



BIOLOGY CENTRE ASCR

Institute of Parasitology

address: Branišovská 1160/31, 370 05 České Budějovice, Czech Republic

IBAN – CZ22 0710 0000 0000 0552 7231 | SWIFT CODE – CNBACZPP | VAT No.: CZ60077344

phone: +420 387 775 403 | fax: +420 385 310 388 | www.paru.cas.cz | e-mail: paru@paru.cas.cz

Supervisor's evaluation of Ph.D. thesis by RNDr. Marie Jalovecká– “Establishment of *Babesia* laboratory model and it experimental application”

(RNDr. Ondřej Hajdušek, Ph.D., Institute of Parasitology, Biology Centre, Academy of Sciences of the Czech Republic)

It was during my postdoc stay in France, where I worked on the Plasmodium-mosquito transmission model, when we, together with Petr Kopáček, started to dream about introduction of a similar model into our laboratory. Ticks, although they do not transmit malaria apicomplexans, are able to transmit whole spectra of pathogens including Piroplasms (Theileria and Babesia), parasites closely related to the Plasmodium species. In Europe, but also in the Czech Republic, tick *Ixodes ricinus* is able to vector several Babesial species. Without any practical and with only limited theoretical knowledge about Babesia we decided to contact people, which could help us to get some of these parasites to establish the model. We were lucky, as we met, during TTP conference in Zaragoza, Dr. Laurence Malandrin (INRA, NANTES), an expert in the Babesia field, which was from the beginning very open for the collaboration.

At that time, a young lady Maruška Jalovecká was giving a seminar about her Cryptosporidium research performed in the laboratory of Dr. Martin Kváč. After the talk, Radek Šíma and Dan Sojka, enthusiastic by her performance, offered Maruška to join our team for a PhD. After small hesitation, Maruška decided to do so, to change the field and to learn some new techniques from the molecular biology. For us, it was very lucky decision, because I must say that without Maruška we could never get so far. Maruška deeply studied available literature, brought the parasites into our lab, learned how to cultivate them, set-up animal infections, and finally fulfilled our dreams and introduced Babesia transmission and acquisition model into our laboratory, to my knowledge the first laboratory Piroplasm model around the world!

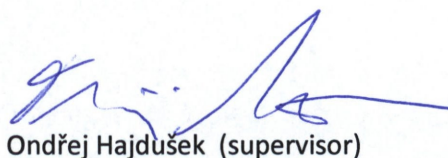
At the beginning of her PhD, Maruška, under supervision of Dr. Petr Kopáček, asked for a French fellowship, which allowed her to travel three times to the laboratory in France to get dual PhD degree - one in France, one in the Czech Republic. Dr. Laurence Malandrin kindly agreed to be her supervisor in France, so she could, already during her first year of PhD, start to work with Babesia in Laurence's laboratory. During her multiple stays Maruška mostly work on the characterization of Babesia divergens CCP genes, genes expressed exclusively in gametocytes. She standardized qRT-PCR method for detection of gametocytes in cultures and tested, how cultivation conditions influence possible infectivity of the parasites. This work has been published in Parasites and Vectors 2016. Together with Laurence Maruška also did deep literature screening about variations in Piroplasm developmental cycles. Review covering this important topic is actually ready for publishing.



During the visits in her home Institution Maruška successfully implemented *Babesia divergens* culturing in bovine blood. However, infection of model hosts (gerbils and mice) shown big deviations and infection of ticks by using animals or artificial feeding system was also unsuccessful. Here we decided to try different *Babesia* species and ordered *B. microti* (US strain) from ATTC. Maruška, together with a Msc. student under here supervision Jiří Ťápal, standardized infections of laboratory mice and found conditions for successful infection of tick larvae. This work was crucial for 7FP EU project Antidote, where we analyzed transcript of the salivary glands from infected and uninfected ticks. Although the infection of larvae (nymphs molting from these larvae) is fluctuating during the year, such infected nymphs can be directly used for transmission experiments upon knock-down or genes or vaccination with candidate recombinant proteins. Maruška's masterpiece was development of *Babesia* acquisition system, where we were able to quantify by qRT-PCR the effect of genes or vaccines on acquisition of *Babesia*, similarly as in the mosquito-*Plasmodium* system. I am sure that we will all profit from this breakthrough in the *Babesia* field during several next year and find many tick genes interacting with the transmission of *Babesia*, and Piroplasms in general. Well, first genes have been already found! I must also mention Maruška's contribution to the tick immunity review published in *Frontiers Cellular and Infection Microbiology* and validation of *Babesia* detection methods published in *Infectious diseases*. Recently, she is, in collaboration with Daniel Sojka, also finishing manuscript covering characterization of the *Babesia* proteasome, possible target for an anti-*babesia* drug treatment.

In a summary, Maruška is first author of one publication and co-author of several others. Obviously, substantial part of her work still await publishing. She has presented her work on several conferences. **I am fully convinced that her PhD study and thesis fulfill all the criteria requested by the Faculty of Science, University of South Bohemia in České Budějovice to be awarded by a title Ph.D.**

In České Budějovice, August 18, 2017



Ondřej Hajdušek (supervisor)



École Nationale
Vétérinaire, Agroalimentaire et de l'Alimentation

Nantes Atlantique

UMR INRA/ONIRIS 1300 BioEpAR
Biologie Epidémiologie et Analyse de Risques
La Chantrerie Route de Gachet
CS 40706
44307 NANTES cedex 03



INRA
SCIENCE & IMPACT

Supervisor's evaluation of Ph.D. thesis by Marie Jalovecka

"Establishment of *Babesia* laboratory model and its experimental application"

(Laurence Malandrin, UMR INRA/Oniris BioEpAR, Nantes, France)

The collaboration between Prof. Petr Kopacek's lab and me started at the Tick and Tick-borne Pathogen Conference in Spain, when Dr. Ondrej Hajdusek contacted me searching a specialist of *Babesia*. They wanted to develop a laboratory model of *Babesia* transmission. I had really bad experiences in this field, with great difficulties in trying to infect and transmit *Babesia divergens* by the tick *Ixodes ricinus*. Anyway, we went on with this project, and Maruska joined the project as a PhD student preparing a double French/Czech diploma. When I say joined the project, you should understand that she carried the project. Thanks to a grant from the French Embassy, she could come in Nantes to work on her thesis project.

As my first experiences with tick and *Babesia* transmission were not successful, we decided that the first part of Maruska's thesis would be to control that the transmission stage of *Babesia divergens* to the tick, namely the gametocytes, were indeed produced and to quantify them. Maruska used the BdCCp gametocyte markers developed in my lab to this achievement. She demonstrated that long term in vitro cultivation indeed affects gametocyte production, and she determined conditions, including stress conditions, that could enhance the transmission stage production by the cultivated parasite. She performed this huge work during her stay in France and this finally led to a publication in Parasites and Vectors.

Back here, she implemented *Babesia divergens* in vitro cultivation, allowing studies on the search of new anti Babesial drugs, namely proteasome inhibitors, to be performed.

Maruska also attempted several experiments to try to infect *Ixodes ricinus* with *Babesia divergens* and get transmission, but again success was not at the rendez-vous. I had the opportunity to come to Prof. Petr Kopacek's lab for three months thanks to a European grant

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from his lab. We tried to infect ticks with cultivated *Babesia divergens* and detect the parasite in the ovaries, but no success.

Faced to this apparently complicated issue to establish *Babesia divergens* transmission cycle, Maruska switched to another *Babesia* species, *Babesia microti*. She implemented a lot of new technics and got nice data that are going to be published.

Maruska came three times in France for a total of 16 months over three years. During this time, and during my stay in Czeck Republic, I really got the opportunity to know her as a scientist and as a person. Maruska is a really great person, she really cares about people, and she always tries to communicate with people. One funny moment was in the culture room in France, with my technician Nathalie trying to explain *Babesia* cultivation in French to Maruska, and Maruska asking questions in English, all this with a lot of gesticulations and drawings. But it worked. To better communicate, she learned French, almost as difficult as her own language. She made a lot of friends in Nantes and the whole lab team has great souvenirs with her. On her side she probably remembers the french administration.. why should it be simple ??

Maruska is scientifically extremely curious, and she is dedicated to her work. She is an extremely hard worker, and her research is always extremely well conducted. She is a great source of scientific discussion, and Maruska is an infinite source of ideas. More, she is always enthousiastic.

To conclude my evaluation : I really have had the pleasure to supervise Marie Jalovecka during her stay in France. I have no doubt that her scientific career will further successfully continue.

I am fully convinced that Marie Jalovecka's PhD. study and thesis fulfill all the criteria requested by the Faculty of Science, University of South Bohemia in České Budějovice as well as those requested by Oniris, Ecole Nationale Vétérinaire, Alimentaire et de l'Alimentation to be awarded by a double title PhD as well as a european label.

de 20 octobre 2017,

L. Malandrin

A handwritten signature in blue ink, appearing to be 'L. Malandrin', with a long horizontal stroke extending to the right.

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