Přírodovědecká fakulta
Faculty of Science

Jihočeská univerzita v Českých Budějovicích
University of South Bohemia in České Budějovice

Evaluation report of bachelor thesis: Understanding the pathogenic lifecycle of *Borrelia duttonii*

Thesis written by Stefan Braunshier deals with life cycle of *Borrelia duttoni*. *B.duttonii* is pathogenic agent causing relapsing fever. The overall aim of the thesis was to gather missing information [redacted] and several molecular-biological techniques. The project was done in laboratory of Dr. Ryan Rego, who was supervising this work. Author studied the transmission of *B.duttonii* [redacted] the [redacted] method and the analysis of [redacted] was also performed.

The thesis consists of several chapters which are in correct proportional length to overall extend of the thesis. Introduction, in range of 6 pages, clearly and sufficiently describes the characteristics of disease of relapsing fever, classification of Borrelia according their vectors and according the geographical distribution and finally ways of Borrelia transmission are outlined. This part is complemented by 4 figures and 1 table. I would only recommend the addition of the paragraph about genome of *B. duttonii* for complete picture about this pathogen. There is a small discrepancy in numbering figures (Lifecycle of soft ticks is shown in Figure 4, not in Fig.5 as is written in text, Figure 13 is mislabeled). Introduction is followed by chapter Aims in which numerous aims are clearly defined.

Methods are described in detail and correctly. However I have several comments to this part: There is not complete information about the source of used material (source of *O. moubata* is missing as well as the information about used antibiotics in medium for Borrelia; dose of used Ketamine to anasthetize an mouse should be stated).
In general, centrifugation conditions are specified by relative centrifugal force expressed in units of times gravity (x g) instead of centrifuge rotor speed (rpm).
Reaction mixture for PCR or cDNA should contain concentration of used components; stating volume is not sufficient (e. g. in Table 5, you added 2ul of RNA but total amount is not stated). This rule is applied in case of recipe for gels as well.

Results and disscusion are written well. They are in range of 14 and 5 pages, respectively. Author compares his results concerning B. *duttonii* life cycle with available literature and also provides possible explanation of obtained negative results. He also gives some suggestion for next experiments. In results section I would recommend short introducing sentence which would say what was done in order to increase the clarity. In Figure 7, it would more proper to express the amount of Borrelia in blood as number of Borrelia per ml of blood. In figure 8, negative control should be also shown (WB).
I recommend to write legend to figures in more detail so the figures would be self-explanatory.

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Finnaly, the conclusion, addressing each aim, should be present as separate chapter at the end of thesis. Also, the acknowledgement is usually at the beginning of thesis not at the end (that differs from the way how scientific papers are written).

Logical structure is good, it is written by very good English using good stylistics and proper scientific terminology. In general, all text is sufficiently understandable. The amount of aims set to be answered is enormous and the most of them were indeed solved. From this point of view thesis is extraordinary. The author used numerous methods, which also increases the merit of this thesis, and gained a lot of valuable information about the condition for *B. duttonii* transmission, about suitability of tick-mouse model and differences in serological proteome.

Questions:

1. Could you clarify the information about human being as only mammalian reservoirs of *B.duttonii*? On page 3, you wrote that chicken and pigs were proposed to be possible reservoir as well.
2. On page 19 you wrote [redacted] for two weeks. What was the reason?
3. You mention that *Borrelia crocidurae* causes erythrocytes aggregation into rosettes. Is it known what mediates this aggregation? Is there correlation between rosetting and virulence in Borrelia causing relapsing fever?
4. You [redacted]
5. [redacted]
7. What was the rational of doing [redacted]

The thesis meets requirements for bachelor thesis and provides valuable information about infectious lifecycle of *B. duttonii*, which are useful for further research concerning this pathogen. I fully recommend thesis of Stefan Braunshier to the defense and suggest the grade excellent.

In Ceske Budejovice, June 8, 2018.

Mgr. Jaroslava Lieskovská, Ph.D.

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