Etiologic Agent of an Epidemic of Cutaneous Leishmaniasis in Tolima, Colombia

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Abstract. American cutaneous leishmaniasis (ACL) has been characterized as a zoonotic disease. However, peri-domestic and domestic transmission have been recorded in at least nine countries in Central and South America. The present study was undertaken to identify the etiologic agent of a peridomestic epidemic of ACL in the Department of Tolima, Colombia. Leishmania isolates were obtained during the diagnosis of 56 patients with ACL who consulted the local leishmaniasis control program in three municipalities in Tolima. Species were identified using monoclonal antibodies and isoenzyme electrophoresis. A total of 53 (94.6%) of 56 isolates were identified as Leishmania (Viannia) guyanensis. Three isolates (5.4%) were identified as L. (V.) panamensis. Leishmania (V.) guyanensis is the probable etiologic agent of the largest epidemic of cutaneous leishmaniasis recorded in Colombia. This species has not previously been reported outside the Amazon and southeastern regions of Colombia, and has not been described in the peridomestic setting or linked with an epidemic.

INTRODUCTION

American cutaneous leishmaniasis (ACL) has traditionally been characterized as a zoonotic disease, primarily affecting males who are occupationally exposed to sylvatic transmission cycles. However, the epidemiologic pattern of transmission of ACL has changed and been accompanied by an unprecedented increase in the number of cases. Evidence of peri-domestic and domestic transmission has been reported in at least nine countries in Central and South America.1-11 Factors contributing to this transition are multiple, but generally associated with urbanization and alteration of ecosystems where the transmission occurs.12

Deforestation and replacement of primary forests by plantations has been frequently associated with the domestication of transmission of ACL. Replacement of primary forests with coffee plantations has coincided with the domestic transmission of Leishmania (Viannia) panamensis in the Pacific coast provinces of Esmeraldas and Pichincha in Ecuador7 and with a peridomestic outbreak of L. (V.) braziliensis in Colombia.11

Alteration of ecosystems has led to changes in the distribution and/or density of sand flies, reservoirs and Leishmania, and in their interactions. Explosive increases in sand fly populations,13 movement of sylvatic vectors to peridomestic settings, and expansion of vector competence for other Leishmania species, even subgenera,14,15 have been reported in Latin America. Although sylvatic mammals continue to be regarded as the principal reservoirs, in some domestic settings dogs have been implicated as a source of infection.10,16-25

Previous studies have reported the geographic distribution and frequency of the etiologic agents of cutaneous leishmaniasis (CL) in Colombia.26-30 Although these studies could be expected to show selection bias related to the origin of patients diagnosed, L. (V.) panamensis consistently predominates (54-80% of cases), followed by L. (V.) braziliensis (10-30%), with other species being infrequent: L. (V.) guyanensis (1-3%), L. (L.) amazonensis (2%), and L. (L.) mexicana (1-5%). Leishmania (V.) braziliensis has been and continues to be widely distributed throughout Colombia, whereas L. panamensis predominates in the northern and southwestern regions and more recently has emerged in the southeastern region.27 Leishmania (V.) guyanensis has been reported only from the southeastern region of Colombia in the Amazon River basin, and L. (L.) mexicana has only been reported from five departments that are located in separate regions of the country.

In Colombia, domestic and peridomestic transmission has been described for L. (V.) panamensis and L. (V.) braziliensis.11,31-34 The importance of domestic transmission is underscored by the high proportion of women and children affected by the disease in leishmaniasis-endemic regions. Leishmania (V.) guyanensis, a characteristically sylvatic species,35,36 has not been previously reported in the domestic setting in Latin America.

Since 2003, the communities of southern Tolima, Colombia, where less than 10 cases per year were reported prior to this date, have been experiencing the largest epidemic ever recorded in Colombia. The epidemic peaked between 2003 and 2004 in the municipality of Chaparral (2,800 cases) and then spread to adjacent municipalities where cases continue to be reported. The high proportion of women and children (30%) and predominance of the endophagic vector Lutzomyia longifirocusa37 indicate that transmission has an important intradomiciliary or peridomestic component. The present study was undertaken to identify the etiologic agent of this peridomestic epidemic and its relationship with Leishmania spp. transmitted in Colombia and neighboring countries.

MATERIALS AND METHODS

Isolation of Leishmania. Leishmania were isolated from patients with a diagnosis of CL within the framework of the local leishmaniasis control programs in the municipality of Chaparral, Tolima during the peak of the epidemic in 2004, and in the municipalities of Chaparral, Ortega, and Rovira during 2006. Lesion aspirates were obtained as part of the regular diagnostic procedures for parasitologic diagnosis, as well as for identification of Leishmania species. Three of the isolates were obtained by the Colombian National Institutes of Health during investigations conducted in Chaparral in 2004. The procedures for isolation and cultivation of parasites

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have been reported. Basic demographic and clinical information was obtained during consultation for diagnosis. All patients with parasitologic diagnosis of CL were referred for treatment with Glucantime® (Aventis Pharma, Sao Pablo, Brazil) (20 mg of Sb/kg/day for 20 days) or Imipavid® (Zentaris, Bordeaux, France) (2.5 mg/kg/day for 28 days) by the leishmaniasis program personnel.

**Study sites.** The municipalities of Chaparral, Rovira, and Ortega are located in southern Tolima, on the western range of the Colombian Andes. Chaparral, the municipality where the epidemic started, is located at 3°43′37″N, 75°29′14″W, and Ortega and Rovira are located at 3°56′20″N, 75°13′7″W and 3°56′20″N, 75°13′27″W, respectively. The altitudes of the three sites range between 600 and 2000 meters above sea level and the average temperature is approximately 21°C. The region consists mainly of intervened dry tropical forest areas and the principal economic activities are agriculture (coffee, maize, beans) and cattle farming.

**Identification of Leishmania species.** Leishmania isolates were initially characterized by an indirect immunofluorescent antibody test using a panel of monoclonal antibodies. Isolates that could not be identified to the species level with monoclonal antibodies were analyzed using isoenzyme electrophoresis in cellulose acetate for five enzymes that enable discrimination of species of the *Viannia* subgenus: (nucleoside hydrolase [NH, E.C.3.2.2.2.], phosphoglomutase [PGM, E.C.2.7.5.1.], phosphoglucomutase [6PGD, E.C.1.1.1.44], glucose-6-phosphate dehydrogenase [G6PD, E.C.1.1.1.49.], and superoxide dismutase [SOD, E.C.1.15.1.1.]). The conditions for electrophoretic analysis of polymorphisms of these enzymes have been described. The following World Health Organization (WHO) reference strains were included in the characterization of patient isolates: *L. (V.) braziliensis* (MAN/BR/1975/M4147), *L. (V.) guyanensis* (MAN/BR/1975/M4147), and *L. (L.) amazonensis* (VEC/BR/1967/PHS).

**Data analysis.** The age and sex distribution of patients was described. Serodemes were identified on the basis of patterns of reactivity with the panel of *Viannia* subgenus and species-specific monoclonal antibodies as described for Colombian strains. Similarly, zymodemes were identified according to electrophoretic polymorphisms of five enzymes used to discriminate species.

### RESULTS

Cultures of lesion aspirates were performed for 102 patients with cutaneous lesions suggestive of CL. A total of 56 strains were isolated from the same number of patients with CL from three municipalities in Tolima. Six isolates were obtained in 2004, at the peak of the epidemic, and the rest were obtained in 2006. Patients had been infected in Chaparral (9), Ortega (19) and Rovira (28), three of the municipalities that have reported cases throughout the outbreak.

All but three of the isolates (94.6%) were identified as *L. (V.) guyanensis* by isoenzyme analysis. Three (5.4%) isolates were identified as *L. (V.) panamensis* by monoclonal antibodies. These three strains had been isolated by personnel of the Colombian National Institutes of Health from adult patients in Chaparral in 2004, and sent to CIDEIM for confirmation of species identity.

**Phenotypic variation.** Serodemes. None of the *L. (V.) guyanensis* strains were reactive with the species-specific B19 antibody for *L. (V.) guyanensis* under the conditions used. Three reactivity profiles with the monoclonal antibody panel for the *Viannia* subgenus were observed for the strains of this species from patients in the epidemic focus in Tolima. These corresponded with serodemes 3B (33 strains), 7 (2 strains), and 8 (18 strains) previously described by Saravia and others (Table 1). All but 2 of the strains of *L. (V.) guyanensis* displayed the *L. Viannia* subgenus epitope recognized by the B2 monoclonal antibody, and 33 of 53 also expressed the epitope recognized by the B12 monoclonal antibody. *Viannia* subgenus and species-specific epitopes were undetectable in two strains of *L. (V.) guyanensis* from Tolima. The three strains of *L. (V.) panamensis* presented the same reactivity pattern, which corresponded to serodeme 1.

**Zymodemes.** All 53 *L. (V.) guyanensis* strains were identified by isoenzyme analysis. Notably, the population of *L. (V.) guyanensis* that causes disease in the human population of Tolima was found to be homogeneous and pertained to a single zymodeme identical to that of the WHO reference.
strain M4147 (Figure 1). The three L. (V.) panamensis strains were identical and pertained to Colombian zymodeme 2.3.

**Age distribution and clinical characteristics of patients.** A total of 32.0% (17 of 53) patients from whom L. guayanensis was isolated were children less than 15 years of age, and 47.1% (25 of 53) were female (Figure 2). Although this sample is not representative because of possible selection bias, the observed age and sex distribution closely resembles that of the population affected by the epidemic over the two-year period in 2004–2006, during which 34% (964 of 2,834) of the patients were children less than 15 years of age and 40% (1,134 of 2,834) were female. *Leishmania (V.) panamensis* was isolated from two females and one male who were 17, 20 and 39 years of age, respectively. All patients in which isolation was attempted presented localized cutaneous lesions.

**DISCUSSION**

*Leishmania (V.) guyanensis* transmission in Colombia, to our knowledge, has not been reported in regions outside the Amazon region including the eastern plains26–28 (Figure 3B) or within the peridomestic setting. Therefore, the results of this study provide further evidence of the changing epidemiologic patterns of transmission of different species of *Leishmania* in the New World1–10 and in Colombia in particular.

The age and sex distribution of cases reported during the southern Tolima epidemic is consistent with domestic/peridomestic transmission. The high proportion of females (40%) and children (34%) contrasts with the exclusive prevlence among occupationally exposed males in cases detected at CIDEIM prior to this epidemic (between 1980 and 2004 *L. guyanensis* was isolated from 26 males between 16 and 56 years of age) and with the high proportion of males (82%) 11–57 years of age in a clinical study of patients who had acquired infection with *L. guyanensis* in the State of Amazonas, Brazil.45 The demographic characteristics of the Tolima epidemic and of CL patients in this study suggest that transmission is taking place in the peridomicile or intradomicile, rather than in the sylvatic context. Domestic and peridomestic transmission of CL had been described and documented for *L. (V.) panamensis*, *L. (V.) braziliensis*, *L. (V.) peruviana*,1–10 and *L. (L.) amazonensis*.14 Concomitantly with the present report, a study conducted in a national park in the Amazonian lowlands of the Department of Cochabamba, Bolivia, reported the sylvatic vector *Lutzomyia shawi* in the peridomestic and domestic settings and the intradomicile. An hsp70 polymerase chain reaction–restriction fragment length polymorphism detected *L. (V.) braziliensis* in 4 of 67 pools from 30–39 female *Lu. shawi* and *L. (V.) guyanensis* in 1 of 67 pools. *Leishmania (V.) braziliensis* also predominated among human cases from whom isolates were obtained, which is consistent with sympatric peridomestic transmission of these two species of the *Viannia* subgenus, and possibly *L. lainsoni*, in areas of Amazonian rain forest.46

The distribution of *L. (V.) guyanensis* in South America is largely confined to the Amazon region (Figure 3A), although some cases have been reported in northern Argentina27 and in the Andes of Ecuador and Peru. In Colombia, the known distribution is limited to the Amazonian lowlands and to the eastern plains26–28 (Figure 3B). A search of the clinical database of CIDEIM showed that 24 of the 26 *L. (V.) guyanensis* cases diagnosed at CIDEIM in years prior to this epidemic

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**FIGURE 1.** Enzyme polymorphisms for World Health Organization reference strains of *Leishmania (Viannia)* species, *L. (Leishmania) amazonensis*, and representative strains from the three municipalities of the Tolima focus (Ortega, Rovira, and Chaparral) for enzymes discriminating species of the *Viannia* subgenus. SOD = superoxide dismutase; NH = nucleoside hydrolase; G6PD = glucose-6-phosphate dehydrogenase; PGM = phosphoglucomutase; 6PGD = phosphogluconate dehydrogenase.
had originated in municipalities in the sub-Andean region in southwestern Colombia (Figure 3B), a finding that differs from the rest of the cases reported, which were from the lowlands of the Amazon region.26–28 The corresponding patients were working-age males who had acquired the disease while temporarily working in the area (military personnel and farmers), and thus are likely to have been affected by the sylvatic transmission cycle. Nevertheless, these sub-Andean municipalities are located at altitudes ranging from 1,000 to 2,000 meters above sea level, which are considerably higher than the sites where *L. (V.) guyanensis* is usually transmitted (0–1,000 meters above sea level), but similar to the altitude of the site of the Tolima epidemic. These observations suggest the expansion of the distribution of *L. guyanensis* from the previously reported foci in the lowlands of the Amazon region of Colombia, to the sub-Andean region and then to the Magdalena river valley in southern Tolima (Figure 3).

The principal (*Lu. umbratilis*) and secondary (*Lu. anduzei* and *Lu. whitmani*) vectors associated with *L. (V.) guyanensis* transmission are prevalent in undisturbed primary forests in the Amazon region of South America (Figure 3A).48,49 Among the reported vectors, only *Lu. umbratilis* has been found in Colombia. If one considers that the ecoepidemiologic characteristics of the site of the epidemic, which consist primarily of dry tropical forest ranging between 1,000 and 2,000 meters above sea level, are strikingly different from the primary tropical rain forests of lower altitudes where the vector is usually found, transmission of *L. (V.) guyanensis* during the southern Tolima epidemic was likely caused by a different sand fly species.

Studies conducted by Pardo and others in Chaparral, the municipality where most cases have been reported, implicate *Lu. longiflocosa* as the most probable vector at this epidemic site.37 This study identified three anthropophilic species (*Lu. longiflocosa*, 81.7%, *n* = 235; *Lu. columbiana*, 3.4%; and *Lu. nunezovari*, 2.1%), but *Lu. longiflocosa* was considered the most likely vector not only because of its relative abundance and predominance but because it was found inside 41.7% (*n* = 46) of the houses sampled.37 *Lutzomyia longiflocosa* was also implicated as the most probable vector in three recent CL epidemic foci, in Huila, Norte de Santander, and Southern Tolima, which are located in the sub-Andean region of Colombia.50,51 Therefore, this species is considered to be the most important vector in this ecologic setting. Unfortunately, the *Leishmania* species responsible for these outbreaks were not identified.

**Figure 2.** Age distribution of 53 patients with cutaneous leishmaniasis caused by *Leishmania guyanensis* from Tolima (2004–2006).

**Figure 3.** A. Current reported distribution of *Leishmania* (*Viannia*) *guyanensis* and its principal vector *Lutzomyia umbratilis* in South America.27–29,40–42,50–54 B. Distribution of *L. (V.) guyanensis* and *Lu. umbratilis* in Colombia, according to published and unpublished data (Colombian National Institutes of Health). C. Map of Tolima showing the municipalities affected by the epidemic and locations where *Leishmania* strains isolated and identified in this study were obtained. Rov = Rovira; Ort = Ortega; San = San Antonio; Cha = Chaparral; Pla = Planadas.
Lutzomyia longiflora has not previously been implicated as a vector of L. (V.) guyanensis either in nature or experimentally. Santamaria and others experimentally infected Lu. longiflcosa with L. (V.) braziliensis and transmitted this infection to an experimental host. However, experimental infection has yet to be attempted for L. (V.) guyanensis.

The homogeneity of the isoenzyme phenotype of the strains of L. (V.) guyanensis isolated from patients in Tolima, their identity with the WHO reference strain for L. (V.) guyanensis originating in the Brazilian Amazon region, and prior documentation of the presence of this zymodeme in the Amazon region of Colombia suggest that the L. (V.) guyanensis population transmitted in Tolima was from the Colombian Amazon region. Migration of human populations or domestic animals exposed to transmission in the Amazon region of Colombia could have introduced this species to Tolima. At least two other zymodemes of L. (V.) guyanensis have been identified among strains from the Colombian Amazon. Antigenic variability in the population of L. (V.) guyanensis transmitted in Tolima was apparent in the reactivity patterns of the strains with species- and subgenus-specific monoclonal antibodies (Table 1). The non-reactivity of L. guyanensis in Colombia with the species-specific B19 monoclonal antibody and two of the three serodemes (3b and 8) observed among L. (V.) guyanensis strains from Tolima had been previously reported among L. (V.) guyanensis strains from the Amazon region that pertain to this zymodeme.

Zymodeme 2.3 of L. (V.) panamensis strains isolated in Tolima corresponds with that found to predominate among strains from the southern Pacific coast region of Colombia (Patía and Mira river basins). Emergence of this zymodeme of L. (V.) panamensis on the eastern side of the Andean mountain range is consistent with introduction by migration of infected reservoirs. The fact that the three cases with L. (V.) panamensis infection were all adults and resided in localities where L. (V.) guyanensis was subsequently found to be transmitted raises the question of whether these were autochthonous cases. Conversely, the conditions that favored extensive transmission in an area where leishmaniasis had only sporadically been reported may have led to transmission of more than one Leishmania species. Sporadic cases of L. (V.) panamensis infection occurred within an outbreak of L. (V.) braziliensis transmission in Dagua, a periurban setting in the Pacific coast region of Colombia in the late 1980s.

Based on these findings, we conclude that L. (V.) guyanensis is the most probable etiologic agent of the largest epidemic of CL in Colombia. The magnitude of the Chaparral epidemic demonstrates the adaptability of transmission to changing ecologic conditions and the potential for sylvatic Leishmania species to be rapidly disseminated within the periendemic setting. Furthermore, in view of the variable clinical response of patients with CL caused by different species to available antileishmanial drugs and uncertainty of the clinical response of patients infected with L. guyanensis in Colombia to available therapeutic options, the findings of this study are relevant to control programs based on case identification and treatment.

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