



Review of the Master thesis submitted by Bc. Hana Velanová

Growth kinetics of the Lyme disease spirochetes in the vector ticks *Ixodes ricinus* and *Ixodes scapularis*.

Supervisor: RNDr. Radek Šíma Ph.D.

Reviewer: RNDr. Zdeněk Franta Ph.D. Faculty of Sciences, University of South Bohemia, České Budějovice

The Master thesis of Bc. Hana Velanová compares the acquisition, growth and the kinetics of two important human pathogens namely *Borrelia afzelii* and *Borrelia burgdorferi* in the tick vectors *Ixodes ricinus* and *Ixodes scapularis*. The thesis has 48 pages and consists of seven parts (introduction, objectives, material and methods, results, discussion, conclusion and references). The thesis reads well and does not contain many typos or other formal problems. The English of the text seems good to me (as far as I can judge).

Introduction:

The introduction is written on 17 pages and guides the reader from the general tick overview towards the biology of genus *Borrelia*, and the disease it causes. The last part of introduction is focused on already established “American (*B. burgdorferi*/*I. scapularis*) and European (*B. afzelii*/*I. ricinus*) transmission models, which are specifically important for this thesis. The chapter contains six figures, which have a good quality and help the reader to orientate and understand the text.

I have two questions / comments to the applicant:

1 – on page three you state that “The adults find a host, they mate, and the adult females feed, drop off, lay eggs and die”. I found this statement a bit incorrect. Are there any other possibilities, when/where tick can mate? Is it possible that the tick female would feed without mating?

2 – Is there anything known about the transmission of *Borrelia* by argasid ticks? These ticks are fast feeders and *Borrelia* cannot really use the advantage of suppressed immune system as known for ixodes ticks.

Material and methods chapter is written on five pages and contain all the techniques the candidate used during her research. I have only one question to this part:

1 – How did you check the quality of isolated DNA. Did you have any internal control to verify the success of the process?

The results are written on ten pages and are supported by eight figures. The chapter has logical hierarchy and shows all the key finding the author acquired during her studies.

My questions / comments to this part:

1 – Both the growth kinetics data (figures 7, 8 and 11) as well as the mean number of spirochetes (figures 9, 12 and 13) indicates the fluctuation of spirochetes in tick vector and are



quite interesting. However the presentation of this data using regular line and bar charts is not the best in my eyes. You do have quite big SEM and it is very hard to say why this might be. Using box plot or violin plot you will get more detail information about the distribution of your data, which can be than connected to any biological phenomena.

2 – Do you have any theory why at least three infected *I. scapularis* ticks are needed to transmit *B. afzelii* CB43.

3 – in the figure 10 you show the detection of *B. afzelii* in mice, however it is not clear to me which organ did you used here (in M & M you state that you dissected joints, bladder and heart)

4 – in figure 14 you detect *B. burgdorferi* N40 in mice tissues using regular and nested PCR. I was wondering what are the other bands here. Would it make sense to do the qPCR instead of nested PCR?

The discussion is on five pages and compares the acquired results with available literature. The author also nicely explain why there is a difference in tick molting, which is most likely associated with the year period and shall be definitely taken into account when designing any comparative tick experiments. Tick seasonality could also have an effect on the pathogen transmission.

Despite all my comments, I believe that Bc. Hana Velanová achieved a high level of scientific competence. The author fulfil all the given objectives and the results gained during her master studies are a valuable contribution to tick borne pathogen research.

Finally, I am glad to conclude that presented work meets all the criteria and I have no hesitation in recommending **to accept** the thesis of Bc. Hana Velanová for Master defense.

In České Budějovice, 03.07.2020

Zdeněk Franta