Thesis evaluation

It is my honor to provide an evaluation of doctoral thesis entitled "Hidden Linkages: Investigating Ixodid Ticks, Tick-Borne Pathogens, and their Presence in Neglected Ecosystems and Unconventional Hosts " by Johana Alaverdyan, supervised by Jiří Černý. The thesis topic revolves around the ticks, their ecology, tick-transmitted diseases and landscape ecology. There is no doubt, that ticks, and mainly *Ixodes ricinus*, represent an important topic among the disease vectors, particularly in the Central Europe. In the Czech Republic itself, research on ticks has more than seven decades history. One would almost say that there is not much to discover. Thesis by Johana shows, that it is fairly not true.

The thesis is submitted in a short form, being based on four published research articles and one submitted MS preceded by a literature review and followed by a discussion and conclusions. Johanna is a first author in two out of four published papers, as well as in the MS submitted to Vector-Borne and Zoonotic Diseases Journal. The quality of results published is undoubtedly proven by review process of respected journals. This fact makes the work of reviewer easy and difficult in the same time. As parasitologist involved in the tick research, I am glad that the published papers already got 47 citations in the WoS (without autocitations), which documents well the importance of studied topic.

The remaining paper entitled „Apparent Link Between Naturalistic Grazing and *Ixodes ricinus* Tick Abundance in Rewilding Sites: A Pilot Investigation" is provided as a submitted MS, so it deserves a bit more attention. The study is described as a „pilot" and its objective was to investigate the effects of grazing on tick abundance, utilizing flagging as the tick collection method, to investigate the potential differences in tick abundance between grazed and ungrazed areas, including assessment of impact of seasonality, temperature, and humidity on tick abundance. Authors observed a pattern of overall reduced tick numbers in grazed sites and discuss its potential reasons. Howsoever simple the study layout seems to be, this is a very difficult study prone to several types of bias. Usability of flagging for estimation of fine differences in tick abundance is a matter of endless debates in many scientific fora. The fact, that grazed habitat has significant impact on all parameters of vegetation cover can greatly impact on the flagging itself and on numbers of harvested ticks. This, in my opinion, deserves some attention in the discussion itself. The same way, the term „rewilding" is not sufficiently explained when discussing its possible impacts on ticks. For instance, can the overpopulation of parts of the Czech Republic by red and roe deer be also considered a rewilding? In Europe and USA, this was repeatedly suggested as one of major reasons of increased tick abundance.

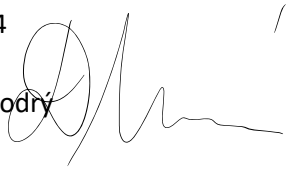
In the discussion, the presence of large mammals as tick host and pathogen reservoirs is discussed as a source of possible tick-born infections. Authors say, "Ticks can introduce tick-borne pathogens (TBPs) to ecosystems, and their prevalence can be one of the driving factors of TBPs. The abundance of ticks (vectors) could, at a first glance, appear to be one of the driving factors of prevalence of TBPs." This part of the text is not really connected to the results itself and also its narrative is not very clear, in part because of improper usage of the term prevalence. This would probably deserve more attention when revising the paper.

In general, the thesis is written in decent English. As I am not native English speaker, I cannot truly appreciate the level of language; however, I noticed only minor issues throughout the text. It is my pleasure to conclude, that thesis of Johana Alaverdyan merits the acceptance as a doctoral

dissertation as it fully meets the requirements for such a study. I can congratulate both the author and her supervisor for very interesting outcomes. However, to satisfy my curiosity and to prove, that I have really read the text, below I have few questions that might be answered during the defense.

7/3 2024

David Modrý



Questions:

? In the grazing study, you observed a pattern of overall reduced tick numbers in grazed sites. The methodology is based on collecting the actively questing ticks in the time of visit. However, questing itself is only a short period of long life of the tick. Isnt it posible that abundace of large mammals shortened the period of questing, reducing the chance to collect such ticks?

? in the same study, you say „Tick collections yielded similar numbers of nymphs and adults. This might be explained by the high prevalence of hosts suitable for adult and nymphal stages of *I. ricinus* like hares, hedgehogs, deer, and megafauna inhabiting the Milovice reserve (cattle, horse, bison)“. In my opinion, in this statement you need to consider the fact, that the questing stages are „products“ of the previous stage/generation. Then, abundace of hosts as you say „suitable for adult and nymphal stages of *I. ricinus“* shouldnt impact on nymphs as such, as they are depending on hosts of the previous stage, which means larvae. On the contrary, abundace of these hosts should be, at least theoretically, reflected in higher number of questing larvae. Can you elaborate on this and explain the original idea?

Last but not least, in the discussed sentence you speak about „high prevalence of hosts…“ which is not a proper term. Prevalence is purely epidemiology term.