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Detection of *Leishmania donovani* and *L. tropica* in Ethiopian wild rodents

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ABSTRACT

Human visceral (VL, also known as Kala-azar) and cutaneous (CL) leishmaniasis are important infectious diseases affecting countries in East Africa that remain endemic in several regions of Ethiopia. The transmission and epidemiology of the disease is complicated due to the complex life cycle of the parasites and the involvement of various *Leishmania* spp., sand fly vectors, and reservoir animals besides human hosts. Particularly in East Africa, the role of animals as reservoirs for human VL remains unclear. Isolation of *Leishmania donovani* parasites from naturally infected rodents has been reported in several endemic countries; however, the status of rodents as reservoirs in Ethiopia remains unclear. Here, we demonstrated natural *Leishmania* infections in rodents. Animals were trapped in 41 localities of endemic and non-endemic areas in eight geographical regions of Ethiopia and DNA was isolated from spleens of 586 rodents belonging to 21 genera and 38 species. *Leishmania* infection was evaluated by real-time PCR of kinetoplast (k)DNA and confirmed by sequencing of the PCR products. Subsequently, parasite species identification was confirmed by PCR and DNA sequencing of the 18S ribosomal RNA internal transcribed spacer one (ITS1) gene. Out of fifty (8.2%) rodent specimens positive for *Leishmania* kDNA-PCR and sequencing, 10 were subsequently identified by sequencing of the ITS1 showing that five belonged to the *L. donovani* complex and five to *L. tropica*. Forty nine kDNA-positive rodents were found in the endemic localities of southern and eastern Ethiopia while only one was identified from northwestern Ethiopia. Moreover, all the ten ITS1-positive rodents were captured in areas where human leishmaniasis cases have been reported and potential sand fly vectors occur. Our findings suggest the eco-epidemiological importance of rodents in these foci of leishmaniasis and indicate that rodents are likely to play a role in the transmission of leishmaniasis in Ethiopia, possibly as reservoir hosts.

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1. Introduction

Leishmaniasis, a group of diseases ranging from self-healing localized cutaneous (CL) to the life threatening visceral form (VL or Kala-azar), is widely distributed in over 88 countries with up to 1.6 million new cases annually (WHO, 2010). Humans are infected by twenty species of the genus *Leishmania* that are transmitted by the bite of phlebotomine sand fly females. The source of infection for humans and parasite circulation is either anthroponotic (transmitted between humans) or zoonotic, where animals serve as reservoir hosts (Desjeux, 2004).

Leishmania species differ in the degree to which they are associated with different host species and reservoirs, among which rodents are considered to be of most importance. However, their role in the transmission cycle as a reservoir host and source of infection for humans differs significantly. For example *Leishmania turanica* is highly infectious and pathogenic to rodents, but human cases are very rare (Guan et al., 1995). In *L. major*, the parasites circulate under natural conditions in rodent populations; nevertheless, they are equally infective to humans and rodents that represent a natural source (reservoir) for human populations (Ashford, 1996, 2000). Cutaneous leishmaniasis caused by *L. tropica* was generally considered to be anthroponotic; however, in some areas hyraxes and rodents could play a role in zoonotic transmission (Jacobson, 2003; Svobodova et al., 2003; Svobodova et al., 2006).

The etiological agent of human VL in the Old World is represented by two closely related parasite species belonging to the *L. donovani* complex: *L. infantum* which circulates as a zoonosis with domestic dogs and wild canids as the main reservoirs (Baneth and Aroch, 2008; Quinnell and Courtenay, 2009), and *L. donovani*, which is believed to be anthroponotic and mainly transmitted among humans (Chappuis et al., 2007).

Visceral leishmaniasis caused by *L. donovani* has claimed the lives of thousands of people in Ethiopia. The main foci are found in the lowland areas of north, northwestern, and southwestern Ethiopia, with some sporadic cases in the central-east part of the country (Hailu and Formmel, 1993; Hailu et al., 2006a). The main potential vectors of VL include *P. orientalis, P. martini*, and *P. celiae* (Hailu et al., 1995; Gebre-Michael and Lane, 1996). The transmission dynamics of VL in Ethiopia and neighboring East African countries is generally believed to be anthroponotic (Chappuis et al., 2007); however DNA of *L. donovani* complex has recently been detected in both wild and domestic animals (Bashaye et al., 2009) and in certain districts of Sudan, rodents are suspected to be reservoirs of the parasite (Chance et al., 1978; Le Blancq and Peters, 1986; Elnaiem et al., 2001). The closely related species, *L. infantum*, has been detected in rodents in Euro-Asian leishmaniasis foci including Portugal (Helhazar et al., 2013), Italy (Gradoni et al., 1983), Greece (Papadogiannakis et al., 2010), and Iran (Davami et al., 2014). In addition, our recent study demonstrated presence of *L. donovani* complex DNA in blood specimens of various domestic animals in the VL endemic foci of north and northwestern Ethiopia (Rohousova et al., unpublished).

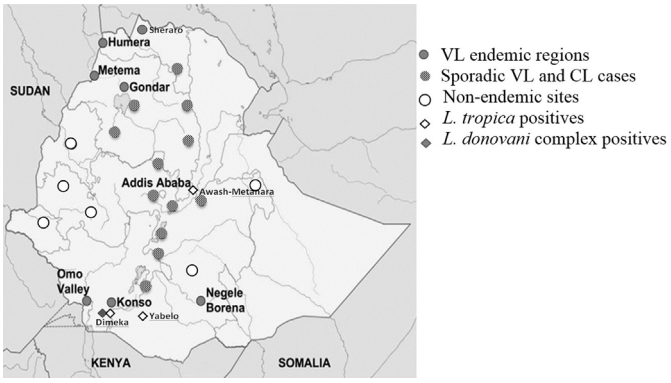

Fig. 1. Rodent trapping sites and their relation to human leishmaniasis foci in Ethiopia. (*Note*: The specific rodent trapping localities were indicated in the supplementary table.)

In Ethiopia, the search for *L. donovani* infection in wild rodents has been going on for many years. Here we focused on the detection of natural *Leishmania* spp. infections in rodents using PCR that targets the kinetoplast (k)DNA and internal transcribed spacer one (ITS1).

2. Materials and methods

2.1. Sample collection

Rodents were trapped in 41 localities (between 2010 and 2013) selected based on altitude, the occurrence of Kala-azar (9 endemic, 18 sporadic and 14 non-endemic), the abundance of sand flies, and the presence of microhabitat features related to *Leishmania* transmission (Fig. 1; Supp. Table S1). Permission to trap rodents was obtained from the Ethiopian Wildlife Conservation Authority (EWCA), Government of Ethiopia.

Supplementary Table S1 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.actatropica.2015.02.006.

Rodents were trapped using Sherman live traps and snap traps baited with a piece of bread with peanut butter or sardine. The traps were placed over-night near houses, animal shelters, around burrows, caves, agricultural fields, termite mounds, under trees, and in other habitats deemed suitable for sand flies. Trapped rodent was photographed and weight, sex, characteristics, and external measurements (lengths of body, tail, hind foot, and ear) were recorded. Rodents captured by live traps were first immobilized in a plastic bag and then humanely euthanized by intra peritoneal injection of ketamine and xylazine, dissected, and a sample of spleen was kept in pure ethanol for subsequent DNA extraction. After removing the viscera, the remaining body was kept in denatured alcohol for further morphological identification.

2.2. DNA extraction

DNA was extracted from spleen samples by QIAamp DNA Mini Kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's instructions or by the guanidine thiocyanate technique (Hoss and Paabo, 1993) with slight modification. Briefly, 10 mg of spleen tissue was homogenized by a grinder in 2 ml eppendorf tube and suspended in 1 ml extraction buffer containing 10 M GuSCN, 0.1 M Tris–HCl (pH 6.4), 0.02 MEDTA (pH 8.0) and 1.3% Triton X-100 and left for overnight agitation in a 56 °C shaker incubator. Then the tissue was boiled for a maximum of 10 min at 94 °C. After centrifugation at 14,000 rpm for 3 min, the supernatant was transferred to a new tube and 1 ml of freshly prepared NaCl solution with 1 μl silica and 1 μl linear acrylamide was added and kept on ice for 1 h with 15 min interval of vortexing. Then the mix was centrifuged at 5000 rpm for 30 s and supernatant discarded. The pellet was washed with washing buffer and then with ethanol and left to air dry. Finally, the pellet was re-suspended in ultra-pure water.

2.3. Host and parasite detection and determination

Confirmation of the morphological identification of hosts was provided by sequencing a fragment of the cytochrome b gene (900 bp). PCR was performed using the following primers: L14723 (forward, 5′-ACC AATGACATGAAAAATCATCGTT-3′) and H15915 (reverse, 5′-TCTCCATTTCTGGTTTACAAGAC-3′) (Lecompte