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# IDENTIFICATION, USING ISOENZYME ELECTROPHORESIS AND MONOCLONAL ANTIBODIES, OF *LEISHMANIA* ISOLATED FROM HUMANS AND WILD ANIMALS OF ECUADOR

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*Abstract.* Six strains of *Leishmania* isolated from wild mammals and humans on the Pacific Coast of Ecuador were identified by isoenzyme electrophoresis and by their reactivity patterns to a cross-panel of specific monoclonal antibodies using a radioimmune binding assay. Single isolates from *Sciurus vulgaris, Potos flavus,* and *Tamandua tetradactyla* were identified as *Leishmania amazonensis.* Three other strains, isolated from cutaneous lesions of humans, were identified as *Leishmania panamensis.*

New World leishmaniases are widely distributed in Central and South America, where they present a considerable public health problem.[1,2] Parasites causing these cutaneous, mucocutaneous, and visceral leishmaniases have been characterized and identified by isoenzyme electrophoresis, monoclonal antibodies, and kinetoplast DNA.[3–5] Since the first human case of leishmaniasis was described in Ecuador in 1920, many additional cases of the disease have been reported.[6] *Leishmania* parasites have been isolated from 3 mammalian species in the country.[7] Until now, however, the identification and taxonomy of these Ecuadorian parasites have been based mainly on their clinical manifestations in humans, epidemiological features, and differing growth patterns in the hamster and in vitro. It is difficult to identify parasites solely on these criteria. Recently, we compared selected *Leishmania* isolates from Ecuador with well characterized WHO reference strains using isoenzyme electrophoresis and monoclonal antibodies. The present paper gives the results of these studies.

## MATERIALS AND METHODS

### *Parasites examined*

Six Ecuadorian *Leishmania* strains were selected for study: strain MSCI/EC/87/G-02 isolated from a liver and spleen homogenate of a squirrel (*Sciurus vulgaris*) captured in Palenque, Department of Los Rios; strain MPOT/EC/87/G-03 from a liver and spleen homogenate of a kinkajou (*Potos flavus*) collected in Palenque, Department of Los Rios; strain MTAM/EC/87/G-04 from a liver and spleen homogenate of an anteater (*Tamandua tetradactyla*) captured in Echeandia, Department of Bolivar; strain MHOM/EC/87/G-05 from a skin ulcer of a human in Quininde, Department of Esmeraldas; strain MHOM/EC/87/G-06 from a skin lesion of a patient in Zapallo Grande, Department of Esmeraldas; and strain MHOM/EC/87/G-07 from a cutaneous lesion of a human in Santo Domingo de los Colorados, Department of Pichincha. The circumstances of these parasite isolations have been described previously.[7,8]

The 6 Ecuadorian isolates were compared with the World Health Organization (WHO) reference strains of New World *Leishmania* listed in Table 1.

### *Isoenzyme electrophoresis*

Cultivation procedures for the promastigotes, preparation of extracts, enzyme activities, and electrophoresis procedures have been reported previously.[9–12] The isolates were characterized twice for up to 17 enzymes, including the 3 enzymes that can accurately identify parasites in

TABLE 1

*Radioimmune binding assay results,* employing* Leishmania *specific monoclonal antibodies, with reference strains and Ecuadorian isolates from humans and wild animals*

| Stock Code | Species | Monoclonal antibodies† | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | M2 | M3 | M7 | M9 | M11 | B3 | B4 | B11 | B16 | B18 | B19 | D3 |
| *Reference strains* | | | | | | | | | | | | | |
| MHOM/BR/73/M2269 | *L. amazonensis* | 34.5 | 22.6 | 6.8 | 26.0 | 34.1 | 1.9 | 0.9 | 1.8 | 1.3 | 1.4 | 1.5 | 1.2 |
| MORY/PA/68/GML3 | *L. aristidesi* | 16.9 | 0.7 | 2.9 | 21.8 | 27.7 | — | — | 1.0 | 1.0 | 2.3 | 1.0 | 0.5 |
| MNYC/BZ/62/M379 | *L. mexicana* | 1.7 | 1.8 | 26.3 | 4.9 | 6.6 | — | — | 1.5 | 2.0 | 1.4 | 1.7 | 1.1 |
| MHOM/PA/71/LS94 | *L. panamensis* | 1.5 | 0.9 | 1.4 | 0.9 | 1.7 | 26.0 | 9.0 | 12.2 | 2.5 | 1.0 | 1.2 | 0.5 |
| MHOM/BR/75/M2903 | *L. braziliensis* | 2.0 | 1.3 | 2.4 | 1.3 | 1.3 | 70.5 | 0.9 | 1.2 | 27.8 | 34.5 | 2.0 | 1.4 |
| MHOM/BR/75/M4147 | *L. guyanensis* | 1.2 | 1.8 | 1.0 | 2.0 | 2.8 | 1.2 | 1.3 | 1.0 | 1.0 | 1.0 | 13.2 | 0.7 |
| MHOM/BR/74/PP75 | *L. chagasi* | 0.4 | 0.4 | 0.7 | 0.4 | 1.2 | 0.9 | 1.0 | 1.0 | 1.0 | 0.7 | 0.6 | 13.0 |
| *Ecuadorian isolates* | | | | | | | | | | | | | |
| MSCI/EC/87/G-02 | *L. amazonensis* | 18.1 | 4.4 | 4.7 | 10.7 | 19.0 | — | — | 1.5 | 1.4 | 1.2 | 1.2 | 1.5 |
| MPOT/EC/87/G-03 | *L. amazonensis* | 31.5 | 11.3 | 11.2 | 25.4 | 31.8 | — | — | 1.5 | 1.2 | 1.1 | 1.3 | 1.2 |
| MTAM/EC/87/G-04 | *L. amazonensis* | 33.8 | 12.6 | 9.9 | 25.6 | 37.9 | — | — | 1.4 | 1.2 | 1.0 | 1.2 | 1.7 |
| MHOM/EC/87/G-05 | *L. panamensis* | 4.1 | — | — | — | — | 12.7 | 13.2 | 12.6 | 1.6 | 2.0 | 3.0 | — |
| MHOM/EC/87/G-06 | *L. panamensis* | 1.5 | — | — | — | — | 15.7 | 14.9 | 10.4 | 1.3 | 2.0 | 3.0 | — |
| MHOM/EC/87/G-07 | *L. panamensis* | 3.0 | — | — | — | — | 32.9 | 30.3 | 27.8 | 1.0 | 0.8 | 2.3 | — |

* Ratio cpm bound monoclonal antibodies/cpm bound control; values >3 were considered positive.

† From hybridoma clones: M2, IX-2H7-E10; M3, IX-5H9-C10; M7, LXVIII-1D7-B8; M9, XLV-2B5-H7; M11, XLV-1D11-E11; B3, VI-4D10-D12; B4, VI-2A5-A4; B11, VI-5G3-F3; B16, XIII-3E6-B11; B18, XIV-2A5-A10; B19, XLIV-5A2-B9; D3, LXXVIII-1F2-A2.

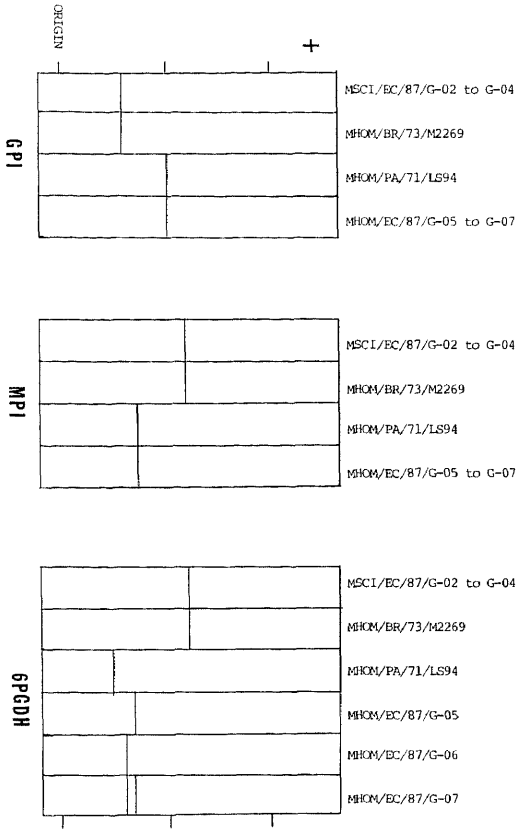


FIGURE 1. Diagrammatic representation of the electrophoretic patterns of GPI, MPI, and 6PGDH distinguishing the 2 groups of Ecuadorian isolates and showing the similarities in the enzyme profiles of each group with, respectively, *L. panamensis* (MHOM/PA/71/LS94) and *L. amazonensis* (MHOM/BR/73/M2269) reference strains.

the genus *Leishmania*: glucose phosphate isomerase (GPI-EC 5.3.1.9), mannose phosphate isomerase (MPI-EC 5.3.1.8), and phosphogluconate dehydrogenase (6PGDH-EC 1.1.1.44).[11] The specific identifications were based on comparisons of the enzyme data from the Ecuador and WHO reference isolates.

### *Monoclonal antibodies and indirect radioimmune assay*

The monoclonal antibodies used in this study, specific for members of the *L. braziliensis, L. mexicana* and *L. donovani* complexes, have been described.[13–16] The promastigotes were cultured in Schneider's medium with 15% fetal bovine serum (FBS). Characterization of the *Leishmania* was performed with an indirect radioimmune binding assay using whole parasite lysates as antigen. Round bottom, 96-well polyvinylchloride plates were coated overnight at 5°C with sonicated homogenates of whole promastigotes which were diluted in PBS containing 0.02% sodium azide ($NaN_3$). The plates were washed 5 times and blocked with PBS containing 0.02% $NaN_3$ and 2% FBS. Culture medium supernatants, containing secreted antibodies, were incubated with the antigen plates overnight at 5°C. After washing, affinity-purified $^{125}I$ labeled rabbit $F(ab')_2$ anti-mouse immunoglobulin ($10^5$ cpm:5–10 $\mu$Ci/$\mu$g protein) was added to the wells and incubated for 1 hr at 0°C. Excess antibody was removed by washing. The plates were air dried and the radioactivity bound to each well was measured using a Packard Auto-Gamma Counter.

### *Parasite classification*

Identification of parasites in this study have followed the simplified nomenclature for the genus *Leishmania,* suggested by Saf'janova[17] and Shaw and Lainson[18] and used by the International Colloquium at Montpellier, France, 2–6 July 1984.[19]

## RESULTS

### *Isoenzyme electrophoresis*

The 3 isolates from wild mammals, MSCI/EC/87/G-02, MPOT/EC/87/G-03, and MTAM/EC/87/G-04, had identical allomorphs (bands of enzyme activity observed by electrophoresis) to each other and to the WHO *L. amazonensis* reference strain (MHOM/BR/73/M2269) for the enzymes GPI, MPI, and 6PGDH (Fig. 1). The latter enzymes are used to separate most *Leishmania*[11] and indicate that the aforementioned isolates are *L. amazonensis.* The 3 isolates from humans, MHOM/EC/87/G-05, MHOM/EC/87/G-06, and MHOM/EC/87/G-07, were similar to each other and to the WHO *L. panamensis* reference strain, MHOM/PA/71/LS94, for the same enzymes. These 3 isolates were polymorphic (more than 1 allomorph in the population) for 6PGDH. The Ecuadorian isolates were also tested for as many as 17 additional enzymes; the data from these enzymes confirmed the original species designations.

*Monoclonal antibodies*

The reactivity of monoclonal antibodies of the 6 Ecuadorian *Leishmania* strains and the WHO reference strains are shown in Table 1. Monoclonal antibodies, previously shown to have a high and consistent qualitative specificity for members of the *L. mexicana* or *L. braziliensis* complex,[4] reacted with the Ecuadorian isolates. The reactive patterns of isolates MPOT/EC/87/G-03, MTAM/EC/87/G-04, and MSCI/EC/87/G-02 were similar to that of the *L. amazonensis* reference strain, MHOM/BR/73/M2269.

The reactive patterns of the human isolates, MHOM/EC/87/G-05, MHOM/EC/87/G-06, and MHOM/EC/87/G-07, were very similar to that of the *L. panamensis* reference strain (Table 1). On the basis of these results, the latter strains were identified as *L. panamensis.*

## DISCUSSION

Six Ecuadorian *Leishmania* strains were identified to the species level by isoenzyme electrophoresis and monoclonal antibodies. The 3 strains isolated from human skin lesions were identified as *L. panamensis.* The 3 parasites recovered from viscera of wild mammals were identified as *L. amazonensis.*

A variety of molecular and biochemical methods have been used to identify and to characterize *Leishmania* parasites.[3-5] Isoenzyme electrophoresis has been commonly used to identify *Leishmania* parasites at species and subspecies levels.[3, 9, 10] A newer approach for parasite characterization and identification is the indirect radioimmune binding assay-monoclonal antibody technique.[4] The high specificity of some monoclonal antibodies permits *Leishmania* parasite identification and provides evidence for the stability of intrinsic molecular characters of the parasite. Further, the results of parasite identification using monoclonal antibodies parallels those of isoenzyme electrophoresis and kinetoplast DNA.[4, 18, 20, 21]

This is the first report on the characterization and identification of parasites isolated from Ecuador. Parasites isolated from humans living on the Pacific Coast of Ecuador were identified as *L. panamensis.* The ecology of this region is similar to that of the Pacific Coast of Colombia, where this same species is highly endemic.[22]

A new finding of this study was *L. amazonensis* visceral infection in *S. vulgaris, P. flavus,* and *T. tetradactyla* captured near the sites where the human isolates of *L. panamensis* isolates were obtained. In these regions, therefore, there are probably at least 2 causative agents of human leishmaniasis, *L. panamensis* and *L. amazonensis.* Other *Leishmania* parasites were also isolated from 3 species of mammals caught in Naranjal, Department of Guayas, Ecuador.[7, 8] These parasites appear to be different from the currently well established New World *Leishmania.*

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