

Review of master thesis

Author of the thesis: Bc. Tereza Pospíšilová

Title: Gene expression and infectivity of *Borrelia afzelii* in the course of tick feeding

Supervisor: RNDr. Radek Šíma, Ph.D.

This master thesis deals with the current issue of changes in the gene expression of *Borrelia burgdorferi* (the bacterium responsible for Lyme disease). The goal of this thesis was to evaluate the expression of selected bacterial genes that influence the survival of *Borrelia* in the tick vector, the transmission of bacteria from the vector to the warm-blooded host, and the induction of the chronic infection in the host. Author of this thesis also examined the possibility of immunizing mice with the injections of intestinal antigens from *B. afzelii* infected ticks taken at various stages of tick feeding. 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. vaccines). Therefore, the chosen topic is of high importance in a field of tick-borne diseases research.

The diploma thesis consists of 29 pages of plain text supplemented by a list of literary sources, which contains 103 papers. The text is logically divided into 6 chapters: introduction, objectives of the work, materials and methodology, results, discussion, and conclusion.

In the introduction part, the author deals with the pathogen causing Lyme borreliosis, with ticks as vectors of this disease, and with pathogen-vector interactions, especially with changes in the expression of *Borrelia* genes during the survival phase inside the tick. A substantial part of the introduction is also focused on *Borrelia* gene expression control necessary for mammalian host colonization, as well as the successful reproduction and dissemination of *Borrelia* in the warm-blooded organism. The text is written comprehensibly in a proper English and it is well supplemented by citations of literary sources.

The objectives are defined clearly.

In the materials and methods chapter, the author describes the way of working with laboratory animals and provides the detailed explanation of the molecular-genetic methods.

The result part is elaborated on 9 pages, supplemented by 13 graphs. The text effectively refers to graphs and is easily readable. The results comprise new priority data concerning the effect of temperature on the expression of the key genes standing behind *Borrelia afzelii* infectivity. In the discussion part, the author critically evaluates the results obtained and compares them with previously published works.

Critical remarks: This master thesis is processed carefully and does not contain more serious shortcomings. I will mention only a few remarks that do not reduce the level of the work.

- In isolated cases, the original sources are not quoted in the Introduction chapter (p. 1 - information on the classification and shape of the *Borrelia burgdorferi* were known from ^{than in} an earlier time than 2008).
- The primers used to detect *Borrelia* 23S rRNA (Bor1-Bor4) have already been published (Eisendle K, Grabner T, Kutzner H, Zelger B. Possible role of *Borrelia burgdorferi* sensu lato infection in lichen sclerosis, Arch Dermatol 2008, 144 (5): 591-598.).
- The methodology of the vaccination experiment lacks the information how many mice were included in each group.
- The publication Wilske et al. (without publication year) is not mentioned in the text. Conversely, citations are given in the text to Pospitch et al. 2017a and b, in the list of literature is only one of them.
- The way of writing a range of pages in the list of literature could be unified.
- The work might contain a list of abbreviations.

Questions to the author:

- Are already known some specific molecules that trigger or inhibit individual pathways regulating the expression of *B. burgdorferi* proteins during tick feeding and then during the host infection?
- p. 17, Fig. 1C: Why does dbpA expression decrease so significantly in the mouse when the dbpA function is to mediate the adhesion to host tissues? An interesting comparison is with Fig. 3C. How do you explain that the expression of dbpA in unfed ticks exposed to different temperatures is so significantly different from the levels of expression seen during tick feeding, when in other monitored genes, the level of expression correlate?
- As well as the author, I think that it would be preferable to use sonicated guts of non-infected ticks instead of PBS as a control in a vaccination trial (filtering the effect of tick's antigens). What anatomical location was the vaccine applied on? Is there any possibility, that the protective effect of the vaccine was caused only by some nonspecific inflammation at the vaccination site (if the ticks were then feeding at the same site)?

Conclusion:

The work unquestionably meets the criteria for the master thesis on the Faculty of Science of the University of South Bohemia in České Budějovice, so I recommend it for the defense.

In Pilsen on 15.5. 2018

A handwritten signature in blue ink, consisting of a stylized 'K' followed by a long horizontal stroke and a loop.

RNDr. Kateřina Černá, Ph.D.



Master thesis of Bc. Tereza Pospíšilová: **Gene expression and infectivity of *Borrelia afzelii* in the course of tick feeding**

Reviewer: Václav Hönig

The topic of the thesis addresses gaps in the knowledge of the transmission course of European *B. burgdorferi* genospecies. In deed, many scientific findings obtained using American *B. burgdorferi* s.s. strains and vector species were automatically accepted as valid for European borrelia and tick species. As shown in several cases before, there are significant differences.

To quickly summarize the main results of the master thesis: Expression dynamics of selected (infectivity-associated) *B. afzelii* genes was determined during feeding of nymphal *I. ricinus* and subsequent infection of the mammalian host. Temperature was confirmed as an important trigger of the expressional changes in culture and in ticks *in vivo*. Furthermore, direct impact of temperature on spirochete infectivity was proven. These results were than applied in a vaccination experiment, which showed some protective level in mice immunized with midgut lysates of infected ticks.

The thesis is written in a very careful and concise way, almost free of any formal or grammar mistakes. The level of English is, according to my opinion, high. Definitely, the work is well comprehensible. The author uses appropriate scientific terms and expressions. The text is logically structured and individual parts nicely connected.

In chapter Introduction, the student summarizes all the important information needed for understanding the experimental part, avoiding any unnecessary details or information unrelated to the topic. She uses appropriate citations, which are correctly listed in section references.

The student was able to master a wide spectrum of methods ranging from work with infectious laboratory animals (borrelia-tick-mouse infection model), assessment of borrelia load in tissue samples (qPCR), analyses of changes in expression of selected target genes (RT-qPCR), to vaccination experiments (preparation of antigen, vaccination, challenge). The methods are well described and the author is apparently familiar with their specifics, as she mentions only important features of the methods. Nevertheless, in the description of some of the experiments, I would appreciate some more detail. For example: How was the mouse tissue sample treated before RNA isolation? How long were the spirochetes cultivated in specific temperatures?

Results are again, described in a logical and concise manner. Apart from presentation of the results, each chapter contains a brief explanation of the rationale of the particular experiment and concise summary of its results. Figures are comprehensive, contain all necessary information and in general well described in captions. In several cases, (e.g. Fig. 1-3) I just miss the information on statistical analysis – were the differences not tested or only found not statistically significant?

In the section discussion, the author correctly compares her results with published findings. The main results are in concordance with published data. Nevertheless, I would suggest considering also the more ambiguous and therefore more interesting results, for example discrepancy in the expression level of *dbpA* in cultivated *Borrelia* and ticks (temperature response experiment). In addition, the results could be put in a wider context e.g. What mechanisms could participate on temperature triggered changes? – heat-shock proteins, role of the Hk1-Rrp1 and Rrp2-RpoN-RpoS pathways? The vaccination experiment is in this regard discussed much better. The author identifies the limitation of using PBS as control instead of non-infected tick gut and suggests alternative design of the experiment to test the hypothesis of non-borrelia associated immune response. The second part, of the discussion is a consideration of the possible future development towards high-throughput transcriptomic analysis. The discussion is the only slightly weaker part of the otherwise high quality work with interesting results.

I would like to ask the student to address following questions/comments:

- 1: Where do *B. burgdorferi* s.l. spirochetes reside in tick after infection to next feeding?
- 2: Could you comment on the selection of *flaB* as a reference gene? There are some indications on upregulation of *flaB* in the early phase of mammalian infection (Hodzic et al. 2013).
- 3: How reliable is the detection of borrelia in ear puncture to consider a mouse borrelia free?
- 4: Temperature triggered changes in the expression levels of the target genes seemed to be less pronounced in ticks than in culture. In case of *dbpA* the expression was downregulated in 20°C in culture, whereas it was slightly upregulated in ticks. Could you speculate on the source of these differences?

The thesis of Tereza Pospíšilová is a nice piece of work bringing new information on the mechanisms involved in the transmission cycles of Lyme disease spirochetes. Furthermore, the new findings provoke new hypothesis to be tested. The critical comments, I have mentioned above, are of minor importance and do not affect significantly the otherwise high quality of the work. The work with living systems is always difficult. Tereza was able to study interactions between three living systems and obtained clear unambiguous results. The thesis fulfils the requirements for a master thesis at the Faculty of Science, University of South Bohemia and I recommend it for defense.

In České Budějovice, 4.5.2018

Mgr. Václav Hönl, PhD.

