Title:

Fish Faecal Xenomonitoring as a potential tool for schistosomiasis transmission monitoring

Abstract:

Background:

Appropriate surveillance methods are needed to detect and monitor schistosomiasis transmission, particularly as ongoing interventions decrease the disease prevalence. *Schistosoma mansoni* is a parasitic trematode causing intestinal schistosomiasis in humans. It has a complex lifecycle depending on two zooplanktonic larvae (cercariae and miracidia) and an intermediate freshwater snail host. Traditionally, environmental transmission monitoring is achieved via malacological surveys where snails are collected and screened for emerging *Schistosoma* cercariae or the parasite DNA. Although informative, these methods can be laborious and insensitive. Other molecular methods, such as detecting environmental DNA (eDNA), could be a more efficient and sensitive tool. Therefore, this study develops a new, potentially more informative, DNA-based approach relying on detection of the *S. mansoni* DNA in the faeces of natural predators – Fish Faecal Xenomonitoring (FFX). To develop this method, we used juvenile cichlid Nile tilapia (*Oreochromis niloticus*) that has previously been demonstrated to consume cercariae and miracidia of *S. mansoni*.

Methods:

We conducted multiple laboratory and microcosm fish feeding experiments under variable conditions, offering 1-900 *S. mansoni* cercariae to juvenile *O. niloticus* fish (SL 2-5 cm). The fish faecal samples were analysed using a multiplex FFX-qPCR assay, targeting *S. mansoni* and *O. niloticus* DNA (internal control), developed and tested as part of this study. In lab feeding experiments, we analysed the effects of fish size, the number of offered larvae, or the availability of alternative fish food sources on the presence of *S. mansoni* DNA in the fish faeces after consumption. Additionally, we tested the gut passage times of the *S. mansoni* DNA and the method's sensitivity.

Results:

S. mansoni DNA was detected in the faeces of 67.5% of the fish offered ≥300 cercariae. When fish were offered cercariae in microcosms and pooled in groups of five during faeces collection, the overall sample positivity increased to 83.3%. Positive detection was achieved when fish consumed ≥1 cercaria, although sensitivity decreased with fewer cercariae consumed. The gut passage experiments showed that the *S. mansoni* DNA is expelled within 24 hours of consumption, with a peak excretion between 12-21 hours after feeding. The analytical sensitivity of the FFX-qPCR, which targets the *S. mansoni* 16S mitochondrial DNA region, was 100% for ≥10 DNA copies/reaction and 71% for 1 DNA copy per reaction.

Conclusions:

Our findings show that *S. mansoni* DNA, from consumed cercariae, is detectable in fish faeces within 24 hours after consumption and that fish readily consume cercariae even in the presence of other food sources. This FFX approach could provide a new, complementary method for schistosomiasis transmission monitoring in endemic settings. *S. mansoni* and *O. niloticus* are co-occurring across Sub-Saharan Africa. Our study may thus not only have implications for xenomonitoring approaches of *Schistosoma* spp., but also indicates that *O. niloticus* may play a role in the biological control of *S. mansoni* and other aquatic parasites. Further studies in field

settings will enable the evaluation of the FFX methodology for the detection of *S. mansoni* transmission.

Authors:

Bartonicek Z.[1,2,3], Dvorak J. [2,4], Allan F. [1,5], Emery A. M. [1], Day J.J. [3], Webster B. L.[1]

[1] Department of Life Sciences, Natural History Museum, London, UK

[2] Center of Infectious Animal Diseases, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences, Prague, CZ

[3] Department of Genetics, Evolution and Environment, University College London, London, UK

[4] Institute of Organic Chemistry and Biochemistry, Prague, CZ

[5] University of St Andrews, St Andrews, UK

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