



### The Reviewer's Report on a Ph.D. Thesis

Author: Mgr. Carlos Díaz

Title: Production of recombinant proteins for veterinary use

Reviewer: RNDr. Jana Prodělalová, Ph.D., Veterinary Research Institute, Brno

African swine fever (ASF) is damaging viral infection of both domestic pigs and wild boars (*Sus scrofa*). The disease occurs worldwide. Due to epidemiological situation in neighbouring countries, namely Poland, Slovakia and Germany, it still pose a serious threat for Czech pig farms. First occurrence of ASF in 2017 was quite surprising, however viral agent was early detected thanks to passive surveillance in wild boars; and strict veterinary measurements have been applied promptly. Within applied measures, the ban of cull at the beginning, followed by extensive shooting in cooperation with Police of the Czech Republic; and cadavers' picking was recognized as the most effective in the core area. The last Czech PCR-positive cadaver was found in the spring 2018. In April 2019, the Czech Republic was declared as ASF free. Recently, veterinary measures concerning both farming and hunting biosecurity, and passive surveillance, are continuously applied. Unfortunately, there is currently no other way of protecting the pig farms, because efficient and safe vaccine is not available yet.

The aim of Ph.D. thesis is production of recombinant ASFV capsid proteins with possible vaccination or diagnostics potential that represents actual topic. On the other hand, development of ASF vaccine is extremely complicated. Thus, I have to appreciate author's audacity to tackle this issue.

The thesis consist of 104 pages. It is divided into theoretical and experimental part as usual. In chapter Materials and methods author clearly describes the experiments that were



carried out in other laboratories. Results are quite surprisingly combined with discussion and consist of twenty pages. The last but not least is the list of 171 references cited.

The work is transparently written, without major formal imperfections. However, I have one comment concerning the small size of some tables and figures that can be read only with difficulties (e.g. Fig. 3 on the page 16 or Fig. 4 on the page 17). Moreover, I would like to mention some minor mistakes that of course did not affect the quality of Ph.D. Thesis:

- At Acknowledgement: Veterinary and Pharmaceutical University (abbrev. VFU) does not exist anymore; recent name is veterinary University (abbrev. VETUNI);
- At Materials and Methods: on the page 40 – there is incorrect description of tables.

Two articles in Journal of Veterinary Research (recent IF 1,744) were published. First, review targeting the most important swine DNA viruses including ASFV was published in 2021. Second, research article summarized results of Ph.D. thesis was published in 2022. Moreover, some results were presented as posters at three international conferences in the Czech Republic and Taiwan.

I have following questions about the Ph.D. thesis:

1. On page 40, source genome sequences for ASFV recombinant protein production are described insufficiently. Used sequence MK333183 was originally obtained from Chinese isolate DB/HLJ/2018, as you can easily find out on GenBank. However, genotype II type strain is ASFV Georgia 2007/1 and its whole-genome sequence is indicated as NC\_044595.2. Did you compare target sequences of both Chinese and type ASFV strains? In addition, is there any reason to choose the sequence of Chinese strain? The same issue concerns sequence U18466. Moreover, this sequence was obtained from attenuated laboratory ASFV strain BA71V that is classified as genotype I.
2. I would be interested in your opinion on the progress of vaccine development to date. What is the situation in Europe and in non-European countries?



3. What is your authorship share in the publications that are part of the Ph.D. thesis?

In conclusion, Ph.D. thesis of author Mgr. Carlos Díaz "Production of Recombinant Proteins for Veterinary Use" meets the requirements for this type of work. The author has demonstrated the ability to work scientifically in the field. Thus, I am allowed to recommend the thesis to defence.

In Brno on 25. November 2022

RNDr. Jana Prodělalová, Ph.D.

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