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April 05th, 2011

To the Chairman of the Thesis committee.

Thesis Defense of Helena Horka entitled "The role of tick saliva and tick salivary cystatins in the transmission of *Borrelia burgdorferi* and the cystatin effects on experimental asthma in mice".

The PhD thesis dissertation by Ms. Helena Horka evaluates the role of tick saliva in the transmission of *Borrelia burgdoferi*; it also attempts to understand structurally members of the cystatin family of proteins and determines how they interfere with experimental asthma in mice. Dissertation is comprehensively discussed and the aims of the study are described in a logical and straightforward manner. The introduction could easily be considered a review to be submitted for publication elsewhere. The experimental approach chosen by the candidate also appears appropriate to answer the most relevant questions that were raised, and also provides a platform to gather expertise in different experimental designs/models which are compatible, and I would say, excels the academic standards needed to acquire the Doctoral degree any where I have worked, in Brazil, France or in the United States.

The quality of this dissertation can be assessed by the publications and impact of Ms. Horka studies, according to 4 original papers which have already been published or submitted for publication. It is obvious that the achievements of the candidate were the result of hard work, ability to collaborate with other groups, creativity and knowledge, and the ability to contextualize the results with the understanding that vector biology and vector-host interactions is a discipline that is at the interface of several research areas which require deep understanding of established concepts.

Accordingly, in the first paper published in the International Journal of Medical Microbiology the student gathered expertise with in vivo transmission studies by tick-infested spirochetes. Ms Horka demonstrated that tick saliva enhances tissue infection by *B. burgdorferi*, and suggested/s that this positive effect in transmission is likely due to the effects of saliva on the host innate immune mechanisms. The next two papers published in the Biochemical Journal and Molecular Microbiology were about solving the structure of immunomodulatory cystatins and understanding their effects in dendritic cell function and *B. burgdorferi* transmission. Finally, in a paper submitted to The Journal of Immunology, Ms Horka demonstrates that a member of the family of tick cystatins (Sialostatin L) prevents experimental asthma, through inhibition of IL-9 production by Th9 cells.

I have some questions regarding the dissertation and papers:
1) What are the typical features and differences between Th2 and Th9 cells and how they may be involved in tick biology and the transmission of tick-borne diseases? What do you know about the epidemiology of tick-borne diseases in Central Europe?
2) Is there anything known about mast cells and saliva interaction except their role in tick resistance development?
3) Sialostatin L has an effect in dendritic cells. How these data associate with your asthma results?
4) What is the status quo in the anti-cytokine therapy for other human diseases?
5) Are there or were there salivary proteins in clinical trial for specific diseases or anti-tick vaccines?
6) Why sialoL2 helps *Borrelia* and sialoL not?

Based on these studies, I am confident that Ms Horka has gathered the expertise needed to receive the PhD degree and to become a leader in here field of research. Therefore, I most certainly support this scientific work by approving her Thesis defense with most of my enthusiasm.

Yours Sincerely,

Ivo M.B. Francischetti, MD, PhD.

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PhD Thesis Review

Candidate	Helena Horká
Thesis title	The role of tick saliva and tick salivary gland cystatins in the transmission of *Borrelia burgdorferi* and the cystatin effect on experimental asthma in mice
University	University of South Bohemia
Reviewer	Professor Pat Nuttall

Overall comments

The thesis is nicely put together and clearly written. It presents the research results as an Experimental Section comprising four publications:
1. Horká et al (2011) submitted to J. Immunol.
2. Kotsyfakis et al (2010) Mol. Microbiol. 77: 456-470
3. Salát et al (2010) Biochem. J. 429: 103-112
4. Horká (2009) Int. J. Med. Microbiol. 299: 373-380

Two of the publications show the candidate as first author, both of which are in internationally leading journals. Publication of the candidate's research obviously demonstrates that her work and the work to which she has contributed have been successfully peer reviewed and are of merit. However, on the face of it the papers are disjointed. For example, publication 3. reports the crystal structure and characterisation of cysteine protease inhibitors from an argasid tick species that has nothing to do with the transmission cycle of the *Borrelia burgdorferi*, publication 2. switches to the North American vector of *B. burgdorferi*, while publication 1. is about the potential of a tick cysteine protease inhibitor to treat asthma.

The candidate needs to demonstrate she has completed a body of work that is commensurate with a PhD research project, providing evidence of her ability to test hypotheses, design appropriate experiments, analyse and synthesise the data, and show how the new knowledge she has generated takes forward our understanding. All the material is present in the thesis to demonstrate these criteria for successful completion of a PhD research project. What is missing is evidence of the candidate's own intellectual contribution. This could be achieved by: (i) including in section 2 (Goals of the project) a rationale for the research undertaken and stating the hypotheses tested; (ii) expanding section 4 (Summary) to include a discussion of the results that brings them together coherently, considers the implications for the tick-host-pathogen triangle of interactions (for example, what insights are there from the asthma model that can be extrapolated to Lyme disease?), and points the way forward to new research questions.

Specific comments

p. 6, line 21-22: "suggesting an antigen replacement in the surface of the spirochetes" as a result of high frequency recombination does not make sense. The meaning needs to be elaborated.
p. 6, line 24: ticks are vectors of pathogens, not of disease.
p. 12, line 20: "driven by exosites" is unclear; requires explanation.
p. 12, line 25-27: "Dendritic cells....overall function" – again the meaning is unclear, possibly because the grammar is incorrect.
p. 14, line 19-20: "saliva-activated transmission" has been superseded by "saliva-assisted transmission."
p. 15, line3-5: The statement that borreliae from salivary glands showed higher infectivity than those from gut needs qualifying as it does not necessarily indicate an important role for tick salivary gland proteins.
p. 21, line 8: RNA interference is not 100% effective and therefore it does not

silence genes.
p. 108 References: incorrectly ordered

Minor comments
There are a few of grammatical corrections required in the Introduction.

Further comments
Two questions were addressed to the candidate to determine her ability to respond to (i) and (ii) above:

Q. Could the lower effectiveness of OmC2 as an immunomodulator be explained by the method of evaluation not being optimal for OmC2? In other systems perhaps it could be highly effective?

Q. Thanks for elaborating your role in the experimental design. I still feel you are expressing the technical input and not explaining how your findings have advanced understanding of the tick-Borrelia-host triangle. Can you provide a synthesis and discussion please?

The candidate provided a well reasoned discussion of the comparative properties of sialostatin L and OmC2, including evidence that she had tried assays with alternative cell types recognising that dendritic cells may be of lesser significance for argasid ticks compared with ixodid ticks. The candidate provided insights into further studies and what these may reveal.

As to the second question, the candidate was able to place her discoveries in the context of previous results, and explain how she has advanced current knowledge of the tick-*Borrelia*-host triangle. It was accepted that the positive results with SialoL in a mouse model of asthma are an exciting "side product" of her research. The underlying result demonstrating that a tick protein has profound effects on IL-9 production, and its implications for tick feeding, are potentially far reaching observations.

Conclusions
My request for evidence of the candidate's ability to test hypotheses, design appropriate experiments, analyse and synthesise the data, and show how the new knowledge she has generated takes forward our understanding, have been fully met by the candidate and by supporting information provided by Dr Kopecky. I am happy to recommend the candidate for the award of a PhD.

P. A. Nuttall

Professor P A Nuttall
14 April 2011