

*Ph.D. Thesis Defence*  
*Faculty of Science, Charles University in Prague*  
*(Ph.D. Program in Parasitology)*  
*Defender: Aysheshm Kassahun Gelaglie*  
*Title: Visceral leishmaniasis in Ethiopia: transmission and variability*

## **Review Report**

by

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### *1. Overall assessment of the thesis*

The thesis is a compendium of six scientific papers, three published in 2013 and three in 2015 (no submitted manuscripts were included). Journals have from mid to elevated impact factor in their fields. The defender (Aysheshm) is the lead author of two papers reporting investigations on *Leishmania* infections in wild mammals from different places of Ethiopia. Looking at the authors' contribution of the remaining four papers, Aysheshm has made a significant contribution to the field collection of samples in different Ethiopian areas, whereas he did apparently not contribute in the study design and writing the articles. These studies were performed by large collaborative groups including several institutions and having different expertise; so that Aysheshm had the great opportunity to work on a broad range of leishmaniasis topics led by reputed specialists.

Such range of studies, having determinants of visceral leishmaniasis transmission in Ethiopia as common denominator, have focused on the diversity of parasites and the detection of asymptomatic *Leishmania* infections by means of innovative methods; their geographical prevalence among humans, domestic animals and wild mammals; and the biological, genetic and vector-competence aspects of geographical populations of the main phlebotomine vector, *Phlebotomus orientalis*.

Approaches underlying each study are scientifically sound and could not be performed without the deep knowledge of the diverse Ethiopian contexts where leishmaniasis is transmitted and/or vectors are present. "Landscape leishmaniasis" has been a traditional discipline that unfortunately is disappearing due to the disproportionate use laboratory-only approaches. For what Aysheshm wrote as a complement to the scientific papers, and the way the thesis has been structured, I believe that he is mastering this complex discipline and has reached sufficient knowledge to continue and complete the studies in Ethiopia.

### *2. Comments and criticism*

A) Paper I, IV, V and VI

The main subject of these papers is asymptomatic leishmaniasis. The concept of “asymptomatic infection”, either in humans or animal hosts, is complex and in my opinion it was not addressed satisfactorily. Immune resistance factors underlying this condition were not discussed, for example in the light of evidence from experimental murine and canine models or from surrogates of protection in humans. For example, why the authors were surprised to find *Leishmania major* circulating in blood of individuals without evidence of cutaneous lesions? In fact, how can strong and permanent reactivity to leishmanin skin test could be explained in exposed individuals with no *L.major* active lesions or scars, as observed by several authors? Furthermore, proven rodent reservoirs harbour these parasites in intact skin and peripheral blood for life, as do asymptomatic resistant dogs infected by *L. infantum*. What mechanisms can be hypothesized for such parasite/host equilibrium?

#### B) Papers V and VI

The studies leaded by Aysheshm in rodents and bats were largely observational. As correctly mentioned by the defender, they could not result in definitive incrimination of infected animals as natural hosts of leishmaniasis, because their infectiousness to vectors was not tested. However a second approach was ignored (i.e. not made, nor mentioned as possible in future), namely the laboratory rearing of target species of wild rodents (for example *Mastomys* and/or *Arvicanthis*, which exhibited highest PCR-positive prevalence) to be infected experimentally with *L. donovani*.

### 3. Question

A) In reference to the last comment above, I would like the defender to briefly design a small study to test the susceptibility, course of infection and infectiousness of a rodent species experimentally infected with *Leishmania*, preferably by the bite of a laboratory-reared sand fly vector.

B) In the defender’s opinion, which human clinical parameters could be taken into consideration to evaluate the significance of an association between genetic make-up of *L. donovani* strains and disease expression/severity?

### 4. Reviewer’s conclusions

For what exposed, scientific merits exceed by far the few critical points. Therefore I believe that the thesis is more than suitable for the award of a PhD title to Aysheshm Kassahun Gelaglie.

Roma, 1<sup>st</sup> December 2015

Luigi Gradoni

