Short Report: Natural Infection of Lutzomyia tortura with Leishmania (Viannia) naiffi in an Amazonian Area of Ecuador

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Abstract. Natural infection of sand flies with Leishmania parasites was surveyed in an Amazonian area in Ecuador where leishmaniasis is endemic. Seventy-one female sand flies were dissected and one was positive for Leishmania protozoa. The species of this sand fly was identified as Lutzomyia (Lu.) tortura on the basis of morphologic characteristics. Analysis of the cytochrome b gene sequence identified the parasite as L. (Viannia) naiffi. We report the distribution of L. (V.) naiffi in Ecuador and detection of a naturally infected sand fly in the Ecuadorian Amazon and natural infection of Lu. tortura with Leishmania parasites in the New World.

Leishmaniasis is a protozoan disease caused by the genus Leishmania. It is distributed worldwide, especially in tropical and subtropical areas, and affects at least 12 million persons.1,2 Disease occurs in three distinct manifestations (cutaneous, mucocutaneous, and visceral), and the clinical forms are largely associated with the Leishmania species responsible.1,2 Parasites are transmitted by female sand flies of the genus Phlebotomus in the Old World and Lutzomyia in the New World.3,4 At present, more than 800 sand fly species are known; however, only a portion of the species can transmit each particular Leishmania species.3,4 Therefore, identification of the prevalent parasite and vector species is important for risk assessment and appropriate treatment.

Currently, little information is available on prevalent parasite and sand fly species in the Ecuadorian Amazon because of difficulty in gaining access to this region. In a previous study, cases of cutaneous and mucocutaneous leishmaniasis were recorded in these areas; however, no parasitologic characterization of the causative agents has been made. Recently, we studied mucocutaneous leishmaniasis in these areas and found that Leishmania (Viannia) braziliensis is prevalent in Amazonian areas.5 The sand fly species involved in the transmission cycle are not well characterized in these areas. In addition, no natural infection of sand flies with Leishmania species has been reported in the Ecuadorian Amazon. In the present study, natural infection of sand flies with Leishmania species was surveyed in an area of the Ecuadorian rainforest where leishmaniasis is endemic.

Sand flies were captured with CDC light traps and protected human bait in tropical rainforests in Arajuno (1°23′S, 77°67′W, Department of Pastaza), Ecuador. In this area, 71 female sand flies were dissected for identification at the species level. On the basis of morphologic characteristics of spermathecae,6 11 species were recognized. The two most prevalent sand flies were Lu. uyulli (28 flies) and Lu. tortura (19 flies). Infection with Leishmania species was examined under a microscope and detected in one anthropophilic sand fly. The positive sand fly was identified as Lu. tortura or possibly Lu. hartmani on the basis of morphologic characteristics (Figure 1). To clarify this finding, we analyzed 18S ribosomal RNA gene sequences of the positive fly. The sequences did not match those of Lu. hartmani. Therefore, the positive sand fly was identified as Lu. tortura. The presence of male Lu. tortura was also confirmed in the same study site, but no male Lu. hartmani was found. Lutzomyia tortura has been reported in other Amazonian areas such as Taisha (2°38′S, 77°50′W, Department of Morona-Santiago) and Cotapino (0°70′S, 77°47′W, Department of Orellana),6,8,9 but not on the Pacific coast subtropical and in Andean areas of Ecuador. This species was reported in the Colombian Amazon,10,11 which suggests that Lu. tortura is distributed mainly in Amazonian regions. No natural infection of Lu. tortura with Leishmania parasites has been reported in the New World.

The dissected sample, including innumerable promastigotes, was fixed in absolute ethanol and genomic DNA was extracted. Using a portion of the DNA as a template, we amplified the leishmanial cytochrome b (cyt b) gene with a pair of primers, L.cyt-S (5′-GTTGTCAGTTGGTGTAGGTTTTAGTYTAGG-3′) and L.cyt-R (5′-TCACACATTACAACTATATATR-CAATT-3′).12,13 The sequence was determined and compared with sequences from 16 Leishmania and 2 Endotrypanum species. A phylogenetic analysis was also performed. The cyt b gene sequence had higher homology with that of L. (V.) naiffi (99.6%) than with others (88–97.9%) and phylogenetic tree analysis supported this result (Figure 2), which indicated that the parasite infecting the sand fly was L. (V.) naiffi. The parasite isolated was identified as ITOR/EC/07/Araj1.

Information on infections with L. (V.) naiffi is limited and most cases have been reported in Brazil. Leishmania (V.) naiffi has been isolated from armadillos, the sand fly species Lu. squamiventris, Lu. paraensis, Lu. davisi, and Lu. hirsuta, and humans with cutaneous leishmaniasis in Brazil.14–19 Outside Brazil, infection with L. (V.) naiffi was reported in a patient with cutaneous leishmaniasis who was infected in French Guiana, Martinique, or Guadeloupe.20 Four human cases of cutaneous leishmaniasis caused by L. (V.) naiffi have been reported: three of the patients were infected in French Guiana or Martinique and one was suspected to be infected in Ecuador or Peru because the patient had been on a seven-week trip to Ecuador and Peru that included four days in the Ecuadorian Amazon.21 This report suggested that L. (V.)
**Figure 1.** Spermathecae (arrows) and ducts of a female Lutzomyia tortura sand fly.

**Figure 2.** Phylogenetic tree of cytochrome b gene sequences among species. The leishmanial cyt b genes were amplified from a patient with cutaneous leishmaniasis (CL patient) and a positive sand fly (Lu. tortura), and the sequences were determined. Phylogenetic tree analysis was performed with sequences obtained and those from 16 Leishmania species and 2 Endotrypanum species as described in the text. Scale bar indicates 0.01% divergence.

naiffi may have spread in Ecuador. Other than this one suspected case, no case of cutaneous leishmaniasis caused by L. (V.) naiffi has been reported in Ecuador, probably because of limited research in the Amazonian regions.

During our field research, one suspected case of cutaneous leishmaniasis was found and tissue material from this person (a four-year-old girl) from an active cutaneous lesion was subjected to DNA extraction and the cyt b gene sequence was amplified. The cyt b gene sequences had the highest degree of homology with that of L. (V.) guyanensis (99.9%), and phylogenetic tree analysis supported the result (Figure 2), indicating that the patient was infected with L. (V.) guyanensis. The presence of L. (V.) braziliensis and hybrid genotypes between L. (V.) panamensis/guyanensis and L. (V.) braziliensis in the Ecuadorian Amazon has been reported.

We have shown that L. (V.) guyanensis is present in the Amazonian areas of Ecuador, but no human cases infected with L. (V.) naiffi were found in the present study. However, this species may be circulating in this area because it was detected in a highly anthropophilic species of sand fly. Further research in Amazonian regions will provide more information on human cases of infection with L. (V.) naiffi.

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