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University of South Bohemia in České Budějovice

Review on diploma thesis

Author: **Bc. Veronika Pavlasová**

Supervisor: RNDr. Radek Šíma, Ph.D.

Title: **Identification of physical and chemical factors regulating gene expression and infectivity of the Lyme disease spirochetes**

Veronika Pavlasová submitted her master thesis dealing with changes in gene expression during *Borrelia* life cycle. Although much has been written about how differential expression affects infectivity, surprisingly not much is known about the control of these procedures. Therefore, I consider the project very interesting.

Formally, the submitted work has 56 pages, usual structure and as far as I can judge, it is very well written and readable (I noticed only a few minor typos or inaccuracies mentioned below). Introduction is nicely supplemented with original Biorender schemes, Aims and Methodology are clear. As a minor comment, a few passages of the Results section would better fit to Discussion.

Formal mistakes (just for future, not to be addressed during the defence):

- missing italics (genera in 1.1.1 and 1.1.2)
- capital letters (Northern Hemisphere, p. 2)
- better "*B. miyamotoi* infection has similar symptoms to LD" (p. 5)
- word order: "During infection of the mammalian host, Rrp2-RpoN-RpoS pathway is activated to promote the expression of genes required in the mammalian host, while repressing genes required in the tick, for example *bbk32*." It sounds like bbk32 is required in the tick (p. 12).
- wrong P value for *bbk32* in the text above Fig. 4 (p. 21)

Questions/comments to be answered during the defence:

1) p. 8: "...spirochetes move relatively quickly through the viscous environment..." How quickly do they move? Are there any data about the actual speed of the spirochetes?

2) p. 11: "complement also promotes B cell differentiation and T cell immunity..." Please describe how complement can help lymphocytes.

3) p. 19: I found interesting that you inoculated mice with tick gut homogenates. Could the presence of gut endosymbionts somehow affect the *Borrelia* transmission/dissemination?

4) Fig. 4 and on: Which sample/calculation corresponds to 100 %?

5) According to Fig. 4, OspC is highly upregulated at 37 °C, but its expression in gut homogenates incubated at the same temperature remained unchanged (Fig. 5). Do you have any explanation for this discrepancy?

6) Fig. 6: Although the differences between experimental groups are not statistically significant, differences between non-injected and water-injected ticks are apparent. Why did you use water for dissolving nutrients instead of using a buffered saline that would be more convenient for hemocoel injections?

7) Although *B. afzelii* primarily does not target joints, have you ever examined ankle joints for the presence of *Borreliae*? If so, how often were the joints colonized in comparison to other tissues?

Conclusion:

Despite a few minor objections, I consider the submitted master thesis of Veronika Pavlasová very good and precise. The author showed nice piece of work both during experiments in the lab and during writing. I **recommend** the thesis for the defence and I suggest the grade "**excellent**" (1).

České Budějovice, May 11th, 2022

RNDr. Helena Langhansová, Ph.D.