LEISHMANIA AMAZONENSI S INFECTIONS IN ORYZOMYS ACRITUS AND
ORYZOMYS NITIDUS FROM BOLIVIA

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Abstract. Three of thirteen Oryzomys acritus, Emmons and Patton 2005 (Rodentia: Muridae: Sigmodontinae) and
3 of 17 Oryzomys nitidus, Thomas 1884, collected from Noël Kempff National Park, Bolivia, from 2002 to 2005, tested
positive for Leishmania (Leishmania) amazonensis or L. (L.) mexicana and negative for Leishmania (Viannia) spp., using
the polymerase chain reaction (PCR). Based on previous records of L. (L.) amazonensis in humans, rodents, and sand
flies from Bolivia, and the geographic distributions of L. (L.) amazonensis and L. (L.) mexicana, it was concluded that
the Oryzomys were infected with L. (L.) amazonensis. These results identify two additional species of Oryzomys as hosts
of L. (L.) amazonensis, and identify an ecological region of Bolivia where L. (L.) amazonensis is enzootic.

INTRODUCTION

Cutaneous and mucocutaneous leishmaniases are endemic in both the Andean highland and the Amazon basin of Bot-
vilia, but visceral leishmaniases is rare.1 Although most human infections have been attributed to L. (Viannia) spp.,
L. (Leishmania) amazonensis also has been reported.2–5 The rice rat Oryzomys capito Jacob (1818) was reported to be a
host in Cochabamba, Bolivia.6 The taxonomy has been re-
vised, and the currently accepted epithet for Western Bolivian specimens of this taxon is Oryzomys perenensis J.A.
Allen, 1901.7 Leishmania (L.) amazonensis also was detected in Akodon sp. Meyen, 1833 (Rodentia: Muridae: Sigmodon-
tinae) and Oligoryzomys sp. Bangs, 1900 (Rodentia: Muridae: Sigmodontinae) and the sand fly Lutzomyia nuneztovari
anglesi (Le Pont & Desjeux, 1984).8,9 The purpose of our investigation was to test the hypothesis that Oryzomys sp. are
reservoirs of L. (L.) amazonensis in Bolivia.10,11

MATERIALS AND METHODS

Field methods. Rodents were captured at two localities in Bot-
vilia, Santa Cruz, Parque Nacional Noël Kempff Mercado: El Refugio Huanchaca (UTM 20L 0712187; 8368228, WGS
84, 220 m elevation) each September from 2002–2005, and Los Fierros (UTM 20 L 0724182; 8387597, 240 m elevation)
in October 2002 and September 2003. The vegetation and ecol-
ogy of the region has been described in detail.12 Small mammals were captured in live traps (H.B. Sherman, Tallahassee,
FL) as part of a project by Emmons to study the faunal com-
munities within the park. Procedures have been approved by
Dr. Emmons' institutional animal care and use protocol. Ani-
mals were transferred from the trap to a cloth bag. One per-
son who was wearing leather gloves restrained the animal by
gripping it firmly behind the head while another performed
other procedures. The area to be biopsied was sprayed with
benzocaine first-aid spray; with this procedure the subjects
showed no signs of distress. Because long-term monitoring is
being continued, most captured animals were marked and
released, although a few were preserved as vouchers. Series of
up to 20 voucher specimens per species, with tissue samples,
have been collected for the localities, and are preserved in the
Musco de Historia Natural Noël Kempff Mercado, Santa
Cruz, with duplicates in the United States National Museum.
In 2002, animals with scars on the basal, dorsal surface of
the tail were chosen for biopsy (Figure 1). A 2-mm punch
biopsy was taken from the edge of the scarred area and from
the rim of each ear, placed in 1.5-mL conical bottom tubes
with screw tops (PGC Scientifics, Frederick, MD), containing
95% Ethanol. Pooled samples of skin from each ear and the
base of the tail were screened by the polymerase chain reac-
tion (PCR) as described below.

During September 11–13, 2003, rodents were captured at El
Refugio Huanchaca and biopsies were taken only from the
base of the tails from 25 rodents with or without scars or
lesions, except that a biopsy from one ear was taken in the
case of three Proechimys longicaudatus that were missing
tails. These were screened by both PCR and culture. In 2004
biopsies were taken only from the tails of rodents with scars;
in 2005 they were taken from all rodents captured in forests.
These biopsies were only screened by PCR.

Culture methods. Unsuccessful attempts were made to cul-
ture Leishmania promastigotes from the tissue samples col-
clected in September 11–13, 2003. They were placed in 1X
medium (GIBCO-BRL, Gaithersburg, MD) supplemented
with 5% (v/v) heat-inactivated fetal bovine serum (GIBCO),
1M Hepes buffer (pH 7.4) (Sigma Chemical Co., St. Louis,
MO), 100X penicillin/streptomycin (GIBCO), 10 mM hypox-
anthin (SIGMA), 0.25% (v/v) bovine hemin (Sigma), and 5
mL human urine cleared at 600 g for 20 minutes in a Cen-
tral CL2 benchtop centrifuge. The cultured medium was sterilized
by passage through a 0.2-µm filter and adjusted to pH 7.4 by
1M Sodium Hydroxide (Fisher Scientific Company, Fair
Lawn, NJ). The cultures were kept at 25°C and monitored at
least three times a week for the presence of promastigotes of
Leishmania.

Primers and probe. DNA was amplified with the universal
Leishmania primers 13A and 13B.13 To identify kDNA se-
quences that were uniquely conserved among L. (L.) ama-
zonensis and L. (L.) mexicana parasites, minicircle DNA se-
quences from L. (Viannia) spp., L. (L.) amazonensis, and L.
(L.) mexicana that had been deposited in GenBank were
aligned using the CLUSTAL software. To detect DNA from
L. (L.) amazonensis or L. (L.) mexicana the forward primer

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Identification of the reference isolates to the subgenus or species level had been determined previously by analysis of sequences but differed from the sequences of L. (L.) amazonensis and L. (L.) mexicana, the universal primers 13A/13B gave a ~120 bp amplicon for all Leishmania strains used to test the specificity of the primers (Table 1).

Polymerase chain reaction screening of Bolivian rodents.

The rodent tissue was lysed and the conserved region of kDNA amplified with the primers described previously in this article. PCR products amplified from two of the animals whose test results were positive were cloned into the plasmid vector pCR®2.1-TOPO® (TOPO TA Cloning® kit, Invitrogen Corporation, Carlsbad, CA). The plasmids were purified by QIAamp® Plasmid Maxi Purification kit (QIAGEN, Valencia, CA), and sequenced at the Advanced Nucleic Acid Core Facility, Department of Microbiology at University of Texas Health Science Center at San Antonio (UTHSCSA). Sequences were compared and aligned using the National Center for Biotechnology Information, U.S. National Library of Medicine, Basic Local Alignment Search Tool (BLAST).  

**RESULTS**

Using a large number of Old World and New World Leishmania isolates the M1.1/13A primers were determined to spe-
specifically amplify DNA from members of the *L. (L.) amazonensis* or *L. (L.) mexicana* (Table 1). Most notably, under the standardized reaction conditions, DNA from members of the *Viannia* subgenus was not amplified by these primers. Furthermore, the *Viannia* subgenus-specific B4/M13 primers did not amplify DNA from *L. (L.) amazonensis* or *L. (L.) mexicana*.

Ultimately 64 individual mammals, 10 species of rodent and two species of opossum, were screened for *Leishmania* (Table 2). Three of thirteen *O. acritus* (Figure 2) and 2 of 17 *O. nitidus* (Figure 3) from El Refugio Huanchaca, and 1 of 2 *O. nitidus* from Los Fierros tested positive for *Leishmania* spp. and negative for *L. (Viannia)* spp., using PCR. Efforts to culture the parasite were unsuccessful. No positive for *Leishmania* was detected in any other mammal species. Both traplines where *Leishmania* positive rodents were collected were in semideciduous tall forest formations.

The sequence of the DNA amplified from *O. acritus* (field no. 444) and *O. nitidus* (field no. 575) compared with a reference kDNA sequence [*L. (L.) amazonensis, MHOM/BR/00/Raimundo (= MHOM/BR/1983/M1132), NCBI Accession number M21326*] is shown below. The primer (13A and M1.1) sequences are underlined and a vertical line indicates identity between the cloned and reference sequences.

Each of the sequenced kDNA fragments was 102 nucleotides in length, including the primer sequences. The sequence of amplified DNA (excluding the primer sequences) from *O. acritus* (field no. 444) and *O. nitidus* (field no. 575) showed 93% and 78% identity to the reference *L. (L.) amazonensis* kDNA sequence, respectively. A similar level of identity was observed with other *L. (L.) amazonensis* and *L. (L.) mexicana* sequences. Notably, the BLAST search did not identify homology to kDNA sequences of parasites of the *Viannia* subgenus, even though more than 40 such sequences of the conserved region of the minicircle DNA are present in the NCBI database.

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La    GTGGGGGAGGGGCGTTCTGCGGAAACTTCATCTCCGAAATCTTCCTCAATAAATGAGGTCGAGGAAAACCGGTTCGTTCTCATATTGGGGAATTTTG

575  GTGGGGGAGGGGCGTTCTGCGGAAACTTCATCTCCGAAATCTTCCTCAATAAATGAGGTCGAGGAAAACCGGTTCGTTCTCATATTGGGGAATTTTG

444  GGGAATTTCGGTCTCGCGGAAACCGGTTCGTTCTCATATTGGGGAATTTTG

La    GGGAATTTCGGTCTCGCGGAAACCGGTTCGTTCTCATATTGGGGAATTTTG

575  TCAAAATTTCGCGGCTCGGCGCGTGAACTGG
Akodon and two collected at this focus were Oligoryzomys for Leishmania. Noel Kempff National Park, Bolivia, from 2002-2005, tested positive based on the similarity of isolates from one comparison with reference strains. Based on isoenzyme electrophoresis and comparison with reference strains, it is noteworthy that both PCR products that were sequenced showed high homology with DNA from parasites collected from humans in Brazil. Previously, eight human stocks from areas where sand flies (Lutzomyia sp.) are abundant, it may also be a reservoir there. According to Eisenberg (1989), oryzomyines are close relatives of the neotomine-peromyscine group of North America, and the tylomyine and nyctomyine of Central America. Based on the information that reservoirs of Leishmania spp. are known from all of these closely related groups, Kerr (2000) suggested that oryzomyine rodents might be reservoirs of Leishmania in South America. Our results support this hypothesis.

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REFERENCES


DISCUSSION

Results indicate that both O. acritus and O. nitidus were infected with either L. (L.) amazonensis or L. (L.) mexicana. It is noteworthy that both PCR products that were sequenced showed high homology with DNA from parasites collected from humans in Brazil. Previously, eight human stocks from the sub-Andean region of LaPaz were identified as L. (L.) amazonensis based on isoenzyme electrophoresis and comparison with reference strains. Leishmania isolates from one Akodon and two Oligoryzomys collected at this focus were identified as L. (L.) amazonensis based on the similarity of kDNA-PCR profiles to that of the human isolates. Isoenzyme profiles of three isolates from the sand fly Lu.(n.)anglesi collected at this focus also indicated L. (L.) amazonensis. PCR using SSU rRNA was used to compare a large number of L. (L.) amazonensis strains from broadly distributed geographical areas. Results indicated that of these two species, L. (L.) amazonensis is the main species occurring in South America, whereas L. (L.) mexicana is primarily confined to North and Central America. Therefore, based on the previous identification of L. (L.) amazonensis from humans, rodents, and sand flies from Bolivia, and the geographic distributions of L. (L.) amazonensis and L. (L.) mexicana, we concluded that the rice rats were infected with L. (L.) amazonensis.

These results identify two additional species of Oryzomys as hosts of L. (L.) amazonensis, and identify Noel Kempff National Park as an ecological region of Bolivia where L. (L.) amazonensis is enzootic. The fact that infections were detected in either O. acritus or O. nitidus each year from 2002–2005, and the lack of infections in other potential reservoirs such as Proechimys longicaudatus, suggest that O. acritus and O. nitidus are reservoirs of L. (L.) amazonensis in this area. Seemingly, Oryzomys is a host, and possible reservoir, of L. (L.) amazonensis or L. (L.) mexicana over a broad geographic range, including Mexico, Central America, and South America. Since Oryzomys spp. also occur in the eastern United States where sand flies (Lutzomyia sp.) are abundant, it may also be a reservoir there. According to Eisenberg (1989), oryzomyines are close relatives of the neotomine-peromyscine group of North America, and the tylomyine and nyctomyine of Central America. Based on the information that reservoirs of Leishmania spp. are known from all of these closely related groups, Kerr (2000) suggested that oryzomyine rodents might be reservoirs of Leishmania in South America. Our results support this hypothesis.

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