



Confidential

Review of USB FFPW PhD Thesis

First name(s), surname, titles of the PhD student: Aleš Pospíchal, Mgr.	First name(s), surname, titles of supervisor: MVDr. Veronika Piačková, Ph.D.
Title of PhD thesis: Susceptibility of cyprinid and non-cyprinid fish species to CyHV-3	
REVIEWER:	
Surname: Haenen	Institution: Wageningen Bioveterinary Research (part of Wageningen University and Research)
Name: Olga	
Titles: Dr.	E-mail: olga.haenen@wur.nl
Please describe your professional relationship to the PhD student: I am a colleague of Dr. Veronika Piačková	Please describe your field of expertise: I have 34 years of experience in multidisciplinary fish disease diagnosis and research, including focus on KHV.

QUESTIONNAIRE

Originality, scientific importance, perspectives and impacts of results presented in the PhD thesis for basic and/or applied research

Evaluate competitiveness of the PhD thesis in the international context and compare its level with the current state of the art in the field (extent ¼ – ½ page):

This thesis on KHVD hits an important subject. KHVD has been listed by OIE and EU since a few years. Delisting of KHVD is a continuous subject within the EU, as it requires much effort and money to keep it listed, and some countries have many small sites for culture of the susceptible fish species, mainly carp. In case delisting will be the case for EU, there must be enough insight into the list of susceptible species and vector species for KHV transmission to protect carp sites against KHVD. This study adds to the knowledge necessary for this. It is economically and scientifically important, it helps carp farmers culturing in polyculture of fishes, it is original as it adds new fish species to the list of tested fishes, proven by the publications in this thesis, and others from this group of scientists. It therefore has a rather good impact on the way professional fish farmers should act regarding polyculture of local fish species. The perspectives given for further studies are sound.



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of Waters

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v Českých Budějovicích
University of South Bohemia
in České Budějovice
Czech Republic

Elaboration of the PhD thesis, objectives of the work and deliverables

Evaluate the overall level of elaboration of the PhD thesis (structuring of the main text, comprehensibility, logicity of the chapters and their ordering) and the originality of the selected approaches to solve the objectives; evaluate publications and whether the results described correspond to objectives of the PhD thesis (extent ¼ – ½ page):

The overall level of elaboration of the PhD thesis is sound and the order of the chapters is logical. It is in general well written. However, I have made quite some corrections on the English, small corrections on the content, and many corrections on the references, please see the marked places in the annex (PDF after Olga Haenen). I think it is necessary that these corrections for precision are taken into account before the final version of the thesis can be presented.

The comprehensibility is rather good. Apart from two English written peer reviewed papers the PhD candidate has written one other in Czech language and 2 as co-author, also in Czech language. In this thesis however, I miss the discussion to less pathogenic variants of KHV strains and their pathogenicity (Engelsma et al., 2013 : Detection of novel strains of cyprinid herpesvirus closely related to koi herpesvirus. Dis Aquat Organ. 2013 Dec 12;107(2):113-20. doi: 10.3354/dao02666.), also related to vaccine development. Also, the overall view if we need to add susceptible species to the EFSA list of susceptible (2008 EFSA Journal, 808: 1-144) and vector (2007 EFSA Journal, 584: 1-163) species and the EU and OIE notifiable disease lists misses.

The originality is good, like given above, and the studies performed correspond well to the objectives of the PhD thesis.



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OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages:

This thesis gives important new information regarding susceptible fish species to KHV and also it gives insight in the value of the recommended test methods, which are well compared. The two peer reviewed publications included are very well, and represent much work.

There are many small corrections to be made in the non published part, the intro and the discussion, and also many in the references. These are marked in the PDF (version _Haenen), and should be corrected. The two publications included are sound and extensive, big studies, comprising the body of this thesis. With the extra publications mentioned in the last pages of this thesis these are sufficient for this PhD defence.

The wording KHV and CyHV-3 is not consequently used, a choice should be made, and officially we talk about CyHV-3 nowadays, but if it is made clear, that KHV is CyHV-3, it may be chosen for KHV in the text. This needs to be adapted throughout the thesis.

The literature review in the introduction is sound, and a good basis for the aims of this thesis. The results are well discussed, with updated literature as reference.

Overall, a nice thesis, which needs more precision in some the wording, and in the referencing. When these corrections are made it is ready as document for the public defence by the candidate Ales Pospichal.

I wish you all luck, and hope to be there to join the session.

Thank you.

Olga Haenen, 1st July 2019

FINAL RECOMMENDATION

PhD Thesis can be recommended for defence (corrections should be made first)

PhD Thesis can be recommended with reservations for defence

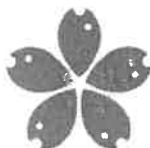
PhD Thesis can not be recommended for defence

Lelystad, 1st July 2019

Date and place

Dr. Olga Haenen

Name and signature



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First name(s), surname, titles of the PhD student: Aleš Pospíchal, Mgr.	First name(s), surname, titles of supervisor: Veronika Piačková, MVDr., Ph.D.
Title of PhD thesis: Susceptibility of cyprinid and non-cyprinid fish species to CyHV-3	
REVIEWER:	
Surname: Palíková	Institution: Veterinární a farmaceutická univerzita Brno
Name: Miroslava	
Titles: doc. MVDr., Ph.D.	E-mail: palikovam@vfu.cz
Please describe your professional relationship to the PhD student: no	Please describe your field of expertise: Fish diseases

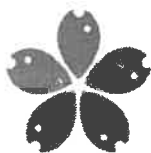
QUESTIONNAIRE

Originality, scientific importance, perspectives and impacts of results presented in the PhD thesis for basic and/or applied research

The dissertation thesis submitted by Aleš Pospíchal is focused on the study of a susceptibility of cyprinid and non-cyprinid fish species to cyprinid herpesvirus 3 (CyHV-3), which causes the koi herpesvirus disease (KHVD).

KHVD is relatively young disease and simultaneously the disease included to the list of compulsory reported diseases as a notifiable disease. Asymptomatic susceptibility of fish species and their role as a potential carrier and the source of infection for susceptible species are very valuable information, therefore, I consider the chosen topic as very actual and the results gained by its solution beneficial for practice.

The research presented in the thesis is of good quality. The studies are well designed and conducted and bring important new knowledge. The results are original and novel.



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Elaboration of the PhD thesis, objectives of the work and deliverables

The PhD thesis is based on two already published scientific papers. It is clearly and logically structured into three main Chapters, contains General discussion, Czech and English summary and further required information (list of publications, training and supervision plan during study and curriculum vitae). First Chapter contains the General introduction, Chapters 2 and 3 present the research papers. The results described in these papers clearly correspond to objectives of the PhD thesis. Overall, the thesis can be assessed as standardly divided with clearly specified and adapted objectives which are published in scientific journals.

OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages:

The Ph.D. thesis is well written and presents interesting and important topic. There is also a good quality of English with only a few errors. It is written on 79 pages and it is structured according to guidelines for writing the Ph.D. thesis at the Faculty of Fisheries and Protection of Waters. In General introduction student clearly in a logical sequence of single chapters presents the current state of knowledge related to the issue using actual literary sources. It includes around 150 references and presents quality literature review. Introduction is written on 22 pages.

Two following chapters contain two student's own results published in journal with IF, namely in Veterinarni Medicina and student is the first author of the both manuscripts. Participation of student on both manuscripts was 60%. In these papers, the study designs, results and interpretation are well presented. They are summarized and put into context with current state of knowledge in Chapter General discussion and Conclusions (6 pages), which includes around 80 references.

From the Training plan it is evident, that he realized research stay at National Reference Laboratory for Viral Disease of Fish in Veterinary Research Institute in Brno and three courses regarding to an agent diagnose by using of molecular methods. Knowledge of these methods is essential for his work.

Comments and discussion questions:

In chapter 1.2. „Loss of natural resistance“, according to my opinion, its content does not match to the name of this chapter. Could the student more elaborate the issue according to the chapter name?

In chapter 1.3.3. you mention that bacteria present in environmental water can directly inactivate CyHV-3 (according to Shimizu et al., 2006). Can you indicate whether it is known which particular bacterial strains are involved in this inactivation?

In the first publication, the presence of CyHV-3 was performed on the 30th day, but not on the 15th day. How you can explain this fact, when the survival fish were kept alone in the aquaria (without infected koi, which were dead on 15 and 20 day, respectively)?



Why were different PCR used in the second experiment (nested PCR in non-stressed fish and real-time PCR in stressed fish)?

It is very important to know the possibility of transmission of CyHV-3 from other fish species to koi. In both experiments you started the secondary challenge in the time, when no positive potential carrier fish were diagnosed. Would not be better to repeat the challenge with really positive fish (after longer time of exposition when simultaneously examined fish are found positive) to find if they are able to transmit the agent?

In abstract is written that the transmission of the virus by topmouth gudgeon was not observed. That is not quite clear, because CyHV-3 DNA was found in one koi-tissue and this fish species is needed to consider as a new potential carrier of this virus although it will be necessary to confirm (as it is also written in the second paper).

In conclusions is recommended to include examined asymptotically susceptible species to CyHV-3 to quarantine. For how long would you recommend quarantine when it comes to asymptomatic carriers? Wouldn't it be appropriate to also attach susceptible fish to rebuke the virus?

In conclusion, succeeded through this research work, I can unequivocally state that the submitted thesis meets the requirements for the dissertation, it is carefully and clearly arranged in terms of content and form, and therefore I recommend it for defense and after successful defense I recommend granting the Ph.D.

FINAL RECOMMENDATION

PhD Thesis can be recommended for defence

PhD Thesis can be recommended with reservations for defence

PhD Thesis can not be recommended for defence

18.6.2019

.....
Date and place

.....
Name and signature

