



December 1, 2015

**The written review of PhD Thesis of Aysheshm Kassahun Gelaglie**

**Supervisor:** Doc. RNDr. Jan Votýpka, Ph.D.

**Title of PhD thesis:** Visceral leishmaniasis in Ethiopia: transmission and variability

**Dear Doc. RNDr. Ivan Hrdy, Ph.D.,**

**Chairman of the Committee**

After the careful evaluation of the PhD thesis of **Aysheshm Kassahun Gelaglie** entitled “*Visceral leishmaniasis in Ethiopia: transmission and variability*”, please find in attachment the assessment report prepared by me. I, with great pleasure, report that this PhD thesis makes a significant contribution to scientific knowledge, makes a significant contribution to the understanding of the subject with which deals, demonstrates the candidate’s capacity to carry out independent research, contains material worthy of six publications, and that the format and literary presentation of the thesis are satisfactory.

I recommend that the candidate be awarded the degree of Ph.D.

Please find in below, a list of my overall assessment, critics and questions according to the rules of The Charles University, Prague, Czech Republic.

Sincerely yours,

A handwritten signature in black ink, appearing to read 'Y. Özbel' with a stylized flourish at the end.

Prof. Dr. Yusuf ÖZBEL, Ph.D.



## Overall assessment of PhD thesis

The studies of this PhD thesis were carried out in Ethiopia where one of the important endemic region for visceral leishmaniasis in the World. The PhD thesis and publications related to the subject of the thesis were read carefully and my assessments are in below:

The general aims of this PhD thesis were defined as follows:

1. *Elucidating the role of symptomatic and asymptomatic L. donovani infected persons in the epidemiology of VL in Northern Ethiopia.*

Almost five thousand dried-blood samples collected from individuals had been tested by qRT-PCR and 14.3% of them had been found positive. ITS1 sequences of PCR products had been revealed 19 *L. donovani* and two *L. major* cases.

2. *Determination of variability and tropism in the vector (P. orientalis) and the parasite (L. donovani).*

Variability studies of *L. donovani* isolates had been carried out using three gene regions, ITS1, cpb and k26. It is determined that the isolates were divided into two clusters based on k26 amplicon size. Regarding vector studies, no significant genetic and susceptibility pattern difference had been observed.

3. *Determination of non-human VL hosts in Ethiopia.*

Domestic animals (cow, dog, sheep, goat, donkey and camels) had been tested for natural infection and 5.8% of them had been found positive for *L. donovani* by molecular methods. In addition to this, 19% and 23% of the animals had been found seropositive for anti-*L. donovani* IgG and anti-*P. orientalis* saliva IgG respectively. A total of 8.5% of the rodent had also been found as positive by PCR and the causative agents were detected as *L. donovani* and *L. tropica*. The samples from bats had been investigated and *L. tropica* and *L. major* DNAs were detected.

In general, the results of this PhD thesis provides new knowledge on the (i) role of non-human host in visceral leishmaniasis epidemiology and (ii) genetic variability of the parasite, *L. donovani*, between two different geographical regions.

In Introduction section, *Leishmania* parasite and its life cycle, the information about visceral and cutaneous leishmaniasis in general and in Ethiopia, reservoir roles of domestic and wild animals in the transmission of VL had been explained briefly.

### Rationale 1

As I understood from the background information, the accurate diagnosis of VL, especially in asymptomatic carriers is an important problem in Ethiopia. The first part his study aimed at elucidating the role of symptomatic and asymptomatic *L. donovani* infected persons in the epidemiology of VL in the new foci by a prospective large-scale cohort study.

### Critical comments

Detection of *L. major* in blood samples obtained from volunteers is really interesting point. In my opinion, there is no doubt about this because ITS1 sequencing is enough for certain diagnosis in species level. I can suggest checking your records about his/her travel story to CL endemic areas.



The PCR tool used in the study is efficient for the diagnosis of VL, but there is no information about the positivity rates of symptomatic and asymptomatic volunteers. Infectivity to sand flies is really crucial point, but in this part of the study he needed to be focused applicable diagnostic tool.

### **Rationale 2**

In this part of his study, for helping or developing a new way for the diagnosis, he looked for a new tool for distinguishing *L. donovani* complex at subspecies level using molecular tools (k26, cpbE/F and ITS1 PCR).

### **Critical comments**

Detection of two genetically distinct populations of *L. donovani* is a new and important finding obtained by this study. In my opinion, this difference can be due to not only host factors but also vector species. In addition to this, the difference in *L. donovani* strains isolated from HIV/VL co-infected patients remains unclear. As written in the thesis, it needs further investigations by different methods.

The number of the strains used is not included in the thesis; it is available only in the published paper. May I suggest including these numbers to the thesis? The further studies on HASPB repeat region will be very interesting and meaningful to incriminate the certain vector species in endemic areas.

### **Rationale 3**

It is known that *P. orientalis* is a potential vector in northern Ethiopia while *P. martini* and *P. celiae* are vectors in southern Ethiopia. In his study, he also worked on the possible differences on the role of vector status of two *P. orientalis* populations that are present in geographically different areas. He aimed to study on the biology and genetics profile of two populations of *P. orientalis*.

### **Critical comments**

The results of this part of the study are very informative scientifically. Because of no differences were found between two *P. orientalis* colonies and no differences can be expected for human populations, living conditions, probably the parasite circulation is very limited in non-endemic area, maybe it is related to micro-climatical or micro-ecological situation. It can also be due to host preference of the vector species as discussed in the paper. To investigate these conditions can be done as further studies.

### **Rationale 4**

In the last part of his thesis, he searched the potential reservoir roles of domestic animals, rodents and bats, and he also worked on exposure of domestic animals to sand fly bite.

### **Critical comments**

The qRT-PCR had been revealed 5.9% positivity for *Leishmania* DNA among domestic animals that are high percentage. In my opinion, this is a very good point to discuss on whether it is a risk factor or a zoonophylaxis? For this reason, their infectivity to sand flies needs to be studied in future as indicated in the thesis. Wild rodents have also epidemiological importance in the



transmission cycle of some *Leishmania* parasites and to include this group of animals to the PhD thesis is a well-directed decision.

Detection of *L. tropica* and *L. major* DNA in bats is successful results. It opens new window for further epidemiological research on leishmaniasis epidemiology. We know that bats and some species of sand flies like caves!

Throughout the studies, I had noticed that he was contributing author in 10 papers published in the valuable journals such as *PLOS Neglected Tropical Diseases*, *Parasite & Vectors*, *American Journal of Tropical Medicine and Hygiene*, etc. in the parasitology field.

### **Some grammatical corrections or typographical errors**

Overall, no important mistake in experimental design of this thesis has been determined. Some errors are listed according to page and line numbers in below:

Page 14, Line 14: “six genera” will be “ six subgenera”

Page 14, Line 26: “...and it closely” will be “... and it’s closely”

Page 15, Line 4: “... of VL recoded” will be “... of VL recorded”

Page 22, Table 3, first row: “*Procavia capensis*” will be italic

Page 24, Line 9: It is better to change the sentence as “In the East African VL endemic regions dogs are accepted as very important risk factors of the disease”

Page 24, Line 10, “seroprevalnca” will be “seroprevalence”

Page 24, Line 14: It is better to use “intermediary host” instead of “intermediate host”. As known, the term of “intermediate host” is using for the hosts carrying immature forms of the parasites.



## General questions

The thesis is well designed, it answered many questions, and more important it opened new gates and served many new research topics to biological science. I would like ask eight questions as indicated in below:

1. What is our study's overall contribution for the solution of VL problem in public health level in Ethiopia? Do you think, this PCR method can be applicable in Ethiopia?
2. Three vector species; *P. orientalis*, *P. celiae* and *P. martini* are present in Ethiopian central Rift Valley but no VL cases. In spite of high LST positivity, how can you explain this situation? What about the status of HIV/VL co-infection cases in the area?
3. What is the factor to increase sand fly-man contact? Do you think a natural zooprophylaxis is present in some areas in Ethiopia and it affects sand fly-man contact?
4. *L. donovani* is causative agent for VL in Eastern Sudan and Indian subcontinent. Could you please explain the differences of the infection between two endemic areas? (in the meaning of vectors, reservoirs, PKDL situation, etc).
5. How can you identify asymptomatic carriers? Which test can be used for this purpose? Why sensitivity and specificity are lower in rK39 test in East Africa?
6. Did you apply questionnaire during blood collection? Can it be essential to explain or comment your results?
7. Did you collect climatic and ecological data to make comments on your results?
8. Do you think, HASPB repeat region can be used instead of direct dissection to detect certain vector species in endemic areas?

## Statement:

The reviewer believes that the PhD candidate, **Aysheshm Kassahun Gelaglie**, has demonstrated her qualification for scientific work at the PhD level. The thesis fulfills qualitative requirements for a PhD dissertation and fulfills also the formal rule as far as the number of published papers in peer-reviewed and impacted journals is concerned. Moreover, the candidate published **six** papers that are more than needed.

In conclusion, I want to congratulate him and his supervisor **Doc. RNDr. Jan Votýpka** for the excellent study. I have found the study of **Aysheshm Kassahun Gelaglie** and his thesis can be defended. I recommend that the candidate be awarded the degree of Doctor of Philosophy.

Sincerely yours,

Prof. Dr. Yusuf ÖZBEL, Ph.D.