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

## OPPONENT'S REVIEW ON BACHELOR/DIPLOMA\* THESIS

Name of the student: Lisa Hain

Thesis title: Transmission Dynamics of Relapsing Fever Spirochete-*Borrelia duttonii*

Supervisor: Ryan O.M. Rego, PhD

Referee: Michael Curtis, PhD

Referee's affiliation: Baylor College of Medicine

	Point scale <sup>1</sup>	Points
<b>(1) FORMAL REQUIREMENTS</b>		
Extent of the thesis (for bachelor theses min. 18 pages, for masters theses min. 25 pages), balanced length of the thesis parts (recommended length of the theoretical part is max. 1/3 of the total length), logical structure of the thesis	0-3	3
Quality of the theoretical part (review) (number and relevancy of the references, recency of the references)	0-3	3
Accuracy in citing of the references (presence of uncited sources, uniform style of the references, use of correct journal titles and abbreviations)	0-3	2
Graphic layout of the text and of the figures/tables	0-3	3
Quality of the annotation	0-3	3
Language and stylistics, complying with the valid terminology	0-3	2
Accuracy and completeness of figures/tables legends (clarity without reading the rest of the text, explanation of the symbols and labeling, indication of the units)	0-3	2
Formal requirements – points in total		18
<b>(2) PRACTICAL REQUIREMENTS</b>		
Clarity and fulfillment of the aims	0-3	3
Ability to understand the results, their interpretation, and clarity of the results, discussion, and conclusions	0-3	2
Discussion quality – interpretation of the results and their discussion with the literature (absence of discussion with the literature is not acceptable)	0-3	3
Logic in the course of the experimental work	0-3	3
Completeness of the description of the used techniques	0-3	3

\* Choose one

<sup>1</sup> Mark as: 0-unsatisfactory, 1-satisfactory, 2-average, 3-excellent.

Experimental difficulty of the thesis, independence in experimental work	0-3	3
Quality of experimental data presentation	0-3	2
The use of up-to-date techniques	0-3	3
Contribution of the thesis to the knowledge in the field and possibility to publish the results (after eventual supplementary experiments)	0-3	3
Practical requirements – points in total		25
POINTS IN TOTAL (MAX/AWARDED)		48 43

**Comments of the reviewer on the student and the thesis:**

As an opponent for the thesis of Lisa Hain, titled "Transmission Dynamics of the Relapsing Fever Spirochete-*Borrelia duttonii*", I thought the project and scientific writing was of high quality. The introduction was written well, the materials and methods were outlined thoroughly, the results were presented clearly, and the discussion/conclusions summarized the results nicely. The student performed an impressive amount of work that resulted in the [redacted] characterization of the [redacted] *B. duttonii* transmission and pathogenesis. Even though the student did not observe transmission of *B. duttonii* from *O. moubata* to *O. moubata*. I think that the work presented here could be publishable with modifications. Therefore, I reviewed thesis and provided constructive feedback for both this work and for the student's future. I have attached Lisa's thesis with my comments scattered throughout, and I have outlined below suggests/questions for the student to answer for the defense.

**Suggestions and questions, to which the student has to answer during the defense.**

As I read the thesis, I made handwritten grammatical corrections and comments throughout the thesis. Unless specified below, those handwritten corrections/comments are to be considered suggestions and up to the student if she wants to implement them. Below are the suggestions/questions I would like Lisa to address during the defense.

- 1) In section 3.15.6, you state that you determined whether or not *O. moubata* ticks were infected by cutting the tick in half and [redacted] for DNA isolation and PCR while the [redacted]
  - a. How were the ticks cut in half? Were the ticks cut along the sagittal (dividing the tick into right and left parts) or transverse (dividing the tick into front (head) and back (hind area)) plane?
  - b. Figures 9-11 demonstrate how well *O. moubata* ticks were able to [redacted] [redacted] in the results section, it was not stated how *O. moubata* infectivity was assessed. Was it through the methods outlined in section 3.15.6? If so, was it through DNA isolation and PCR, culturing or both? Did PCR and culturing produce the same results?
- 2) In section 4.8, [redacted] *B. duttonii* dissemination to different mouse tissues. In this section, you did not state how the mice were inoculated and if/how you determined whether or [redacted] *B. duttonii*. I realize that you explained that the mice were needle inoculated and seroconversion was used to confirm mouse infection in Section 3.15.13. However, it would make section 4.8 much clearer if you briefly restate

- how the mice were inoculated and whether or not you confirmed infection.
- a. Same for Section 4.9. State how the mice were inoculated and how you deemed them to be infected.
- 3) You mentioned that previous studies in the [redacted] [redacted] by *O. moubata*. I was wondering if you know if there was anything different between your experiments and the previous experiments that showed transmission?
    - a. [redacted] in both experiments? Were the cultures at similar passages when used?
    - b. Were the *O. moubata* ticks the same in your experiments and the previous experiments?
  - 4) You did a great job thoroughly demonstrating [redacted] that *O. moubata* are infected with infection [redacted] by inoculating [redacted] with tick [redacted]. [redacted] that tissue is critical for *B. duttonii* to colonize in [redacted] order to be transmitted within the 60 minute feeding time? How is this different from Lyme disease *Borrelia*? What experiments can you do to show that *B. duttonii* has successfully colonized that tissue in *O. moubata*?
  - 5) You should cite "Experimental Infection of *Ixodes scapularis* Larvae by Immersion in Low Passage Cultures of *Borrelia burgdorferi*" by Policastro and Schwan for the method on how to surface sterilize the ticks in section 3.1.1 "[redacted] of *Borrelia* from *O. moubata*".
  - 6) In section 3.16.2 [redacted] describe how you generated antibodies in rabbits against saliva of *O. moubata*, however, did not see those results in the thesis. Did I miss it somewhere in the thesis? Are you still running those experiments? I am curious to know if you were able to generate antibodies specific to *O. moubata* saliva, and what you think will be future directions for this experiment.

**Mistakes, which the students should avoid in the future:**

- 1) When you write journal articles in the future, it is nice to very briefly restate the how you performed an experiment when you describe the results. This makes your results section much clearer to the reader. This is a very brief summary of the methods, just to allow the reader to know how the experiment was performed while they are looking at the results. You did this well for some sections your results in section 4.1, but not in other sections such as section 4.8 and 4.14.
- 2) When starting a sentence with a number, write out the number ("One" instead of 1).
- 3) Anytime that you write a document, internal consistency is important. Journals may require you to have different formats, but it's important to remember to at least be consistent. For example, on your "Content" page, you have some titles with all of the words capitalized and others where only the first word is capitalized. Unless specified in your thesis requirements, I think either one would be appropriate, but just make sure to be consistent throughout.
- 4) Another example of internal consistency regards the phrase in vivo/in vitro. In some instances, you wrote "in vitro" and in others you wrote "in-vitro". I have always been taught to italicize Latin phrase such as "*in vivo*" and "*in vitro*"; however, I have seen journals that require those phrases to not be italicize. Again, I think this is up to you and your thesis requirements, but just make sure it is consistent.

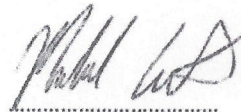
Conclusion:

In conclusion, I

recommend / ~~do not recommend\*~~

the thesis for the defense and I suggest the grade A <sup>2</sup>

In date



1/16/19

signature

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<sup>2</sup> You can suggest a grade, which can be modified during the defense based on the presentation. However, if the reviewer is not present at the defense, the grade will not be counted. Grades: excellent (1). Very good (2), Good (3), Unsatisfactory/failed (4).