

## OPPONENTS'S REVIEW OF THE DISSERTATION THESIS

Hidden Linkages: Investigating Ixodid Ticks, Tick-Borne Pathogens, and their Presence in Neglected Ecosystems and Unconventional Hosts

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The thesis consists of quite short, yet well written, introduction and literature review. I found it informative, logically flowing and inviting to the main part of the thesis, which is based on the four published articles with IF and one manuscript.

Both review articles thoroughly investigated and discussed the topics of tick and disease mitigation strategies and the presence of tick-borne pathogens in zoo animals. The strategies against ticks and TBDs are in general intrusive, aiming on control tick populations, pathogen prevalence or both at the same time. What I miss in the whole concept are options of adaptation to the given situation. However, I understand that it is beyond the scope of the review as such schemes are not to be made solely by biology-oriented research but in an interdisciplinary effort between social and natural sciences. Zoo animals can be in the roles of either reservoirs of unexpected new pathogens/vectors and facilitate spread of disease or a naïve hosts exposed to local pathogens resulting in disease or death. Screening for locally known threats is not sufficient in such setting and having a concise review on what can be expected in exotic animals is very useful to zoos, exotic animal breeders but also veterinary authorities involved in decision making in regard to exotic animals in human care.

The study of *Borrelia* presence in exotic animals in human care provided several interesting results. Possibility of vertical transmission of *Borrelia* in the eland that could explain the observed high prevalence in young animals in habitats not supporting ticks is interesting and should be investigated in more detail in future. The single animal with *B. miyamotoi* positivity was ostrich, any singular result has to be evaluated very carefully and critically but may be pointing to a more general issue.

The analysis of tick activity and *Borrelia* prevalence in abandoned place with ongoing habitat succession and city park is a relevant addition to understanding of value and identifying risks emerging from the „new wilderness“ that is forming in vicinity or directly within the most urban areas. The observed *Borrelia* prevalence was low compared to usual results from Czechia. If the result was not due to methodology (e.g. assay sensitivity) it would be interesting to repeat the study and get a longer time frame to check whether this is a permanent status, or the prevalence increases in time as the habitat succession advances.

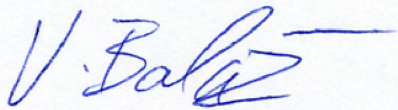
The prepared manuscript covers a very interesting topic of “rewilded country” and effects of wild large herbivores on tick abundance. The manuscript still needs some fine tuning. I found a reference error in the introduction, where authors state that “*ecology of I. ricinus has been extensively studied, but ongoing changes in land management, climate etc.*“ with a reference to publication Nolan et al. (2022) that was aimed on American tick species and mostly on the effect of urbanisation on prevalence of different TBPs. It was likely just erroneous placement of a reference but should be corrected before submission. The figure 1. illustrates the abundance of ticks in value of absolute numbers of ticks collected, while that should be corrected to



sampling effort and changed to ticks/sampling hour or similar. If I understand the figure 1 correctly, the tick activity was inverted between the two types of habitat: when ticks were present at grazed, they were completely absent in ungrazed and vice versa. Was that so? The observed lack of larvae is likely biased by the sampling method, I can't imagine collecting larvae from a "fabric with 1 cm hair length", thus I would suggest to exclude the larvae counts from the analyses.

**After reviewing the doctoral dissertation of Johana Alaverdyan, I gladly *recommend* it to defence and expect successful fulfilment of all requirements to finish the doctoral study of the author.**

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Mgr. Vojtech Baláž, Ph.D.

I look forward to being personally present at the defence. Here are few of my thoughts and questions that I would like to be part of the final discussion:

The overall issue of Czech landscape is de-fragmentation, over-utilisation, exploitive monoculture forestry and at the same moment abandonment and spontaneous overgrowing of habitat fragments and ecotones. There is significant motivation to support landscape biodiversity by increasing the fragmentation of the agricultural landscape, involve public in management of deserted places, increase extensive pastoralism by domestic or even wild herbivores. What would you expect from TBDs and ticks in general in future if such changes are successful on broader scale?

How much is known on survival of tick larvae/nymphs/adults in relation to TBD pathogens?  
Does infection cause any additional cost to the tick?