To the Chairman of the Thesis committee.

Evaluation of PhD dissertation by Mr. Jan Kotal entitled:

"Functional analysis of tick salivary serine and cysteine protease inhibitors".

The PhD dissertation by Mr. Jan Kotal evaluates the role of tick salivary protease inhibitors belonging to the cystatin and serpin families on host defense mechanisms, focusing on inhibition of inflammation, immune cell activation, infiltration, complement activation, and blood coagulation. The dissertation is comprehensively discussed, and the aims of the study were well designed and described in a logical and straightforward manner. The introduction section is written well, and the content flows in an understandable and logical way. It is followed by two review manuscripts that nicely complement the literature presented in the dissertation's introduction, adding up more knowledge on the role of tick saliva and salivary cystatins/serpins in the tick-host interaction. The experimental approaches chosen by the candidate were key to address the hypotheses and also provided a platform to gather expertise in different experimental designs/models. All in all, the dissertation excels the academic standards needed to acquire a PhD degree at any university in the United States.

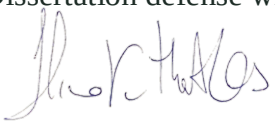
The impact of Mr. Kotal´s publications speaks volumes about quality of his dissertation. It encompasses three published scientific manuscripts as well as one extra manuscript that seems to be ready for submission; all of which has Mr. Kotal as first author. Accordingly, in the first research paper (Manuscript 3) published in the Cellular and Molecular Life Sciences, the PhD applicant gathered expertise with biochemical and immunological assays. Mr. Kotal demonstrated that a tick salivary cystatin named Iristatin is capable of carrying out a broad spectrum of immunomodulatory effects in the tick´s host, which can possibly be related to its protease inhibitory properties. The second research paper (Manuscript 4) characterized an anti-hemostatic serpin expressed in the tick salivary glands. The authors performed a detailed characterization of the serpin biochemical properties and link them to the observed anti-coagulation activity.

All these achievements are a testament of Mr. Kotal's 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.

In regards to Mr. Kotal's dissertation body of work, I have a few questions:

1) What can be a potential therapeutical use of Iristatin??
2) How do you explain contradictory effects on host immunity between Iristatin and whole tick saliva?
3) What is the role of protease inhibitors in tick physiology? Are there also proteases present in tick saliva?
4) As far as Manuscript #4, what else needs to be done in order to submit this manuscript for publication?

Based on this dissertation and presented studies, I am confident that Mr. Kotal has gathered the expertise needed to receive the PhD degree. Therefore, I most certainly support this scientific work by approving his Dissertation defense with most of my enthusiasm.



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