



Institut Pasteur



Gerald Späth, Ph.D.
Professor, Institut Pasteur
Head, Molecular Parasitology and Signaling Unit

Paris, 31/01/2025

Dear colleagues,

It is my pleasure to submit this report on the PhD thesis presented by Kamal Eddine BENALLAL entitled 'Phlebotomine sand flies in transmission cycles of leishmaniasis in Algeria'.

Leishmania infection is a major public health problem across the world causing important human suffering and imposing economic hardship on endemic countries that stalls their development. Both these challenges are highly relevant to Algeria, which ranks second to Afghanistan for the global incidence for cutaneous leishmaniasis (CL) with over 6,000 annual cases observed in recent years. The complex interplay between ecological, social, and health factors contributing to this alarming situation calls for in-depth analysis of the CL epidemiology and risk factors in Algeria. This thesis is directly responding to this shortcoming addressing a major knowledge gap on the fauna of sand flies in Algeria, interaction between *Leishmania major*, its major insect vector *Phlebotomus papatasi*, and two rodent hosts (i.e. *Meriones shawi*, *Gerbil/us amoenus*).

Combining a series of complementary approaches, including DNA barcoding, protein MALDI-TOF analyses, experimental infections and xenodiagnosis, the candidate made several key discoveries relevant to *L. major* transmission, asymptomatic infection and treatment failure in Algeria, including (i) the description of a new sand fly species *Sergentomyia (Sergentomyia) imihra* n.sp, (ii) the determination of a surprisingly large range of mammals (i.e. cattle, camel, goat, sheep, dog, chicken and human) as the source of blood meals for the sand flies *Ph. perfiliewi*, *Ph. pemiciosus*, *Ph. papatasi*, *Ph. bergeroti*, and *Ph. alexandri*, (iii) the identification of *G. amoenus* as a novel *L. major* reservoir, (iv) the experimental demonstration of an important role of asymptomatic *M. shawi* in parasite dissemination, and finally (v) the role of *Ph. papatasi* to transmit antimony resistant *L. major* strains in the field.

This strong body of work sheds new light on the risk of zoonotic parasite transmission in Algeria provide valuable information for local authorities to improve disease control strategies and identifies new aspects of the parasite infection cycle such as silent chronic infection that needs to be considered by epidemiologists when modelling the disease.



The candidate's scientific productivity translated into 6 published reports, three of which are signed by the candidate as first author. The thesis document follows a pragmatic, paper-based thesis structure. A brief Introduction on leishmaniasis and some of the techniques used (13 pages) is followed by the description of the Objectives (1 page). The Results section consists of the pdf versions of the candidate's 6 publications. The rest of the thesis document is then dedicated to a short discussion (4 pages), a 1-page conclusion on the major achievements, a list of publications and the candidate's CV.

In conclusion, this thesis and the published reports represent an important advance in our limited understanding of the transmission dynamics in Algeria. As a result, Mr Kamal Eddine BENALLAL fulfills the criteria to obtain the PhD degree.

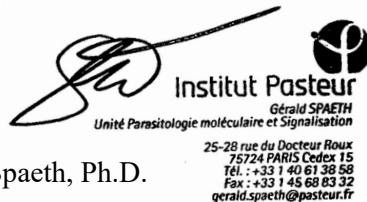
Remarks to be considered during the defense and when preparing the final version of the thesis document:

- 1) Throughout the thesis, the candidate often refers to 'disease transmission by the sand fly'. The sand fly does not transmit any disease but only the parasite that causes the disease. I suggest correcting this statement throughout the document.
- 2) Given the paper-based thesis structure, I am very surprised that the individual contributions of the candidate to the 6 publications is not highlighted in detail. I strongly recommend adding for each publication a short bullet point list that clarifies the candidate's implication.
- 3) The wrong impression is given that only PKDL is a consequence of the immune response. In fact, all types of leishmaniasis are immuno-pathologies, not only PKDL. The latter one has been described in the context of drug treatment, with dermal tropism being considered an escape strategy.
- 4) Page 12, last two lines correct two typos (at least on my pdf version): '12eishmaniasis' and 'L. Major'.
- 5) Page 14: it is unclear when and where metacyclic stage parasites develop.
- 6) I expected the thesis to include at least a draft version of the manuscript that is currently under review.
- 7) The Conclusion section lacks a paragraph on the scientific/societal impact of the results obtained by the candidate and a brief discussion on the key gaps and new questions opened by his work.

8) Questions:

- i. Can you critically assess the depth of your literature search for the PLoS NTD review? Are there any sources that remain to be explored? Can you estimate the error rate of the techniques used in these reports to assess the sand fly species?
- ii. What additional approaches (aside this literature search) would you suggest for a more complete assessment of regional variations in sand fly distribution and their role in parasite transmission?
- iii. How did (and still does) the sand fly population change during the studied period until today?
- iv. What are the current and newly emerging risk factors you can identify that will impact the sand fly distribution across the Maghreb?
- v. What is known about climatic adaptation of insects, their mechanisms of fitness gain and how may this be relevant in times of climate change in the Maghreb region?
- vi. Does Leishmania-phlebovirus co-infection occur and how does this affect transmission and disease (caused by both pathogens)?
- vii. How does Leishmania infection affect the sand fly feeding behavior and host preference for blood feeding?
- viii. How stringent is sand fly species determination using morphological criteria? Are the morphological characteristics showing any overlap between species or are they strictly species-specific?
- ix. How do you explain the failure of blood meal host determination (MS-based and DNA-based) observed for several sand flies?
- x. Has bovine or goat leishmaniasis been described and what is the risk that these animals are an undeclared reservoir?

Sincerely,



Gerald F. Spaeth, Ph.D.