



Review on diploma thesis

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Title: **Analysis of genetic diversity of *Borrelia burgdorferi* sensu lato and *Borrelia miyamotoi* across Central and Eastern Europe using multilocus sequence typing (MLST) in range of Slovakia and data from the MLST database with focus on Central and Eastern Europe**

Veronika Morávková submitted her master thesis trying to extend current data about distribution and multilocus sequence analysis of *Borreliae* in questing ticks in Slovakia. Generally, the current state of knowledge in this field, with so many genospecies, subtypes and isolates of Lyme disease spirochetes worldwide is rather confusing and I hope such kind of research would be helpful.

Formally, the submitted work has usual structure and unusual length of 159 pages. As far as I can judge, language and level of various grammar or stylistic mistakes is tolerable. Although the literature overview is logically structured, it is way too long (31 pages), too detailed and even a focused reader cannot hold the attention from start to finish. Methodology describes many PCR-based techniques and several applications of sequences. Some results are presented only in text format (with graphic expression in appendix) which makes reading more difficult. In Discussion, I miss possible explanations of the obtained results that are not in correlation with previously published data (e.g. overall mean prevalence of *B. burgdorferi* sensu lato in Slovakia or absence of *B. burgdorferi* sensu stricto in majority of sampled areas). Although the topic of the thesis is complex, more than 400 (!) references rather mean that the student was not able to select the most relevant information for her project. As for appendices, some of them would better fit directly into introduction (after redesign, Appendix 1), some of them contain apparent mistakes (e.g. Appendix 2: same proteins listed twice), some of them are completely irrelevant (e.g. Appendix 5 with precise dates of the tick collection). Generally, almost all the tables would need better design in order to occupy less space.

Questions to be answered during defence:

- 1) PCR-RFLP is one of the methods used for molecular typing of *Borrelia* spp. Could you please describe the principle of this technique?
- 2) According to chapter 4.1, all the ticks were collected in 2017. What was the time schedule of the whole sample procedure (nucleic acid extraction, PCR, sequencing, MLST etc.) and, more importantly, what was your role/part in each step?
- 3) Ad 4.2 and 4.3: What is the principle, advantages and disadvantages of described DNA extraction using ammonium hydroxide, in comparison to other DNA extraction methods? What was the success rate of this extraction method when applied on your samples?
- 4) p. 9: "There is no evidence whether infected humans may establish transmission of *Borrelia* to feeding tick." Describe the conditions when/how the human→tick transmission could work.



5) *Borrelia burgdorferi* sensu stricto was found only in questing ticks from Košice. Was the habitat in Košice somehow different from other localities?

6) Could you please explain the third article of the discussion? ("The chi-squared statistic (84.26) computed from the observation was significantly higher than the expected value of chi-squared distribution with 21 degrees of freedom (32.665). Therefore, we can safely reject the null hypothesis and conclude that there exists an association between *Borrelia* genospecies and its locations.")

7) What is the greatest contribution of your project and how can your data improve the general knowledge about Lyme disease?

To conclude, the submitted work is inadequately long which makes rather bad impression on me. I am also not convinced that the student fully understands the principles of used methods. However, I **recommend the thesis for the defence** and I hope Veronika is able to prove her knowledge and clarify the controversial issues.

České Budějovice, May 16th, 2022

RNDr. Helena Langhansová, Ph.D.