



OPPONENT'S REVIEW ON BACHELOR THESIS

Name of the student: Ana Cetković

Thesis title: Determination of tick-pathogen interactions during acquisition and transmission of *Borrelia duttonii* by *Ornithodoros moubata*

Supervisor: Ryan O. M. Rego, Ph.D.

Referee: RNDr. Helena Langhansová, Ph.D.

Referee's affiliation: Dept. of Medical Biology, Faculty of Science, University of South Bohemia

	Point scale ¹	Points
(1) FORMAL REQUIREMENTS		
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	3
Graphic layout of the text and of the figures/tables	0-3	2.5
Quality of the annotation	0-3	3
Language and stylistics, complying with the valid terminology	0-3	2.5
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	0.5
Formal requirements – points in total		17.5
(2) PRACTICAL REQUIREMENTS		
Clarity and fulfillment of the aims	0-3	2
Ability to understand the results, their interpretation, and clarity of the results, discussion, and conclusions	0-3	1
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	2
Completeness of the description of the used techniques	0-3	3
Experimental difficulty of the thesis, independence in experimental work	0-3	3

¹ Mark as: 0-unsatisfactory, 1-satisfactory, 2-average, 3-excellent.

Quality of experimental data presentation	0-3	0.5
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		20.5
POINTS IN TOTAL (MAX/AWARDED)	48	38

Comments of the reviewer on the student and the thesis:

The investigation of relapsing fever *Borrelia* has not been as intense as Lyme disease research so far and there is a substantial lack of knowledge about vector – host – pathogen interactions. Therefore such a research is beneficial and this bachelor thesis could have big contribution in the field.

The proposed thesis has classic and balanced structure. I have no major remarks/comments about Introduction and Materials and methods up to section 3.12 (Mouse experiments) on. This section would have been much more comprehensible if the design of animal experiments was outlined in diagrams. I had to draw my own diagrams in order not to get lost in results. The arrangement of experiments is not logical and does not correspond with the arrangement of results. Several important facts were not explicitly described (e.g. full spectrum of antibodies means active and relapsing infection). Some proposed results are missing. As for gene expression, Ana claims unsolved problems with RNA isolation and/or reverse transcription (4.7.), which led to lack of expression data. However, supposed/virtual results are extensively discussed: “*The hypothesis that Vmp expression is continuously switched on in unfed ticks was confirmed. Additionally, it was proven that Borrelia does not need tick to start feeding in order to change the Vmp expression.*”

The proposed thesis is experimentally demanding since work with three living entities (bacteria, ticks, mice) together is always difficult. I also understand that some experiments were performed in suboptimal numbers of mice/ticks for practical, financial or availability reasons. However, the presentation of obtained data should be definitely better and hopefully will be better during defense.

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

- 1) Can RF *Borreliae* be present in other tissues except for blood stream of a mammalian host?
- 2) Blood samples for serological confirmation of infection were collected by retro-orbital bleeding. Why was this particular technique used?
- 3) What was the origin of *O. moubata* ticks and *B. duttonii* isolates?
- 4) Regarding serological proof of infection by WB, how can you distinguish between active relapsing infection and antibody response to dead/non-infectious *Borreliae*?
- 5) What is your explanation of different growth curves in different sera? How would human serum presumably work? Were the animal sera heat-inactivated?

Conclusion:

In conclusion, I r e c o m m e n d the thesis fo
good (2).

In České Budějovice, September 10, 2020

RNDr. Helena Langhansová, Ph.D.