In search of an identification technique for Bulinid snails and their parasites

The role of southern African bulinid snails (Mollusca: Gastropoda) in transmission of human and animal schistosomiasis and other trematode infections

Detailed project information

Background
Snail-borne diseases affect more than 300 million people worldwide but also lead to economic losses and mortality in livestock. Several bulinid snails that transmit human and animal schistosome species are prevalent in Zimbabwe. Although some of the previous studies used allozymes, diagnosis of the snails has largely been morphological. Resource constraints have limited molecular diagnosis although it is imperative that modern diagnosis needs to be more reliable as bulinid snails are difficult to identify morphologically.

Aims
The aim of this study was to investigate the role of bulinid snails in the transmission of schistosomes and other trematodes among humans, livestock and fishes in Zimbabwe, with a focus on lake Kariba and Mazowe. More specifically, we wanted to apply different methods, both molecular and morphological, to identify bulinid snail species and their parasites in order to identify putative transmission sites. In order to identify the parasite species within snail DNA, we had to optimize and compare different PCR techniques.

Material and Methods
After snail collection and cercarial shedding experiments in Zimbabwe, snails (and the cercariae if present) were preserved in absolute ethanol for molecular diagnosis in Belgium. For snail identification, the partial cox1 fragment of the mitochondrial DNA was amplified and sequenced. In order to identify snail infection, different PCR assays were tested on the extracted snail DNA and optimized. Lastly, partial parasite cox1 mtDNA and 18S rDNA fragments were amplified and sequenced.

Results

Based on morphological analyses we found the following snail species: *Bulinus globosus, Bulinus truncatus, Bulinus forskalii, Biomphalaria pfeifferi, Lymneae natalensis, Lymnaea sp.* and *Physa acuta*. Molecular analyses confirmed B. truncatus, *B. forskalii*, *Lymnaea sp*. and *Physa acuta*. The RD-PCR that was originally developed for free-living schistosome species (Van den Broeck et al. 2011) proved to work equally well to detect schistosome mtDNA in snail tissue. This method is therefore a perfect tool to diagnose snail infection and at the same time inform about the species status as it can differentiate between *Schistosoma haematobium, S. mansoni* and *S. bovis.*

Also the DRA PCR of Amarir et al. (2014) proved to be successful; this assay can differentiate between *S. haematobium* and *S. bovis*. Sequence analysis of the cercariae isolated from *B. truncatus* from Mazowe showed them to be closely related to the fish parasite *Petasiger phalacrocoracis*. Another species was identified as a species similar to *Echinostoma paraensei*, another echinostome species affecting mammals. Echinostomiasis is an important food-borne, intestinal, zoonotic disease, affecting humans, livestock and wildlife animals.

Conclusions

This study has proven the potential of simple and cost-effective PCR assays to diagnose snail infection and therefore an ideal tool to identify areas that pose infection risk for human and animal trematode parasites. The assay can identify the schistosome cercariae up to species level; in case of other trematode infections, sequencing is needed. Once we sequenced all existing trematode species in Zimbabwe, we can develop a RD PCR similar to the one we developed for schistosome species, thus precluding expensive sequence analysis. This will ultimately lead to quicker diagnosis of the infections even under field conditions.