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Copies: Lucie Lichtenbergova  
Hana Vlckova, Science and Research Administration Division

### Review of the thesis submitted by Mgr Lucie Lichtenbergova

This thesis presents original work on *Trichobilharzia regenti*. This parasite is unusual among other trematodes in that it migrates in the nervous system of mammals rather than in the blood system as do, e.g., the well-studied schistosomes. Research on *T. regenti* is internationally very rare, and this thesis comes from the team leading this field. Hence, this thesis has a particular scientific merit, particularly since it is conceptually and experimentally well performed. The scientific basis of the thesis are three publications, 2 of which with the candidate as first author, and each published in one of the best international journals in the field of parasitology („Parasite Immunology“ and „Experimental Parasitology“). A third publication with the candidate as second author is submitted to the international journal „Parasitology Research“. Based on these publications, the scientific merit of the candidate's work is certainly suitable for the award of a Ph.D.

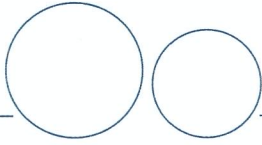
The Introduction of the thesis is a well-researched literature review on the parasite *T. regenti*, and places this in the context of other species of the same genus and of the related schistosomes, which have a different biology, different migration behaviour in the host and cause a different pathology. In fact, each of the sub-chapters of the introduction contains an interesting comparative overview over specific aspects of trematodes:

- basic, but diverse life cycles;
- complex process of entering the host's skin;
- first clinical manifestations in the form of dermatitis;
- complicated interactions of the anti-parasite immune response with the parasite itself, the resulting inflammatory reactions with interacting parasite and host molecules;
- unique immune evasion strategies of schistosomes, for which these have been best studied among trematodes
- involvement of the CNS, which greatly varies among several helminths with respect to clinical relevance, to parasite migratory behaviour, to the specifically involved immune reactions and – as the final result – to pathology.

It is a pleasure to note that the references are, in most cases, well researched into the past decades, and they demonstrate that the candidate has a sound scientific understanding of her research area. The general orientation of the thesis and its aims are strongly immunologic in nature and study the host's antibody response against *T. regenti*, parasite antigens and antigenic structures, and immune involvement in parasite destruction, but also host pathology. Nevertheless – and somewhat surprisingly – the title of thesis does not mention the immunologic context.

The Introduction needs some critical remarks. The English language of the Introduction is flooded with a very disturbing multitude of non-use, mis-use or wrong use of articles. This could/should have easily been corrected. Unfortunately, this lack of diligence gives an unnecessary negative impression to a scientifically very good work.

In the following, comments labelled with \* might be dealt with during the defence.



- \*p.6 If „miracidia hatch directly within the host tissue“, how would they get *out* of the tissue? For schistosomes, tissue migration occurs with the egg within the granuloma; I would expect the miracidium of *T. regenti* to be sensitive to immune killing, and not to be resistant as is an egg.
- \*p.7 While talking about visceral trichobilharzia, line 3 states that the parasites „frequently“ develop in the veins and tissue of the gut: Where else than in the veins would they develop, and would „tissue“ imply an extra-vascular location? Or is this formulation wrong?
- Figs 2 and 3 are not referred to in the text.
- \*p.18-19 The schistosome surface has a double unit membrane and this is its key feature in dealing with immune evasion (resistance to complement and cellular attack; stability). This property is, however, not dealt with explicitly, but only indirectly. Other functions on p.19 appear as rather minor to me.
- \*p.19 there is an apparently wrong concept (or at least confusion) about the schistosome surface: „antigenic variation – fast changes of antigen expression“ certainly does *not* occur in schistosomes. These are immune evasion mechanisms of, e.g., trypanosomes. Schistosomes rather acquire host antigens (or proteins) (as stated on p.19 and 22), and this mechanism of mimicry was a revolution when detected originally by Smithers and Terry in the late 60ies (they are not cited). The candidate mentions „expression of surface epitopes identical or similar to host molecules“ (5th line from bottom). This formulation could be interpreted as meaning that these epitopes are being synthesized by the parasite, which is the case in antigenic variation by trypanosomes, but definitely not for host antigens of schistosomes.
- p.23 It is said that „human schistosomes may reach the CNS“ after egg laying starts. It is certainly not the worm, but only their eggs, which could do this.

#### Questions to the publications:

##### Parasite Immunology:

\* Figure 4, lane 2 shows an IgG1 response 10 days after infection. In my understanding of the development of the immune response, I would not expect a detectable reaction at this very early time. How is this compatible with the time needed according to textbooks for antibody production?

\* A 34 kD protein is described as a major cercarial immunogen of *T. regenti* and is hypothesized to be Cathepsin B. This enzyme was also discussed on p. 9 of the Introduction. It would have been useful to refer to the extensive work performed with schistosome Cathepsin B, which was first identified in the mid 80ies, as being immuno-dominant and having about 34 kD.

##### Experimental Parasitology

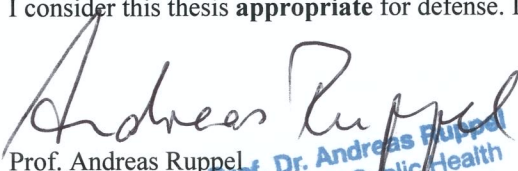
\*This report did not detect paralysis in immuno-competent hosts, which contrasts with the previous work of Horak, where paralysis was described for normal mice. The number of schistosomula are supposed to play a role, but the claim is not substantiated. What exactly were the cercarial numbers applied in both reports? Reference in this respect could also be made to the submitted manuscript.

##### Parasitology Research

This manuscript is submitted. The English language will need improvement.

\*In Fig 2, the staining is not easily visible for me in the lower 2 pictures (a, b, c and d are not labeled). The candidate could be asked to explain the staining during the defence.

I consider this thesis **appropriate** for defense. Its quality reaches the level **sufficient** for the Ph.D. degree

  
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