

## Review of Dissertation thesis

RNDr. Kateřina Černá

Tick saliva activated transmission of *Borrelia burgdorferi*.

Reviewed thesis is addressed to the „hot topic“ in the research of tick-borne diseases. Only exact comprehension of the vector-pathogen interactions, pathogen transmission and the establishment of the infection in the host can lead to the development of an effective preventive strategies (e.g. anti-tick vaccines). That is why I think that a chosen topic is of high importance in a field of tick-borne diseases research.

Dissertation thesis is written clearly and comprehensible in a proper English. Small misspellings and mistakes don't affect the good quality of the work. It consists of short but clear and well written introduction and literature survey. Objectives are explicitly stated and followed by summary of results and discussion of four papers presented in the thesis. The missing part of the thesis is the list of abbreviations, that would make reading of the thesis much easier since author uses many abbreviations, for some readers possibly unknown. I am also curious how big was the contribution of Dr. Černá to the papers where she is not the first author.

Immunomodulatory activity of tick saliva plays an important role in the facilitation of pathogen transmission from the tick to the host. In the first paper the author studied the effect of tick saliva and salivary gland extract on the borreliacidal activity of the complement. Moreover, the effect of the SGE and tick saliva on the phagocytosis of borrelia by macrophages as well as production of cytokines and NO by macrophages was analyzed. The inhibitory effect of SGE on the borrelial killing by the alternative pathway of the complement was observed. Difference between the different borrelial species was detected. According to the previously published results by Kurtenbach et al. different borrelial genospecies have different sensitivity to the complement from different hosts. Even though calf serum shows the strongest borreliacidal activity and should lyse all borrelia, differences between genospecies were observed in the experiment. My questions to this part of the thesis are: *Do you*

think, that you would get different results using the sera from different hosts in your experiments? Which molecules might be responsible for the anticomplement activity in the *I. ricinus* (Isaac group?) and do you plan to further study their effect on the borrelial as well as different tick-borne pathogen transmission (e.g. *Anaplasma*, Tick-borne encephalitis virus)? How do you explain different effect of the SGE and tick saliva in NO production by macrophages? Second paper studies the effect of tick-saliva activated transmission (SAT) of *Borrelia burgdorferi* sensu stricto to the experimental mice. SAT is very important phenomenon that facilitates the transmission of tick-borne pathogens from vectors to the host and has been previously demonstrated for several tick-borne pathogens. Moreover, authors focused to describe the effect of tick saliva or SGE on the multiplication of borrelia in different murine tissues and organs in the early stage on infection and their impact on the transmission of *Borrelia* from mice to feeding uninfected ticks. They confirmed that tick saliva or SGE facilitates borrelial transmission to the mice. Furthermore, the prevalence of spirochetes in ticks feeding on mice infected with the mixture of spirochetes and SGE or tick saliva were higher. Surprisingly, only SGE significantly increased spirochete load in the mouse skin at the inoculation site at days 4 and 6 post infection. Authors hypothesises that it might be due to the effect of protease inhibitors in the treated SGE. Do you have an idea about the design of experiment that would elucidate the effect of protease inhibitors?

Last part of the thesis deals with the experimental infection of ticks with the specific strains of *B. burgdorferi* sensu lato complex. The percentage of infected ticks using immersion method were comparable with the results obtained by feeding ticks on infected mice. Moreover, higher percentage of ticks with the *B. afzelii* were obtained using immersion method. Production of infected ticks using this method is fast, easy technique that is less laborious than feeding the ticks on infected mice or capillary feeding. This method can have very broad implementation in many experiments where a large number of ticks infected with a known borrelial species are needed. The prevalence of *B. afzelii* in experimental ticks was lower than percentage of ticks infected with *B. garinii* and *B. burgdorferi* s.s. Author stated that it might be due of different virulence and infectivity of various strains. Did you tested more strains of one species? Do you plan to use this method also for different tick-transmitted pathogens?

The obtained results bring new insights into the research on the mechanisms of innate immunity against *Borrelia*. Moreover, Dr. Černá in her dissertation thesis analyzes the effect of saliva activated transmission of spirochetes and presents effective, fast and simple method of artificial infection of ticks, that can be implemented in many laboratories. All this information are important to better understand the transmission of the pathogen and the invasion of the host.

In conclusion I allege that RNDr. Kateřina Černá met all the criteria and I agree to award her a PhD (Philosophiae Doctor) title.

Bratislava, 17.4.2009

  
MVDr. Markéta Derdáková, PhD.



Centers for Disease Control  
and Prevention (CDC)  
National Center for Zoonotic, Vector-borne  
and Enteric Diseases (NCZVED)  
Division of Vector-Borne Infectious Diseases  
3150 Rampart Rd, Foothills Campus  
Fort Collins, Colorado 80521

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Dr. Jan Kopecky  
Institute of Parasitology  
Centre of Biology, AS CR  
Branisovska 31 370 05  
Ceske Budojovice  
Czech Republic

Jan,

Here is my review of Katerina Cerna's thesis:

1) Have you found an original and creative intellectual input of the student? Yes - the work demonstrating *I. ricinus* SGE inhibiting the alternative complement pathway, decreased phagocytosis of spirochetes by mouse macrophages are novel findings regarding saliva effects on the innate immune system. Also the SAT effects on *Borrelia* load in specific tissues is unique, as well as the effects of saliva and SGE causing higher infection rates in feeding *I. ricinus* ticks. I found the literature review within the Introduction to be complete and well written, flowing well into the resulting hypothesis testing and results of the studies. Katerina's first author publication in the refereed journal, *Journal of Medical Entomology*, is well written and explores some very interesting aspects of innate immune response(s) to SGE and/or saliva.

2) Cannot answer the question re: autonomy here.

3) I have found the results of this project very original, in terms of looking at the alternative complement pathway as well as saliva-activated uptake of spirochetes into *nadve* ticks. This work will set the stage for future studies to look at specific molecules in *I. ricinus* SGE and/or saliva, since the assay systems are straight-forward and are well thought out.

4) This PhD thesis, in terms of the literature review, would be adequate for a PhD degree in the U.S. What is needed here is a bit more dialogue regarding the actual meaning of the results and what future studies would be needed to answer questions raised by this work.

5) Among the best 10% I have seen.

6) This thesis should meet the criteria for conferring the PhD degree.

Specific questions for K. Cerna:

1) What effect(s) if any would increases in IL-4 have on IFN-g, with Th2 polarization?

2) Why do ticks and other medically important blood feeding arthropod vectors contain molecules to promote SAT when most vectors in nature are uninfected (no pathogen harbored)?

3) Why the contrast in results, i.e. SGE increased production of NO by

macrophages, whereas saliva inhibited NO production? Why were these studies conducted using cells derived from BALB/c mice, when most of the in vivo transmission studies were done in C3H mice? Was there ever a comparison to C3H mice done?

4) Both mice fed upon by immersed larvae or fed upon by nymphs molting from immersed larvae (infected) were negative for anti-CB53 antibodies - why? Were Western blot assays attempted with different dilutions of serum, and why is there a discrepancy in results between the J. Med Micro and the Folio Parasitologica studies - in terms of anti-borrelia antibody results? Were lesions ever detected in both bladder and heart where mice were sero-negative?

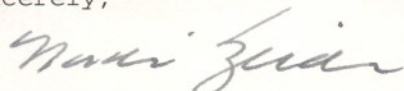
Comments:

1) Pg. 9: "presence of competent vector saliva on the contrary enhances the percentage of surviving spirochetes". Not technically correct - think the killing was just significantly less. There was some killing using competent vector saliva, just not statistically significant compared to controls and probably due to pilocarpine contamination.

2) P. 24: shouldn't the figure legend(s) read, "heat inactivated serum" served as a negative control?

If you need anything else do not hesitate to ask.

Sincerely,



Nordin Zeidner, D.V.M., Ph.D.  
Chief, Vector-Host Laboratory  
Centers for Disease Control and Prevention  
Division of Vector-Borne Infectious Diseases  
Bacterial Diseases Branch  
3150 Rampart Road, Foothills Campus  
Fort Collins, Colorado, 80521  
970.221.6495  
970.225.4257 (FAX)

## Review of Ph.D. Thesis

### Tick saliva-activated transmission of *Borrelia burgdorferi*

by Kateřina Černá

The Thesis submitted deals with microbe – vector – host interaction which is very interesting and complicated topic from scientific point of view. Focusing on the transmission of borreliosis by ticks is of great practical importance with regard to current epidemiological situation. The immunosuppressive effect of tick saliva is already known long time but not yet fully understood. The thesis was worked out in the laboratory with long-time experience in the field and with international significance. Many valuable results concerning biological activities of tick saliva were already obtained here and the work of K. Černá represents their significant enrichment.

The Thesis is based on 4 publications in international peer-reviewed journals. K. Černá is the first author of one of them. Publications mentioned above represent one part of the thesis, the other part comprises Introduction (outline of current knowledge), Objectives, Summary of results and discussion, Conclusions and list of more than 200 references. Besides of several small formal faults (p. 12 - Fig. 3 instead of Fig. 2; Fig 3 and 4 - heat activated serum instead of heat inactivated; p. 34 - Paper VI instead of Paper IV), the work is very carefully proceeded. The results obtained fully meet the defined aims. Appropriate and modern methods and mouse model were used in the study. The sophisticated experimental work required large manual skill.

The results comprise new priority data concerning the effect of tick saliva on innate immunity of the host (mainly on complement activation and phagocytosis), describe proliferation and distribution of *Borrelia burgdorferi* in mice after tick born infection, characterize the importance of tick saliva for infection of ticks during feeding on infected mice, did not found a substantial protective effect of vaccination of mice with salivary proteins of ticks. A method elaborated for defined and standard infection of tics with *borrelia* can be fully exploited in the future work.

The interesting results evoke some questions:

- It seems the effect of tick saliva is not only local in the close vicinity of tick feeding but also systemic even in remote places of the host. What concentration of active substances could be supposed in these localizations, what is the catabolic rate of these factors, how long after tick release, the effect of saliva factors can be seen?

- Is it possible to suppose the future practical exploitation of immunosuppressive effect of tick saliva?
- Expression of some saliva immunoregulatory factors is upregulated after the beginning of feeding. How quick is this upregulation? After what time, maximal effect of saliva is reached? What time after starting tick feeding is the optimal for the infection transmission?
- Under certain conditions (p.33), antibodies were not detected in infected mice. Does it mean saliva is suppressive also for antibody formation?
- What animal was used for feeding 100 larvae “that completed their feeding on one animal”? (p. 35)

### **Conclusion**

The thesis of. K. Černá brings interested and important results some of them of priority importance. The author proved her experimental skill, theoretic knowledge in the field, competence and criticism in result interpretation and capability of the independent scientific work. I recommend the thesis presented as a basis for PhD graduation.



Prof. Ludmila Prokešová, MD, PhD  
Charles University in Prague  
First Faculty of Medicine