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**Reviewer's evaluation of PhD thesis of Mgr. Ján Štěrba entitled „Glycobiology of Ticks and Tick Born Pathogens“**

The Ph.D. Thesis of Ján Štěrba has been submitted in the format recommended by Faculty of Science of South Bohemian University in České Budějovice. It has a form of „Ph.D. Thesis Series, 2011, No.3“ booklet having a total of 182 pages, and the usual Ph.D. Thesis summary. It is based on 6 papers published in prestigious international peer reviewed journals with impact factor. Such a form of Ph.D. Thesis simplifies the role of the referee from the beginning since the major parts of the work already went through the rigorous peer review procedure assuring its scientific quality and precluding the occurrence of major mistakes or shortcomings.

The presented Thesis is based on the combination of two very topical subjects of the contemporary biomedical research: the biochemistry of invertebrates focused on studies of chemical processes and reaction in hitherto not so well investigated species (here ticks and their bacterial pathogens), and contemporary carbohydrate biochemistry (glycobiology) including the use of modern methods of glycomics and mass spectrometry of glycans. The introductory part of the Thesis provides a detailed and well argued summary covering the biology and biochemistry of invertebrates with a special emphasis on ticks, and described our current knowledge on the synthesis of glycoproteins. This is discussed both in general perspectives, and then with special emphasis on the known processes occurring in invertebrates. This part is then concluded with a nice summary on carbohydrate-binding proteins (lectins) and their biological roles in invertebrates. There follows an equally well written part on the used methodologies with a special emphasis on modern techniques such as electron microscopy or mass spectrometry.

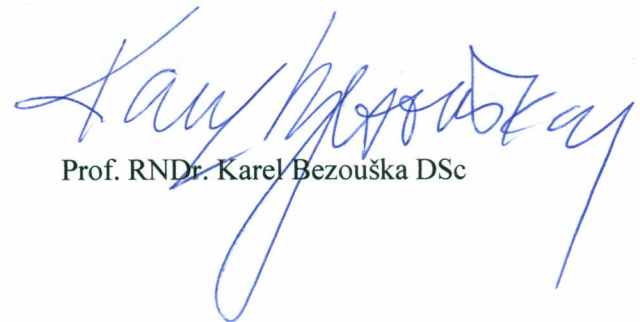
All planned specific aims were fully accomplished, which is well documented by a number of presented results, both published in the individual papers and so far unpublished. The amount of experiments performed by the candidate and the amount of experimental technique that he had to cover is certainly remarkable, and present Ján Štěrba as a very talented and diligent young scientist with a good perspective of further professional growth as a prominent member of the research team of Professor Libor Grubhoffer. I have been particularly impressed by the detailed bioinformatical evaluation of the genes coding glycan synthesizing enzymes using the freshly available data from the complete sequencing of the genome of tick *I. scapularis*. Also, a detailed description of the glycosylation of the 640 kDa lectin Dorin M from tick hemolymph represent a pioneering work based on the application of modern mass spectrometry methodologies. Important for the large glycobiology community are the findings published in the recognized journal *J. of Bacteriology* that corrects certain previously published data and proves them to be incorrect. Certainly in the coming times correcting the scientific literature plagued by a huge number of artefacts and incorrect finding is fast becoming one of the major tasks of every serious scientist. Although the great amount of tiny everyday work performed using the classical biochemical techniques such as tissue extractions, electrophoretical separations in combination with specific staining, Western blotting, and other techniques is worth emphasizing. The results of these numerous studies have already been published in the major journals in the field of parasitology. Modern experimental techniques have been used in

order to obtain evidence for lectin type interactions including SPR (surface plasmon resonance). The discussion is detailed and outlines the major directions for future research. Based on the above considerations, **I fully recommend** the presented Ph.D. Thesis of Mgr. Ján Štěrba for defence and further evaluation leading to the awarding of the title Ph.D. (Doctor of Philosophy) in accordance with the valid legislature.

I have the following remarks and questions for the candidate:

- (a) The English of the Thesis is mostly appropriate, although there are occasionally too „verbose“ formulations at the expense of good understanding. Such parts should be avoided in scientific language.
- (b) The availability of the full reprints of the published papers directly in the Thesis are welcome since the reader can fast get to the original findings of the study. From this point of view I do not find the „cutting“ of the principal paper published in *European Journal of Mass Spectrometry* (of which there remains only the title and the abstract) too appropriate.
- (c) The technical question concerns the glycan permethylation procedure (e.g. p.117) in which the use of NaOH beads is cited. Can candidate specify the exact nature of these beads and their purpose in the procedure?
- (d) It would appear reasonable to complement the assignment of individual glycans based on MALDI MS measurements by additional techniques, such as exoglycosidase digestion or fragmentation mass spectrometry. Were such analyses performed, and what was the experience of the candidate with these approaches.
- (e) In the glycobiology community a great use of technologies that have become widely available through the international Consortium of Functional Glycomics is now widespread. Did the candidate use any of these possibilities, and what is his experience with CFG or other international collaborations?
- (f) I would be interested to know the role of sialic acids in the interactions between the tick and its bacterial pathogens. Is there any well documented role for this interaction? Do ticks have an endogenous synthesis of sialic acids, and if yes, which particular forms of this carbohydrate are produced?

Prague, 11th March 2012



Prof. RNDr. Karel Bezouška DSc



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Examiner's Report on Mgr. Ján Štěrba's PhD Thesis:

#### **Glycobiology of Ticks and Tick-Borne Pathogens. Glycans, Glycoproteins, and Glycan-Binding Proteins.**

This study describes an investigation focused on one side on identification of glycans of both ticks and tick-borne pathogens and, on the second side on identification of novel glycan-binding proteins and their structural and topological characterization. Specifically, the individual aims covered (i) the analysis of N-linked glycans in tick organs; (ii) identification of novel fibrinogen-related proteins with lectin activities in tick hemolymph, (iii) structural characterization of tick lectin Dorin M, and (iv) the analysis of the glycosylation of Lyme borreliosis spirochetes with the focus on previously reported glycoproteins of *Borrelia burgdorferi*.

The thesis starts with extensive introduction which in its first part includes the paragraphs describing tick taxonomy, tick-borne pathogens and aspects of tick feeding process. As for the latter the anatomy of tick salivary glands is depicted and salivary proteins important for both blood-sucking event and counteraction of host defense mechanism are mentioned. This part of introduction is then ended by description of tick circulatory system involving structural and functional information on currently known hemolymph proteins.

The second part of the introduction deals with protein glycosylation. First the biological importance of protein glycosylation is briefly outlined followed by description of both N- and O- glycosylation enzymatic pathways. Then there is a sum of accessible information on glycosylation in arthropods and arthropod-borne pathogens. The last paragraph summarizes current biological and biochemical protocols exploited for glycan purification, identification and structural characterization.

Globally the introduction is well written up and helps the reader to follow the logic of the candidate's argument as he constructs the rationale for the study, describes its design, procedures and the methods required for its analysis.

The result section is opened by bioinformatic data documenting the presence of appropriate glycosyltransferases in tick genome databases. Furthermore the expression of  $\alpha$ 2,3- sialyltransferase in *Ixodus ricinus* cell line was confirmed by PCR. In accordance with this information incorporation of sialic acid in salivary glands and gut of fed and partially fed *I. ricinus* females was found. Functioning of glycosyltransferases in tick was further underpinned by identification of core-fucosylated glycans in saliva.

The second part of experimental section is also very interesting to read. It characterizes the N-glycan structures of two lectin molecules. The first one Dorin M has three classical N-glycosylation sites. Two of them are modified high-mannose type glycans, the third one contains from four to five mannose residues and a fucose attached to N-acetylglucosamine. The second lectin molecules a 290 kDa hemelipoglycoprotein bears high-mannose and complex N-linked glycans. Another group of potential lectins with fibrinogen domains were detected in the tick hemolymph using sera against Dorin M and the hemagglutination activity of the ticks *R. appendiculatus* and *D. marginatus*.

The last results concern the detection of N-glycosylation of the major outer membrane proteins and the flagellar proteins isolated from *Borrelia burgdorferi*. Surprisingly both the indirect procedures as well as direct mass spectrometric approaches did not confirm the previous information on N-glycosylation of these proteins.

Based on this section it can be concluded that Ján Štěrba clearly demonstrates his skillfulness and intellectual ability to undertake and solve the difficult tasks associated with the analyses of glycosylation in ticks. This type of study necessitates the application of very demanding methodological approaches and the fact that Ján Štěrba was able to exploit them for his research strategies is a great achievement for PhD student.

The Discussion and Conclusion sections summarize these successful attempts and put them into the context of current knowledge surrounding the tick glycobiology.

**Curiosity-driven questions:**

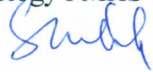
Based on presented data, there are two curiosity-driven questions related to this study:

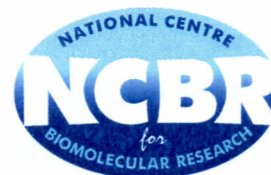
- The analyses presented in the thesis are all focused on the identification of N-glycans. It would be also interesting to analyze O-linked glycans. Have you already performed such type of study? Have you designed a protocol for purification of O-glycans ?
- You have identified several interesting lectin molecules in ticks; however their real biological function is not known. Do you plan any investigation in this area? Are you going to prepare deletion or insertion mutants for the genes encoding these proteins and test the biological impact of these mutations?

**Conclusions and recommendation**

Ján Štěrba thesis represents a first class work presented in a well-written standard format which brought significant advancement in the field of the tick glycobiology. The author has already published four papers in well recognized international journals specialized in this topic. Moreover, two additional manuscripts highlighting current results described in his PhD thesis were submitted. Given the quality of Ján Štěrba's work, I fully recommend this thesis to be accepted as the fulfillment of the requirement for awarding PhD degree to the candidate according to the law §47 section 4.

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### Reviewer's report on Ph. D. thesis of MSc. Ján Štěrba

#### **Title: Glycobiology of ticks and tick-borne pathogens. Glycan, Glycoproteins, and glycan-binding proteins.**

The submitted work has been completed in the joined laboratory under University of South Bohemia and Biological Centre AS CR. The subject follows a long-term objectives studied in the Parasitology laboratory of Ján Štěrba's supervisor, Prof. Libor Grubhoffer. The thesis is written in English and English language used throughout the thesis is at the corresponding quality level; the text is clear and there is only a minimum of grammatical errors in the text. There are 182 numbered pages in total including all the necessary parts of the thesis together with CV, documents on professional experience and a list of publications.


The main goal of the thesis was studying of carbohydrate-binding proteins from ticks and tick-borne pathogens and to understand glycosylation of tick saliva and salivary glands. The scope of the thesis is very wide, however Ján Štěrba was able to extract all important information and to prepare exhaustive introduction to the field. The thesis cites almost 440 references, and introduction enables to subtract all important information necessary to understand the thesis topic. Introduction counts about 70 pages, and give really detailed overview about ticks and tick-borne pathogens, their physiology, art of knowledge in the field of tick glycosylation, proteins involved in as well as methodology necessary to study whole glycoms and glycosylation profiles. It may look too detailed concerning general introduction of the field, however, other students and scientists interested in the glycofield can find very quickly very useful citations concerning different aspects of the discipline. Method part introduces only techniques not described in the enclosed Ján Štěrba's publications so this part is very straightforward without any unnecessary mess, which could be seen in this part. Results are partially based on unpublished as well as already published results. It is divided according problems studied; each chapter starts with unpublished results followed by a short description of published results and by the publication(s) itself/themselves. This allows to follow

the basic ideas of the study including a potential for further results publishing. The main part of the thesis is finished by Conclusion and perspectives.

The submitted thesis is based on 5 publications where Ján Štěrba is two times as the first author, three times reported as a corresponding author or co-author. I would appreciate if all publications are present in the thesis. The publication of Ruzek *et al* is only mentioned in the list of publications, the abstract only is shown form the paper of Man *et al*.

Ján Štěrba's involvement in teaching duties at University of South Bohemia is visible and an important part of his higher education. He has supervised 3 Master and 9 Bachelor theses, which shows his manager and science organizing abilities. He also participates in several course teaching.

Altogether, the formal quality of the thesis is excellent and processing of theoretical and experimental data into the presented form gives an evidence of scientific skills and a creative potential of the author. The subject is not easy and results presented in the Ján's thesis give a clear testimony to the fact that he proved his ability to suggest, conduct and present independent research projects. Therefore the presented thesis fulfils all the requirements for awarding the degree of doctor of philosophy. I, therefore, recommend the thesis for defence, and congratulate the candidate for his work. After a successful accomplishment of the defence I suggest to award Ján Štěrba by Ph.D. degree.



Brno, March 13, 2012

Doc. RNDr. Michaela Wimmerová, Ph.D.