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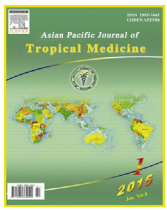

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Preliminary study on investigation of zoonotic visceral leishmaniasis in endemic foci of Ethiopia by detecting *Leishmania* infections in rodents

Wossenseged Lemma[1✉], Asrat Bizuneh[2], Habte Tekie[3], Habtamu Belay[2], Hirut Wondimu[2], Aysheshm Kassahun[4], Welelta Shiferaw[2], Meshesha Balkew[5], Ibrahim Abassi[6], Gad Baneth[7], Asrat Hailu[2]

[1]*Department of Medical Parasitology, School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, University of Gondar, Gondar, Ethiopia*

[2]*Department of Microbiology, Immunology and Parasitology, School of Medicine, Addis Ababa University, Addis Ababa, Ethiopia*

[3]*Department of Zoological Sciences, College of Natural Science, Addis Ababa University, Addis Ababa, Ethiopia*

[4]*Department of Parasitology, Faculty of Science, Charles University in Prague, Vinicna 7, 12844, Prague 2, Czech Republic*

[5]*Aklilu Lemma Institute of Pathobiology, Addis Ababa University, Addis Ababa, Ethiopia*

[6]*Department of Microbiology and Molecular Genetics, The Institute for Medical Research Israel-Canada, The Kuvin Centre for the Study of Infectious and Tropical Diseases, The Hebrew University – Hadassah Medical School, Jerusalem, Israel*

[7]*School of Veterinary Medicine, Hebrew University, P.O. Box 12, Rehovot, 76100, Jerusalem, Israel*

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ABSTRACT

Objective: To investigate the zoonotic visceral leishmaniasis (ZVL) by identification of the most probable reservoir hosts using parasite isolation and analysis of a possible transmission dynamics of the disease in extra-domestic agricultural fields and rural villages.

Methods: Rodents were collected from selected study sites in kala-azar endemic areas based on information for localities of kala-azar cases for screening of *Leishmania* infections using parasitological, serological and polymerase chain reaction (PCR) from March, 2013 to January, 2014. Ketamine (Clorketam Veterinary) was used to anaesthesize the rodents according the prescribed dosage (average 2 mg/kg for intra-venous route). The blood obtained using sterile needle was dropped into sterile filter paper and allowed to air dry before sealing in plastic bags. The tissues from liver, spleen and skin were macerated in Locke's solution before transferring them into NNN medium. Blood and touch smears of liver, spleen, skin and bone marrow were prepared for fixing using methanol and staining by Giemsa stain for microscopy. These tissues were also used for DNA extractions and PCR amplification of *Leishmania* infection.

Results: A total of 335 rodents (13 species) were analyzed by sampling internal organs. The infection rate by PCR was 11.1% (6/54) for *Arvicanthis nilothicus* compared to 17.6% (3/17) and 12.5% (2/16) for *Acomys cahirinus* and *Tarera* (*G*) *robustus* respectively. Almost all the infections were found from bone marrow samples (8/48 or 16.7%) compared with 1/91 (1.1%) liver, 2/87 (2.2%) spleen and 0/87 (0%) skin. In all study sites with past human VL cases, rodents and proved vectors shared similar habitats.

Conclusions: *Leishmania donovani* might circulate among different species of rodents in kala-azar endemic lowlands and valleys of Ethiopia by *Phlebotomus orientalis* and *Phlebotomus martini*. Detailed studies to substantiate the preliminary data on the possible role of these rodents are urgently needed.

✉First and corresponding author: Wossenseged Lemma, Department of Medical Parasitology, School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, University of Gondar, Gondar, Ethiopia.
E-mail: wossensegedlemma@yahoo.com

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1. Introduction

In Ethiopia, the annual incidence of visceral leishmaniasis (VL) is around 4000–7000 from the estimated 3.2 million people at risk for getting this disease [1,2]. The disease is believed to have a zoonotic transmission except during the man to man epidemics situation in Libo-Kemkem district [2–4]. Intrusion into a zoonotic cycle in extra-domestic environments has been indicated as the source VL infections in the major foci in Southern and northwestern Ethiopia [1,3–7]. However, the reservoir hosts of kala-azar have not been found conclusively. Direct agglutination test (DAT), enzyme linked immuno sorbent assay (ELISA) and polymerase chain reaction (PCR) indicated the presence of *Leishmania donovani* (*L. donovani*) infection and salivary protein of *Phlebotomus orientalis* (*P. orientalis*) (vectors of VL) in serum of cow, dogs, sheep, goats and donkeys in the northwestern Ethiopia [8,9]. The role of domestic animals such as cow, sheep, goat, donkey, camel and dogs in northwest Ethiopia might be related only to blood meal sources for the vector of VL [7–9]. A study in Sudan suggested Egyptian mongoose as probable reservoir host [10] while other studies considered rodents as possible reservoirs [11–14]. The fact that this carnivorous mongoose feed on rodents might lead to rodents' origin of the *Leishmania* infection. *Leishmania* screening of the rodents and bats in Ethiopia, including those samples for the study of animal wildlife in the country, indicated only rodents in the VL endemic areas harbored *L. donovani* infections [14,15].

Experimental *L. donovani* infection in *Arvicanthis nilothicus* (*A. nilothicus*) in Sudan indicated the susceptibility of this species to *Leishmania* infection but parasitemia decreased with time indicating that rodents may not be the reservoir host of VL [16]. Hoogstraal and Heyneman [11], on the other hand, found chronic natural of the infection in *A. nilothicus* and considered this rodent species as the most probable reservoir host of VL in Sudan, where the *Leishmania* parasites isolated from the rodents were found to be identical with parasites from VL patients and *P. orientalis* vectors. The objective of this study was to describe zoonotic visceral leishmaniasis in Ethiopia by investigating the role of rodents as reservoir hosts of VL in endemic lowland areas of Ethiopia.

2. Materials and methods

2.1. Study area

In Western Tigray Zone (Kafta Humera district), extra-domestic agricultural fields and tickets of *Acacia seyal* near Baeker (14°01′N, 36°59′E) and May Kadra towns (Gelanzeraf) (13°59′N, 036°31′E) were used for trapping the rodents in addition to agriculture fields in Adijamus village in Welkit districts. In Tahtay Adiyabo district (around Shiraro town; Western Tigray Zone), rodents were sampled from Ademiti, Chameskebet and Mayhas villages. In Raya Azebo district, southern Tigray zone, rodents were collected from pre-domestic areas around houses with previous VL cases in a village located 7 km away from Mehoni town, where the administrative center of the zone was located. In southern Ethiopia rodents were trapped from Galga-village (900–1300 m.a.s.l) and Segen Valley (<700 m.a.s.) in Aba-Roba kebele (5°15′0″N; 37°16′0″E) in Konso district. In Guji Zone of the Oromia Region, Negele Borana (or Neghelle) (5°20′N39°35′E, 5.333°N 39.583°E; 1475 m altitude) is the largest town in the zone. Rodents were sampled from Gofe Ambo (Enso) and Kobadi villages that were located at about 20 km west of Negele Borana town. The inhabitants in these two villages were semi-pastoral communities. The surroundings of clustered huts (tukuls) were bushy with big termite mounds serving as habitats for rodents and *Phlebotomus martini*, the proved vector of kala-azar in southern Ethiopia [17], similar to Galga village. Algude and Cherkeka (026°34′E; 05°65′N; 550 m altitude) villages were the other sites for rodent sampling in Omo valley in Hamer district of South Omo zone. These villages had similar vegetation with Segen valley, Galga and Negele Borana areas.

2.2. Study design

The rodent sampling sites in villages and extra-domestic habitats were selected based on information on document obtained from VL patients in treatment centers in Humera, Gondar, Arbaminch and Negele Borana Hospitals. Rodents were trapped for screening of *Leishmania* infection by parasitological, serological and molecular techniques before the species of the rodents were identified by morphological characters.

2.3. Study period

Rodents were sampled from sampling sites in Humera-Shiraro lowlands (March, April September and December, 2013), Mehoni lowland (June, 2013), Galga village (July, October, 2013), villages in Hamer (August, 2013), villages in Negele Borana (August, 2013) and Segen Valley (January, 2014).

2.4. Rodent trapping and tissue sampling techniques

Rodents were captured by Sherman live traps baited with peanut butter and placed overnight in the sampling sites. The rodents were anaesthetized for tissue biopsies from liver, spleen, bone marrow and tip of the nose (skin) for screening of *Leishmania* infections. Ketamine (Clorketam Veterinary) was used to anaesthesize the rodents according the prescribed dosage (average 2 mg/kg for intra-venous route). The blood obtained using sterile needle was dropped into sterile filter paper and allowed to air dry before sealing in plastic bags. The tissues from liver, spleen and skin were macerated in Locke's solution before transferring them into NNN medium. Blood and touch smears of liver, spleen, skin and bone marrow were made on microscope slides and allowed to air dry before fixing using methanol and staining by Giemsa stain for microscopy. Rodents were trapped from the sampling sites after permission was obtained from Ethiopian Wildlife Conservation Authority (EWCA), Government of Ethiopia.

2.5. Serological test of the blood samples on filter paper

Five mm disks from the dried blood samples on the filter papers were punched out before eluting in 125 μL DAT buffer in each well of a micro-plate row B which was incubated for overnight at 4 °C. Fifty micro-liter DAT diluent (physiological saline (0.9% NaCl) containing 0.78% β-mercaptoethanol) was dispensed into every well of the vertical rows A, B, C, D, E, F, G

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