infected, disease reduction programs may need to examine chemotherapy or immunotherapy to control manifestations in previously infected persons.

Given the low overall incidence rates observed in this study (6.6 and 0.47 per 100 person-years respectively for infection and disease) community-based primary prevention programs may not be warranted. Rather, secondary prevention programs should control the progression of clinically manifest lesions and primary prevention should be targeted toward individuals at risk.

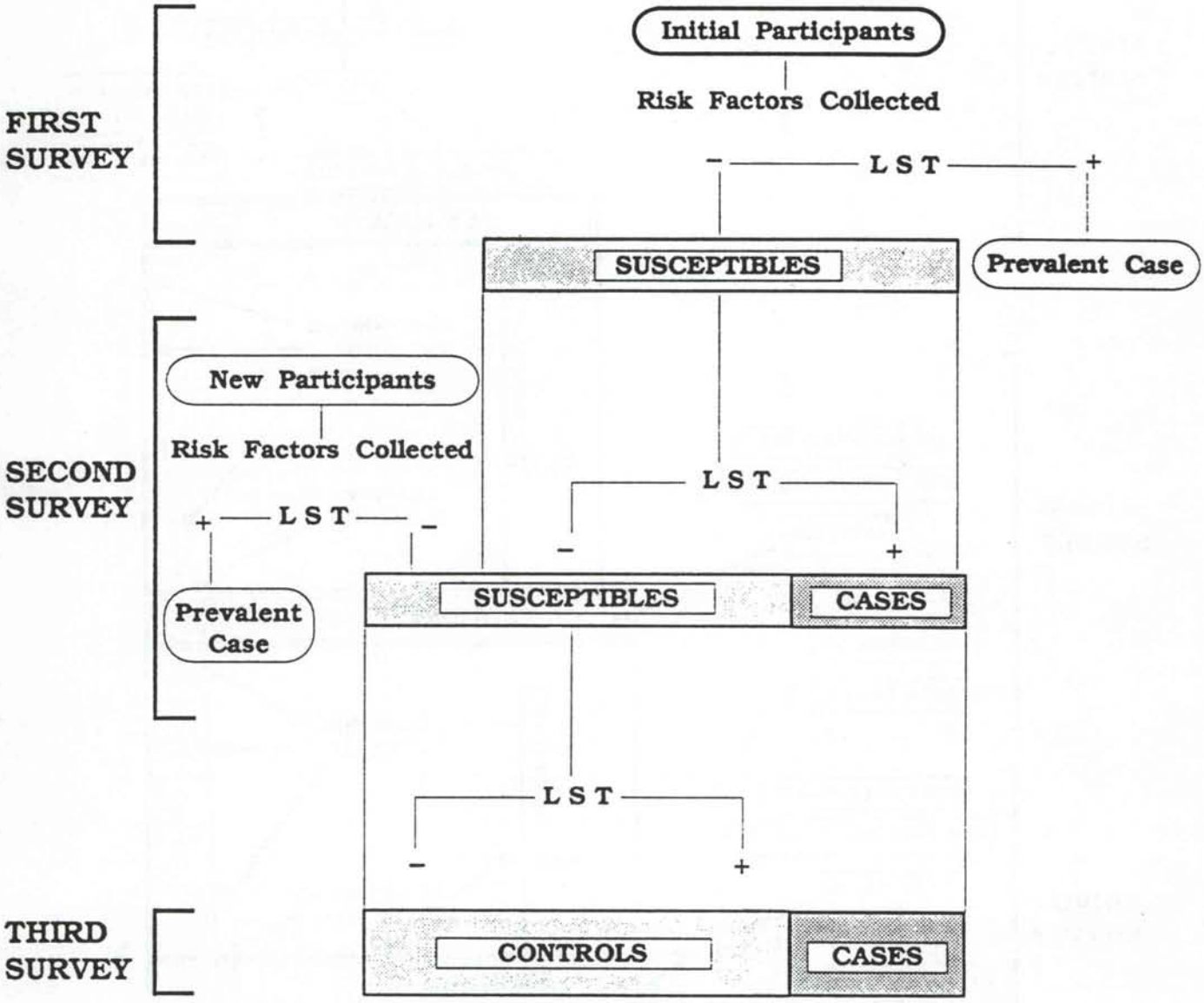
II. Risk Factors for leishmanis Infection and Disease

Cross sectional studies and case series have indicated that populations with frequent contact with the habitat of infected sandflies are at an increase risk of ATL. Such studies have focused primarily upon adult men and occupational groups. After a review of existing literature and a pilot-study we sought to examine risk factors that might be associated with intradomestic, peridomestic, and occupational tranmission. Based on our pilot study and reported cases we believed that transmission in the study area was primarily peri-domestic and extra-domestic. However, we also examined risk factors related to intra-domestic transmission. Conduct of a population based study permitted the examination of multiple transmission sites. We chose a prospective design in order to avoid recall bias and examine separately risk factors for infections and risk factors disease. The analysis strategy was designed to estimate the size of the increased risk conferred by a particular factor. Because the number of non-infected individuals were so numerous, and the rates of infection and ATL relatively low, a nested case-control analysis proved most efficient.

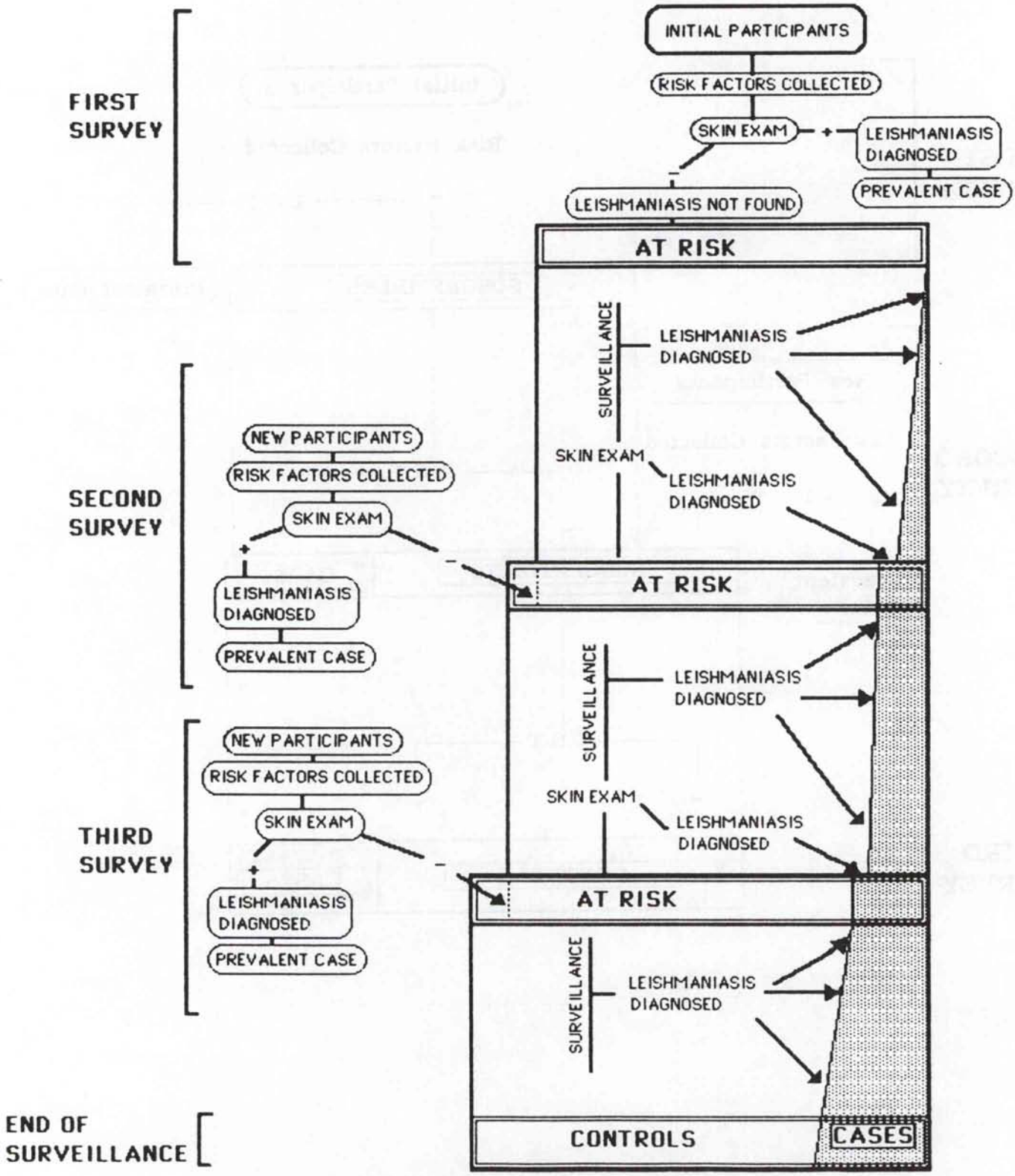
Methods

Study Design and Definitions: Cases of Leishmania infection and disease and their respective controls were drawn from the longitudinal study described above. Essentially two cohorts were defined, one for evaluation of risk factors for infection and another for risk factors for disease, as described in Figures 4 and 5. The evaluation of risk factors for infection was restricted to persons initially LST negative at the time of enrollment and who participated in two or three of the sequential surveys. Cases of infection were defined as persons who converted from an initially negative LST reaction to a positive LST during a later survey or diagnostic evaluation. Controls were randomly

STUDY DESIGN RISK FACTORS FOR INFECTION



STUDY DESIGN
RISK FACTORS FOR TEGUMENTARY LEISHMANIASIS



chosen from those who remained skin test negative, matched with a case in the village of residence and year of skin test conversion. The cohort defined for the evaluation of risk factors for clinically apparent leishmaniasis excluded the 43 prevalent cases of leishmaniasis that were diagnosed during the enrollment survey. Incident cases of leishmaniasis, defined as described in the previous methods section, could meet either parasitological, clinical-lab, immunological, or likely diagnostic categories. Criteria for controls were: 1) Active diagnosis i.e., typical or suspect leishmanial lesions were not observed during the three serial physical exams nor during active surveillance; 2) They were skin tested during at least two of the three surveys and remained skin test negative; 3) Residence in the same village as the case; 4) Skin examination undertaken before and after the date on which the case was diagnosed. Three unique controls were randomly chosen for each clinically apparent case based on the preceding criteria. In total 89 participants were excluded from the control group because they presented typical Leishmania lesions that were not diagnostically confirmed.

Data collection: Instruments were developed, and pilot tested prior to the first survey. First, recognized and likely risk factors were listed. The study was discussed with community leaders and former leishmaniasis patients in the study area, to ascertain the daily habits of the residents and become familiar with local terms and phraseology regarding risk factors of interest. The questionnaire was prepared in Spanish and revised by repeated testing with native Colombians in Cali. Three instruments were employed to collect risk factor information:

1) The Individual Questionnaire concerning individual characteristics and behaviors was answered in person by each family member; mothers or immediate caretakers responded for minors.

2) A Household Questionnaire obtained information from the head of the household regarding risk factors common to the household. The head of household was defined as the person responsible for the economic support of the household. These two questionnaires were completed through personal interviews.

3) After observing and measuring the ecological and physical characteristics of the residence and its surrounding, this information was recorded on the Household Observation Form. The Household Questionnaire and Household Observation Form were completed during subsequent surveys for individuals who had moved to a new residence.

Data Management and Statistical Analysis: Data was entered using a defined screen format which included range checks and double entry features (Data Entry/SPSS). Frequency distributions of each potential risk factor were examined for cases and controls combined. Categories and recoding of variables were based on logical categories or distributions. Variables lacking sufficient variability were excluded from subsequent analyses. Initially, cases were compared to controls, by comparing frequencies of

categorical variables (Chi^2 test) or means of continuous variables (Student's T Test). The strength of the association between a risk factor and either Leishmania infection or disease was estimated by the odds ratio (OR) = odds of being a case given risk factor is present/odds of being a case given risk factor is absent. Variables likely to be associated with the case control status ($P < .15$) were evaluated in logistic regression models, which controlled for age and sex. To determine the amount of risk incurred beyond that related to farming, these likely risk factors were also assessed in models adjusted for sex, age, and farming occupation. Because the association between age and infection risk was not linear, age was included as a categorical variable for the adjusted analyses. Prevalence of risk factors was approximated by the distribution of the factor in the control group.

Results

Risk factors for infection: Table 7 demonstrates that older age, male gender, and years of residence were associated with leishmania infection. No association was observed between race and infection, however insufficient variability existed to evaluate race as a risk factor. Farming was a very prevalent risk factor; involving 33.5% of the controls. Overall being a farmer implied a nine fold increase in risk of infection (Table 8) and a 2.41 fold increase in risk compared to non-farmers of the same sex and age group (Table 11). A marked dose effect was observed between number of days of farming per week and infection. Certain farming activities appear to augment the risk associated with farming; in particular farmers who prepared for cultivation by clearing land of trees and brush incurred a greater risk of infection than farmers who did not clear land, O.R. = 13.36 versus 6.92, respectively. Although hunting was associated with infection (Crude O.R. = 4.3, adjusted O.R. = 2.91) this activity is infrequent; 6.2% of controls reported having hunted or accompanied someone hunting during the 12 months preceding enrollment. Lumbering was also an infrequent occupation, 3.5% of controls reported this exposure which is associated with Leishmania infection, (Crude O.R. = 4.66, adjusted OR = 2.36). Fishing activities were reported by 12.8% of controls and was associated with Leishmania infection to a moderate extent (crude O.R. = 2.36, adjusted O.R. = 1.56). Forest activities were frequently reported by both cases and controls and most were strongly associated with infection (Table 9). The factors displayed in Table 9 concern entering or passing through the forest for any reason, whereas specific reasons for entering the forest are evaluated in Table 10. For each additional hour spent in the forest the risk was increased by 1.26 (adjusted O.R., Table 11). Also being in the forest during non-daylight hours was associated with a marked increase in the risk of infection. (Entering forest after sunset, crude O.R. = 44.3; adjusted O.R. = 13.27). Residents of the study area entered the forest for many reasons besides farming, hunting, fishing, and lumbering (Table 10). Collection of wood and fruits increased the risk of infection associated with entering the forest but other forest related activities had no effect on the amount of risk associated with entering the forest. In some localities homes were surrounded by the forest such that all travel away from the homestead involved walking through the forest. For these individuals forest exposures were not separable. Adjusted

Table 7: RELATIONSHIP BETWEEN SOCIODEMOGRAPHIC CHARACTERISTICS AND L. braziliensis ssp INFECTION*

CHARACTERISTICS	CASES N=227	%	CONTROLS N=227	%	UNADJUSTED O.R.	(95%C.I.)
Age (years)[1]						
<10.0 - No. (%)	15	(7)	109	(48)	1	
10.0 - 29.9	82	(36)	76	(33)	7.8	(4.2-16)
≥30	130	(57)	42	(18)	22	(11.8-43)
Age (years)[1]	35.8 ± 19.9		16.9 ± 18.7		N.A.	N.A.
Male Sex - No. (%)[2]	138	(61)	107	(47)	1.7	(1.20-2.5)
Race						
Black or Indian - No.(%)	213	(94)	215	(95)	1.0	
Mixed and Caucasina-No.(%)	14	(6)	12	(5)	1.2	
Length of residence[1] in study area (years)						
<1 No. (%)	19	(8)	34	(15)		
1 - 5.9	40	(18)	83	(37)		
6 - 10.9	32	(14)	46	(20)		
11- 20.9	57	(25)	32	(14)		
> 20	79	(35)	30	(13)		
unknown	0	(0)	2	(1)		
Length of residence[1] in study area (years)	4.4 + 5.4		3.4 + 6.4			

± = Indicates standard deviation
1 = P<0.001
2 = P<0.01
* = Frequency distribution compared by Chi^2 test, means compared by student's T test

Table 8: RELATIONSHIP BETWEEN FARMING ACTIVITIES AND L. braziliensis ssp INFECTION*

ACTIVITY	CASES N= 227 No.	(%)	CONTROLS N=227 No.	(%)	Unadjusted O.R.
Farming occupation: Yes[1]	187	(82)	76	(33)	9.3[2]
No**	40	(18)	151	(66)	1.0
Cleared land of trees in last year[1]	117	(51)	33	(14)	13.4
Did not clear land of tree in last year	70	(31)	42	(18)	6.9
Planted cocoa in last year[1]	109	(48)	34	(15)	12.1
Did not plant cocoa in last year	77	(34)	42	(18)	6.9
Planted crop other than cocoa[1]	156	(69)	55	(24)	10.7
Only plated cocoa	31	(14)	21	(9)	5.4
Worked on additional farms[1]	142	(63)	58	(26)	10.1
Only worked own farm	43	(19)	16	(7)	9.2
Went to a farm in last year[1]	218	(96)	156	(69)	11.0[3]
Did not go to a farm in last year	9	(4)	71	(31)	1.0
Goes to the farm every day[1]	17	(7)	1	(0)	134.1
4-6 days per week	93	(41)	42	(18)	17.5
1-3 days per week	85	(37)	72	(32)	9.3
less than weekly	23	(10)	41	(18)	4.4
Never in last year	9	(4)	71	(31)	1.0

* = Frequency distributions compared by Chi^2 test
** = Reference category for farming activities
1 = P<0.001
2 = 95% C.I. (OR) = 5.99 - 14.41
3 = 95% C.I. (OR) = 5.14 - 24.41

Table 9: RELATIONSHIP BETWEEN ENTERING FOREST AND L. braziliensis ssp INFECTION

ACTIVITY***	CASES N= 227 No.	(%)	CONTROLS N=227 No.	(%)	Unadjusted O.R.
Entered forest frequently[1]	190	(84)	89	(39)	33.4
infrequently	34	(15)	91	(40)	5.8
never**	3	(1)	47	(21)	1.0
Hours per day in the forest: >7[1]	107	(47)	22	(10)	76.2
5 - 7.0	60	(26)	50	(22)	18.8
0.1 - 4.9	57	(25)	108	(48)	8.3
Entered forest before dawn[1]	38	(17)	7	(3)	85.0
Entered forest but not before dawn	186	(82)	173	(76)	16.8
Entered forest after sunset[1]	79	(35)	26	(11)	47.6[2]
Entered forest but not after sunset	145	(64)	154	(68)	14.7[3]
Entered forest at night[1]	17	(7)	6	(3)	44.4
Entered forest but not at night	207	(91)	174	(77)	18.6
Hours per day in the forest[1]	6.2 + 2.4		4.0 + 2.3		1.5[4]

* = Frequency distributions compared by Chi^2 test, means compared by student's test
** = Reference category for forest activities
*** = Reported for prior 12 months
1 = P<0.001
2 = 95% C.I. (OR) = 13.66 - 165.90
3 = 95% C.I. (OR) = 4.49 - 48.44
4 = O.R. per hour in forest, 95% C.I. = 1.36-1.60

Table 10: RELATIONSHIP BETWEEN SPECIFIC FOREST ACTIVITIES AND L. braziliensis ssp INFECTION*

ACTIVITY**	CASES N= 227 No.	(%)	CONTROLS N=227 No.	(%)	Unadjusted O.R.
Entered forest to obtain water[1]	91	(40)	69	(30)	20.7
Entered forest, but not to obtain water	133	(59)	111	(49)	18.8
Did not enter the forest***	3	(1)	47	(21)	1.0
Entered forest to collect wood[1]	164	(72)	79	(35)	32.5
Entered forest but not to collect wood	60	(26)	101	(44)	9.3
Entered forest to gather fruits	52	(23)	28	(12)	29.1
Entered forest but not to gather fruits	172	(76)	152	(67)	17.7
Entered forest to visit relatives or friends[1]	127	(56)	100	(44)	19.9
Entered forest, but not to visit relatives or friends	96	(43)	80	(35)	13.5
Entered forest to travel to another town[1]	116	(51)	77	(40)	23.6
Entered forest but not to travel to another town	108	(48)	103	(45)	16.4
Entered forest to travel to school[1]	19	(8)	10	(4)	29.8
Entered forest but not to travel to school	205	(90)	170	(75)	18.9
Entered forest to defecate	87	(38)	68	(30)	20.0
Entered forest but not to defecate	137	(60)	112	(49)	19.2
Entered forest to bathe	50	(22)	41	(18)	19.1
Entered forest but not to bathe	174	(77)	139	(61)	19.6

* = Frequency distributions compared by Chi_2 test
** = Activity during preceeding 12 months
*** = Reference category
1 = P<0.001

Table 11: Relationship between selected individual characteristics and behaviors and L. braziliensis infection, after adjustment.

Variable	Age and sex adjusted O.R.	(95% CI)	Age, sex, farming adjusted O.R.	(95% CI)
Male sex	1.9[1]	(1.2 - 2.9)	1.3	(0.8 - 2.2)
Age (years):				
< 10.0	1.0[1]		1.0[1]	
10 - 29.9	7.9	(4.2 - 14.9)	4.5	(2.2 - 9.2)
> 30.0	23.3	(12.2 - 44.7)	9.3	(4.0 - 21.7)
Farming occupation	2.8[1]	(1.5 - 5.2)	2.8[1]	(1.5 - 5.2)
Hunting*	2.4[3]	(1.2 - 4.9)	2.0	(1.0 - 4.1)
Lumbering*	2.4	(1.0 - 5.7)	2.0	(0.8 - 4.7)
Fishing*	1.6	(1.0 - 2.7)	1.4	(0.8 - 2.4)
Hours per day in forest*	1.3[3]	(1.1 - 1.4)	1.2[1]	(0.4 - 3.6)
Entered forest after sunset	13.3[1]	(3.4 - 51.2)	11.5[1]	(2.8 - 44.8)
Entered forest, but not after sunset	6.8	(1.9 - 23.3)	6.8	(1.9 - 24.4)

* = Activity reported for preceding 12 months.
1 = P < .001.
2 = P < .01.
3 = P < .05.

Table 12: Relationship between ecological, environmental conditions, and *L. braziliensis* infection*.

Condition	Cases N = 227 No.	%	Controls N = 227 No.	%	Unadjusted O.R. (95% CI)
Tall trees within 10m of residence[3]	129	(57)	104	(46)	1.6 (1.1 - 2.2)
Trees with exposed roots within 10m of residence[3]	76	(33)	53	(23)	1.6 (1.1 - 2.5)
Tall trees, surrounding farm (yes)[3] and visited farm in last 12 months	170	(78)	134	(59)	2.1 (1.4 - 3.1)
No swamp within 10m of residence[3]	171	(75)	152	(67)	1.5 (1.0 - 2.3)
Distance from next house > 15m[3]	115	(51)	92	(41)	1.5 (1.0 - 2.1)
Distance from next house (meters)**	37.8 ± 103		31 ± 32		N.A.
Distance of floor above ground = 0.3-1m (reference < .3m or > 1)[2]	133	(59)	100	(44)	1.8 (1.2 - 2.6)
Distance of floor above ground (meters)	0.6 ± .41		0.6 ± .48		N.A.
Wall construction:					
Wood, bricks, or zinc	87	(38)	100	(49)	1.0
Wood and bamboo	33	(14)	33	(14)	1.1
Bamboo or thatch	107	(47)	94	(41)	1.3
Floor material:					
Dirt, cement, or wood	185	(81)	198	(87)	1.0
Palm, bamboo, other open	42	(18)	29	(13)	1.5 (0.9 - 2.6)
Roof material:					
Tile, zinc, carton, closed	117	(52)	147	(65)	1.0
Thatch	109	(48)	78	(35)	1.8 (1.2 - 2.6)

* = Frequency distributions compared by Chi^2 test, means by Students t-test.
** = 1. $P < .001$.
2 = $P < .01$.
3 = $P < .05$.

Table 13: Relationship between ecological, environmental conditions and *L. braziliensis* infection, after adjustment.

Variable	Age and sex adjusted O.R.	(95% CI)	Age, sex, farming adjusted O.R.	(95% CI)
Tall tree within 10 m of residence	1.7[2]	(1.1 - 2.6)	1.7	(1.1 -2.6)
Trees with exposed roots within 10 m of residence	1.7[2]	(1.1 - 2.8)	1.7	(1.0 - 2.7)
Tall trees surrounding farm	0.8	(0.4 - 1.3)	0.7	(0.4 - 1.3)
No swamp within 10 m of residence	1.6	(1.0 - 2.6)	1.6[2]	(1.0 - 2.7)
Distance from next house > 15 m)	1.5	(0.9 - 2.3)	1.5	(0.9 - 2.3)
Distance of floor above ground 0.3-1.0 m	1.6[2]	(1.0 - 2.5)	1.6	(1.0 - 2.5)
Floors bamboo, palm, open	1.2	(0.6 - 2.1)	1.1	(0.6 - 2.1)
Roof thatch, palm leaves	2.0[1]	(1.3 - 3.1)	2.0[1]	(1.3 - 3.2)

1 = P < .01.
2 = P < .05.

Table 14: RELATIONSHIP BETWEEN SOCIODEMOGRAPHIS CHARACTERISTICS AND TEGUMENTARY LEISHMANIASIS

Characteristics	CASES N = 34 No.	(%)	CONTROLS** N = 102 No.	(%)	Unadjusted O.R.
Age (Years)[1]					
<10.0	4	(11.8)	48	(47.1)	1.00
10.0 - 29.9	20	(58.8)	35	(34.3)	6.86
> 30	10	(29.4)	19	(18.6)	6.32
Age (Years)[3] mean ± s.d.[1]	23.6 ± 17.1		16.3 ± 17.5		
Male Sex[3]	25	(73.5)	55	(53.9)	2.57
Race					
Black or Indian	31	(91.2)	99	(97.1)	1.00
Mixed of Caucasian	3	(8.8)	3	((2.9)	3.10
Length of residence (years) In study area	14.3 ± 14.9		9.3 ± 12.2		N.A.

* = Frequency distributions compared by Chi^2 test means by student's test
** = lacked infection and any suspect lesions
1 = P<0.001
2 = P<0.01
3 = P<0.05

Table 15: RELATIONSHIP BETWEEN OCCUPATIONAL AND BEHAVIORAL FACTORS AND TEGUMENTARY LEISHMANIASIS*

FACTOR		CASES N = 34 No.	(%)	CONTROLS N = 102 No.	(%)	UNADJUSTED O.R.
Farming occupation	yes[1]	27	(79)	35	(34)	7.4
	no	7	(21)	67	(66)	1.0
Hunting***	yes	5	(15)	14	(14)	1.1
	no	29	(85)	88	(86)	1.0
Lumbering***	yes[3]	5	(15)	3	(3)	5.7
	no	29	(85)	99	(97)	1.0
Fishing***	yes[3]	11	(32)	15	(15)	2.8
	no	23	(68)	87	(85)	1.0
Hours per day in forest***[1] mean ± s.d.		59 + 2.4		4.3 + 2.5		N.A.
Entered forest after sunset***[2]		11	(32)	15	(15)	24.9
Entered forest but not after sunset		23	(68)	70	(69)	11.2
Dented entering forest		0	(0)	17	(17)	1.0
Visited a farm***	yes2	32	(94)	73	(72)	6.4
	no	2	(6)	29	(28)	1.0

* = Frequency distributions compared by Chi2 test, means by Student's test
** = lacked infection and any suspect lesions
*** = Activity reported for preceding 12 months
1 = P<0.001
2 = P<0.01
3 = P<0.05

Table 16: RISK FACTORS FOR PATHOGENECITY

VARIABLE	DISEASE* N = 27 No.	(%)	INFECTED** N = 207 No.	(%)	UNADJUSTED ODDS RATIO
Highly endemic village	13	48.1	81	39.1	1.52
Moderately endemic village	8	29.6	69	33.3	1.10
Low endemic village	6	22.2	57	27.5	1.00
Age (Years)[1]					
<10	4	14.8	15	7.2	4.57
10.0 - 29.9	16	59.3	72	34.8	3.81
> 30	7	25.9	120	58.0	1.00
Age (Years) mean ± s.d.	22.3 + 16.3		36.25 + 20.3		
Farming occupation					
yes	20	74.1	168	81.2	0.66
no	7	25.9	39	18.8	1.00
Went to a farming last year					
yes	25	92.6	199	96.1	0.50
no	2	7.4	8	3.9	1.00
Hunting in last 12 months					
yes	4	14.8	48	23.2	0.58
no	23	82.5	159	76.8	1.00
Fishing in last 12 months					
yes	9	33.3	49	23.7	1.61
no	18	66.7	158	76.3	1.00
Lumbering in last 12 months					
yes	5	18.5	26	12.6	1.58
no	22	81.5	181	87.4	1.00
Goes to farm every day	2	7.4	17	8.2	2.71
Goes to farm 1-6 days per week	22	81.5	159	76.8	3.18
Goes to farm less than weekly	1	3.7	23	11.1	1.00
Cleared land of trees					
yes	14	51.9	102	49.3	0.76
no	6	22.2	66	31.9	0.51
Doesn't farm	7	25.9	39	18.8	1.00

* = Diagnosed cases of leishmaniasis initial. LST negative
** = Cases of infections without leishmaniasis of active lesions
[1] = $p < 0.01$

analyses for sociodemographic and occupational risk factors are displayed in Table 14. In general, associations observed by crude analyses persisted after adjustment for age and sex, although the strength of associations decreased. In addition, we adjusted by sex, age and farming occupation but found that controlling for farming changed the O.R. for various other occupations and behaviors very little. Hence, it appears that risks were conferred by activities such as entering the forest were independent of farming status.

Evaluation of risk factors obtained from the Household Questionnaire and Household Observation Form are summarized in Tables 12 and 13. Much of the information and the associations obtained through the household questionnaire duplicated that observed using the individual questionnaire. The importance of farming as a risk factor for infection was verified. However, most characteristics of the families' farm such as crops grown, size of farm and ecological conditions surrounding the farm were not associated with an increased risk of infection. In these analyses all persons were assigned the exposure characteristic of their household farm regardless of whether the individuals actually participated in agricultural activities, thereby misclassifying actual exposure. Also, insufficient variability was present to assess the effect of many exposures. The presence of large trees, or trees with exposed roots nearby residences increased risk of infection moderately.

Risk Factors for Tegumentary Leishmaniasis: Risk factors for leishmanial lesions resembled those observed for infection, Tables 14 and 15. Due to the smaller number of cases, estimates of the risk asssociated with infrequent activities, such as hunting and lumbering, are imprecise. No factors were associated with disease that had not been associated with infection.

To determine whether there were any risk factors associated with pathogenicity, we utilized an additional case control design. Cases of leishmaniasis who were initially LST negative at enrollment were compared to "controls" consisting of persons who LST converted without developing clinical manifestations. To avoid misclassifications, persons with any suspect of diagnosed skin lesion were excluded from these controls. Young age appeared to be associated with an increased pathogenicity (Table 16). This parallels the observation that the maximal incidence of infection was between 40 and 60 years of age whereas the highest illness rate was observed between 10 and 29 years of age. Gender did not impact on pathogenicity, nor did environmental and behavioral factors related to vector contact.

Discussion

The major risk factors for both Leishmanis infection and disease and occupations are behaviors that presumably increase contact with the forest habitat of Leishmania laden sandflies. The increased risk associated with increasing age and male gender are consistent with finding of others (15,18,19) and are probably due to the occupations, farming, hunting, etc. that predominate in males and adulthood. A clear dose response

was observed with hours spent in the forest. In contrast to observations in Guatemala (19) we observed that activities that lead to presence in the forest after nightfall were especially hazardous. These findings indicate the types of behavioral intervention that may be most appropriate to this setting. Although the most common risk factor, farming, is not amenable to intervention, other activities, such as hunting and fishing, could be curtailed. Avoidance of the forest during night could be encouraged and may also impact on other vector-borne diseases such as malaria. Complete avoidance of the forest is obviously impossible in these communities due to the multiple forest related activities encountered. However, the dose response relationship that we observed would predict that even a reduction in forest activities would prove beneficial.

The second application of the findings is to identify the individuals for whom both behavioral and vector-control measures would prove most beneficial. Clearly adult male agricultural workers who spend long hours in the forest and need to enter the forest after nightfall could be targets for self-protection measures such as repellents, repellent soaps, or repellent clothing (3). The indicates for insecticides in less clear. Nearly all homes report insecticide spraying, so we could not determine whether insecticides reduced domestic transmission. Insecticide spraying and forest clearing in the periphery of farms may also reduce risk associated with farming. The observation that similar risk factors were found for infection and disease may indicate that the host is the major determinant of pathogenecity. This hypothesis is supported by the increased pathogenecity observed in younger individuals.

Summary - Implications for Control

Studies of human L. braziliensis ssp infection in the municipality of Tumaco, Narino have yielded a wealth of information about the natural history of leishmaniasis caused by this species complex, and the methodologic bases for similar studies in other foci of transmission. Incidence, prevalence and risk factors for infection and disease were defined for the inhabitants of 15 villages within a focus of transmission of primarily L.b. panamensis, and L.b. braziliensis. Such information on disease burden is essential to the development and implementation of strategies for the reduction and prevention of leishmaniasis.

Among the communities studied, prevalent infection and disease were the source of a large portion of the morbidity recorded over the three years of observation. Thus, many incident cases of leishmaniasis derived from already infected individuals. Infection was more common than disease with an overall ratio fo 10:1. Prevalent disease exceeded incident disease over the three years of observation, reflecting the accumulation of both infection and disease over years past. The incidence of illness was correlated with the rate of infection in each village, indicating a relatively constant rate of pathogenicity throughout the study area. In foci such as these, secondary prevention of the progression of disease and infection to disease, among the individuals at risk, is an important consideration in reducing the disease burden. Chemotherapy and immunotherapy might

be indicated, particularly in more highly endemic settings, in order to control disease manifestations in previously infected indivdiuals.

Risk factors for infection and disease were found to be the same: occupational and/or behavioral exposure to the forest; male gender; and increasing age up to 30 years, at which time risk reached a plateau. Risk associated with contact with the forest was clearly dose dependent, increasing with the number of hours spent in the forest. Activities undertaken in the forest after nightfall were particularly hazardous. Pathogenicity was greatest among younder individuals with the highest incidence rate for disease being observed between 10 and 29 years of age. In this or similar foci, potential preventive interventions include reduction of exposure to the forest especially nighttime incursion, use of some form of repellent by male agricultural workers, and clearing of the forest around farms.

Based on these observations, reports of others, and WHO recommendations it appears that control strategies must be tailored to the epidemiology of ATL in a given community. Three major considerations are 1) the relative role of new infections or pre-existing infections in disease burden; 2) Site (S) of transmission; 3) High risk populations. Where numerous chronic or recurrent cases are found attention to efficient surveillance, diagnosis and case management is appropriate. Where intra-domestic transmission occurs, insecticide impregnated curtains or bednets may reduce transmission. Peridomestic transmission could be reduced by resevoir control, perimeter clearing and possibly insecticide spraying. Where risk is concentrated in agricultural settings personal protection measures, perimeter spraying or clearing and education regarding riskier circumstances should be evaluated. For high risk occupational groups such as road construction workers or lumbermen personal protection and risk reduction education may prove beneficial. None of the above mentioned measures have been evaluated sufficiently to warrant their wide adaptation. However pilot intervention trials that measure efficacy, cost to benefit ratio and community acceptance are needed. This project has developed methodologies and an awareness of the design issues inherent in such trials.

The selection of communities for intervention trials is quite challenging. Community interest and collaboration, a pre-exisiting primary care infrastructure and community leadership and cohesion could facilitate such trials. The rates of end points to be impacted upon, such as sandfly biting, leishmania infection, and leishmaniasis cases must be high, otherwise sample size and the length of the trial will be prohibitive. Logistical consideration and the support of local health care providers are integral to planning such trials. The observation that incidence rates are highest in sparse settled forested areas indicates logistical planning is crucial to such trials.

Another important consideration for these intervention trials is the selection of an appropriate design. Clearly the results of randomized trials are often less ambigous than those of a before and after study or interventions in "matched" settings. Quasi-

experimental designs may falter if year-to-year rates of leishmania transmission are quite variable. The unit of randomization, such as community, household or individual must also be considered carefully. Each unit has inherent advantages and disadvantages.

Because we and others (15,18,19) have observed relatively low incidence rates of ATL, the use intermediate endpoints of interventions such as sandfly counts, biting counts, and LST conversion may provide more rapid answers to comparisons of intervention methods. Once interventions are demonstrated to effect intermediate endpoint, study of their impact on new cases of ATL would be appropriate.

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