



Přírodovědecká
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Faculty
of Science

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

OPPONENT'S REVIEW ON BACHELOR/DIPLOMA* THESIS

Name of the student: Zuzana Dvorníková

Thesis title: Identification of glycosylated tick proteins containing immunogenic epitopes

Supervisor: RNDr. Ján Štěrbá, Ph.D.

Referee: Ryan O. M. Rego

Referee's affiliation: Institute of Parasitology BC CAS and Faculty of Science, University of South Bohemia, Ceske Budejovice

	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	2
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	3
Formal requirements – points in total		18
(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	1
Logic in the course of the experimental work	0-3	2

* Choose one

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

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	2
Contribution of the thesis to the knowledge in the field and possibility to publish the results (after eventual supplementary experiments)	0-3	1
Practical requirements – points in total		17
POINTS IN TOTAL (MAX/AWARDED)	48	35

Comments of the reviewer on the student and the thesis:

The Master's work by Zuzka Dvornikova is unique in nature as it attempts to identify using various methods, tick proteins that are glycosylated by using tick cell lines. The methods used are important and the student has shown a fair amount of independence in carrying out the aims of the project. The thesis is written in the English language and for the most part is well written. There are some mistakes in construction of sentences, some mentioned below which should be avoided. The thesis is 79 pages long with 5 more pages of Appendix. All the diagrams that the student created using BioRender are well created and much appreciated in the introduction. The rest of the figures related to the data obtained are well described. The overall potential of this work is very promising.

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

Mistakes, which the students should avoid in the future:

1. The title states 'identification of glycosylated tick proteins having immunogenic epitopes'. Would it not be correct in just saying 'identification of glycosylated tick proteins'? Was any effort made to see if the proteins identified have immunogenic epitopes?
2. The introduction appears to not really mention any other work outside of *Drosophila* looking at glycosylated proteins in arthropods especially blood feeding vectors. If this is true then this work is truly the first of its kind and certainly should be stated in the introduction/discussion. Could the student elaborate on this?
3. On page 9 second to last paragraph, it is not rare for ticks to feed on reptiles. Also ticks transmit the pathogen and not the disease. This is an important difference.
4. On page 12, in the first paragraph the *I. ricinus* lifespan is put from 2 to 6 years yet *I. scapularis* at 2 years. Given that they are very similar Ixodidae ticks I would appreciate a more correct set of years for the lifespans to be stated.
5. Page 12 first paragraph, the description of *I. ricinus* in the last line is very strange. Describing birds and sheep only, when it is well known that the ticks have a host of animals that they feed on is strange as there are no more sentences after this. The presence of this sentence is unwanted and such 'awkward' sentences need to be avoided.
6. This project used cell lines to identify glycosylated proteins. Since the aim is to identify potential glycosylated proteins that may be immunogenic would it not be more appropriate to have worked with tick saliva and look at proteins that are glycosylated in

the saliva. Such proteins would probably be potentially better immunogenic candidates. What are the students' thoughts on this and why look at all glycosylated proteins in a tick cell line ?

7. Page 25, the primary antibodies that are mentioned for use in the Western Blot, how were they chosen i.e., based on what criteria/previous publications?
8. The MS analysis was able to determine a couple of proteins each, for the different glycosylations, that were common between the two hard tick cell lines. No real effort was made to analyse the potential function of these proteins, are they secreted in the saliva or do they have some other important role? Can the student provide more information on the relevant proteins in other arthropods/vectors? Also could they provide an example/s of where bioinformatic identification of potential glycosylation sites was proven true.
9. In the Discussion, page 65. It is stated that the reason for the absence of any similarities between soft and hard ticks is hard ticks are more likely to spread diseases than soft ticks. This is confusing and needs an explanation.
10. One can understand that a pathogen can multiply within a mammalian host after being transmitted by the tick and hence lead to antibody production based on recognition of the surface proteins of the pathogen. My question is, would the secretion of glycosylated proteins in the saliva of an individual tick have the effect of being immunogenic in humans/other mammalian hosts?
11. Finally, what possible application can be visualized by understanding the glycosylated proteins being secreted by the tick?
12. Examples of mistakes in the writing within the thesis:
 1. Page 1, second to last line it is 'mass spectrometry analysis' and not 'mass analysis'.
 2. Page 2, first line ' by ' and not 'by of'
 3. Page 3, second paragraph, the sentence ends with 'in fact' which is never how any sentence ends.
 4. Section 4.2.1 the sentence in the first paragraph says 'As for OME...' it is abrupt and makes no sense.
 5. Page 31, third line 'processed' and not 'process'

Conclusion:

In conclusion, I

~~recommend / do not recommend*~~

the thesis for the defense and I suggest the grade 1 .²

In Ceske Budejovice

date 18.5.2023

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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. Grades: excellent (1). Very good (2), Good (3), Unsatisfactory/failed (4).