



Reviewer's report on a PH.D. Dissertation thesis (Author: Ing. Johana Alaverdyan)

The dissertation thesis of Ing. Johana Alaverdyan examines the complex relationships between tick-borne pathogens, ticks, their neglected habitats and unusual hosts. It mainly focuses on exotic animals bred in the Czech Republic and the effect of TTBDs on them. It also deals with TTBDs in used pastures, reclaimed areas and post-industrial landscapes. It is written on 125 pages and contains 6 chapters and a CV (introduction, literature review, publications, discussion, conclusion and references) and has a good quality of English without typing and spelling errors.

The first chapter, named Introduction has 4 pages and the author describes the issue of ticks and emphasizes the need for a One Health approach. It gradually explains the influence of climate change and human action on the population of ticks, the occurrence of diseases as well as the financial impact on treatment. This chapter clearly describes the issue of the diploma thesis.

Chapter Literature review clearly deals first with the history of ticks, their biology and ecology, reproduction and feeding, as well as hosts and pathogens and their transmission. The review is very comprehensive and detailed, there is also a section dedicated to the environment where ticks are found and its changes that affect ticks.

Chapter Publications contains five publications: two reviews and three original works. Four publications have already been published in peer-reviewed journals, one is under review. The first two publications are devoted to the management of ticks and the prevention and breeding of exotic animals in the Czech Republic in connection with the occurrence of ticks and pathogens. Three original works are devoted to the occurrence of ticks in rewilding locations and post-industrial locations and the occurrence of *Borrelia* in exotic animals.

Chapter Discussion: This chapter offers a comprehensive look at the impact of climate change and other factors on the occurrence and ecology of ticks and pathogens. It is written very clearly and gradually covers all aspects of the issue.

Finally I have some questions:

1. In the abstract and in the introduction, you mention the necessity of the One Health approach. Could this approach be used in your work as well? You are dedicated to animal health, environmental health - vectors. But there is a lack of publicly available data on people's health. How could they be used?
2. In the second review – chapter 2, you compare the serological results of antiborrelia antibodies in exotic animals from Germany and the Czech Republic. How do you explain the lower seroprevalence in animals in Germany compared to animals in the Czech Republic? Both states occur in the same endemic area. Can it only be due to



Státní zdravotní ústav

- the very low number of examined animals in the Czech Republic or does another factor play a role here?
3. How do you explain the high PCR positivity of *Borrelia* in the blood of exotic animals in chapter 3, when almost no ticks were found on the pastures or around the pastures? Detection of *Borrelia* in the blood of animals is much more difficult than from tissues. The prevalence of *Borrelia* DNA in the blood of 13 to 67% is more than surprising, especially when comparing serological results from Germany in chapter 2, which reached similar prevalences. How do you explain that?
 4. Why did the author not use qPCR to detect *Borrelia* but rather chose nested-PCR (chapter 3)?
 5. Did you use uracil instead of thymine and Uracylglycosylase in the reactions to eliminate amplicon contamination?
 6. Did you use the same methodology for detecting *borrelia* in ticks as in the publication devoted to the post-industrial landscape (chapter 5)? The percentage of positivity here is rather lower at 10.12 and 4.3%, which correlates well with similar publications.
 7. How do you explain your conclusion from the publication regarding the greater occurrence of ticks on ungrazed areas (chapter 4) in the context of the result of the publication on exotic animals (chapter 3), where you did not find many ticks even around pastures (I assume that around pastures was not grazed)?

From the comments and questions above, it is clear that Johana has dealt well with a complex issue and mastered many methods and obtained a relatively large number of results. In my opinion, Johana Alaverdyan's dissertation meets all the requirements of the Faculty of Tropical AgriScience of CZU, and therefore I recommend it for defense.

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Katerina Kybicova Ph.D.

National Reference Laboratory for Lyme Borreliosis
Centre for Epidemiology and Microbiology

E-mail: katerina.kybicova@szu.cz

National Institute of Public Health
Šrobárova 49/48
100 00 Prague 10
Czech Republic
www.szu.cz