Abstract. The status of American cutaneous leishmaniasis was investigated from 1985 to 1991 to provide an epidemiologic characterization of the disease in Bergantin, a rural community in the northeastern part of Anzoátegui State, Venezuela. The study revealed the presence of the infection during the period analyzed, with an annual incidence of 50.2 cases per 10,000 inhabitants and this number has increased 1.5 times during the last two years. Three villages where clinical cases had been recorded were selected for a comparison of their prevalence data. These villages comprise the human population in the high and low altitude limits of Bergantin. Immunologic assessment of the inhabitants used two different antigen preparations to examine responses to parasites associated with the cutaneous and visceral forms of the disease. The leishmanin skin test (LST) was used in a sample of 276 individuals (46.3% of the inhabitants) and resulted in an overall positivity of 16.7%. The percentage of LST positivity varied with age and sex, yet analysis of this response and the prevalence for each village reflected the specific characteristics of these localities. La Montaña, situated at 800 meters above sea level, had the highest prevalence (800 cases per 10,000 inhabitants) and the most positive LST response (21.2%) in comparison with the two other villages situated at a lower altitude (300 meters above sea level).

Venezuela is one of the South American countries where American cutaneous leishmaniasis (ACL) is increasing. However, the epidemiologic characteristics of the disease are mostly unknown. Estimates of the number of cases are frequently unreliable due to local difficulties in diagnosis and the frequency of subclinical infections. In general, a better understanding of the epidemiology of ACL is important so that effective control measures may be applied.

The geographic distribution of ACL in Venezuela covers 21 of its 23 territorial divisions. It has been shown that these ACL-affected regions present foci with different degrees of endemicity, but only the infections detected in the western and central regions have been systematically described. Presently, little is known about the epidemiology of leishmaniasis in the eastern region of the country.

The general purpose of the study was to determine the distribution, prevalence, and risk factors for the disease within the endemic areas of the Turimiquire Mountains in the northeastern states of Venezuela.

MATERIALS AND METHODS

Study area. This study was carried out in the territory of Bergantin located in the southwestern area of the Turimiquire Mountains between latitudes 10°03′30″′ and 10°00′17″N and longitudes 64°20′18″ and 64°23′20″W in the Anzoátegui State of Venezuela. Bergantin comprises 36 villages within a total area of 630 km². Of these, approximately 61 km², including the villages of La Montaña (800 meters above sea level), La Panela (300 meters above sea level), and El Poblado (300 meters above sea level) (Figure 1), were studied. These villages are situated at the high and low altitude range where cases of leishmaniasis are known to occur. Tropical rain forests and climatic zones with high humidity (at altitudes higher than 800 meters above sea level) and low humidity (less than 300 meters above sea level) characterize the area of the three villages, with an average annual rainfall of 1,330 mm and an annual mean temperature of 17.8°C. Bergantin is mainly an agricultural community with coffee production and several small cultivated fruit and vegetable plots as primary sources of livelihood.

Population survey. A preliminary retrospective investigation of incidence data for ACL in Anzoátegui State between 1985 and 1991 was carried out. The data were obtained from the Dermatology Service files of the Hospital Luis Razetti in the State’s capital city, Barcelona. To establish the annual incidence for the period, we considered the information relative to the age of the skin lesions, the parasitologic confirmation diagnosis, and the address of all patients registered in the medical records.

The field work was conducted in Bergantin throughout 1992. We chose the villages of La Montaña, La Panela, and El Poblado where the incidences, calculated on the basis of the hospital records, were extremely high. A survey of the population and the follow-up of the active case of ACL were done with the assistance of local medical personnel. After approval of the study protocol by the local authorities of the Ministry of Health and the institutional authorities of the Universidad de Oriente, all inhabitants were invited to the principal medical center, where specific information was provided regarding the procedure to be followed for the application of the skin test, emphasizing the aspects related to the risks and the benefits involved in this process and the methodology used in the preparation and control of the novel antigen.

The 300 individuals (50% of the study area’s population) to be surveyed between May and August 1992 attended interviews and gave individual informed consent to participate. Data regarding age, sex, location of residence, and personal history of leishmaniasis were compiled to establish the personal characteristics of inhabitants subjected to epidemics of leishmaniasis. All participants who voluntarily accepted in writing to participate in the study and met the require-
ments of the Declaration of Helsinki (Finland) were given the leishmanin skin test (LST).

**Leishmanin skin test.** A novel polyvalent antigen for the LST was obtained from the Laboratory of Immunobiology of the Instituto Venezolano de Investigaciones Científicas (IVIC), which was prepared by O’Daly and others following the instructions recommended and published in 1980 by the World Health Organization for the production of biological materials for use in humans. For the development of amastigotes, parasites were grown at the transformation temperature (30–34°C) in a synthetic culture media supplemented with 5% fetal bovine serum (Gibco/BRL, Gaithersburg, MD). They were then collected at the stationary growth phase and washed twice with phosphate-buffered saline (PBS), pH 7.4, by suspension and centrifugation at 800 g for 20 min at 4°C. For the purification of the proteins composing the leishmanin, the parasites were killed after incubation at 30°C for three days in Eagle’s minimal essential medium (MEM; Gibco/BRL) containing 150 μg/ml of N-p-tosyl-L-lysine-chloromethyl ketone, washed twice by centrifugation at 12,000 × g for 10 min at 4°C, and treated for 30 min at 4°C in Eagle’s MEM supplemented with 0.12% (v/v) NP40 (Sigma, St. Louis, MO). The particulate antigens were collected and washed twice by centrifugation as before, resuspended in PBS, and sonicated for 5 min at 4°C in a Sonifier Cell Disruptor (Model W185; Heath-Systems-Ultrasonic, Inc., Plainview, NY), at the microtrop limit of the output control at 50 W. The final antigen preparation contained an equal quantity of protein of each strain of parasites used, and the protein content was adjusted to 40 μg/ml by the method of Lowry and others.

As previously described, sterility control procedures were carried out for each batch of antigen and the batches were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) to ensure consistency in the pattern of *Leishmania* protein bands.

Two kinds of antigens were obtained: 1) antigen A, prepared from amastigotes of *L. amazonensis* (La: IFLA/BR/67/PHB), *L. venezuelensis* (Lv: MHOM/VE/80/H16), and *L. brasiliensis* (Lb: MHOM/VE/75/H27), and antigen B, prepared from amastigotes of *L. chagasi* (Lch: MHOM/BR/74/PP75). The preparations were stored at 5°C until use. Both antigens were composed of eight major proteins, as revealed by SDS-PAGE analysis.

For the immunologic analysis, each individual was simultaneously injected intradermally with 0.1 ml of antigen A (4 μg of protein) into the alcohol-cleansed volar surface of the left arm and with an equivalent quantity of antigen B into the right forearm. Of the 300 individuals treated, 276 returned to have the reaction measured. The size of the induration was measured by the ball point pen technique after 48 hr. An induration of 5 mm or more at one or the two antigens sites was regarded as a positive test result. The absence of significant false-positive reactions with these antigens was previously evaluated in a population of 400 individuals of a nonendemic area for leishmaniasis and in individuals affected by American trypanosomiasis, lepra, malaria, and other tropical diseases (O’Daly J, IVIC, unpublished data).

**Analysis of data.** Proportions were compared by the Z value for incidence and prevalence data and by chi-square tests for locality-, age-, and sex-stratified samples. The analysis of the differences between average induration measures was done by the Student’s *t*-test.

**RESULTS**

A review of the dermatology records of the main hospital treating leishmaniasis in Anzoátegui State showed that approximately 25% of the ACL cases (172 of 747) recorded between 1985 and 1991 were from Bergantin. The incidence data showed an increase in diagnosed cases over the past seven years, with an average of 50.2 cases per 10,000 inhabitants (Table 1). A statistically significant increase in cases can be seen for the last two years of this period (*P* < 0.005), corresponding to an incidence index of 1.5 (Figure 2). The plotting of the cases for 1992 was compared with the mean value calculated for the 1985–1991 period (Figure 3), and these results showed April to July 1992 as an epidemic period, with 33% of this year in a situation of high risk for leishmaniasis infection. Furthermore, it showed the seasonal variations of the infection with a sustained endemic character throughout the year.

Together, the retrospective incidence data (Table 1) and the total number of cases registered for 1992 (Figure 3) were used to calculate and compare the prevalence of ACL in three villages in Bergantin for the period 1985–1992 (Table 2). The results revealed a difference among localities; La
TABLE 1
Annual incidence of American cutaneous leishmaniasis between 1985 and 1991 in Bergantin, Venezuela

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of inhabitants*</th>
<th>No. of cases†</th>
<th>Incidence (cases per 10,000 inhabitants)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1985</td>
<td>4,993</td>
<td>4</td>
<td>8.0</td>
</tr>
<tr>
<td>1986</td>
<td>5,045</td>
<td>25</td>
<td>49.6</td>
</tr>
<tr>
<td>1987</td>
<td>5,097</td>
<td>24</td>
<td>47.1</td>
</tr>
<tr>
<td>1988</td>
<td>5,149</td>
<td>23</td>
<td>44.7</td>
</tr>
<tr>
<td>1989</td>
<td>5,201</td>
<td>24</td>
<td>46.2</td>
</tr>
<tr>
<td>1990</td>
<td>5,253</td>
<td>40</td>
<td>76.2‡</td>
</tr>
<tr>
<td>1991</td>
<td>5,305</td>
<td>41</td>
<td>77.3‡</td>
</tr>
</tbody>
</table>

Annual average§ 50.2

* Census results were obtained from the Oficina Central de Estadistica e Informatica (OCEI).
† Number of cases recorded in the Dermatology Service of the Hospital Luis Razetti, Barcelona, Anzoátegui State.
‡ Z value demonstrated the statistically significant differences obtained for incidence in 1990 and 1991 (Z = 2.6; P < 0.005).
§ Calculated as the quotient between the cases for the total period and population in 1988.

Montaña, situated at a high altitude (800 meters above sea level), had the highest prevalence (P < 0.0001) in comparison with the entire territory of Bergantin and its surroundings (630 km²) and with those recorded for the villages of La Panela and El Poblado, both located at a lower altitude (300 meters above sea level). These differences were also reflected in the LST responses. La Montaña had the highest percentage of LST positivity (P < 0.01) (Table 3).

The frequency of LST positivity increased with age. In general, the older age group (> 20 years of age) had a percentage of response more than four times greater than the younger group (< 10 years of age). Gender differences were also seen in the locality of La Montaña, where these percentages were much larger in males (χ² = 5.2, P < 0.025; Figure 4).

For the immune response examination we used two kinds of leishmanin, one composed of antigens obtained from parasites related mainly to cutaneous disease (antigen A) and the other from parasites associated mainly with visceral leishmaniasis (antigen B). In view of the existence in the area of the vectors for parasite species causing cutaneous and visceral infections, we compared the responses to both antigens in these communities. Most participants responded to both antigens, probably as the result of cross-reactivity, while a lesser proportion reacted with antigens A or B. Of the total individuals tested, only 8.7% responded only to antigen B (Table 4). Irrespective of these differences, the average size of the reaction was similar for both antigens (Table 3).

DISCUSSION

This study is the first epidemiologic characterization of an area endemic for leishmaniasis in Anzoátegui State, Venezuela. The lack of previous information concerning factors, such as the geographic extent of the disease and the relationships between prevalence and ecology of vectors and parasites in the area, encouraged us to pursue this investigation. Analysis of the retrospective data revealed the impact of the disease in Bergantin, where there was a considerable increase in the incidence between 1985 and 1991 (Table 1 and Figure 2). For this area, the control of cutaneous leishmaniasis and treatment of patients with this disease has been

![Figure 2](image2.png)

**Figure 2.** Variations in annual incidence (○-○) and incidence index (■■■) for cutaneous leishmaniasis in Bergantin between 1985 and 1991. The incidence index (**) was calculated as the quotient of the annual incidence and the average value obtained for the period (50.2).

![Figure 3](image3.png)

**Figure 3.** Seasonal behavior of American cutaneous leishmaniasis in Bergantin during 1992. The bar (*) represents the mean ± SD number of cases (1.6 ± 1.5) calculated for the period 1985–1991.

![Figure 4](image4.png)

**Figure 4.** Variations in annual incidence (○-○) and incidence index (■■■) for cutaneous leishmaniasis in Bergantin between 1985 and 1991. The incidence index (**) was calculated as the quotient of the annual incidence and the average value obtained for the period (50.2).

![Figure 5](image5.png)

**Table 2**

Comparison of the prevalence for American cutaneous leishmaniasis in the localities studied within Bergantin between 1985 and 1992

<table>
<thead>
<tr>
<th>Localidad</th>
<th>Prevalencia (casos por 10,000 habitantes)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bergantin</td>
<td>344.5</td>
</tr>
<tr>
<td>La Montaña</td>
<td>800.0‡</td>
</tr>
<tr>
<td>La Panela</td>
<td>392.2</td>
</tr>
<tr>
<td>El Poblado</td>
<td>370.0</td>
</tr>
</tbody>
</table>

* Values were calculated as the quotient between the number of the total of cases for the period and the population of each locality in 1992 (including the cases recorded during the survey).
† Including all of the territory of Bergantin and its surroundings (630 km²).
‡ Z value demonstrated the statistically significant differences obtained for prevalence in this locality (Z = 3.9; P < 0.0001).
assumed by the Dermatology Service of the Hospital Luis Razetti, where for more than 15 years a passive surveillance system had existed. Therefore, we believe that the increase of the annual incidence is not primarily related to an improvement in the availability of health resources, but to a real increase in disease transmission. On the other hand, as a result of our presence in Bergantin in 1992, this traditionally passive surveillance system was modified in favor of a more active one that accomodated people who would not regularly go to the hospital in search of medical attention. This situation may be related to the increase in the number of cases of leishmaniasis seen for the period April to July of the year being studied when compared with the average number of cases recorded during the period 1985–1991. These observations reveal the problems that can arise as a result of a deficiently passive surveillance systems in the control of leishmaniasis, similar to those reported by other investigators.\textsuperscript{13, 14}

More than 16\% of the total of the people examined were LST positive. The magnitude of this response is similar to that recorded by other investigators in the central and western endemic areas of ACL in Venezuela.\textsuperscript{1, 8} According to the classification of Pifano, the LST response in this study, ranging from 10\% to 20\%, would classify the region as one of high endemicity.\textsuperscript{15} As described elsewhere,\textsuperscript{1} most people (32 of 46) with a positive LST result were healthy, without scars or a history of leishmaniasis. This may be related to the occurrence of subclinical infections associated with repeated low-dose exposure, a situation frequently seen in endemic regions.\textsuperscript{1, 16, 17}

The territory of Bergantin is situated on the southeast side of the Turimiquire mountain range, in proximity to the territory of Naricual, where endemic foci of visceral and cutaneous leishmaniasis that are not characterized coexist. The main objective of this investigation was the study of the epidemiology of ACL in Bergantin, but the proximity to Naricual induced us to investigate the LST responses to antigens purified from amastigotes of parasite species associated mostly with the cutaneous and the visceral forms of the disease. When one considers that most of the individuals tested were positive for both antigens, we cannot as yet access a difference in this respect, but we believe that responses seen only to one antigen or to another antigen (rows 1 and 2 in Table 4) cannot be ignored in view of the possibility that situations of more frequent contact with one or another parasite species could arise. With the purpose of clarifying the significance of these findings, we are presently conducting an entomologic investigation of the taxonomic identification of vectors and their natural infections in these regions.

The analysis of the LST response to both antigens showed a clearly positive response beginning with adolescence; this finding, together with the tendency for higher LST responses in groups of older males, suggests that transmission is taking place mainly outside their dwellings. In La Montaña, these gender differences associated with the LST response were statistically significant and can probably be associated with the marked distribution of working activities by gender that we found in this locality (Garcia A and others, Universidad de Oriente, unpublished data). These activities placed men in a condition of higher exposure to sandfly bites due to the agricultural nature of their work.\textsuperscript{18}

Our findings of incidence and prevalence identify La Montaña as the locality with the highest risk of leishmaniasis in the study area. This must be related to the specific characteristics of its geographic location; that is, a high altitude

\begin{table}[h]
\centering
\caption{Leishmanin skin test (LST) results in the localities studied in Bergantin, Anzoátegui State, Venezuela}
\begin{tabular}{|l|c|c|c|c|}
\hline
Locality & Individuals* & Positive LST result (\%) & Antigen A\textsuperscript{1} (mean ± SD)\textsuperscript{3} & Antigen B\textsuperscript{1} (mean ± SD)\textsuperscript{3} \\
\hline
La Montaña & 104 (52.0\%) & 21.2§ & 10.1 ± 4.0 & 9.7 ± 3.1 \\
El Poblado plus La Panela & 172 (44.0\%) & 14.0 & 9.1 ± 2.7 & 9.9 ± 3.1 \\
Total & 276 (46.3\%) & 16.7 & 9.6 ± 3.1 & 9.8 ± 3.0 \\
\hline
\end{tabular}
\* Values in parentheses are the corresponding percentages of each locality’s population. \textsuperscript{1} For specifications of antigens, see Materials and Methods. \textsuperscript{3} The comparison of the mean values was not significant. § The chi-square test showed a significant difference between data of both localities analyzed ($\chi^2 = 6.7; P < 0.01$).
\end{table}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{comparison.png}
\caption{Comparison of leishmanin skin test (LST) responses by age and sex in the localities studied in Bergantin, Anzoátegui State, Venezuela. The number above the bars indicate the number of individuals tested.}
\end{figure}
and a jungle environment that favors the presence of vectors and reservoirs available for the transmission of these infections.\(^3\)

Acknowledgments: We thank the personnel of Bergantin’s ambulatory medical care center for cooperation and Cipriano Aponte for helping with the field work. We also thank Drs. E. Villarroel and A. Lander for contributing the information for epidemiologic analysis and Duwia Otero, Pedro Medina, and Carlos Zamora (IVIC) for help in preparing the drawings and photographs. Our special gratitude is given to Corina Grey for the English language revision of the manuscript.

Financial support: This study was supported by the Consejo de Investigación-Universidad de Oriente (Project CI-1-009-00648194), the Fundación Para El Desarrollo de la Ciencia y la Tecnología (Fundacite-Anzoátegui) (Project P1-013-93), and the Ministerio de Sanidad y Asistencia Social.

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REFERENCES


<table>
<thead>
<tr>
<th>Antigen A</th>
<th>Antigen B</th>
<th>LST results (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>–</td>
<td>23.9</td>
</tr>
<tr>
<td>–</td>
<td>+</td>
<td>8.7</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>67.4</td>
</tr>
</tbody>
</table>

* The responses were classified as positive (+) for an induration ≤ 5 mm and negative (−) for smaller values.
† For specifications of antigens, see Materials and Methods.