Opponent's Review of Master Thesis

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Title: Growth kinetics of the Lyme disease spirochetes in vector ticks *Ixodes ricinus* and *Ixodes scapularis*
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The presented master thesis deals with the current topic of transmission of Lyme disease spirochetes by a tick vector to a model host (mouse). Both phases are monitored, infection of the vector, and subsequent transmission to the host. The results for the American strain of *Borrelia burgdorferi* sensu stricto and the European strain of *Borrelia afzelii* in two different tick vectors, namely *Ixodes scapularis* (America) and *Ixodes ricinus* (Eurasia), are compared.

Formal arrangement:
The diploma thesis consists of 38 pages of text supplemented by a list of literary sources, which includes 99 references. The work is logically structured into an introduction, objectives, materials and methodology, results, discussion and conclusion.
In the introduction part, the author first describes in detail the ticks, their systematics and life cycles, and emphasizes their role in the transmission of infectious diseases. The next part focuses on the pathogen causing Lyme disease, the spirochete *Borrelia burgdorferi*, where the author summarizes recent knowledge about its systematics, ecology and molecular mechanisms that enables *Borrelia* to adapt to different environments as the tick's intestine and vertebrate's body. The text is legible and it is well complemented by citations from literary sources.

Objectives are defined clearly.
In the chapter Materials and methods, methods are well explained and the materials used are described in detail.
The result part takes up 10 pages supplemented by 6 clearly processed graphs, 1 table and two pictures, in the discussion part, the author on 5 pages critically evaluates the obtained results and compares them with previously published works.

Critical remarks: The work is formally carefully processed, doesn't show any serious shortcomings, and contains a lot of interesting information and original data processed into a well-understood complex. After completing the experiment with *B. burgdorferi* N40 and *I. scapularis* larvae, it will certainly be the basis for publication in an impact journal. I will mention here only a few details that do not reduce the level of the presented work:
• In the methodological part - primers used for detection of *Borrelia* 23S rRNA (Bor1-Bor4) were published before 2005.
• In the result part - Fig. 10 and 14: It would be appropriate to describe the molecular ladder in the figure (the methodology states that it is a GeneRuler 100 bp DNA from Thermo Fisher Scientific, but it is necessary to browse the work to find this information, moreover the ladder used here is probably GeneRuler 100 bp Plus), at least its range (100 - 1500 bp?). The photos are not very clear and especially in Fig. 14 it is harder to find out which product is the specific one.
• The publication Schwaiger et al. 2001 is missing in the bibliography, it is mentioned only in the text.
• The way of writing the range of pages in the bibliography should be unified.

Questions for the author:

• Why were these specific *Borrelia* strains selected?

• p. 26: In an experiment with the transmission of *B. afzelii* using *I. scapularis* ticks, the methodology describes that mice were tested for infection 4 weeks after tick feeding in 3 different tissues (ear, bladder and heart). In the graph 10, however, the results are shown only from 1 tissue, it is not written from which. Can the author specify this?

• Since *B. afzelii* has an affinity for the skin, wouldn't be appropriate to test, for example, the skin from the site of tick feeding? Moreover, the infection was detected just in 3 of the 5 mice used in the experiment, but the tissues were tested only by standard PCR. Didn't you try to test negative mouse tissue using more sensitive nested PCR, which was then used in the second experiment (when the standard PCR was negative/inconclusive)?

• Table II summarizes the results of the transmission experiment with *I. scapularis* and *B afzelii* – wouldn't the results be different if more sensitive detection methods were used not only in mouse tissues but also in nymphal ticks (nested PCR, real time PCR)? How long after molting were the nymphs used in the transmission experiment? Based on the results of Fig. 8, all nymphal ticks tested 6 weeks after molting were *Borrelia*-positive.

• Given the ability of *I. scaularis* to transmit *B. afzelii* infection to the host, do you think this infection is already present in America but has not been detected yet?

Conclusion:

The thesis undoubtedly fulfills the requirements for a master thesis on the Faculty of Science of the University of South Bohemia in České Budějovice, so I recommend it for the defense.

In Plzeň on 7.7. 2020

RNDr. Kateřina Černá, Ph.D.