



Přírodovědecká
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Faculty
of Science

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

STATEMENT OF THE BACHELOR/DIPLOMA* THESIS REVIEWER

Name of the student: Brian Ringhofer

Thesis title: Establishing a tick-mouse model for the relapsing fever pathogen *Borrelia duttonii*

Supervisor: Ryan O.M. Rego, PhD

Reviewer: Alejandro CABEZAS-CRUZ, PhD

Reviewer's affiliation: UMR BIPAR, Animal Health Laboratory, INRA, ANSES, ENVA, Maisons Alfort Cedex, France.

	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	2
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	1
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	1
Formal requirements - points in total		15

(2) PRACTICAL REQUIREMENTS

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	3
Discussion quality - interpretation of the results and their discussion with the literature (absence of discussion with the literature is not acceptable)	0-3	2

* Choose one

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

Logic in the course of the experimental work	0-3	3
Completeness of the description of the used techniques	0-3	3
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 (40) ²

Comments of the reviewer on the student and the thesis:

The bachelor thesis has good scientific quality. The author established a model of Tick-borne Relapsing Fever using *Borrelia duttonii* and *Ornithodoros moubata*. This model will be relevant to study tick-host-pathogen interactions. I consider this thesis as a good contribution to the study of Tick-borne Relapsing Fever. The formal part of the thesis, however, was poorly developed. More effort should have been put in the graphic layout of the final document. In general, the figures are of poor quality and the legends are not informative enough. There are many misspellings, grammatical errors and the sentences and ideas are difficult to follow. This thesis represents an amazing experimental effort with a poor presentation. In the future, Brian's career, if science is chosen, will benefit from a better attention to the presentation of scientific results. A detailed account of things that should be improved is provided below.

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

- 1- Microscopy-based methods of pathogen detection and quantification in blood are not very accurate. Low pathogens levels can be easily overlook using microscopy. Why a more sensitive method of pathogen quantification was not used to follow *B. duttonii* infection in mice? For example, qPCR.
- 2- Previous studies showed that *B. duttonii* strain Ly did not infect outbred ddY mice (Microbiol Immunol. 2000; 44(12):1071-4.). Similar results were observed in this thesis using C3H/HeN and BALB/c mice. Please, discuss the possible reasons for why *B. duttonii* strain Ly is not infective for mice? The *B. duttonii* strain Ly genome is available (PLoS Genet. 2008; 4(9):e1000185) and several genes of *B. duttonii* strain 1120K3 are available in GenBank (e.g. *p66*, *groEL*, *glpQ*, *flaB* and *recC*). Are there genetic differences between these two strains that may explain the variability in mice infectivity among these two *B. duttonii* strains?
- 3- In the sentence 'LBRF has an epidemic potential... whereas TBRF is called to be

² Enter the number of points awarded.

- endemic.' it is not clear the distinction between 'epidemic potential' and 'endemic'. Does it mean that TBRF does not have 'epidemic potential' or that LBRF cannot be 'endemic' in some regions? Please, explain.
- 4- The method used to quantify the bacteria for experimental inoculation of mice is missing. Please, detail this method.
- 5- Do the infected mice suffer from episodes of 'relapsing fever' as observed in humans?

Mistakes, which the students should avoid in the future:

Several formal mistakes and misspellings were found of which some examples were provided below.

1- Introduction

- 3.1 -In the introduction is missing a presentation of the animal models used currently to study Lyme borreliosis (LB). In addition, the models used previously to study transmission of *B. duttonii* by *O. moubata* should be presented in the introduction (e.g. Tabuchi, N., et al. 2008. Vector-Borne Zoonotic Dis. 8, 607-614.). The *B. duttonii* strains used in the study should be also presented. For example, there is a lot of literature about *B. duttonii* strain Ly (e.g. Int J Syst Bacteriol. 1999; 49: 1793-1799; Microbiol Immunol. 2000; 44(12):1071-4 and PLoS Genet. 2008; 4(9):e1000185.)
- 3.2- Page 1: The first letter in the name of a phylum (i.e. Spirochaetes) goes in capital letters.
- 3.2- Page 1: This sentence '...by arthropod vectors like ticks and the mammalian hosts.' reads as if the 'mammalian hosts' were also 'vectors' which is not the case. Please, reword.
- 3.3- Page 2: delete 'evolved to' in the second line. In biology, 'evolution' refers to 'change in the heritable characteristics of biological populations over successive generations'. Not the case here.
- 3.4- Page 2: Change 'end host' to 'dead-end hosts'
- 3.4- Page 3: In the sentence 'The disease pattern containing ...can be explained from the changes in these proteins.' it is not clear what proteins are being referred to. Please, mention some.
- 3.6- Page 4: Here, 'old world' is defined as 'Africa, European and Asian regions'. However, it is not clear whether all the times 'old world' is mentioned below; it includes all these three regions. Please, specify.
- 3.7- Page 5: Three ways of naming USA are used here: 'USA, United States and U.S', please, be consistent and use one of them in the entire thesis.
- 3.8- Page 6: No need to put 'Hard-Tick-Borne Relapsing Fever', the abbreviation 'HTBRF' was already introduced above in the text.
- 3.9- Page 7: This title 'Soft ticks vs. /hard ticks' should be modified to, for example, 'Tick vectors of Borrelia'... Also, a brief introduction to ticks should be provided.
- 3.10- Page 7: Family names (i.e. Ixodidae and Argasidae) do not go in Italics.
- 3.11- Page 7: The name of the genus is '*Ornithodoros*', no 'Ornithodoroinae'.
- 3.12- Page 7: Taxonomic levels are badly used, for example in 'Morphologically, even though both tick species...', it should say 'Morphologically, even though both tick families...' This mistake is repeated many times in the thesis.
- 3.13- Page 8: The title of table 2 should read 'Differences'. Also, if 'ixodid' will be used

to name 'hard ticks', then use 'argasid' to name 'soft ticks'. Do not mix 'Ixodid and soft' or 'argasid and hard'.

3.14- Page 8: The first sentence after the table is not very clear. A 'Two or three-host life cycle' is also a multiple-host life cycle. Please, be more precise.

3.15- Page 8: Not sure what is the meaning of 'time-dependent' in the second sentence. Nor, what would be a time-independent life cycle for argasid ticks.

3.16- Page 10: What the abbreviation 'CSIC' in Figure 6 stand for?

2- Objectives

4.1- Objectives should start with 'measurable verbs'. For example, in Objective 1, 'To identify *B. duttonii* strains using'

4.2- Objective 1 reads as if were the primers that are going to be 'sequenced' and 'analyzed'. Please, reword.

4.3- Objective 3 is not very clear. Please, be concise and clearer.

4.4- Objective 4. Change to 'To evaluate *B. duttonii* transmission by *O. moubata* ticks in mice.'

3- Methods

General

Information about city and country should be provided for machines, reagents and kits used in the study.

3.1- Page 12: This is the first time the species *Escherichia coli* is mentioned in the text, therefore the full name should be written. In addition, DH5 α is a strain of *E. coli* used as competent cells. Here, it is written as if "DH5 α " and '*E. coli*' were different things.

3.2- Page 14: It should be specified the expected size of the *glpQ* and *flaB* fragments to be amplified.

3.3- Page 14: Please, instead of '&' use 'and'. It applies to the entire document.

3.4- Page 18: Change the title to 'Genetic identification of *B. duttonii* strains'. The name of the species in *B. duttonii*, no *B. duttoni*.

3.5- Page 18: In table 8 is not clear whether these are accession numbers of the sequences obtained in this study for these genes or accession numbers of the sequences to which the amplified fragments were identical to.

3.6- Page 18: What is '7 eight' ??

3.7- Page 18: Please, specify how 'anesthesia' was achieved.

3.8- Page 19: These (e.g. 'Chapter 3.12') are not chapters but subsections. In addition, subsection '3.12' above is not 'Immunoblotting', but 'SDS - PAGE'. Please, correct.

4- Results

4.1- Page 22: Change 'E. Coli' to '*E. coli*'.

4.2- Page 22: Not clear what 'Ly glpQ' or '1120K3 glpQ' mean.

4.3- Page 22: What 'no result' means in Figure 7. No sample was applied in this well?

4.4- Page 23: 'query' or 'covery'?

4.5- Page 23: '100%' is the maximum identity two DNA fragments can have. It is impossible to get 'more than 100%' identity.

4.6- Page 24: Not clear what is 'embedded in the corresponding strain'.

4.7- Page 24: The title of '4.2' reads like 'Methods'. It should be renamed to 'Acquisition of *B. duttonii* by *O. moubata* ticks'

4.8- Page 24: Before mentioning whether ticks acquired the infection or not, more details should be provided on mice infection after syringe inoculation of bacteria. This appears to

be described below, but following the logic of the infection model, it should appear in the following order:

1- Mice infection by syringe inoculation with all details.

2- Acquisition of *Borrelia* sp. by ticks after feeding on infected mice.

3- Transmission of *Borrelia* sp. infection from infected ticks to naive mice.

4.9- Page 24: The third line of '4.2' reads as if the mice were infected with the two strains simultaneously. Was it the case? If not, please, reword.

4.10- Page 25: In '...inoculated with Ly showed to be poorly or even non-infective...' is no clear how 'poorly' was defined. Low levels of bacteria after counting? If yes, why this was not considered as 'infected'? Also, change 'non-infective' to 'non-infected'.

4.11- Page 26: After how many days the 'peak of spirochetemia' was expected? In methods it is mentioned that '3rd day post-inoculation, when the spirochete peaked (3.11)'. If the peak occurred within the first 5 days, why then the infection was followed for 21 days?

4.12- Page 26: Before, it was mentioned that 'infection analysis of the mice was carried out over a span of 21 days'... then, it was mentioned that 'the first 9 days were used...'. Please, clarify and make simpler.

4.13- Page 26: Information is missing in the legend of Figure 11. It seems that the information is in the main text. However, figures should be self-explanatory and all the information needed to understand a figure should be provided in the legend of that figure. In addition, in the main text, it is mentioned that 'Mouse 10-14 C3H/HeN', but only 10 mice appeared in Figure 11. There are also lines and symbols that are not represented in the colored legend.

5- Discussion

5.1- Page 29: Diseases do not 'become' 'neglected'. Diseases are (or not) 'neglected' by human politics.

5.2- Page 29, line 6: Non sequitur: this sentence is not logically connected with the one before.

5.3- Page 30: Not clear what is meant by 'after receiving the ... passages'

5.4- Page 30, line 4: 'withby' ???

5.5- Page 31, line 4: 'preserved' ???

6- Conclusions

6.1- 'Conclusion' section is missing.

7- References

There are some mistakes in the reference list and the main text. For example:

1.1- The name of the manuscript in reference '3' is in capital letters and it's not the same for the rest of the references.

1.2- Not clear the pages numbers for reference '18'.

1.3- In some references the name of the journal is fully written (e.g. 'Frontiers in Public Health', reference '25') and in others, the abbreviation is provided instead.

1.4- For some references the 'doi' is provided (e.g. '32'), for others no.

1.5- Not clear the rule followed for reference '35'

1.6- References are not in order in the main text. For example, from reference '8' in page 2, it jumps to references '31,33'.

8- Figures and tables

Figures have very low resolution. It is not easy to read them. In addition, all figures

should be cited in the main text. For example, Figure 1 is not cited in the main text. Same as for figures, all tables should be cited in the main text. Not the case for example for 'Table 1'.

In conclusion, I

recommend / do not recommend*

the thesis for the defense and I suggest the grade 2.³

In Paris date 05.06.2018


.....
signature

³ 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).