Universidade Nova de Lisboa
INSTITUTO DE HIGIENE E MEDICINA TROPICAL

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Dear Doc. RNDr. Ivan Hrdý
Katedra parazitologie
Predseda of Mrg Jan Drahota PhD thesis

I am sending you my written report regarding PhD Thesis of **Mgr. Jan Drahota** "**Antigens in the sandfly saliva and antibody response of the bitten hosts**"

Report

Leishmaniases are major vector-borne diseases caused by parasites of *Leishmania* genus transmitted by the bite of phlebotomine sandflies. Leishmaniases are endemic in 98 countries being the most important emerging and resurging vector-borne protozoan disease second only to malaria in terms of numbers of people affected. Leishmaniasis caused by *L. infantum*, is the only tropical vector-borne disease that has been endemic in southern Europe for decades and most of the reported cases are due to zoonotic visceral leishmaniasis, the most dangerous form of *Leishmania* infection, being lethal when untreated. Dogs are the main reservoir hosts for human leishmaniasis and therefore, the evaluation of vector-reservoir/host contact should be monitored in order to evaluate the risk of *Leishmania* transmission and to assess the efficacy of control measures.

The work developed by Mrg Jan Drahota´s thesis represents a new and valuable tool for the evaluation of the effectiveness of anti-*Leishmania* vector campaigns. Mrg Drahota analysed the antigenicity of salivary proteins of *Phlebotomus perniciosus*, the most important vector of *L. infantum* in sourthern Europe, and *P. papatasi* the main vector of *L. major* responsible for cutaneous leishmaniasis human cases in the Old World. Furthermore, he developed several recombinant salivary antigens and tested their efficacy to detect antibodies against sandfly saliva. The ELISA method proved to be an important and novel tool to measure the exposure of hosts (especially dogs) to sandflies, and indirectly to assess the risk of *Leishmania* transmission.

In conclusion, the work developed in his thesis might be integrated as a strategic tool to enhance sandfly surveillance, in order to interrupt or limit the spread of sandfly-borne pathogens with the final goal of protecting humans and animals from these threats.

Nevertheless, I would like to rise some points for Mrg Drahota consideration.

- In abstract, you wrote that leishmaniases are also present in North America, do you consider this subcontinent endemic for these parasitic diseases and if you do, of which *Leishmania* species?

- In introduction, page 8, you mentioned that in Europe new reservoir hosts of the current *Leishmania* species have been recorded. Can you tell us which indicators should be fulfil in order to identify a host as reservoir?

- In section 1.2, you said that in Europe, seven proven *Leishmania* vector species are present. Can you tell us which requisites should be fulfil in order to identify a sandfly species as a proven vector?

- In the same section you also mentioned that the susceptibility of many European sandfly species to endemic and non-endemic/sporadically endemic *Leishmania* species had not been identified yet. As you know, in the last years the role of some *Sergentomyia* species in the transmission of human pathogenic *Leishmania* sp. have been debated. What do you think it should be done in order to determine, for instance, if *S. minuta*, the most spread species in the Mediterranean Basin, can play as a vector of *Leishmania* species that are endemic in this geographic area (i.e. *L. infantum, L. major, L. tropica*)?

- In section 1.3, and regarding the Spanish study made during the outbreak of HumL in in Madrid in the years 2009-2012, why do you think that the prevalence of leishmaniasis in dogs, the main domestic reservoir of *L. infantum*, was so low?

- Regarding your ELISA results and taking into account the bibliographic revision that you made in the introduction (section 2), do you think that the chosen *P. perniciosus* antigens used to test dog´s sera can be applied to test human samples? In addition, and taking into account that in Europe there are five proven vectors of *L. infantum*, all belonging to *Larroussius* subgenus, do you expect to have some antigenic cross-reactions when testing human/dog samples obtained from regions where more than one vector is present? If yes, do you think that has any epidemiological consequence?

As a minor comment I would like to clarify/point out that regarding canine leishmaniasis (CanL) prevalence in Portugal, two studies carried out in whole continental country are missing, namely:

1. Cortes et al. 2012. Risk factors for canine leishmaniasis in an endemic Mediterranean region. Vet Parasitol. 189(2-4):189-96.
2. Cardoso et al. 2012. Prevalence of *Dirofilaria immitis, Ehrlichia canis, Borrelia burgdorferi* sensu lato, *Anaplasma* spp. and *Leishmania infantum* in apparently healthy and CVBD-suspect dogs in Portugal--a national serological study. Parasit Vectors. 5:62.

Moreover, it was not in the study performed by Branco et al. 2013 that the CanL prevalence in Torres Novas was determined (the correct reference is Cortes et al. 2012).

Finally, I would like to state that I consider the thesis suitable for the defense and that its quality fulfils the criteria necessary for Mrg Jan Drahota to obtain the PhD degree.

Yours sincerely,

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