LEISHMANIASIS CONTROL STRATEGIES

A CRITICAL EVALUATION OF IDRC-SUPPORTED RESEARCH

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Leishmaniasis control strategies

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

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Community and Environmental Risk Factors Associated with Cutaneous Leishmaniasis in Montebello, Antioquia, Colombia

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Introduction

The establishment of measures to control zoonotic diseases such as leishmaniasis requires understanding of the mechanism of transmission within the natural focus of infection. The identification of the parasite species present in the endemic focus, the vector species and reservoir animals are the three basic elements of transmission. Studies of vector population dynamics, which determine the focus of the endemic zone, are of fundamental interest (1).

Delimiting the focus, identifying the population at greatest risk of becoming ill, determining the time of year when infection is most likely to occur and the location, with respect to human dwellings, where humans come into contact with infected vectors, are points that must be addressed to design rational measures of epidemiological surveillance and control.

Pioneering studies such as those of Professor J.A. Rioux have adapted the ecoepidemiological method of analysis for the calculation of risk of contracting leishmaniasis in endemic foci in the Old World (2).

In Latin America, the study of leishmaniasis is complicated by the large number of species of the parasite that affect humans, the variety of species of phlebotomine sandflies and reservoirs, and the diversity of habitats where parasites are endemic, ranging from Amazon forests to deserts, from sea level to Andean mountain valleys and chains, and from rural zones to urban areas in some cities (3,4,5).

The present study of an endemic focus of cutaneous leishmaniasis caused by Leishmania braziliensis was carried out in Montebello, Colombia, a small town in the Andes Mountains. Our goal was to determine the epidemiological risk of contracting leishmaniasis within this focus.

The main objective of the study was to establish the biological basis of the modes of transmission of cutaneous leishmaniasis in Montebello, by determining the prevalence of the disease, identifying the species of parasite, the vectors and reservoirs present, and relating these findings to ecological characteristics of the region.

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Materials and Methods

Description of the zone studied

Montebello is a town located 50 km from the city of Medellin, Colombia. Data were collected from the surrounding rural area from August 1986 to January 1989. Montebello is located at 5°, 57' north and 75°, 32' west on the west slopes of the Andes Mountains, at an elevation ranging from 800 to 2200 metres above sea level (m.a.s.l.) (Fig. 1).

The region has been inhabited since the beginning of the nineteenth century by farmers; the main cash crops are coffee and fruit. The current population of the rural area is 2,101 inhabitants.

In 1984 there was an outbreak of cutaneous leishmaniasis caused by L. braziliensis in Campoalegre and La Merced boroughs on the outskirts of Montebello (6). The epidemic followed the arrival, in June 1983, of two persons with a total of 11 active cutaneous leishmaniasis lesions between them. Two months later, the first cases were identified in persons who had not travelled beyond these boroughs. In subsequent months there was an exponential increase in the number of cases, which had reached 56 by January 1984 (26% of the population in these boroughs). Patients ranged in age from 8 months to 69 years; 32 (57%) were males, and 24 (43%) females. The greatest number of cases was observed in patients aged 15 to 44 years, however, the highest incidence (number of cases per age group) was found in children younger than 4 years (43% and 50% in males and females respectively). Treatment with antimony derivatives brought the epidemic under control. Since 1984, sporadic cases have been reported to the Colombian health service (7).

Study population

To calculate disease prevalence we actively searched for cases through the study period. Lesions compatible with cutaneous leishmaniasis were sampled with a smear, and the exudate was cultured on NNN medium; in addition, biopsies were taken from the margin of the lesion. Indirect immunofluorescence and Montenegro tests were performed.

To determine the degree of human contact with the parasite, we administered the Montenegro test via automatic syringe (Dermojet) to a population of schoolchildren in the study zone.

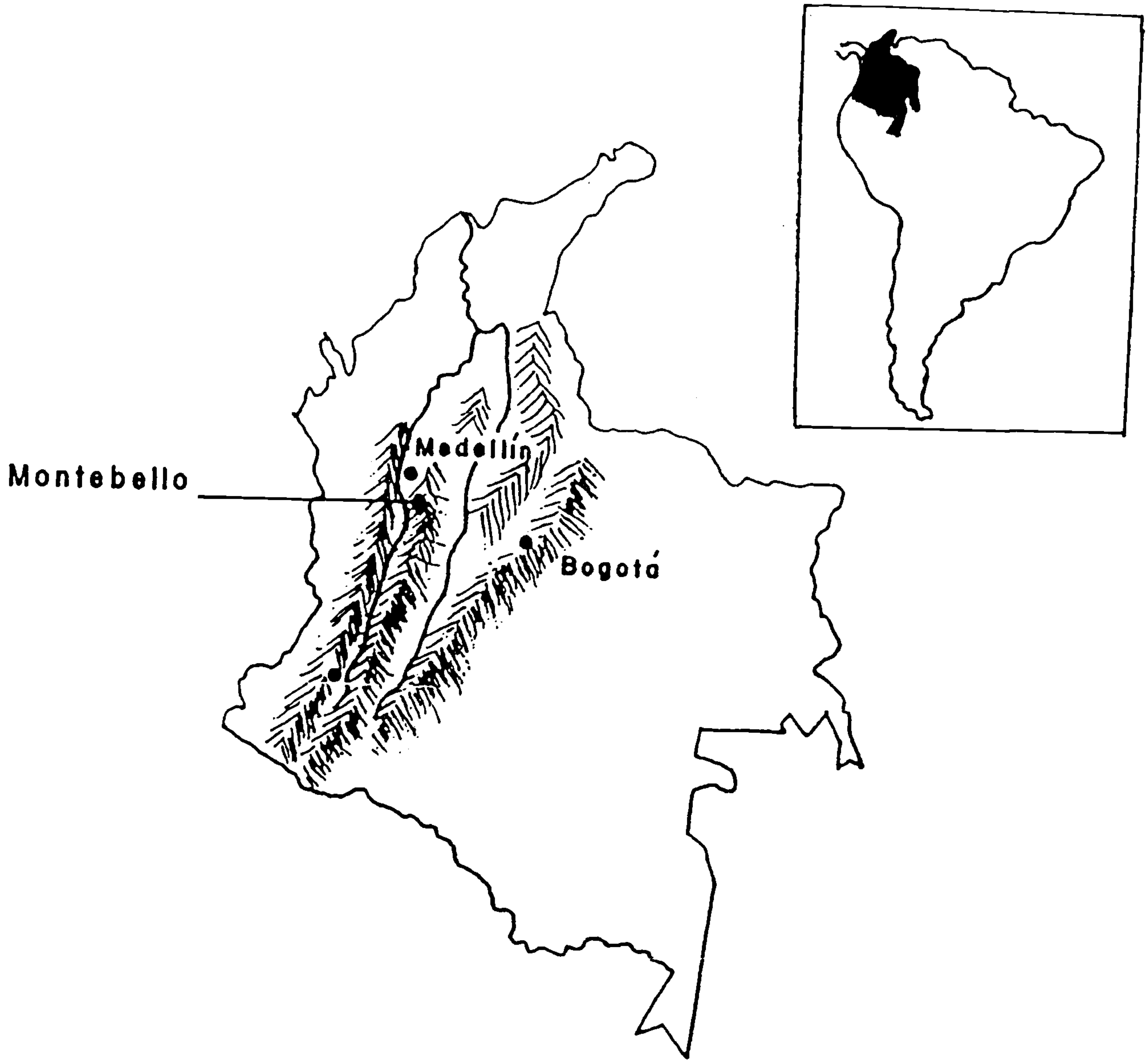


Figure 1. : The Montebello, COLOMBIA site of a cutaneous Leishmaniosis focus

Phlebotomine sandfly fauna

We used seven capture points located at different elevations between 800 and 2200 m.a.s.l., and separated by a distance of approximately 200 metres. During 30 months, the authors visited the zone every 15 days to collect phlebotomine sandflies, which were captured as follows:

a) **Paper traps impregnated with castor oil:** Sheets of white bond paper measuring 20 by 20 cm were impregnated with castor oil and placed on bamboo stands, as described by Rioux (8). At least 20 such traps per station and per visit were placed in domestic (corridors and rooms), peridomestic (independent buildings that housed domestic cattle and fowl, located within a radius of 100 m from the human dwelling), and sylvatic sites (caves, rock crevices and tree hollows located more than 100 m away from the human dwelling). The traps were replaced every 15 days. All phlebotomine sandflies captured were detached from the traps with a small brush soaked in 90% alcohol, and preserved in alcohol for subsequent clearing and identification. We determined the species present, their relative density per square meter of oiled paper in each dwelling, at each capture point and at each season of the year.

b) **CDC type light traps, Shannon traps and oral suction:** To determine the hours of peak activity of each species (circadian variation) we used CDC type light traps at domestic and peridomestic sites. At the same time sandflies were captured by oral suction from Shannon traps and resting sites. Phlebotomine sandflies were identified according to the scheme proposed by Lewis et al. (9).

Reservoir studies

In areas where leishmaniasis had been found in humans, domestic and wild animals were tested in search of reservoir species. All horses, mules (Equus equus and E. equus x Equus asinus) and dogs (Canis familiaris) were clinically examined, and indirect immunofluorescence test was also performed on serum samples from dogs.

Wild animals were trapped with metallic (Tender and Manufrance) traps, observed for 2 to 3 months and then killed under general anaesthesia. Smears and cultures of the skin, liver, spleen and bone marrow were examined for Leishmania spp.

Results

Human cutaneous leishmaniasis

During the study period 14 cases (8 males, 6 females) of cutaneous leishmaniasis were diagnosed (8 in 1987, 4 in 1988 and 2 in 1989). No cases of mucocutaneous or visceral leishmaniasis were detected. The annual prevalence of the disease was 3.81%

in 1987, 1.9% in 1988 and 0.95% in 1989. Table 1 summarizes the distribution of patients by age group and sex.

Table 1: Distribution of patients by age and sex

Age Group (years)	Male	Female	Total
0 - 14	0	0	0
5 - 14	0	4	4
15 - 44	5	1	6
45 +	3	1	4
TOTAL	8	6	14

Of the male patients, 7 were farmers and 1 was a public employee; of the female patients, 2 were housewives, 3 were schoolgirls and one was a baby girl. The patients had from 1 to 5 lesions: 9 had 1 lesion, and 5 had 2 to 5 lesions. Among all patients we observed 26 lesions distributed as follows: trunk 10, arms 9, legs 6, face 1. Most lesions were ulcerous; of 20 such lesions, 11 were crusted, 6 wet, 2 verrucous and 1 purulent. There were 4 nodular and 2 plaque-like lesions. At the moment of examination, the lesions had been present for periods ranging from 15 weeks to 1 year.

All patients were treated with meglumine antimoniate (Glucantime) at doses of 10-20 mg Sb 5/kg/day until all lesions had resolved. Mean duration of treatment was 20 days, with a range of 10 to 24 days.

The elevation of the site of residence of all patients was between 800 and 1300 m.a.s.l., most patients (66%) living at approximately 1000 m.a.s.l.

Montenegro tests were performed in 156 schoolchildren between 6 and 12 years of age. Of this sample, 101 children (65%) were positive (induration $\geq$ 5 mm.).

Identification of the parasite

The strains of Leishmania spp., isolated from patients were identified with monoclonal antibodies (mAbs) and isozyme electrophoresis at the CIDEIM Foundation in Cali, Colombia, with monoclonal antibodies provided by Dr. C. McMahon Pratt. All strains reacted at a dilution of 1:1000 with mAbs B16, B18, B21 and B12, and were therefore typified as Leishmania braziliensis.

Thirteen enzyme standards were used in electrophoresis studies (MPI, G6PD, NH, 6PGD, ASAT, ALAT, PEP-D, ACP, PGM, PEP-1, ES, GPI and SOD). All strains were identified as L. braziliensis differing from variant of reference strain M2903 by enzymes MPI (Mannose phosphate isomerase) and PEP-1 (aminopeptidase 1), and corresponding to Cali zymodene 11.

Phlebotomine sandfly fauna

Of the 10 species of phlebotomine sandfly captured and identified, 9 belonged to the genus Lutzomyia (Lu), and one to the genus Warileya (Wa.). The flies were distributed through a range of elevations from 800 to 2000 m.a.s.l., as shown in Table 2 and Figure 2, and were active throughout the year.

Table 2: Distribution of species of phlebotomine sandfly captured in Montebello, Colombia at different elevations

SPECIES	ALTITUDE (m.a.s.l.)
Lu. rosabali (Fairchild & Hertig, 1958)	800
Lu. gomezi (Nitzulescu, 1931)	800-1300
Lu. venezuelensis (Flock & Abonnenc, 1948)	800-1300
Lu. lichyi (Flock & Abonnenc, 1950)	800-1300
Wa. rotundipennis (Fairchild & Hertig, 1951)	800-1300
Lu. hartmanni (Fairchild & Hertig, 1957)	800-1600
Lu. dubitans (Sherlock, 1962)	800-1800
Lu. nuneztovari (Ortiz, 1954)	1000-1850
Lu. youngi (Feliciangeli & Murillo, 1987)	1000-1850
Lu. moralesi (Young, 1979)	2000

The most abundant species was Lu. gomezi (715), followed by Lu. venezuelensis (14%) and Lu. youngi (8.5%) (Table 3). Lu. gomezi was the species most frequently captured in the act of biting humans (92%), followed by Lu. youngi (6%).

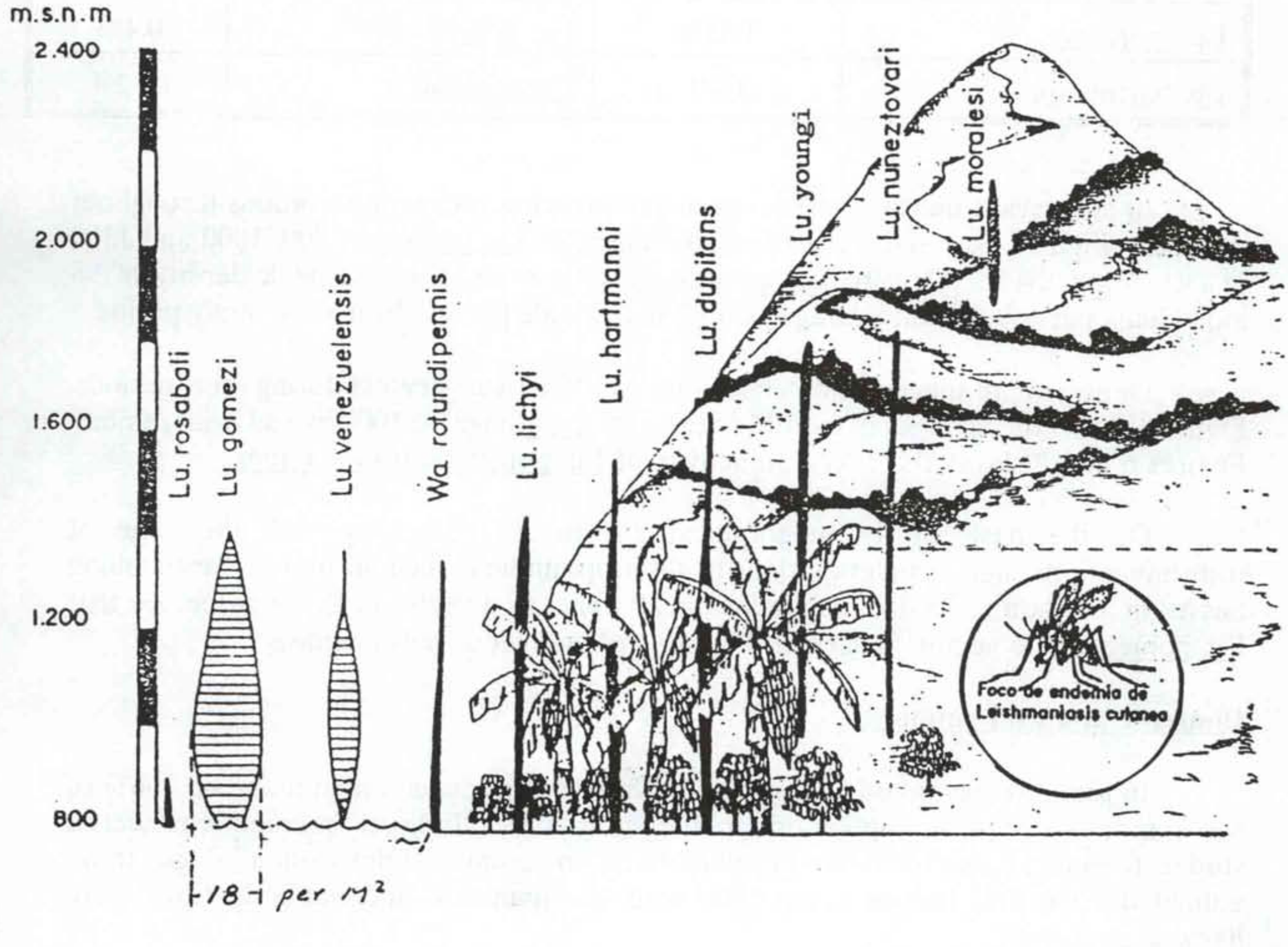


Figure 2. : Altitudinal distribution and density of phlebotomine sandflies in Montebello, COLOMBIA

Table 3: Percentage capture of phlebotomine sandflies with paper traps soaked with castor oil

Lu. gomezi	71.3%	Lu. dibitans	0.8%
Lu. venezuelensis	14.1%	Lu. nuneztovari	0.6%
Lu. youngi	8.5%	Wa. rotundipennis	0.56%
Lu. moralesi	1.85%	Lu. lichyi	0.4%
Lu. hartmanni	1.7%	Lu. rosabali	0.12%

In all species, density per square meter varied at different elevations throughout the year. Figures, 3, 4 and 5 illustrate the density of Lu. gomezi at 800, 1000 and 1300 m.a.s.l. respectively. Density was greatest at 1000 m.a.s.l., with a peak density of 98 individuals per m^2, and an average of 15.9 individuals per m^2 during the study period.

Density of all species of phlebotomine sandflies was greatest during rainy periods. Figure 4 shows the changes in monthly density of Lu. gomezi at 1000 m.a.s.l. with rainfall. Figures 6 and 7 shows the circadian activity of Lu. gomezi and Lu. youngi.

On the basis of its geographical distribution coinciding with the zone of transmission, its high population density, anthropophilic behaviour and domestic biting habits, in addition to its documented capacity to act as a vector (10), we suspected that Lu. gomezi is the vector species in this focus of cutaneous leishmaniasis.

Domestic and wild animals

In total, we examined 34 dogs and 20 horses and mules, which made up 100% of the canine and equine populations in the study area. Indirect immunofluorescence studies to detect Leishmaniasis were performed on serum samples from all dogs. In no animal did we find lesions compatible with leishmaniasis, and antibody titres were likewise negative.

The following wild animals were captured in peridomestic areas near dwellings whose occupants had leishmaniasis: 10 Didelphis marsupialis, 8 Rattus rattus, 17 Mus musculus and 1 Sciurus granatiensis. None of these animals showed clinical signs of lesions compatible with leishmaniasis, and parasitological analyses failed to reveal the presence of Leishmania in any of them.

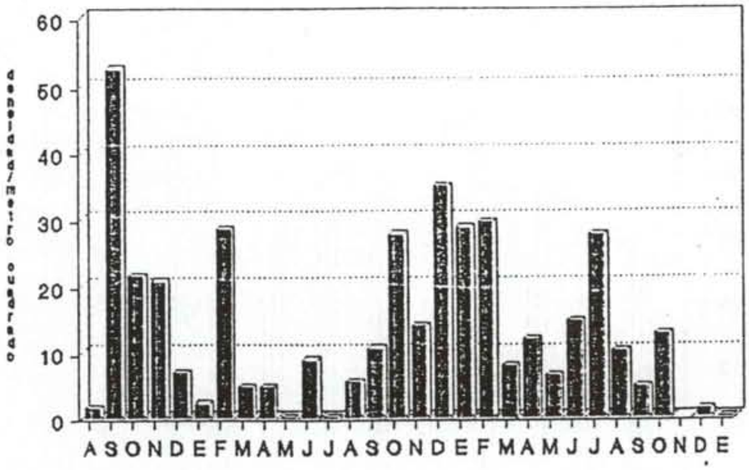


Figure 3. : Density of *Lu. gomezi*/m² at 800 m.a.s.l.

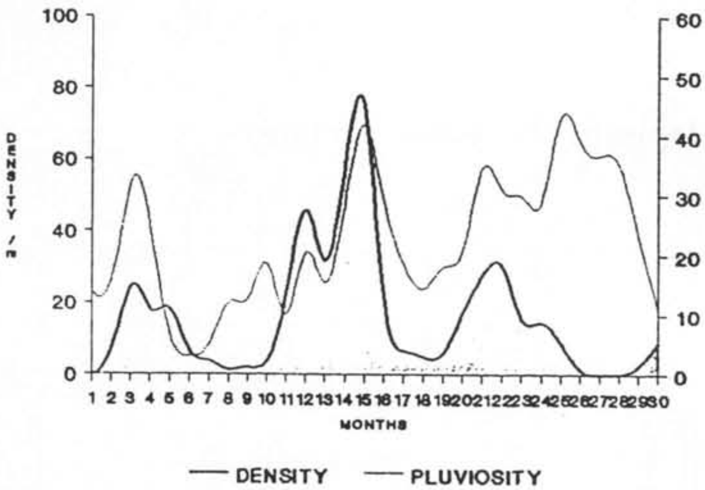


Figure 4. : Pluviosity and *Lu. gomezi* at 1.000 m.a.s.l.

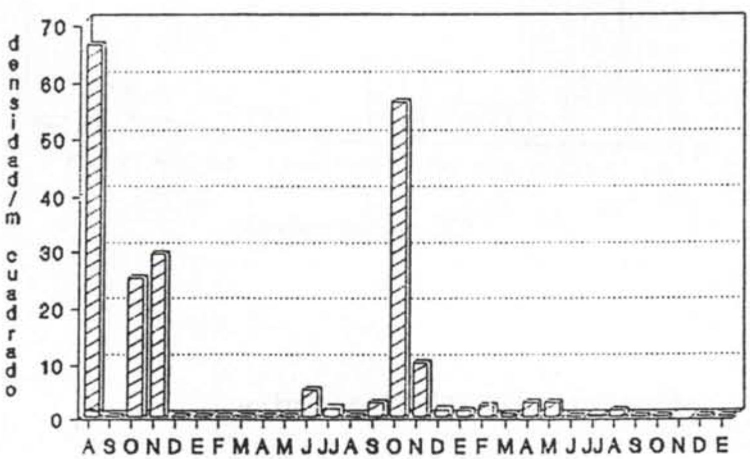
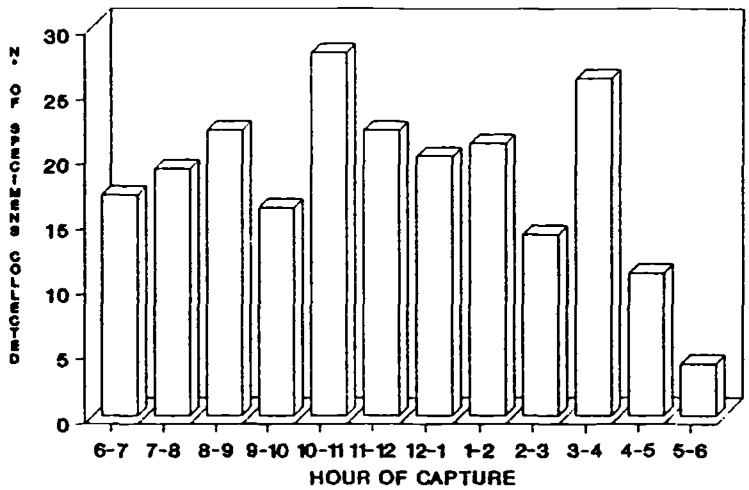
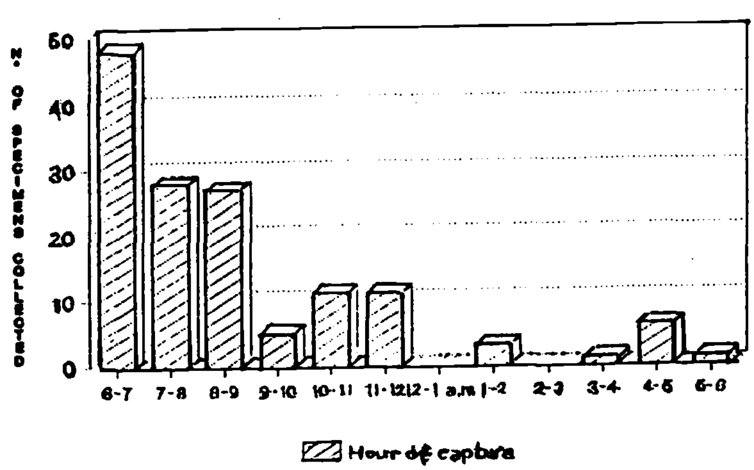


Figure 5. : Density of *Lu. gomezi*/m² at 1.300 m.a.s.l.



<u>Figure 6.</u> : *Lu. gomezi* activity



<u>Figure 7.</u> : *Lu. youngi* activity

Discussion

Thanks to active searching and early treatment of patients the prevalence of cutaneous leishmaniasis in the Montebello region decreased from 3.81% in 1987 to 0.95% in 1989. However, the 1989 figure was still higher than the corresponding prevalence in regions where the disease was known to be most common: 0.75% and 0.76% respectively in the Rio Magdalena Valley and Bajo Cauca Valley regions.

All patients diagnosed in this study lived at elevations ranging from 800 to 1300 m.a.s.l., i.e., the same range as that where Lu. gomezi was found to be most common. Moreover, most patients lived at elevations where the population densities of this species was highest (approximately 1000 m.a.s.l.).

Given the probable domestic nature of transmission, the disease affected all members of the family regardless of age or sex. The type of lesions and their anatomical distribution matched those classically described in association with American cutaneous leishmaniasis, however, it should be pointed out that although the disease is caused by a variant of L. braziliensis, the mucosa were not affected in any of the patients we studied, including those who had been ill several years before the study was performed.

The high rate of positive results on the Montenegro test among schoolchildren (65%) reveals that contact between humans and the vector occurred in domestic and peridomestic areas, and that infection occurs within the first few years of life at an incidence of around 10% per year. Much infection must be cryptic.

The elevation distribution of the probable vector species limits the focus to areas between 800 and 1300 m.a.s.l., and facilitates the rational planning of measures to eradicate the disease, which should be concentrated at these elevations although phlebotomine sandflies are found at elevations of up to 2000 m.a.s.l.

The presence of 10 species of phlebotomine sandflies in the study region reflects the variety of phlebotomine fauna in Colombia, where 127 different species have been reported to date. The present study represent the first report of Lu. youngi in Colombia.

The endemic presence and epidemic outbreaks of cutaneous leishmaniasis in Montebello, where all members of the population are at risk of infection, led us to suspect the presence of a domestic animal reservoir. The absence of anti-Leishmania antibodies in dogs, and of lesions compatible with cutaneous leishmaniasis in dogs, horses and mules, bolsters the notion that humans themselves act as a reservoir during an epidemic outbreak (11). This would give rise to a peridomestic cycle which would maintain the endemic in the study region, with Lu. gomezi serving as the vector and as yet unidentified organism acting as a reservoir. Epidemics may occur by a domestic, anthroponotic cycle set in motion when the number of active cutaneous lesions in the focus rises sufficiently; in epidemics the vector species is also Lu. gomezi.

The decrease in prevalence during the three year study period may be explained by early diagnosis and suitable treatment, which prevented the spread of the disease and transmission by new vectors.

Conclusions

The findings of the present ecoepidemiological study allowed us to determine the epidemiological risk of contracting cutaneous leishmaniasis within the region limits of Montebello, Colombia.

The risk zone, where most cases of cutaneous leishmaniasis were detected, is situated between 800 and 1300 m.a.s.l., at elevations where Lu. gomezi is distributed. Risk is highest at an elevation of 1000 m.a.s.l., where the density of this species of phlebotomine sandfly is greatest. The period of greatest risk is towards the end of the rainy season, when the Lu. gomezi population increases. The human population at risk comprises all persons living at elevations between 800 and 1300 m.a.s.l., regardless of age, sex, and occupation. The risk of contracting cutaneous leishmaniasis is greatest in domestic areas, where contact between humans and the vector is most intense, especially after 1800 hours and throughout the night.

The determination of epidemiological risk allows us to formulate control measures, which should be based on two main aspects. The first aspect deals with the control of the vectors. This requires the evaluation of measures aimed at preventing Lu. gomezi bites in the population; such measures should be implemented at elevations between 800 and 1300 m.a.s.l., in domestic and peridomestic areas, and should be especially concerted at the end of the rainy season. All persons should avoid being bitten, although efforts should be concentrated on protecting children. Among the measures worthy of consideration are the planting of trees and shrubs containing natural pesticides, the domestic and peridomestic application of long-acting pesticides, the use of pesticide-impregnated mosquito nets and the application of insect repellents before bedtime.

The second aspect involves control of human activities. Since humans seem to act as reservoirs for the parasite, the disease should be diagnosed and treated promptly, to spare the patient as much suffering as possible, alleviate morbidity, and to reduce the spread of the disease and the appearance of new epidemics.

These suggestions involve intervention at three different levels:

1) Community education about leishmaniasis, its clinical appearance, etiology, mode of transmission, diagnosis and treatment, so that inhabitants will recognize the disease, seek medical treatment promptly, and participate in control measures.

2) Continuing education courses for private and public health service doctors, to enable them to recognize cutaneous leishmaniasis despite its clinical polymorphism, and to teach them to collect and forward samples correctly for parasitological analysis, the effectiveness of which depends to a large extent on the sensitivity of the test used.

3) Training rural health officers to actively search for cases, provide correct treatment, and cooperate with community health education programs. These officers in turn would maintain community-wide motivation to actively participate in campaigns aimed at controlling the vector.

Acknowledgements

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