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Dear Professor Nováková,

I am pleased to provide the following review and comments on the PhD thesis submitted by your candidate, Mr. Hynek Mazanec: *Isolation and characterisation of extracellular vesicles of parasitic helminths*.

Mr. Mazanec research has described for the first time the secretion of EVs in different tapeworms, the aquatic *Schistocephalus solidus* and the terrestrial *Hymenolepis diminuta*. It has explored EVs secretion at different developmental stages of *S. solidus*, and in case of *H. diminuta* proteomic analyses and ultrastructural observations has been used to identify the main biogenesis pathways behind EVs secretion and establish comparison of EVs generation in both tapeworms. As a result of this research, four scientific studies have been published, which gives the results presented in the thesis validity and reliability.

I recommend that the thesis be defended as planned. The document is well developed and the candidate Hynek Mazanec has demonstrated a sufficient body of work for a PhD.

On the attached pages, I have provided specific comments and questions about the thesis, to form the basis of further discussion with the candidate at the defence.

Thank you very much for participating in the review of the thesis and I hope to be in contact with you in the future.

Sincerely,

MARIA
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VILLANUEVA
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María Trelis Villanueva

Detailed review and comments

Introduction

Comments: The introduction covers the existing literature on extracellular vesicles in general (biogenesis and proteomics) and on their role in pathogen-host communication. Specifically, an exhaustive review is made of all those parasitic helminths in which EVs have been described, as well as the functions attributed to them and the challenges that remain to be solved.

An extensive and updated literature review has been carried out.

Chapter I

Comments: This article is an important contribution to the world of extracellular vesicles as it describes for the first time the presence and secretion of EVs in adult *H. diminuta*. Interestingly, two distinct subtypes are observed and high quality images are provided to demonstrate the release process of the tegument-derived vesicles. Regarding the proteomics of EVs, proteins of interest in future studies on treatment, vaccination, etc. are found.

It would be interesting to test the immunomodulatory capacity of EVs to assess their possible application for anti-inflammatory purposes, especially in this case where you have an experimental model that allows maintenance of infection and ensures a source of EVs.

Questions for the candidate:

- In the conclusions of the article the authors make the following comment "Furthermore, we would like to observe internalisation of host-origin EVs by tapeworms' tegument and the internalisation of parasite-origin EVs by intestinal cells of the host, to help elucidate their roles in the host-parasite relationship". How would you set up an assay to observe the internalisation of host EVs by the cestode?

Chapter II

Comments: On the results of this study, it is interesting to note the demonstration of several biogenesis mechanisms of EVs and their cargo enrichment. The data presented also suggest a novel mechanism behind the formation of beaded protrusions and the subsequent release of corresponding EVs. As for the proteomics results, it is shown a highest functional complexity in the fractions obtained after 24h of cultivation with an enrichment of gene ontologies, indicative of higher viability of the organism and a decrease in secretory activity after 48h caused by a lack of stimulation from the external environment.

Chapter III

Comments: The most remarkable finding of this article is the fact that it has demonstrated the secretion of EVs at all parasitic stages of *Schistocephalus solidus* (hexacanth, proceroid, plerocercoid, and adult) an aquatic tapeworm with a complex life-cycle including three different host types (invertebrate, fish and bird).

Questions for the candidate:

- Have you considered what functions the EVs secreted by the larval stages might have?
- Have you read about the possible functions of these vesicles in the intermediate host?

Chapter IV

Comments: This work addresses real problems and needs in the day-to-day study of helminth-derived EVs.

Questions for the candidate:

- In terms of the proposals made in the paper, which ones do you think could be really useful to achieve a breakthrough in this type of studies?

Extra questions for the candidate:

1. As mentioned in the thesis, *H. diminuta* is a possible candidate for helminthic therapy trials in the treatment of autoimmune diseases.

As you have already set up the obtaining of vesicles and you have characterized them at the proteomic level, do you think it is possible to carry out tests of anti-inflammatory capacity of these EVs in murine models?

