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NOTA BREVE / SHORT NOTE

BIOMEDICINA

A NEW RECORD OF *Leishmania* (*Viannia*) *guyanensis* (Trypanosomatidae) FROM THE PACIFIC COAST OF COLOMBIA

Un nuevo registro de *Leishmania* (*Viannia*) *guyanensis* (Trypanosomatidae) de la costa pacífica de Colombia

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ABSTRACT

The aim of this study was the identification of *Leishmania* species that causes cutaneous leishmaniasis in a patient from Buenaventura, Valle del Cauca, on the Pacific coast of Colombia. Clinical samples were obtained from a 29 years-old male who presented a distinct ulcer with raised borders on his neck. Samples were taken for direct microscopic examination, parasite culture, and molecular identification of the infecting *Leishmania* species by sequencing of the cytochrome b gene. Direct examination was positive for amastigotes of *Leishmania* but the culture was negative. The infecting parasite species was identified as *L. (V.) guyanensis* by means of the nucleotide sequence of a 509 bp fragment of the cytochrome b gene. We report the presence of *L. (V.) guyanensis* in rural areas of Buenaventura in Valle del Cauca, and the expansion of the geographical distribution of this species in the Pacific region of Colombia.

Keywords: cutaneous leishmaniases, cytochrome b, epidemiology, parasites.

RESUMEN

El objetivo de este estudio fue identificar la especie de *Leishmania* causante de la leishmaniasis cutánea en un paciente de Buenaventura, Valle del Cauca, en la costa Pacífica de Colombia. Se obtuvieron muestras clínicas de un varón de 29 años de edad que presentó una úlcera distintiva con bordes levantados en el cuello. Se tomaron muestras para examen microscópico directo, cultivo de parásitos e identificación molecular de la especie infectante de *Leishmania* mediante secuenciación del gen del citocromo b. El examen directo fue positivo para amastigotes de *Leishmania* pero el cultivo fue negativo. La especie parasitaria infectante se identificó como *L. (V.) guyanensis* por medio de la secuencia de nucleótidos de un fragmento de 509 pb del gen citocromo b. Con este reporte notificamos la presencia de *L. (V.) guyanensis* en zona rural del municipio de Buenaventura en el Valle del Cauca y la expansión de la distribución geográfica de esta especie en la región Pacífica de Colombia.

Palabras clave: citocromo b, epidemiología, leishmaniasis cutánea, parásitos.

The leishmaniases are diseases caused by obligate intracellular protozoans of the genus *Leishmania*. The geographical distribution of leishmaniasis is limited by the distribution of the vectors, and the wild and domestic animals that serve as reservoirs for the parasites (Rotureau, 2006). Although *L. (Leishmania) infantum* has been relatively well characterized as the etiological agent for visceral leishmaniasis in multiple areas, the species responsible for the cutaneous form of the disease remains unknown in many Colombian foci, whereas the existing distribution records come from the characterization of a very limited number of human isolates (Martinez *et al.*, 2010; Salgado-Almario *et al.*, 2019). Studies carried out to date have established that *L. (V.) braziliensis* Vianna, 1911 and *L. (V.) panamensis* Laison and Shaw, 1972 have the broadest distribution throughout the country, while *L. (L.) mexicana* Biagi, 1953 is present in all geographical regions of Colombia with the exception of the Caribbean plain.

Other species exhibit limited distribution; *L. (L.) amazonensis* Lainson and Shaw, 1972 has been reported in the departments of Antioquia, Chocó, Meta, Norte de Santander, Tolima, and Santander, *L. (V.) lainsoni* Silveira, Shaw, Braga and Ishikawa, 1987 was reported in Antioquia and Putumayo, *L. (V.) colombiensis* Kreutzer *et al.* 1991 was found in Santander, and *L. (V.) equatoriensis* Grimaldi *et al.* 1992 in Antioquia (Corredor *et al.*, 1989; Gore Saravia *et al.*, 2002; Ovalle *et al.*, 2006; Ramírez *et al.*, 2016). On the other hand, *L. (V.) guyanensis* Floch, 1954 was originally recorded in the Orinoco and Amazon river basins (Corredor *et al.*, 1989). However, over the last decade has also been reported in the departments of Tolima, Valle del Cauca, and Sucre (Rodríguez-Barraquer *et al.*, 2008; Figueroa *et al.*, 2009; Martinez *et al.*, 2010), outside of what was traditionally considered to be the natural focus of transmission of this species. Here, we report the presence of *L. (V.) guyanensis* in rural area of Buenaventura, on the Colombian Pacific coast. This provides further evidence for a more extensive geographical distribution of *L. (V.) guyanensis* in Colombia.

Clinical samples were obtained in January 2011 from a 29 year-old male from Bajo Calima, a village within the municipality of Buenaventura, in the department of Valle del Cauca, Colombia. The autochthonous nature of the infection was determined by an epidemiological survey, in which sociodemographic aspects and sites visited during the three months before diagnosis were analyzed. The patient presented a distinct ulcer with raised borders on his neck, which had appeared approximately a month earlier according to the information provided by the patient. The patient was examined at Laboratorio de Salud Pública at Departamento Administrativo de Seguridad Social en Salud de Sucre (DASSSALUD) during a short visit to the city of Sincelejo, in the department of Sucre. Samples from the lesion were taken for parasite culture and molecular genotyping of the infective species. Samples for parasite culture were taken by aspiration biopsy from the ulcer with a fine needle. Macerated tissue was inoculated into Novy-Nicolle-MacNeal (NNN) culture medium (Walton *et al.*, 1977), and incubated at 26 °C. The culture was examined daily during four weeks to detect the flagellate forms of the parasite.

A clinical sample for species identification was obtained by scraping the border of the lesion with a scalpel. This material was deposited in 1.5 mL microtubes containing 0.5 mL of lysis buffer (Tris-HCl [10mM], EDTA [10mM] and SDS [10mM]). Genomic DNA extraction was performed as described previously using a salting out procedure (Martinez *et al.*, 2010). For identification of the parasite species, a fragment of 866 bp of the Cytochrome b gene of *Leishmania* was amplified by polymerase chain reaction (PCR) with primer pair Lcyt-S (5'-GGT GTA GGT TTT AGT YTA GG-3') and Lcyt-R (5'-CTA CAA TAA ACA AAT CAT AAT ATR CAA TT-3') (Kato *et al.*, 2007). The amplicon of the expected size was purified and directly sequenced on both strands of DNA with these primers.

The sequences obtained were edited using MEGA 5.22 software (Tamura *et al.*, 2011), in order to generate a consensus sequence. This consensus sequence was subjected to a preliminary analysis of similarity against sequences available in GenBank using BLASTn (Basic Local Alignment Search Tool) (Altschul *et al.*, 1990). Multiple alignments were carried out with the Clustal W software (Thompson *et al.*, 1994) incorporated in MEGA 5.22. The sequences of other clinical isolates of *Leishmania* from Colombia (Martínez *et al.*, 2010), as well as those of *L. (V.) guyanensis* reference strains available in GenBank (LC153220, LC153193), were analyzed together with the sequence obtained from the patient sample. The genetic relationships within this group of sequences were inferred by the Maximum Parsimony method in MEGA 5.22 program using the Subtree-Pruning-Regrafting (SPR) algorithm and a thousand bootstrap were performed to assess branch support (Felsenstein, 1985; Tamura *et al.*, 2011). The patient participated voluntarily and provided written informed consent.

Direct parasitological examination of dermal scrapings revealed the presence of *Leishmania* amastigotes. The parasite was identified as *L. (V.) guyanensis* by the analysis of the Cytochrome b gene. The characteristic nucleotide polymorphisms of *L. (V.) guyanensis* described by Martínez *et al*. (2010) were observed within the nucleotide sequence of the Cytochrome b fragment amplified from the sample from Bajo Calima. This allowed distinguishing this species from other *Leishmania (Viannia)* species that may be found at this region. In the phylogenetic tree the sample from Bajo Calima clustered with high confidence (bootstrap 97 %) with the reference strains sequences of *L. (V.) guyanensis* (Fig. 1). On the other hand, parasite culture was considered to be negative after four weeks follow-up, during which no promastigotes were observed.

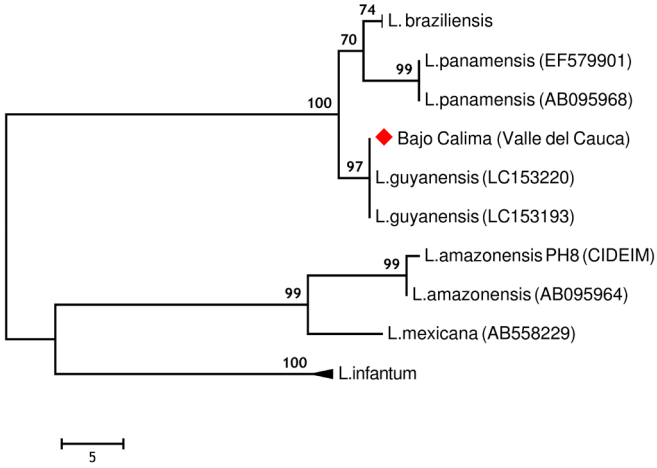

Figure 1. Most parsimonious tree (lenght=101 steps, consistency index= 0.949495, retention index= 0.983713) of the Cytochrome b partial sequences from *Leishmania* species. The analysis involved 18 nucleotide sequences and a total of 509 positions in the final dataset. Numbers above each branch represents the bootstrap support and the red diamond denotes the sample from Bajo Calima, Buenaventura

This study records the presence of *L. (V.) guyanensis* in a patient from Bajo Calima, in the department of Valle del Cauca, on the Pacific Coast of Colombia. In this region *L. (V.) panamensis* has been widely recognized as the main causal agent of leishmaniasis (Gore Saravia *et al.*, 2002). Figueroa *et al.* (2009) previously reported the isolation of *L. (V.) guyanensis* from two patients with cutaneous leishmaniasis in Guacarí and Vijes municipalities on the Andean region of Valle del Cauca. The Vijes leishmaniasis focus is best known for the presence of *L. (V.) panamensis*, which has been isolated from humans and the sloth *Choloepus hoffmanni* (Loyola *et al.*, 1988), and *L. (V.) braziliensis*, which has been isolated from dogs (Travi *et al.*, 2006).

Although in Brazil and Bolivia *L. (V.) guyanensis* is associated with both cutaneous and mucocutaneous leishmaniasis, in Colombia this parasite has been found to be associated mainly with cutaneous leishmaniasis. One possible hypothesis is that parasite strains circulating in Colombia lack the virulence factors that characterize metastatic *L. (V.) guyanensis* parasites from those countries where mucocutaneous manifestations in patients are frequent. Additionally, it is also possible that the genetic background or other intrinsic factors related to human population may play a role in the appearance of the mucocutaneous form of the disease.

Until the past few years, *L. (V.) guyanensis* was reported mainly in the Amazon and Orinoquia regions of the country (Grimaldi *et al.*, 1989). The finding of this species in both the Andean and Caribbean regions (Rodríguez-Barraquer *et al.*, 2008; Martínez *et al.*, 2010; Hernández *et al.*, 2014), together with this record from the Pacific Coast, supports the existence of wider scenarios for *L. (V.) guyanensis* transmission cycles in Colombia. Those changes in the geographical distribution of *L. (V.) guyanensis* could be attributed to forced displacement of human populations by the internal conflict, and perhaps due to the migration of reservoirs, or the appearance of new epidemiological actors (Martínez *et al.*, 2010; Ferro *et al.*, 2011). In Colombia, only two sand fly species have been found infected with *L. (V.) guyanensis*, i.e., *Lutzomyia umbratilis* Ward and Fraiha, 1977 (*Nyssomyia* subgenus) in the department of Amazonas, and *Lutzomyia longiflocosa* Osorno-Mesa, Morales, Osorno and Muñoz, 1970 (*Verrucarum* group) in the department of Tolima (Young *et al.*, 1987; Ferro *et al.*, 2011). However, to date, there are no reports of these sand fly species in the Colombian Pacific region. Therefore, ecoepidemiological studies are required to incriminate the vector of this parasite in the region since we cannot rule out the role of other phlebotomine sand fly species, including those from *Nyssomyia* subgenus or the *Verrucarum* group. Regarding the potential reservoirs, in addition to humans, canines and cotton rats are the vertebrates found infected with *L. (V.) guyanensis* in this country (Vásquez-Trujillo *et al.*, 2008; Santaella *et al.*, 2011; Ocampo *et al.*, 2012). Among these, it has been suggested that canines could act as reservoirs for this parasite, but their infective capacity to sandflies has not been proven so far.

Furthermore, the establishment of transmission cycles in new areas may have been facilitated by the presence of other phlebotomine sand fly species already known to be vectors of *Leishmania* parasites. Although this could explain epidemic outbreaks of the disease (Rodríguez-Barraquer *et al.*, 2008), the distribution of *L. (V.) guyanensis* in the country may also be wider than previously assumed, since the *Leishmania* species involved in vast majority of cases of leishmaniasis are not usually typed. Furthermore, there is a possibility that isolates of *L. (V.) guyanensis* have been mistyped or misidentified as *L. (V.) panamensis*, given the close genetic relationship between these two species, thus contributing to the underestimation of the former's geographical distribution in the country. In conclusion, the presence of *L. (V.) guyanensis* in the Colombian Pacific region has been confirmed, which demonstrates the expansion of the geographical distribution of this parasite species in Colombia, and reveals the complexity of the transmission cycle in this area of the country, where other *Leishmania* spp. also circulate.

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DISCLOSUREOF INTEREST

The authors declare that they have no conflicts of interest

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