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Man-biting sand fly species and natural infection with the *Leishmania* promastigote in leishmaniasis-endemic areas of Ecuador

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ABSTRACT

A countrywide surveillance of sand flies was performed to obtain information on their geographical distribution and natural infection by *Leishmania* protozoa in Ecuador. A total of 18,119 sand flies were collected by human landing collections during 32 years from 1982 to 2014, and 29 species were recognized. The most prevalent 10 species were *Lutzomyia gomezi*, *Lu. robusta*, *Lu. hartmanni*, *Lu. shannoni*, *Lu. trapidoi*, *Lu. panamensis*, *Lu. maranonensis*, *Lu. ayacuchensis*, *Lu. tortura* and *Lu. yuilli yuilli*, and their topographical and vertical distributions were identified. Among all the sand flies, only 197 (1.09%) flies of four *Lutzomyia* species, *Lu. gomezi*, *Lu. trapidoi*, *Lu. tortura* and *Lu. ayacuchensis*, were positive for *Leishmania*. *Endotrypanum*, a flagellate parasite not pathogenic to humans, were detected in five *Lutzomyia* species, *Lu. robusta*, *Lu. hartmanni*, *Lu. trapidoi*, *Lu. panamensis* and *Lu. yuilli yuilli*, suggesting wide vector-ranges of *Endotrypanum* species. These data on the genus *Lutzomyia* and their natural infections with *Leishmania* and *Endotrypanum* will be useful for transmission studies and surveillance of leishmaniasis.

1. Introduction

Leishmaniasis is one of the most important vector-borne parasitic diseases prevalent mainly in tropical and subtropical countries around the World, affecting at least 12 million people in 98 countries and regions (Alvar et al., 2012). Human leishmaniasis [cutaneous (CL), mucocutaneous (MCL) and visceral forms (VL)] is caused by infection with about 20 *Leishmania* species transmitted by the bite of female sand flies of the genus *Phlebotomus* in the Old World and *Lutzomyia* in the New World. There are approximately 800 sand fly species in the world; among them only 10% have been reported as vectors of the man-infecting *Leishmania* spp. (Munstermann, 2004; Kato et al., 2010).

The presence of countrywide American cutaneous leishmaniasis (ACL) is associated with several species of the genus *Leishmania* in Ecuador, and the absence of detailed information focused on the distribution of man-biting species of the genus *Lutzomyia* led the authors to compile data accumulated over a period of more than 30 years from 1982 to date. In Ecuador, ACL, which was reported for the first time by Valenzuela in 1928 (Rodriguez, 1974), is currently reported as an endemic disease with notification in 21 of 24 provinces of the country, especially those in the Pacific Coastal, Andean and Amazonian regions. Only three provinces, Carchi and Tungrahua in the Andes and the Galapagos Islands, are free from the disease. In the Pacific Coastal and Amazonian region, ACL primarily affects males of productive age, particularly farm workers, but in the Andean region, it afflicts children less than 10 years of age, especially those under 5 years, suggesting intra-domiciliary transmission of the disease in the area (Gomez and Hashiguchi, 1991; Hashiguchi and Gomez, 1991; Hashiguchi et al., 1991).

First reports of sand fly vectors in Ecuador were published in the 1950s (Rodriguez, 1950, 1953, 1956), and then followed by other works (Arzube, 1960; Young, 1979; Young and Rogers, 1984; Alexander et al., 1992; Jones et al., 2010). Thereafter, following an indepth review, there are currently 76 sand fly species reported in Ecuador (Galati, 2010).

We have been conducting investigations on the epidemiology of leishmaniasis countrywide, mainly aiming at disclosing the specific relationships between parasites (*Leishmania* spp.) and vector sand flies (*Lutzomyia* spp.) at given leishmaniasis-endemic areas

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of Ecuador. With regard to the sand fly fauna and morphological taxonomy of the country, part of our study has already been published (Alexander et al., 1992), in addition to other vector-related studies (Hashiguchi et al., 1985; Takaoka et al., 1990; Gomez and Hashiguchi, 1991; Kato et al., 2005, 2007, 2008a,b, 2010, 2013). Further, a part of our more recent data at Andean slope was included in this study, as complementary data (Gomez et al., 2014). In this study, summing-up the data we accumulated over the period from 1982 to date, the man-biting species of sand flies and their natural infections with flagellates, *Leishmania* and *Endotrypanum*, are discussed. The data on sand flies and those on the natural flagellate infections were arranged by Ecuadorian provinces, three topographical regions (Pacific Coastal, Andean and Amazonian regions) and altitudes at both sides of the Andes.

2. Materials and methods

2.1. Sand fly collections

Sand fly collections were performed at 43 localities distributed in 18 provinces of Ecuador during 32 years from 1982 to 2014. All these sites were areas where leishmaniasis is endemic, and sand fly collection was conducted mainly to identify sand fly species responsible for transmission of *Leishmania* protozoa. To date, no control efforts of sand flies have been done at any endemic areas of the country. All the collection sites and their elevation (above sea level) were shown in Fig. 1. All the sand flies were captured as they landed on protected human volunteers (human landing collection), using an insect-aspirator (capture tube). The collections were usually performed between 18:00 and 21:00. In order to obtain data on the hourly biting activity of several sand fly species, captures were also carried out by human landing collection from 19:00 to the next morning 06:00.

2.2. Dissection and identification of sand flies

All the sand flies collected were kept in capture tubes and taken back to the field laboratory for dissection soon after collection at each leishmaniasis-endemic area. Female sand flies were dissected using the method reported previously (Hashiguchi et al., 1985). The gut of each sand fly was isolated and placed on a slide, covered with a cover slip, and then examined microscopically using ×400 and ×1000 magnifications. Spermathecae and cibarial armatures of the sand flies dissected were also observed for species identification, mainly based on the reports of Young and Duncan (1994).

2.3. Identification of flagellate species

Dissected sand fly guts were examined microscopically for flagellates. If a sand fly was positive for parasites, the gut contents including promastigotes were inoculated into golden hamsters and/or transferred into culture tubes containing defibrinated rabbit blood agar for further studies. In general, it was easy to differentiate microscopically the flagellates/promastigotes between *Leishmania* and *Endotrypanum* based on their numbers, morphology, rosette formation and localization in the sand fly gut. The most marked points for differentiation were: the abundance of promastigotes in sand fly guts; if positive for *Leishmania*, they were numerous with a rosette formation and slender shape with a long flagellate. On the other hand, in the case of flies positive for *Endotrypanum*, there were few parasites and they were round with a relatively short flagellate. They were mainly localized in the posterior triangle and hind-gut and were also frequently found in Malpighian tubules.

Some specimens were fixed individually in absolute ethanol for molecular analysis as described previously (Kato et al., 2005, 2008a,b, 2010, 2013). In brief, each ethanol-fixed specimen was lysed in DNA extraction buffer [150 mM NaCl, 10 mM Tris–HCl (pH 8.0), 10 mM EDTA, and 0.1% sodium dodecyl sulfate (SDS)] in the presence of proteinase K (100 μg/ml). The flagellate cytochrome *b* (*cyt* b) gene was amplified by PCR, and the sequence was analyzed (Kato et al., 2005, 2008a,b, 2010, 2013).

3. Results

3.1. Man-biting sand fly species in the provinces of Ecuador

A total of 18,119 sand flies were collected at 43 sites in 18 provinces in Ecuador by human landing collection. They were then dissected to check for flagellate parasites and to identify individual insects. The results are arranged by province for each man-biting species of the genus *Lutzomyia*, as shown in Table 1. Twenty-nine man-biting species of sand flies were identified. The figures in Table 1 do not necessarily indicate the abundance of each sand fly species because they depend on the frequency of capture trials at given study sites.

Periodical sand fly collections were conducted at several sites, such as Huigra (17 nights in total), Chanchan (14 nights), Alausi (13 nights), Ocaña (25 nights), and Paute (22 nights), and therefore the numbers of *Lu. ayacuchensis* in Chimborazo and Azuay, and *Lu. hartmanni* and *Lu. trapidoi* in Cañar were extremely high. No marked difference in sand fly abundance, distribution and species composition was observed during over a 32 year span, which is similar to our recent findings comparing sand fly fauna between 1994 and 2012 conducted in several fixed points (Gomez et al., 2014). Further, no marked environmental changes, such as de-forestation and urbanization were noted in the above-mentioned collection sites of Ecuador.

From the present results of capture and dissection of sand flies in 18 provinces, the following 10 species are listed as the most prevalent and/or important man-biting species of the genus *Lutzomyia* in Ecuador: *Lu. gomezi* (collected in 13 of the 18 provinces tested), *Lu. trapidoi* (11 provinces), *Lu. hartmanni* (11 provinces), *Lu. robusta* (10 provinces), *Lu. panamensis* (8 provinces), *Lu. shannoni* (7 provinces), *Lu. maranonensis* (4 provinces), *Lu. yuilli yuilli* (3 provinces), *Lu. tortura* (5 provinces) and *Lu. ayacuchensis* (2 provinces). Of these, the first two and the last two species have already been confirmed as vectors of *Leishmania* spp. in Ecuador. Sand fly species were for the first time recorded in Imbabura and Cotopaxi; *Lu. hartmanni*, *Lu. trapidoi*, *Lu. panamensis* and *Lu. robusta* from Imbabura and the first two species and *Lu. shannoni* from Cotopaxi.

Lu. castanea, an extremely large specimen, captured for the first time in Ecuador by human landing collection at “Valle Hermoso” in Palanda, Zamora Chinchipe province, is listed in Table 1. The provable participation of *Lu. castanea* in *Leishmania*-transmission should be evaluated thoroughly in the near future since the sand flies were collected in the surroundings of a *Leishmania*-positive patient’s house.

3.2. Natural infections of man-biting sand fly species with *Leishmania* flagellates

The sand flies positive for the *Leishmania* promastigote were found in the following species: *Lu. gomezi* flies from Los Rios, *Lu. trapidoi* from Pichincha, *Lu. tortura* from Orellana and *Lu. tortura* from Pastaza, and *Lu. ayacuchensis* from Chimborazo and Azuay. There were 6 *Leishmania*-positive provinces in total, one Pacific Coastal province, three Andean provinces and two Amazonian provinces. When the number of *Leishmania*-positive sand flies was totalled excluding the *Lutzomyia* species, the infection rate was 1.09% (197 positives per 18,119 flies).