GEOGRAPHIC DISTRIBUTION AND CLINICAL DESCRIPTION OF LEISHMANIASIS CASES IN PERU

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Abstract. Studies were conducted from 1986 through 1993 to further define the geographic distribution and relative importance of different species of Leishmania as a cause of leishmaniasis in Peru. Patients with a clinical diagnosis of cutaneous and/or mucosal or diffuse cutaneous leishmaniasis were enrolled at the Naval Medical Research Institute Detachment (NAMRID) Laboratory in Lima, the Tropical Disease Clinic at San Marcos University Daniel A. Carrión, the Central Military Hospital, and a Ministry of Health hospital in Cusco, Peru. Clinical features, lesion aspirates, and biopsy tissue were obtained from each patient. All specimens were collected and assayed separately, including multiple specimens from some of the same patients for Leishmania parasites by inoculating aliquots of either aspirates or biopsy tissue suspensions onto Senekji's blood agar medium. Stocks of Leishmania isolates were used to prepare promastigotes to produce extracts for identifying the Leishmania species by the cellulose acetate electrophoresis enzyme technique. A total of 351 isolates of Leishmania were obtained from 350 patients who were infected primarily in the low and high jungle of at least 15 different Departments of Peru. Of the 351 isolates, 79% were identified as L. (V.) braziliensis, 7% as L. (V.) guyanensis, 10% as L. (V.) peruviana, 2% as L. (V.) lainsoni, and 1.7% as L. (L.) amazonensis. The clinical form of disease varied depending on the species of Leishmania, with L. (V.) braziliensis being associated most frequently with cutaneous, mucosal ulcers and mixed cutaneous and mucosal disease, and L. (V.) peruviana, L. (V.) guyanensis, L. (V.) lainsoni with cutaneous lesions. Leishmania (L.) amazonensis was isolated from six patients, three with cutaneous lesions, one with mucosal lesions, and two with diffuse cutaneous lesions. Among all of the leishmaniasis cases, males were affected more frequently, and cases occurred among patients less than 10 to more than 51 years of age. These data further defined the geographic distribution and the relative frequency of Leishmania species associated with different clinical forms of leishmaniasis in Peru.

Leishmaniasis is a major public health problem in Peru where the endemic range spans approximately 74% (951,820 km²) of the country. The endemic distribution pattern of cutaneous and mucosal leishmaniasis extends throughout the Andean and inter-Andean valleys for the cutaneous form, and the tropical rain forest (low jungle and high jungle) for the mucosal form. Current estimates of the actual number of human cases were not available, but in 1992 the Peruvian Ministry of Health indicated that there were approximately 10,000 cases of severe disease in the country (Chumbe AW, Navincopa FM, unpublished data).

Studies conducted in Peru in the late 1980s showed that most cases of both clinical forms of leishmaniasis were caused by Leishmania (Viannia) braziliensis or Leishmania (V.) peruviana (Braga VJI, unpublished data). Leishmania (V.) guyanensis, L. (V.) lainsoni, and L. (Leishmania) amazonensis and were associated with human disease in the Amazon forest (Braga VJI, Minaya G, Lucas C, unpublished data). This report describes the results of studies conducted from 1986 through 1993 that further defined the geographic distribution and the relative importance of different Leishmania species as causative agents of different clinical forms of leishmaniasis in Peru.

PATIENTS, MATERIALS, AND METHODS

Patients and specimens. Patients with a clinical diagnosis of cutaneous (CL), mucosal (ML), or diffuse cutaneous leishmaniasis (DCL) were enrolled in this study from 1986 through 1993 at the NAMRID Laboratory, the Clinic for Tropical Disease at San Marcos University Daniel A. Carrión, the Central Military Hospital in Lima, or at the Ministry of Health hospital in Quillabamba and Cusco, Peru for medical care and treatment. Clinical histories, informed consent, and lesion aspirates and biopsy tissue were obtained from each patient. All specimens were collected and assayed separately, including multiple specimens from the same patient.

The research protocol using human subjects in this study has been reviewed and approved by the Naval Medical Research Institute’s Committee for the Protection of Human Subjects.

Assay of specimens. Specimens were assayed for Leishmania parasites by inoculating aliquots of either aspirates or biopsy tissue suspensions onto Senekji’s blood-agar medium. The blood agar medium was overlayed with saline containing 1% gentamicin sulfate (10 mg/ml [w/v]; Sigma Chemical Co., St. Louis, MO) and 1% 5-fluorocytosine (5mg/ml [w/v]; Sigma Chemical Co.). Isolates of Leishmania parasites were propagated in Schneider’s Drosophila medium (Gibco-BRL Life Technologies, Inc. Gaithersburg, MD) supplemented with 20% heat-inactivated fetal bovine serum (FBS) (Sigma Chemical Co.). Stocks of Leishmania isolates were stored in vials at -190°C in RPMI 1640 medium (Sigma Chemical Co.) tissue culture medium supplemented with 10% dimethylsulfoxide (Mallinckrodt, Inc., Paris, KY) and 20% heat-inactivated FBS.

In vitro cultivation. Stocks of Leishmania isolates were used to prepare promastigotes to produce extracts for identifying the Leishmania species by the cellulose acetate electrophoresis technique. Promastigotes were harvested during the log phase of growth in Schneider’s Drosophila medium and centrifuged at 2,200 × g at 4°C for 10 min, and then washed twice in phosphate-buffered saline (pH 7.2). The pelleted promastigotes were suspended in an equal volume of deionized water with 1 mM EDTA, e-aminocaproic acid.
(Sigma Chemical Co.) and dithiothreitol (Sigma Chemical Co.), and then lysed by repeated rapid freezing and thawing as described by Saravia and others.6 Suspensions of the extracts were stored at -85°C until analyzed for isoenzymes.

**Isoenzyme electrophoresis.** Cellulose acetate electrophoresis analysis of the isoenzyme profiles of the *Leishmania* isolates were performed according to previously described methods.5,6 Extracts were analyzed for isozymes, mannosse phosphate isomerase (MPI) and glucose phosphate isomerase (GPI); oxidoreductases, including malic enzyme (ME), phosphogluconate dehydrogenase (6PGDH), glucose-6-phosphate dehydrogenase (G6PDH), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), isocitrate dehydrogenase (ICD), and glutathione reductase (GSR1 and GSR2); transferases, including aspartate aminotransferase (ASAT and GOT) and alanine aminotransferase (ALAT); phosphoglucomutase (PGM), hexokinase (HK), 6-phosphofructokinase (PFK); hydrolases, including acid phosphatase (ACP), and lysases, including fumarate hydratase (FUM).

**Identification of *Leishmania* isolates.** The Peruvian *Leishmania* isolates were identified by comparing their isoenzyme profiles with those of selected World Health Organization (WHO) reference strains. These WHO reference strains were *L. (V.) braziliensis* MHOM/BR/84/LTB300, *L. (V.) panamensis* MHOM/PA/71/LS94, *L. (V.) guyanensis* MHOM/BR/75/M4147, *L. (L.) mexicana* MHOM/BZ/82/BEL21, *L. (L.) amazonensis* MHOM/BR/73/M2269, and *L. (L.) pifanoi* MHOM/VE/57/LL1.1. A non-WHO reference strain, *L. (V.) peruviana* MHOM/PE/87/PAB2880, isolated from a male patient who acquired his cutaneous disease in Yauyos-Lima, was used. *Leishmania* isolates were identified by comparison of their isoenzyme profiles for GPI, MPI, and 6PGDH with those of reference strains. Additional enzymes (G-6PDH, ME, ASAT, ALAT, and PGM) were used to confirm the identifications made with GPI, MPI, and 6PGDH enzymes. Isolates that could not be identified with the reference strains were analyzed further using other enzymes, including ACP, FUM, GOT, HK, ICD, LDH, MDH, GSR1, GSR2, LP, PFK, and PK.

**RESULTS**

A total of 351 isolates of five different *Leishmania* were obtained from 350 patients who were infected in at least 15 different departments in Peru (Table 1). Of the 351 isolates, 79.5% were typed as *L. (V.) braziliensis*, 6.8% as *L. (V.) guyanensis*, 10.0% as *L. (V.) peruviana*, 2.0% as *L. (V.) lainsoni*, and 1.7% as *L. (L.) amazonensis*. The distribution of *Leishmania* isolates by Departments of Peru is presented in Figure 1, and the distribution by the two major ecologic regions, the Andes Mountains and Amazon rain forest, is presented in Table 2.

The clinical forms of leishmaniasis were primarily cutaneous or mucosal with some patients presenting with simultaneous cutaneous and mucosal lesions (Table 3). There were three cases of diffuse cutaneous leishmaniasis, all of which were previously reported.5,6,8,11

*Leishmania* (*V.*) *braziliensis* was associated most frequently with cutaneous and mucosal ulcers and mixed cutaneous and mucosal disease (Table 4). Of the 35 *L. (V.) peruviana* isolates, 33 were obtained from patients with cutaneous lesions who apparently acquired the infections in the Departments of Ancash, Cajamarca, Huanuco, and Lima (Table 1). The two other cases included a patient from Oyon in the Andean region of Lima, with cutaneous lesions that extended from the facial area to the mucosa, and a patient with diffuse cutaneous lesions who apparently was infected approximately 22 years ago in the Andean region of the Department of La Libertad (Miranda H, unpublished data).

Among the 24 isolates of *L. (V.) guyanensis*, one was obtained from a mucosal nasal lesion of a 22-year-old woman who apparently acquired the infection in the Department of Junín (Table 4). All other strains were obtained from patients with cutaneous lesions who apparently acquired the infections in the Departments of Amazonas, San Martin, Ancash, Casco, Junín, Huanuco, and Ucayali. One patient, who worked for an oil company in the Department of Pucallpa yielded an isolate of *L. (V.) guyanensis* from a cutaneous ulcer; one year later, the same patient presented with another cutaneous lesion from which *L. (V.) braziliensis* was isolated.

*Leishmania* (*V.*) *lainsoni* was diagnosed among seven patients with primary cutaneous lesions who acquired the disease in the high jungle of the Amazonian forest in the departments of San Martin, Huanuco, Pasco, Ayacucho, and Cusco (Table 4). The identity of four of the isolates were verified by DNA hybridization.

*Leishmania* (*L.*) *amazonensis* was isolated from six patients in the Departments of Amazonas, Ucayali, Junín, and Ayacucho; three had cutaneous lesions, one a mucosal lesion, and two cases presented with diffuse cutaneous lesions (Table 4). The latter two patients apparently acquired the disease in the Department of Junín (Minaya G, Lucas C, unpublished data).3 Two of the isolates, LEH6337 (DCL) and LEH1646 (ML), yielded amplification products at high stringency using the *L. mexicana* complex primers, thus verifying that they belong to this complex. Further analysis of the isoenzyme profiles identified the isolates as *L. (L.) amazonensis*.

Among all cases of leishmaniasis caused by the five different *Leishmania* species, males were affected more fre-
FIGURE 1. Geographic location of leishmaniasis cases by departments in Peru. Each symbol represents the location where one or more cases occurred per department.

### DISCUSSION

The cases of leishmaniasis described in this report were diagnosed among persons who resided at altitudes less than 400 meters above sea level in the tropical rain forest or the low jungle, at 400–1,000 meters above sea level in the high jungle, and between 900 and 3,000 meters above sea level in the Andean and inter-Andean valleys. Our findings extended the geographic distribution of the *Leishmania* isolates to 15 other Departments of Peru. Studies conducted in Ecuador, Colombia, Brazil, and Bolivia revealed diverse subspecies of *Leishmania* as well as several new species. Our results indicated that at least five *Leishmania* species were associated with cases of cutaneous, mucosal, and diffuse cutaneous leishmaniasis in Peru.

-Lucius and others
Analysis of isoenzyme profiles by cellulose acetate electrophoresis has been useful for the identification of different *Leishmania* strains. However, the strains isolated from patients with self-healing cutaneous lesions (*L. (V.) peruviana*) could only be distinguished from the two WHO reference strains of the *L. (V.) braziliensis* by using the MPI and ME enzymes.

Studies conducted in Ecuador, a neighboring country of Peru that has similar Andean geographic characteristics, failed to detect any cases associated with the *L. (V.) peruviana*, the species that causes Uta in Peru.10, 21

*Leishmania (V.) braziliensis*, a species found mostly in the tropical forest that extends from the Department of Loreto in northern Peru to the Department of Puno in southern Peru, was the of cause of 98% of the cases of mucosal leishmaniasis. The remaining 2% of cases were associated with patients (age range = 2–31 years) who were infected in Ambo and Yanas in the Andes Mountains in the Department of Huanuco (Table 2). The occurrence of mucosal infection among both children and adults in the Andes Mountains is not understood, but may reflect transmission from workers who contracted infection in the jungle and returned to their hometowns. Although the mechanism of transmission is unknown, the anthropophilic sandfly species, *Lutzomyia teijadae* has been suggested as a possible vector of *L. (V.) braziliensis* (Fernandez R, unpublished data).

Of 279 *L. (V.) braziliensis* isolates, 61% were isolated from mucosal cases, 31% from cutaneous cases, and 8.0% from mixed cutaneous and mucosal cases (Table 4). Among our isolates that were classified among the *Vianna* group, the GPI isoenzyme profile of 99.0% corresponded with that of WHO reference strain LTB300. However, a variant strain of *L. (V.) braziliensis* was isolated from 1.0% (n = 3) of the patients. In these variants the MPI band was more anodal, MPI had two strong bands, not just one, and the G-6PDH band was more cathodal compared with the bands of the subspecies *L. braziliensis*.

*Leishmania (V.) guyanensis* is endemic in the northern and central jungle regions of the Departments of Amazonas, Ancash, San Martin, Huanuco, Ucayali, and Junin. In 1987, two strains of this parasite were isolated from cutaneous lesions of a man from the Department of Loreto (Braga VJ, unpublished data). Except for one patient, all isolates were obtained from cutaneous lesions. In Colombia, this species has been associated with mucosal disease.13 This parasite has a very wide distribution in Peru, extending from the western region of the inter-Andean valleys of Ancash through the low jungle region of the western part of the Department of Ucayali, with most of the cases apparently being acquired in high jungle region (Table 3).

*Leishmania (V.) lainsoni* was previously reported by Silveira and others20 in the State of Para, Brazil. Our isolation of this species was the first association with human disease in Peru, and extended the distribution to the Departments of San Martin, Huanuco, Ayacucho, and Pasco. These Departments are located in the eastern Andean zone, or the high jungle area between 600 and 2,000 meters above sea level.4 The seventh isolate was obtained from a patient who probably acquired the infection in La Convencion locality of the Department of Cusco (Tables 1 and 2). This species was shown to have the same sequence homology as the reference strain M6426 of *L. (V.) lainsoni*.

Cellulose acetate electrophoresis with MPI and ME enzyme markers appear to distinguish strains of *Leishmania* from cutaneous and mucosal cases. The site of acquisition of the parasites were mostly between 1,300 and 2,300 meters above sea level (Table 3). Although we studied only patients who were referred to medical care centers, most of the active cases were among children less than 10 years of age, as reported previously (Miranda H, unpublished data and Llanos-Cuentas A, Campos M, unpublished data). *Leishmania*
(V.) peruviana has not been associated with cutaneous cases in Ecuador, even though the Andean geographic characteristics are similar to those in Peru.11

Leishmania (L.) amazonensis cases have been reported to present with a wide range of clinical manifestations.14,22 Similarly, our patients included two cases with diffuse cutaneous lesions, three with cutaneous lesions, and one with mucosal leishmaniasis. The patient with mucosal disease was a 33-year-old woman who acquired the disease in the high jungle of the Department of Junin; her disease affected the palate, uvula, and pharynx, with infiltration of nasal fossae and septum. The strain was isolated from nasal septum and a positive smear was obtained from the palate. The identity of the two Leishmania isolates from DCL cases were also confirmed by DNA hybridization analysis.

The results of this study indicated that leishmaniasis was contracted in two main regions of Peru, the Andes mountains and the tropical rain forest. Males were affected most, which was possibly due to their more frequent outdoor occupational activities as compared with females. Finally, our findings extended the known geographic distribution of Leishmania species, and further described the clinical manifestations associated with human cases of the different forms of leishmaniasis in Peru.

Acknowledgments: We are grateful to Drs. Nancy Saravia and Iris Segura (Centro Internacional de Investigaciones Medicas [CIDIELM], Cali, Colombia) for training provided on the cellulose acetate electrophoresis technique to the NAMRID staff. Also, our sincere appreciation goes to Dr. David Carrizales (Hospital Militar Central, Lima, Peru), Dr. José Echeverría (Western Geophysical Company, Ucayali, Peru), Dr. Elaine Chavez (Asociacion Beneficia Cristiana, Programa de Desarrollo Integral, Cusco, Peru), and Hermana Teresa Ruíz Ruiz De Gauna (Hospital Antonio Lorena, Cusco, Peru) for assistance with the enrollment of patients and collection of specimens.

Financial support: This work was supported by the Naval Medical Research and Development Command, Work Unit No. 62787A 870AN.

Disclaimer: The opinions and assertions contained herein are the private ones of the authors and are not to be construed as official at large.

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Reprint requests: Editorial Assistant, U.S. NAMRID/Unit 3800, American Embassy, APO AA 34031.

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