Title:

Schistosomes and how to find them – testing novel methods of *Schistosoma mansoni* molecular environmental monitoring in Lake Albert

Introduction:

Sensitive and specific surveillance methods are needed to detect and monitor schistosomiasis transmission, particularly as interventions decrease disease prevalence. *Schistosoma mansoni* causes intestinal schistosomiasis and is endemic in Sub-Saharan Africa, parts of South America and the Caribbean. Its lifecycle depends on zooplanktonic larvae (cercariae and miracidia) and intermediate freshwater snail hosts, all of which are a part of freshwater food webs.

Traditionally, environmental transmission monitoring is achieved via malacological surveys where snails are collected and screened for *Schistosoma* cercariae or DNA. Although informative, these can be laborious and insensitive, and new, more sensitive monitoring methods are needed.

This study field-tested a new molecular approach – Fish Faecal Xenomonitoring (FFX), detecting *S. mansoni* DNA in the faeces of juvenile *Oreochromis niloticus*, a natural fish predator of cercariae and other zooplankton, and compared it to traditional and environmental DNA (eDNA) methods.

Methods:

After laboratory tests of the FFX method showed promising results confirming that *S. mansoni* DNA can reliably be detected in faeces of *O. niloticus* that consumed cercariae, the method was tested in *S. mansoni* - endemic north-eastern Lake Albert (Uganda).

Across ten sampling sites, juvenile (1 - 4 cm standard length) *O. niloticus* were caught and pooled in groups of five. Fish were fed and kept in aquaria for 18 hours, after which they were released back into the lake. All fish faeces were collected from aquaria, stored in ethanol, and subsequently molecularly analysed for the presence of *Schistosoma* DNA by qPCR. The FFX results were compared to the detection of transmission by traditional xenomonitoring, where *Biomphalaria* and *Bulinus* snails were collected and checked for emergent schistosome cercariae. Alongside, we also tested *Schistosoma* detection using eDNA methods, where the water from tested sites was filtered through 0.45 µm Sterivex PVDF filters and subsequently tested by qPCR. For FFX and eDNA, samples were analysed using a newly developed multiplex qPCR assay targeting the 16S region of African *Schistosoma* species and also *O. niloticus* as an internal control.

Results:

All tested methods (FFX, eDNA and shedding) were in agreement at 4/10 sites tested. The FFX approach was most sensitive, indicating *Schistosoma* presence at 9/10 sites, followed by eDNA (5/10 sites) and shedding analysis, which identified 4/10 inspected sites with patently infected snails.

These results clearly demonstrate that *Schistosoma mansoni* transmission is ongoing in Lake Albert and that molecular methods are more sensitive than traditional shedding analysis.

Conclusions:

Our findings show that juvenile *O. niloticus* readily consume *Schistosoma* cercariae with DNA detectable in their faeces post-consumption. We also demonstrate that both the FFX end eDNA methods can detect schistosomiasis transmission in water bodies such as Lake Albert, perhaps with higher sensitivity than traditional methods, enhancing schistosomiasis transmission monitoring.

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