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Review of the Ph. D. Thesis "Development of surface and body musculature of the bird schistosome *Trichobilharzia regenti*" by Mgr. Jana Bulantova

To whom correspond

I consider that the thesis constitutes an excellent piece of work. The thesis is well written and the topics covered are of interest. Moreover, the experimental design and the methodology are adequate for the purposes of the study.

In relation to the results obtained, I think that they are sound and of novelty. This affirmation is based on:

- Development of methodologies for in vitro cultivation of *T. regenti*: this is one of the main gaps in the development of experimental Parasitology. Thus, I consider that this kind of study is of great interest, particularly considering that the most of the culture media employed are commercially available which will facilitate the reproducibility of these methods and their use in future studies, even by other research groups.

- Studies on the ontogenic development of helminths are needed: The development of helminths and the changes induced by the growth has been poorly studied. In this context, the present study constitutes an important contribution focused on the surface and musculature of the parasite, together with important implications in the immune response.

- Characterization of antigenic structures in different developmental stages of *T. regenti*: Although it has been shown that antibody responses against adult stages of several helminths species is ineffective, an increasing spot of interest in the current immunoparasitology is the fact that these responses may be able to control the helminths at the larval stage. Thus, characterization of the target of antibody responses in larval stages such as schistosomules may be useful for providing of new tools for the control of helminths infections.

Although, the thesis is of great quality, its reading has raised several questions and comments to be answered by the defendant:

- I feel that the author repeatedly confuses the terms "immunization" with "infection" in the paper by Chanova et al. (2011): In this study the authors used sera from mice experimentally infected with *T. regenti* but not immunized. The term "immunized" should be reserved for the stimulation of the immune system by inoculation of antigens by a different ways than those that occurs in natural conditions. This is not an important problem since I think that in this way the response is more similar to that occurring in nature and, thus, more useful for the study, but the terms should be used correctly.

- Another question in relation to this paper is about the use of control sera from non-infected mouse. Why did the authors only used sera collected from a single non-infected mice? I feel that this may be an important problem in the interpretation of the results and a higher number of different sera should be used even either individually or as a pool of sera.

- In this same context, I wondered why the authors used sera from repeatedly infected mice, but not from mice after a single infection. A single infection did not generate a response of enough intensity?

- The loss of glycocalyx in the schistosomule stage is known, but I miss in the study further mention about the presence of glycocalyx (or at least lectin-binding sites) in the adult stage. It is known that in the surface of adult human schistosomes, there are several surface carbohydrates that act as target of antibody responses. Is it known something about this topic in *T. regenti* or in a related species?

- A striking feature in the study is that sera from infected mice did not recognize the adult stage. This can be explained by the fact the parasite does not reach the adult stage in mice. However, this would imply that the antigen renewal in the pass from schistosomule to adult stage is complete and the adult does not retain any of the antigenic molecules present in the schistosomule which, apparently, seems to be unlikely. It is possible to issue a hypothesis about this feature?

- In the latest years, the work of several authors such as Dr Horak or Kolarova has served to significantly increase our knowledge on the immunobiology of bird

schistosomes and the cercarial dermatitis. However, I feel that further studies on the characterization of antigens are required. Currently, and as far as I know, only GADPH and GST has been identified. Have you projected any study on this topic?

- One of the most striking features in the thesis is the presence of bubbles (or blebs) on the surface of almost all the stages of *T. regenti*. I am somewhat confused about the origin of these blebs. Is it known anything about the origin of the blebs?, i.e., osmotic pressure, tegumental breakage, extracellular vesicles…

Finally, I would like to note that the thesis constitutes an excellent work and absolutely merits the award of a Ph. D.

Signed: Rafael Toledo