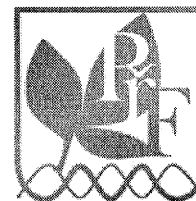




UNIVERSITY OF SOUTH BOHEMIA
IN ČESKÉ BUDĚJOVICE

Faculty of Science



STATEMENT OF THE BACHELOR/DIPLOMA* THESIS REVIEWER

Name of the student: Bc. Martin Strnad
Thesis title: Localization of Lyme disease spirochetes *Borelia Burgdorferi* in ticks
Ixodes ricinus
Supervisor: RNDr. Marie Vancová, PhD
Reviewer: Ing. Jana Nebesářová, CSc.
Reviewer' affiliation: Biology Centre of ASCR, v.v.i.

	Point scale ¹	Points
(1) FORMAL REQUIREMENTS		
Extent of the thesis (for bachelor theses min. 18 pages, for masters theses min. 25 pages), balanced extents of the thesis divisions (recommended extent of the theoretical part is max. 1/3 of the total extent), 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	3
Adequacy and clarity of the results and conclusions	0-3	3
Quality of the annotation	0-3	3
Language and stylistics, complying with the valid terminology	0-3	3
Accuracy and completeness of figures/tables legends (clarity even without reading the rest of the text, explanation of the symbols and labeling, indicating the units)	0-3	2
Formal requirements – points in total		22
(2) PRACTICAL REQUIREMENTS		
Clarity of the aims	0-3	3
Fulfillment of the aims	0-3	3
Discussion quality – interpretation of results and their discussion with the literature	0-3	3
Logic in the course of the experimental work	0-3	3
Completeness of the description of the used techniques	0-3	2

* Choose one

¹ 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	1
The use of up-to-date techniques	0-3	3
Contribution of the thesis to the knowledge in the filed and possibility to publish the results (after eventual supplementary experiments)	0-3	3
Formal requirements – points in total		24

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

- 1/ Gold nanoparticles are very often used as a markers for the electron microscopy part of CLEM. How large nanoparticles are detectable in the high resolution SEM working in BSE mode and is it possible to use the method of silver enhancement in this case?
- 2/ Can you further explain changes in staining protocols, which you mentioned in the last paragraph on the page 34? Do these changes relate to the staining protocol for fluorescence microscopy or SEM?
- 3/ In the study a number of technically demanding procedures is used such as cryo-sectioning. Were these procedures performed by your own hands? If not, why this assistance is not mentioned?
- 4/ How precisely can be the number of spirochetes *B.burgdorferi* determined in salivary glands of ticks using the CLEM method presented in your thesis?
- 5/ Are the images shown in Figures 6, 9 and 10 taken on the same sample?

Eventual mistakes, which have to be corrected:

- P.16 – In the sentence “CLEM is one of the most intensively developing electron microscopy techniques” to delete the word electron.
- To enlarge the size of Figures 7,9,10, where nothing is possible to recognize.
- To increase the number of microscopy images!
- I don't agree with the author's assertion on the page 32, that CLEM is recently emerging technique. First attempts were made already in 1960.
- It would be useful to present results in tables. This would facilitate the orientation for readers.

Eventual additional comments of the supervisor on the student and the thesis:

**Conclusion: In conclusion, I r e c o m m e n d the thesis for the defense and
I suggest the grade e x c e l l e n t .²**

In C.Budejovice date 26.5.2013


signature

² 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.



Přírodovědecká
fakulta
Faculty
of Science

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

STATEMENT OF THE DIPLOMA THESIS REVIEWER

Name of the student: Bc. Martin Strnad

Thesis title: Localization of Lyme diseases spirochetes *Borrelia burgdorferi* in ticks
Ixodes ricinus

Supervisor: RNDr. Marie Vancová, PhD.

Reviewer: MVDr. Markéta Derdáková, PhD.

Reviewer` affiliation: Institute of Zoology, Slovak Academy of Sciences, Bratislava

	Point scale ¹	Points
(1) FORMAL REQUIREMENTS		
Extent of the thesis (for bachelor theses min. 18 pages, for masters theses min. 25 pages), balanced extents of the thesis divisions (recommended extent of the theoretical part is max. 1/3 of the total extent), 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	2
Adequacy and clarity of the results and conclusions	0-3	3
Quality of the annotation	0-3	2
Language and stylistics, complying with the valid terminology	0-3	3
Accuracy and completeness of figures/tables legends (clarity even without reading the rest of the text, explanation of the symbols and labeling, indicating the units)	0-3	3
Formal requirements – points in total		21
(2) PRACTICAL REQUIREMENTS		
Clarity of the aims	0-3	3
Fulfillment of the aims	0-3	3
Discussion quality – interpretation of results and their discussion with the literature	0-3	2
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	3

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

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
Formal requirements – points in total		47

POINTS IN TOTAL (MAX/AWARDED)

51

0-51²

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

For the artificial tick feeding you have used the ram or cattle blood. I understand that it is probably the most commonly used and the most easily obtained source of blood for the experimental studies. However *B. burgdorferi* s.s. is only partially resistant to the serum complement from cattle (for reference see: Kurtenbach et al. 2002). Do you think that if you would use other source of blood f.e. rodent that is natural reservoir host and *B. burgdorferi* s.s. is fully resistant to its serum complement could this affect some of your results?

Can you please mention the papers or studies (if there are any) where the transtadial transmission of *B. burgdorferi* in the salivary glands was studied? At page 12. You stated “Ecdysteroid is also common insect molting hormone, which is probably the reason why **many scientists** believe that salivary glands degenerate during the juvenile ticks molt to such an extent that it cannot transpass the *Borrelia burgdorferi* spirochetes into the next developmental stage.” There is no reference in this statement, so I am curious if they were studies done on this topic. This is known phenomenon that *Borrelia* (unlike the TBE virus) are usually not present in the salivary glands of the ticks at the beginning of the feeding. So I think that the experiments to study the extend of salivary glands degeneration during the molting process of the ticks done in the diploma thesis of Bc. Strnad has a high value for the knowledge of potential transovarial transmission in the salivary glands of ticks. So what is your opinion on transtadial transmission of *Borrelia* in the salivary glands? As I understand from your results you did not find the borrelia in the salivary glands after the molt but you did not rule out the possible transmission?

Eventual mistakes, which the students should avoid in the future:

I found some formal mistakes, typos, some references missing.

- Reference Bacon et al. 2008 refers only to the number of cases in the USA not in Europe.
- Reference describing the *Borrelia burgdorferi* as a new species – you should cite papers Burgdorfer and Barbour 1982 or Johnson et al. 1984
- Currently 19 genospecies are present not 18 as you cite.
- Sometimes different font is used throughout the text (italics, page 5, last three lines of the page etc)
- Correct species name is “*Anaplasma phagocytophilum*” not “*Anaplasma phagocitophylum*” page 10.
- Types IV acini are found also in males of *I. ricinus*

Eventual additional comments of the supervisor on the student and the thesis:

² Enter the number of points awarded.

Conclusion:

The diploma thesis has a high quality. Author in the experimental part uses up to date techniques that some of them are very laborious and their application for the borrelia visualization is rather complicated. I hope that the experiments will be followed up in the future, since this information can bring new insights on the tick-borrelia interaction and transmission to the host.

In conclusion, I r e c o m m e n d the thesis for the defense and I suggest the grade A.

In Bratislava date 24.5.2013

A handwritten signature in black ink, appearing to be 'M. L. ...', written over a dotted line.

signature