Differentiating new reproductive schistosomes from surviving adult worms following praziquantel treatment using microsatellites

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Introduction

- Schistosomiasis is a neglected tropical disease, infecting over 240 million people globally.
- Infected humans shed *S. mansoni* eggs in their faeces. Eggs hatch into miracidia in freshwater. Miracidia infect snails, reproduce asexually to produce cercariae. Humans get re/infected by contacting freshwater with cercariae in.
- Schistosomiasis is treated using praziquantel (through mass drug administration), however the drug is ineffective against juvenile schistosomes and does not prevent re-infection.
- Adult schistosomes are inaccessible, so diagnosis is by proxy through egg detection in stool using Kato-Katz microscopy or in urine using Circulating Cathodic Antigen tests, to detect worm antigens. However, all available diagnostics cannot detect adult worms directly and cannot differentiate new from surviving adult worms post treatment.
- Microsatellites are neutral genetic markers made of tandem repeats of 1–6 nucleotides. Microsatellite profiles have high mutation rates and can be used to understand parasite population size, structure, gene flow, mating systems, sibship reconstruction, and parentage determination, especially when only offspring are available, such as for schistosomes.

Aim

- Our aim was to estimate sibship and parentage of miracidia, using microsatellites, to distinguish between new and surviving adult worms following praziquantel treatment.

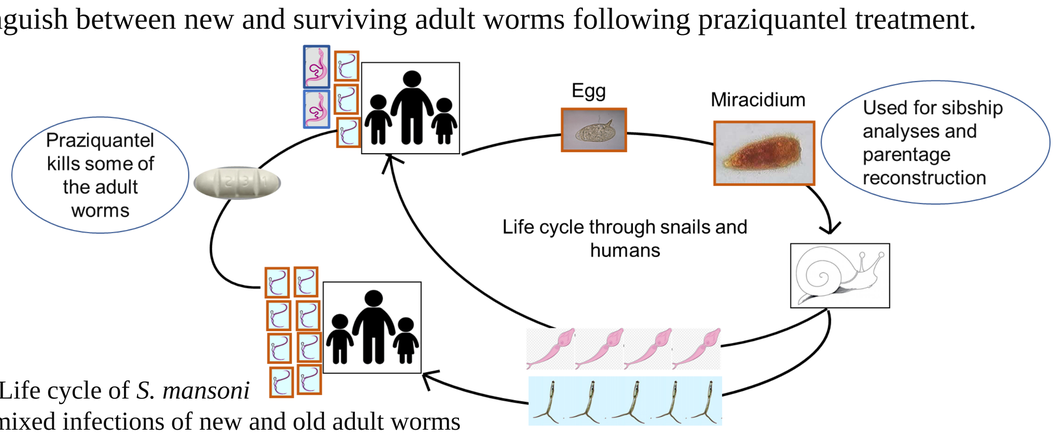

Figure 1: Life cycle of *S. mansoni* showing mixed infections of new and old adult worms that survived praziquantel treatment producing eggs.

Methods

- A child with a light infection (56 eggs per gram of stool (EPG)) was selected for this study.
- *Schistosoma mansoni* miracidia (hatched from eggs excreted in human stool) were collected pre-treatment and over 22 weekly time points post-treatment.
- DNA was extracted from 471 miracidia individually.
- Two multiplex microsatellite PCRs (17 microsatellites in total).
- Gel electrophoresis for visualisation of amplification and selection for allele sizing.
- Allele sizes were scored using Geneious Prime software Version 2020.2.
- COLONY (full likelihood method) used to identify non-, half-, and full-sibling miracidia

Results

Sibship analysis at each time point

- Pre treatment: 54.3% (82 miracidia) were full-siblings, 13.3% (20 miracidia) half-siblings, and 32.4 % (49) non-sibling miracidia among 151 miracidia successfully analyzed.
- 320 miracidia were successfully analyzed from 3 to 22 weeks post-treatment.
- 5.7% to 48.8% of post treatment miracidia were full siblings within each time point.
- Half-siblings were observed at 11, 19 and 21 weeks post-treatment with percentage compositions of 10.8%, 27.9 % and 51.3% respectively.
- As reproducing adult worm pairs produce 100s eggs/day, the presence of non-siblings within a time point, indicates that we have likely, not fully represented, all off-spring from all adult worms (i.e. sampling saturation was not achieved at any time point).

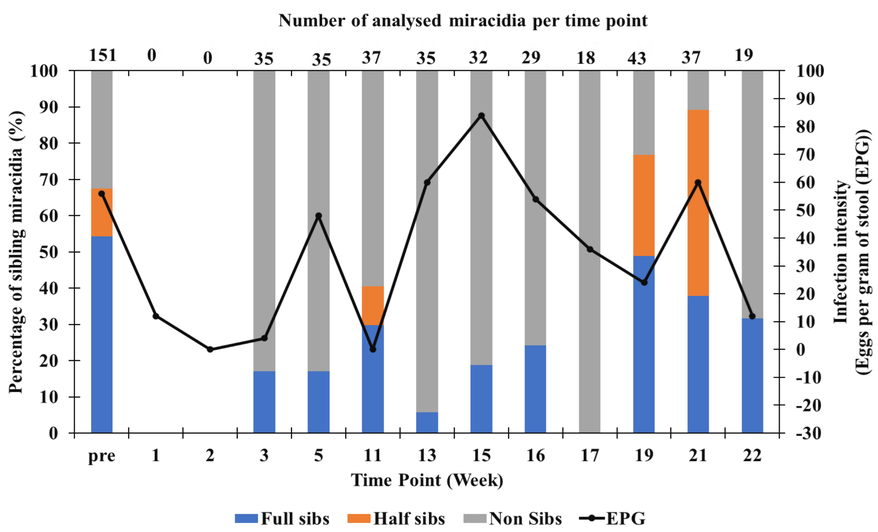

Figure 2: Proportions of siblings and infection intensity at different time points

Sibship analysis between pre- and post-treatment miracidia to determine adult worm survival

- ✓ Post-treatment, up to 60% of miracidia were full- or half-siblings with pretreatment miracidia.
- ✓ Individuals and adult worm pairs survived praziquantel and went on to produce offspring.
- ✓ A large proportion (40.0% to 86.7%) of post treatment miracidia were non-siblings with pretreatment miracidia indicating new reproductive schistosome infections.

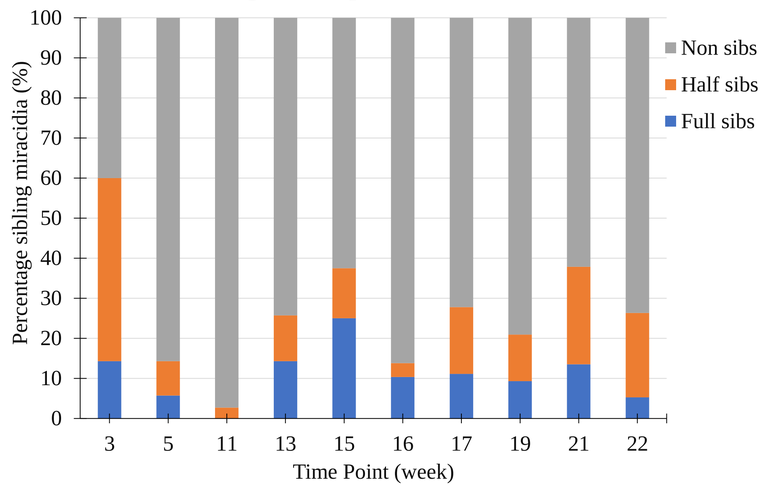

Figure 3: Proportions of miracidia post treatment that are siblings with pre-treatment miracidia

Discussion

- We have likely not fully saturated sampling at each individual time point.
- Non-siblings post treatment are probably from new infections, but as we did not saturate our sampling pre treatment, some may be full-or half-siblings, which would be detected if sample sizes were increased.
- Natural variations in praziquantel susceptibility exist among schistosomes hence individuals and pairs of adult worms survive treatment, even if the majority are killed.
- Despite infection intensity decreasing post treatment, when eggs start to be excreted again several of them are from surviving adult worms.

Conclusion

- Sibship and parentage analysis using microsatellites distinguishes between new reproductive schistosomes and surviving adult worms following praziquantel treatment.
- *S. mansoni* individuals and worm pairs survived praziquantel treatment, and went on to produce full- and half-sibling miracidia, representing adult worms surviving praziquantel.
- To achieve saturation and increase power to detect siblings and new infections, miracidia sample sizes at all timepoints need to be increased, even in this low intensity infection.

Summary

- In hyperendemic schistosomiasis regions, humans are continuously reinfected with schistosomes despite praziquantel MDA resulting in a complexity of both new and old (surviving praziquantel) adult worms, co-existing in the same human host.
- We have demonstrated the potential of sibship and parentage analysis using microsatellite markers in miracidia to distinguish between new reproductive and surviving adult worms (both pairs and individual worms) following praziquantel.

Acknowledgement

- Six year old boy in Bugoto for donating stool samples over 22 weeks.
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