

Advisor opinion on Mgr. thesis of Bc. Havran Jiri "Detection of Lyme disease spirochetes in clinical samples by PCR-based methods and optimization of conditions of borrelia cultivation from samples of patients with LB symptoms".

The work presented by Bc. Jiri Havran was conducted in Laboratory of molecular biology of vectors and pathogens and represents an extension of long term research led in the lab for the past 5 years. After defense of his Bc. thesis that was devoted to analysis of *Ixodes ricinus* gene involved in anti-oxidative response, Jiri decided to change the field of his further research and was involved in newly started collaborative project with Department of Pediatric Infectious Diseases, Faculty of Medicine of Masaryk University in Brno. For those who ever worked with clinical samples of human origin or was involved in collaboration with hospitals it must be clear that Jiri's choice was rather brave. Existing ethical rules accepted by hospitals, impossibility to choose the type of samples personally or to affect the schedule of their delivery to the lab, the lack of basic information due to the hospitals privacy policy as well as general complexity of work with human samples that were NOT primarily provided for scientific project, aggravated the project from the very beginning. It was a lucky combination of Jirka's patience, persistence, responsibility, ability to work, ability to accept and respect advices and complete lack of emotions that brought this project to the point where it is now.

Using the PCR based techniques Jiri was able to detect the presence of Lyme disease spirochete in almost "wasted clinical material". Using traditional laboratory methods together with virtual techniques which contribution to identification of correct borrelia species cannot be under evaluated, Jiri confirmed the presence of *B. burgdorferi* sensu stricto in 70% of positive samples, unidentified by serological methods. Negative serological findings in a hospital might be related to the use of primarily, *B. afzelii* as antigen, the most distributed species in Europe, which genetic profile might differ markedly from *B. burgdorferi* sensu stricto or any other species that might possibly be involved in Lyme disease in Europe. *B. afzelii* was identified ONLY in cases of co-infection, never as a single species, which confirms the above mentioned.

Another worth to mention Jiri's achievement was detection of *B. bissettii* that confirms our statement (2 publications: JCM, 2008 and FEMS Letters, 2009) about involvement of this species in European Lyme borreliosis. Recent publication "Genetic diversity of *Borrelia burgdorferi* and detection of *B. bissettii*-like DNA in serum of north-coastal Californian residents" by group of Prof. Robert Lane (JCM, December 2010, in press) proves that *B. bissettii* is a causative agent of Lyme disease worldwide.

Detection of two new spirochete species in European patients, *B. carolinensis* and *B. americana*, that were described by us in 2009 and 2010 once again rise a question "How restricted is territorial distribution of spirochetes around the world?". *B. carolinensis* was already detected in *I. ricinus* in Western France, so, results presented in this thesis are the first detection of this species in human. *B. americana* is a bird associated species and it was found as co-infection with *B. burgdorferi* sensu stricto, the species which worldwide distribution by migratory birds was confirmed by multiple publications of European and

American scientists. It is just a matter of time when the presence of this species in Europe will be confirmed by other groups.

I would like to state that, according to my opinion, Jiri Havran did his best to solve the aims of the project working with complex material in difficult conditions. He managed to begin the spirochete cultivation from clinical samples that is extremely valuable for us. As far as I know no one else in our Institute succeeded in this field for rather long time.

I would like to state that results obtained by Jiri Havran during his Mgr. project represent a significant and valuable part of the lab results on identification of *Borrelia burgdorferi* sensu lato species involved in Lyme disease in Europe that we are planning to publish in a nearest future.

I would like to add one more thing that I hope Jiri will accept and will understand. It is known for a long time that "Brevity is the soul of wit"...but it is known as well, that excess of brevity can make more harm than one can imagine. I am sure that some reasonable verbalism and emotions will be very helpful in Jiri's future work, communication with colleagues as well as for presentation and sharing of his achievements.

I would like to recommend this work for defense.

January, 23, 2011

Natasha Rudenko

A handwritten signature in black ink, appearing to read 'Natasha Rudenko', written in a cursive style.