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*Author-formatted, not peer-reviewed document posted on 15/02/2024*

DOI: <https://doi.org/10.3897/arphapreprints.e120910>

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# Development of a Field Diagnostic Tool for *Schistosoma mansoni* Praziquantel Resistant Markers in Selected Endemic Communities

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## Abstract

Schistosomiasis is a neglected tropical disease that affects more than 200 million people and 45% of infections have been shown to occur in school-aged children. A large percentage of the disease burden lies in Africa. In 2012, the WHO outlined a roadmap for the elimination of schistosomiasis by 2020; however, this was not achieved. Treatment for schistosomiasis is by the use of Praziquantel, a drug in use for over 30 years and there is a concern for emerging drug resistance. There are several species of the genus *Schistosoma* causing infection in humans. For this study, *Schistosoma mansoni* which causes intestinal schistosomiasis will be investigated. There are reports of lowering cure rates and suboptimal response to praziquantel following several cycles of mass drug administration (MDA). Praziquantel resistance has also been reported in some countries and laboratory-bred schistosome experiments. To address the concerns of resistance, this study aims to employ a two-part approach to assess the prevalence of *S. mansoni* praziquantel resistance among school-aged children in schistosomiasis endemic communities in Ghana and develop a diagnostic tool to aid in field assessment of infections. To achieve this, the study will attempt to answer the following research questions: 1. Is there developing *S. mansoni* praziquantel resistance in communities that have undergone several mass drug administrations? 2. Is there an interplay between intermediate host exposure to praziquantel and the development of praziquantel drug resistance in the definitive host?

## Keywords

NTDs, Neglected Tropical Diseases, PZQ Resistance, Diagnostic Tool, Schistosomiasis, MDA (Mass Drug Administration), CRISPR/Cas 9

## Introduction

Infection by blood flukes causes schistosomiasis. Five species of the genus *Schistosoma* infect humans: *S. intercalatum*, *S. japonicum*, *S. mekongi*, *S. haematobium*, and *S. mansoni*. *Schistosoma haematobium*, *S. mansoni*, and *S. japonicum* are the most prevalent species causing human infection. Sites of infection and symptoms depend on the *Schistosoma* species, which can occur in the intestines, bile ducts, and bladder (Nelwan 2019).

According to the World Health Organization (WHO), infection occurs with exposure to schistosome cercariae-infested fresh water while engaging in mundane household, economic, recreational and agricultural activities (WHO 2013). Over 54 million people are infected annually with school-aged children having the highest rates of infection and severity in the Middle East and Sub-Saharan Africa. In 2019, an estimated 236.6 million individuals needed treatment for schistosomiasis, which ranks second after malaria in human parasitic diseases (Mnkugwe et al. 2020). Approximately 280,000 deaths annually in Africa, Asia, South America and some Caribbean Islands are due to schistosomiasis (Aula et al. 2021).

The life cycle of the parasite is asexual and sexual, depending on two hosts (freshwater snails and mammals). Beginning with miracidia transformation into sporocyst then developing into cercariae to end the asexual stage in the snail. When mammals come in contact with cercariae, it penetrates the skin and sheds off the bifurcated tail, becoming schistosomula which then migrates via venous circulation to the lungs, develops into adult worms and exits the liver via the portal vein system. Male and female worms copulate and reside in the mesenteric venules, the location of which varies by species. The females deposit eggs in the small venules of the portal and perivesical systems (Nelwan 2019). Many of these eggs are moved progressively toward the lumen of the intestine (*S. mansoni*) and the bladder and ureters (*S. haematobium*), through the walls of the blood vessels, and intestines, to be passed out of the body in feces or urine. The eggs hatch in contact with fresh water and release miracidia, which penetrate specific snail intermediate hosts.

The host's tissue secretes enzymes that trigger inflammatory and granulomatous reactions around eggs lodged in the liver and gut, resulting in intestinal, urogenital, or hepatosplenic and neurological schistosomiasis (Colley et al. 2014; Ross et al. 2015). This study focuses on *S. mansoni* infection (intestinal Schistosomiasis) with symptoms of abdominal pain, blood in stool and diarrhea (Anderson and Enabulele 2021). The detection of *S. mansoni* infection is by identification of parasite ova using Kato-Katz technique (Katz et al. 1972).

In Ghana, information on *S. mansoni* is sparse due to under-reporting by infected people at health centers. Armoo et al. (2020) suggest that surveillance via Kato-Katz could underestimate the disease prevalence and intensity and thus more sensitive tools are required. They state that molecular-based and serological methods exist for the detection

of *S. mansoni* infection, however, these require highly skilled personnel and showed the effective use of circulating cathodic antigen test as an alternative.

The WHO recommends the use of praziquantel (PZQ) for the treatment and control of schistosomiasis. Preventive chemotherapy is done by annual/ bi-annual Mass Drug Administration (MDA) programs in a dose of 40mg/kg. The use of PZQ for MDAs in Sub-Saharan Africa began in 2003 and in Ghana in 2008 and over 30 years in other parts of the world (Cunningham et al. 2020) . Current efforts to eliminate schistosomiasis, a public health problem, have seen the augmentation of MDA campaigns which could increase drug pressure favoring resistant phenotype selection. The basis of PZQ resistance, still lacks concord however, there are emerging reports of reduced drug efficacy, low cure rates, induced laboratory resistance, emphasizing the need to investigate PZQ resistance ( Summers et al. 2022).

## Background

### ***Schistosoma mansoni* infection**

*S. mansoni* infection in humans begins with bifurcated-tailed cercariae penetrating the skin and transforming into schistosomulum. This causes a host-enzymatic reaction, resulting in dermatitis. The larvae pairs, male and female, move from the skin and the worm matures in the liver, where they lodge in the mesenteric venules (Skelly 2013). The female produces approximately 300 eggs daily. The eggs cross the intestinal mucosa, reaching the lumen, and are eliminated with faecal material. The worms survive for up to 15 years, relying on host immune evasion mechanisms. The eggs that pass out hatch in freshwater and penetrate snails, the intermediate host. In the snail, asexual reproduction occurs, and cercariae emerge (Boros 1989).

Infection can be acute or chronic. Acute schistosomiasis occurs during a new infection and may be void of clinical manifestations. However, some people may have symptoms like fever, anorexia, weakness, diarrhea, and bloody stool. Symptoms can easily be mistaken for other tropical diseases, like typhoid fever. The chronic phase occurs due to the parasite's ability to lodge in organs like the liver and intestines. Granulomas are observed when an acute phase progresses to a chronic phase (Silva et al. 2005).

Since 1954, there has been documented evidence of *S. mansoni* in Ghana. Infection was thought to be confined to Tarkwa and Bogoso in the west, Bawku and Wiaga near Navrongo, and Nyive and Atikpui in the Volta region. McCullough (1965) suggested that the disease would spread in the absence of strict control measures (McCullough 1965). Presently, schistosomiasis can be identified across Ghana. Armoo et al. (2020) detected *S. mansoni* in Tomefa, Torgakope, and Manheam in the south of Ghana, near the Weija dam, with a prevalence as high as 90% in some communities. Anyan et al. (2019) also detected dual infections of *S. haematobium* and *S. mansoni* in Tomefa, with a *S. mansoni* prevalence of approximately 23%. In a study by Asuming-Brempong et al. (2022) investigating IgE responses to PZQ treatment, they observed that a few individuals in their cohort remained infected after treatment, and though they could not fully ascertain the

cause, they hypothesized that elevated levels of IgE could be linked with susceptibility to reinfection.

There is clear evidence of *S. mansoni* infection in Ghana with such serious pathological outcomes, and this disease should be eliminated urgently. Despite ongoing MDA programmes with PZQ, researchers continue to detect *S. mansoni* in different study areas. It will be important to investigate if there is a developing resistance and suboptimal response to PZQ.

### Reported Reduced Praziquantel Efficacy

The success of experimental induction of resistance raises concerns over drug efficacy. Alonso et al. (2006) show that the standard PZQ treatment failed to clear schistosomiasis in two Spanish travelers. It has also been reported that patients from parts of Egypt and Senegal show high tolerance for PZQ in a passage experiment, concluding that some level of PZQ resistance may occur. This highlights the need for surveillance as the use of PZQ intensifies (Doenhoff et al. 2002).

Crellen et al. (2016), reported reduced efficacy of PZQ in *S. mansoni* infections correlating with multiple rounds of MDA. In a study in Uganda, analysis of egg reduction rates (ERR) showed significantly reduced cure percentages in school-aged children that had received higher rounds of MDA. They conclude that increased MDA exposure could jeopardize the efficacy of schistosomiasis control initiatives. Gryseels et al. (2001), on the other hand, reviewed the poor response to PZQ in Senegal and observed low cure rates of about 30% despite increasing PZQ dosage. They, however, stated that the reduced efficacy of PZQ could be based on pre-treatment intensity or age-related factors and concluded that the evidence for PZQ resistance is not persuasive. Though studies report reduced ERR, clearly stated evidence for PZQ resistance is lacking. Exploring the mechanism of PZQ action and the cause of reduced ERR could elucidate the phenomenon of suboptimal response, reduce cure rates, and/or PZQ resistance.

### Mechanism of action of PZQ

The mechanism of action of PZQ at the molecular level remains unknown, though it is widely used for schistosomiasis treatment. Praziquantel has been suggested to play a role in the muscular contraction and calcium influx of schistosomes (Angelucci et al. 2007). Park et al. (2019), also suggest that PZQ results in calcium influx leading to paralysis of the adult worm and show that PZQ activates the schistosome transient receptor potential channel, consistent with known effects of PZQ.

Considering the wide use of Praziquantel as the exclusive treatment essential to reducing morbidities associated with schistosomiasis, emerging resistance is perturbing. Experimentally, resistance of *S. mansoni* to PZQ can be induced and demonstrated in the field, though this mechanism remains unclear (Wang et al. 2012). There are gaps in knowledge on its mechanism of action, and continuous use has led to the notion of possible resistance, though not clearly stated.

## Molecular Determinants of PZQ resistance

To study PZQ resistance, it is essential to investigate genes that play a role in resistance mechanisms. Messerli et al. (2009), examine the relational effect of P-glycoprotein expression in *S. mansoni* and PZQ; they show an increased expression of SMDR2 RNA and anti-P-glycoprotein immunoreactive protein in an Egyptian *S. mansoni* isolate with a suboptimal response to PZQ. They conclude that in PZQ resistance, multidrug resistance proteins could be implicated. Research by Park and colleagues, as well as Clec'h and team, identified a transient receptor potential melastatin ion channel (TRPM<sub>pzq</sub>) as the target of PZQ in *S. mansoni*. They stated that this could be an important approach to monitoring PZQ resistance in field settings and could be employed in schistosome elimination programs (Clec'h et al. 2021).

Pereira et al. (1998) compared the genetic differences between PZQ-resistant and susceptible strains of *S. mansoni* and found that SMDR2 was not overexpressed in the PZQ-resistant strain; however, they found SCOX1 to be overexpressed about 5-fold in the resistant strain. Messerli and colleagues in 2009 postulated that increased SMDR2 expression could be linked to PZQ resistance in *S. mansoni*. It could be argued that in 1998, available tools to study differential gene expression were not advanced, and some observations could be missed.

Abou-El-Naga et al. (2019), investigated *S. mansoni* decreased sensitivity to PZQ at the proteome level. Morphologically, eggs from PZQ-resistant worms were spherical and smaller when compared to those from PZQ-susceptible worms. Pinto-Almeida et al. (2015), also use a proteomic approach in investigating PZQ-resistant and susceptible strains. They found some proteins present in parasites not exposed to PZQ but absent in parasites exposed to PZQ. They concluded that the role of these proteins in resistance is uncertain and needs to be verified.

Reduced sensitivity to PZQ and resistance in *S. mansoni* can be observed at the morphological and molecular level, with several genes and proteins hypothesized to play roles in these mechanisms. However, the challenge remains accurately using this information to monitor emerging resistance in the field.

## Diagnostic approaches for PZQ resistance

There exist various methods that aid researchers in identifying laboratory-induced PZQ resistant strains. Liang et al. (2010), report a method where tail-shedding cercariae can be detected with a dissecting microscope in a time-dependent manner following PZQ treatment. They reveal that male cercariae shed their tails quicker than females, and this is not seen in PZQ-resistant strains. Melman et al. (2009), in a study in Kenya, employed an in vitro assay where they measured the length of adult worms to identify susceptible and resistant populations. They state that there is a potential for resistance, and it should be critically examined for treatment programs. Tushabe et al. (2020), on the other hand, investigated a schistosomiasis hotspot in Uganda enrolled in an MDA program using the

Egg Reduction Rate (ERR) technique based on a 3 sample Kato-Katz and CAA dipstick and found that the ERR was over 80% and concluded that there was no exact evidence of reduced PZQ efficacy but encouraged continuous monitoring. According to Botros and Bennett (2007), although there is almost no field evidence that schistosomes have gained reduced sensitivity to PZQ, less than 100% cure rates are apparent; additionally, the sensitivity of available egg-counting techniques may be overestimated. Thus, PZQ effectiveness should be routinely assessed. Aside from morphological techniques, there are few molecular tools developed for the detection of PZQ resistance.

## Aim

Assessing the prevalence of praziquantel-resistant *S. mansoni* among school-aged children in schistosomiasis-endemic communities in Ghana and also developing a diagnostic tool to aid in field assessment of drug resistance.

## Objectives

1. Screening and validation of genes associated with Praziquantel resistance in an *in-silico* analysis; *S. mansoni* field isolates and cercariae shed from field-captured *Biomphalaria* sp.
2. Identify markers of interest in laboratory-induced resistant schistosomiasis models for intermediate (snails) and definitive hosts (mouse model).
3. Investigate the effects of PZQ resistance via mediated genome editing with functional markers from Objective 1.
4. Molecular diagnostic tool development with selected resistant markers from Objective 1 and 3
5. Assessing the developed diagnostic tool sensitivity in the detection of *S. mansoni* and PZQ-resistant *S. mansoni* strains.

## Problem Statement and Rationale

In 2012, the WHO came up with a roadmap for eliminating Neglected Tropical Diseases (NTDs), schistosomiasis. This was, however, not achieved, and a new roadmap for 2030 has been drawn (WHO 2020). This indicates a continued disease presence, and with no vaccine available, praziquantel remains the sole drug for treatment. Praziquantel has been in use for over 30 years, and suboptimal responses and some scattered cases of drug resistance have been reported (King et al. 2011). In addition, the mechanism of action of praziquantel remains unknown, and concerns about the decline in the effectiveness of MDA keep increasing (Vale et al. 2017). Apart from measuring egg cure rates, there are few reported molecular markers to measure PZQ resistance.

Complete cure rates following PZQ treatment are rare, and field data on schistosomes becoming less sensitive to treatment increases (Lamberton et al. 2017). Considering the sensitivity of the majority of diagnostic methods, including Kato Katz (gold standard), the

information on cure rates in endemic communities may be exaggerated. The effectiveness of PZQ needs to be routinely assessed, and the incidence, epidemiological, genetic, and mechanism of resistance ascertained by investigating field isolates. The best approach to tackle this is summarized in Fig. 1.

## Methodology

### Study Design

#### ***In-silico* search for genes associated with praziquantel resistance**

An online search strategy to obtain genes associated with praziquantel resistance would be adopted. PubMed and google scholar databases would be used. The results will be reviewed to eliminate unsuitable hits. Table 1 shows the search strategy to be adopted.

After selection, genes would be screened to ascertain functional validity with bioinformatics tools such as: DynaMut (Rodrigues et al. 2018), Mutplot (Zhang et al. 2019), and Uniprot (The UniProt Consortium 2021). Primers would be designed from the selected genes employing a multiplex approach. The selected resistant gene panel would be used to screen *S. mansoni* field isolates and cercariae shed from *Biomphalaria sp.*

### Ethical Approval

Ethical clearance would be obtained from the Institutional Review Board (IRB) of the Council for Scientific and Industrial Research—Water Research Institute (CSIR-WRI) and the Ethics Committee for Basic and Applied Sciences (ECBAS), as well as from the Ghana Health Service.

### Study population and sample collection

A full outline of the study is summarized in Fig. 2. The study would be conducted in two communities (Torgakope and Tomefa) in the Ga South Municipality of the Greater Accra Region. The sample population would be school-aged children. A written consent will be obtained from participants and guardians for the provision of stool samples. Incentives would be provided for study participants. Participants would be resampled 12 months after community-wide treatment, during which there would be an annual MDA. This sampling period would be used to develop the diagnostic tool. To ensure successful community participation, durbars would be organized in conjunction with community elders, school heads, and the Ghana Health Service to educate the communities on schistosomiasis and explain the project in detail.

A total of 634 school-aged children (300 from Tomefa and 334 from Torgakope) would be sampled based on *S. mansoni* prevalence rates (78% and 30%, respectively) in the Cunningham et al. (2020) study. A single fecal sample would be collected at each sampling time and divided into three aliquots kept in cryovials and stored at -80 °C for downstream



analysis. *Biomphalaria* sp. would be collected from water contact points in the communities. Snails would be kept under optimal conditions and fed, as well as induced to shed miracidia.

### **Identification of resistance markers in lab-generated resistance models for intermediate (snail), definitive host (mice), and eggs**

Kato-Katz and PCR will be run to determine *S. mansoni* positivity. DNA extraction (from stool samples and cercariae shed from snails) and qPCR will be run for *S. mansoni* and resistant genes detection. Isolated eggs from Kato-Katz-positive samples and shed miracidia would be used for downstream analysis.

Snails that do not shed cercariae after 5 to 6 weeks of exposure will be infected with *S. mansoni* miracidia and treated with PZQ. Infected snails and non-infected snails treated with PZQ would be compared for possible resistance markers. Eggs from Kato-Katz positive samples would be put in a 96-well plate and treated with different concentrations of PZQ. Mice would be infected with cercariae to generate Schistosomiasis definitive host models and treated versus untreated group compared utilizing eggs isolated from fecal material and adult worms from intestines of sacrificed mice. Groups of susceptible versus resistant would be compared with a gene panel from selected genes using qPCR as well as RNA-seq to identify novel differentially expressed genes for both in vitro and in vivo assays.

### **Investigation of effects of PZQ resistance via CRISPR-Cas9-mediated genome editing with functional markers from objective 1**

Gene Knock-in/ Knock-out into the genome of the egg of *Schistosoma mansoni* will be executed via CRISPR-Cas9 with selected resistance markers, and successful transfection will be assessed. Edited schistosome eggs will be hatched into miracidia, which will be used to infect snails and subsequently produce cercariae. The cercaria will be injected into the tails of experimental mice, and comparisons will be made between treated and untreated mice following PZQ treatment or its absence to gain a preliminary insight into the mechanism of PZQ resistance.

### **Molecular diagnostic tool development with selected resistant markers**

Colorimetric Loop-mediated Isothermal Amplification (LAMP) assay using six primers with one generic *S. mansoni* and 2 resistance gene markers would be developed. Validation of the generic outer primers will be done via PCR with CRISPR-Cas9 strains, crude stool extracts, and purified extracted DNA. Primers will be designed with Explorer version 5. The Colorimetric assay will be achieved by adding phenol and hydroxy naphthol blue with a pH change resulting in a color change as an indication of positivity.

## Field testing and assessment of the developed diagnostic tool in selected schistosomiasis-endemic communities

Crude egg extract from Kato-Katz detection on the field during the second sampling period would be used to carry out the field-testing assessment. Samples would also be collected and confirmed in the laboratory. Resistant samples would be sequenced to ascertain which genes mediate resistance, and PCR with a resistance gene panel would also be carried out.

### Data Analysis

The prevalence of *S. mansoni* will be determined with its corresponding 95% confidence interval. The intensity of the *S. mansoni* infection will be determined by WHO guidelines (WHO 2002). The arithmetic mean egg count will be calculated as the average egg count of the two Kato-Katz smears. Demographic data will be presented as frequency and proportions of population of subjects in each category. The prevalence and intensity of *S. mansoni* infection will be stratified by age, gender, and community using the Chi-square, t-test, and Mann-Whitney tests, where appropriate.

PZQ-resistance would be scored as a binary trait such that parasites that recover from drug treatment are resistant and those that fail to recover are designated as susceptible. Field applicability of the developed tool would be measured via comparison to Kato Katz to determine positivity of crude samples using the tool and PCR with resistance gene panel.

The complete workflow for the proposed methodology is summarised in Fig. 2.

### Expected Outcomes

1. This study would reveal the prevalence of praziquantel resistance in *S. mansoni* infection at the study site.
2. The presence of specific genes linked to PZQ resistance as well as additional genes which play a role would be investigated.
3. A field diagnostic tool for praziquantel resistance detection would be developed and evaluated in both laboratory and field settings.

### Author contributions

Maame Ekuia Acquah: Conceptualization, Writing and Drafting, Methodology, Idea development

Frank Twum Aboagye: Methodology, Review, Editing and Feedback, Idea development

Yvonne Ashong: Methodology Review and Feedback, Review and Editing , Contribution to Background and References

Lydia Mosi: Review and Feedback

## Conflicts of interest

The authors have declared that no competing interests exist.

## References

- Abou-El-Naga I, Amer E, Boulos L, El-Faham M, Abou Seada N, Younis S (2019) Biological and proteomic studies of *Schistosoma mansoni* with decreased sensitivity to praziquantel. *Comparative Immunology, Microbiology and Infectious Diseases* <https://doi.org/10.1016/j.cimid.2019.101341>
- Alonso D, Muñoz J, Gascón J, Valls ME, Corachan M (2006) Failure of standard treatment with praziquantel in two returned travelers with *Schistosoma haematobium* infection. *Am J Trop Med Hyg* 74 (2): 342-4.
- Anderson TC, Enabulele E (2021) *Schistosoma mansoni*. *Trends in Parasitology* 37 (2): 176-177. <https://doi.org/10.1016/j.pt.2020.06.003>
- Angelucci F, Basso A, Bellelli A, Brunori M, Pica Mattoccia L, Valle C (2007) The anti-schistosomal drug praziquantel is an adenosine antagonist. *Parasitology* 134 (9): 1215-1221. <https://doi.org/10.1017/S0031182007002600>
- Anyan WK, Pulkkila B, Dyra C, Price M, Naples J, Quartey J, Anang A, Lodh N (2019) Assessment of dual schistosome infection prevalence from urine in an endemic community of Ghana by molecular diagnostic approach. *Parasite Epidemiology and Control* 9 <https://doi.org/10.1016/j.parepi.2019.e00130>
- Armoo S, Cunningham LJ, Campbell SJ, Aboagye FT, Boampong FK, Hamidu BA, Osei-Atweneboana MY, Stothard JR, Adams ER (2020) Detecting *Schistosoma mansoni* infections among pre-school-aged children in southern Ghana: a diagnostic comparison of urine-CCA, real-time PCR and Kato-Katz assays. *BMC Infect Dis* 20 (1). <https://doi.org/10.1186/s12879-020-05034-2>
- Asuming-Brempong E, Ayi I, der Puije Wv, Gyan B, Larbi A, Ashong Y, Otchere J, Frempong NA, Quartey J, Jones F, Wilson S, Dunne D, Boakye D (2022) Increased ShtAL1 IgE responses postPraziquantel treatment may be associated with a reduced risk to re-infection in a Ghanaian *S. haematobium*-endemic community. *PLOS NEGLECTED TROPICAL DISEASES* <https://doi.org/10.1371/journal.10.1371/journal.pntd.0010115>
- Aula OP, McManus D, Jones M, Gordon C (2021) Schistosomiasis with a Focus on Africa. *Tropical Medicine and Infectious Disease* 6 (109). <https://doi.org/10.3390/tropicalmed6030109>
- Boros D (1989) Immunopathology of *Schistosoma mansoni* Infection. *Clinical Microbiology Reviews* 2 (3DOV L. BOROS): 250-269.
- Botros S, Bennett SJL (2007) Praziquantel resistance. *Expert Opin. Drug Discov* 2.

- Clec'h WL, Chevalier F, A. Mattos AC, Strickland A, Diaz R, McDew-White M, Rohr C, Kinung'hi S, Allan F, Webster B, Webster J, Emery A, Rollinson D, Djirmay AG, Al Mashikhi K, Yafae SA, Idris M, Moné H, Mouahid G, LoVerde P, Marchant J, Anderson TC (2021) Genetic analysis of praziquantel response in schistosome parasites implicates a transient receptor potential channel. *Science Translational Medicine* 13 <https://doi.org/10.1126/scitranslmed.abj9114>
- Colley D, Bustinduy A, Secor WE, King C (2014) Human schistosomiasis. *The Lancet* 383 (9936): 2253-2264. [https://doi.org/10.1016/s0140-6736\(13\)61949-2](https://doi.org/10.1016/s0140-6736(13)61949-2)
- Crellen T, Walker M, Lamberton PL, Kabatereine N, Tukahebwa E, Cotton J, Webster J (2016) Reduced Efficacy of Praziquantel Against *Schistosoma mansoni* Is Associated With Multiple Rounds of Mass Drug Administration. *Clinical Infectious Diseases* <https://doi.org/10.1093/cid/ciw506>
- Cunningham LJ, Campbell SJ, Armoo S, Koukounari A, Watson V, Selormey P, Stothard JR, Idun B, Asiedu M, Ashong Y, Adams ER, Osei-Atweneboana MY (2020) Assessing expanded community wide treatment for schistosomiasis: Baseline infection status and self-reported risk factors in three communities from the Greater Accra region, Ghana. *PLoS Negl Trop Dis* 14 (4). <https://doi.org/10.1371/journal.pntd.0007973>
- Doenhoff M, Kusel J, Coles G, Cioli D (2002) Resistance of *Schistosoma mansoni* to praziquantel: is there a problem? *Transactions of the Royal Society of Tropical Medicine and Hygiene* 96 (5): 465-469. [https://doi.org/10.1016/s0035-9203\(02\)90405-0](https://doi.org/10.1016/s0035-9203(02)90405-0)
- Gryseels B, Mbaye A, De Vlas SJ, Stelma FF, GuisseÂ F, Lieshout LV, Faye D, Diop M, Ly A, Tchuem-TchuenteÂ LA, Engels D, Polman K (2001) Are poor responses to praziquantel for the treatment of *Schistosoma mansoni*. *Trop Med Int Health* 6 (11): 864-873. [In English]. <https://doi.org/10.1046/j.1365-3156.2001.00811.x>
- Katz N, Chaves A, Pellegrino J (1972) A simple device for quantitative stool thick-smear technique in *Schistosomiasis mansoni*. *Rev Inst Med Trop Sao Paulo* 14 (6): 397-400.
- King CH, Olbrych SK, Soon M, Singer ME, Carter J, Colley DG (2011) Utility of repeated praziquantel dosing in the treatment of schistosomiasis in high-risk communities in Africa: a systematic review. *PLoS Negl Trop Dis* 5 (9). <https://doi.org/10.1371/journal.pntd.0001321>
- Lamberton PHL, Faust CL, Webster JP (2017) Praziquantel decreases fecundity in *Schistosoma mansoni* adult worms that survive treatment: evidence from a laboratory life-history trade-offs selection study. *Infect Dis Poverty* 6 (1). <https://doi.org/10.1186/s40249-017-0324-0>
- Liang Y-, WW, Dai J-, Li H-, Tao Y-, Zhang J-, WL, Zhu Y-, Coles GC, Doenhoff MJ (2010) Susceptibility to praziquantel of male and female cercariae of praziquantel-resistant and susceptible isolates of *Schistosoma mansoni*. *J Helminthol* 84: 202-207. [In English]. <https://doi.org/10.1017/S0022149X0999054X>
- McCullough F (1965) A note on intestinal schistosomiasis and the snail hosts in Ghana. *Annals of Tropical Medicine & Parasitology* 59 (3): 312-319. <https://doi.org/10.1080/00034983.1965.11686313>
- Melman S, Steinauer M, Cunningham C, Kubatko L, Mwangi I, Wynn NB, Mutuku M, Karanja DS, Colley D, Black C, Secor WE, Mkoji G, Loker E (2009) Reduced Susceptibility to Praziquantel among Naturally Occurring Kenyan Isolates of *Schistosoma mansoni*. *PLoS Negl Trop Diseases* 3 (8). <https://doi.org/10.1371/journal.pntd.0000504>

- Messerli S, Kasinathan R, Morgan W, Spranger S, Greenberg R (2009) Schistosoma mansoni P-glycoprotein levels increase in response to praziquantel exposure and correlate with reduced praziquantel susceptibility. *Molecular & Biochemical Parasitology* 167: 54-59. <https://doi.org/10.1016/j.molbiopara.2009.04.007>
- Mnkugwe RH, Minzi OS, Kinung'hi SM, Kamuhabwa AA, Akillu E (2020) Prevalence and correlates of intestinal schistosomiasis infection among school-aged children in North-Western Tanzania. *PLoS One* 15 (2). <https://doi.org/10.1371/journal.pone.0228770>
- Nelwan ML (2019) Schistosomiasis: Life Cycle, Diagnosis, and Control. *Curr Ther Res Clin Exp* 91: 5-9. <https://doi.org/10.1016/j.curtheres.2019.06.001>
- Park SK, Gunaratne GS, Chulkov EG, Moehring F, McCusker P, Dosa PI, Chan JD, Stucky CL, Marchant JS (2019) The anthelmintic drug praziquantel activates a schistosome transient receptor potential channel. *J Biol Chem* 294 (49): 18873-18880. <https://doi.org/10.1074/jbc.AC119.011093>
- Pereira C, Fallon PG, Cornette J, Capron A, Doenhoff MJ, Pierce RJ (1998) Alterations in cytochrome-c oxidase expression between praziquantel-resistant and susceptible strains of *Schistosoma mansoni*. *Parasitology* 117 (1): 63-73. <https://doi.org/10.1017/S003118209800273X>
- Pinto-Almeida A, Mendes T, Armada A, Belo S, Carrilho E, Viveiros M, Afonso A (2015) The Role of Efflux Pumps in *Schistosoma mansoni* Praziquantel Resistant Phenotype. *PLOS One* <https://doi.org/10.1371/journal.pone.0140147>
- Rodrigues CH, Pires DE, Ascher DB (2018) DynaMut: predicting the impact of mutations on protein conformation, flexibility and stability. *Nucleic Acids Research* 46 <https://doi.org/10.1093/nar/gky300>
- Ross AG, Olveda RM, Chy D, Olveda DU, Li Y, Harn DA, Gray DJ, McManus DP, Tallo V, Chau TN, Williams GM (2015) Can mass drug administration lead to the sustainable control of schistosomiasis? *J Infect Dis* 211 (2): 283-9. <https://doi.org/10.1093/infdis/jiu416>
- Silva LCD, Chieffi PP, Carrilhoa FJ (2005) Schistosomiasis mansoni – Clinical features. *Gastroenterol Hepatol* 28 (1): 30-39. <https://doi.org/10.1157/13070382>
- Skelly PJ (2013) The use of imaging to detect schistosomes and diagnose schistosomiasis. *Parasite Immunol* 35 (9-10): 295-301. <https://doi.org/10.1111/pim.12040>
- Summers S, Bhattacharyya T, Allan F, Stothard JR, Edielu A, Webster B, Miles M, Bustinduy A (2022) A review of the genetic determinants of praziquantel resistance in *Schistosoma mansoni*: Is praziquantel and intestinal schistosomiasis a perfect match? *Frontiers in Tropical Diseases* 3 <https://doi.org/10.3389/fitd.2022.933097>
- The UniProt Consortium (2021) UniProt: the universal protein knowledgebase in 2021. *Nucleic Acid Research*. URL: <https://doi.org/10.1093/nar/gkaa1100>
- Tushabe JV, Lubyayi L, Sserubanja J, Kabuubi P, Abayo E, Kiwanuka S, Nassuuna J, Kaweesa J, Corstjens P, Dam Gv, Sanya R, Ssenyonga W, Tukahebwaa EM, Kabaterene N, Elliott A, Webb8 , (2020) Does Intensive Treatment Select for Praziquantel Resistance in High-Transmission Settings? *Parasitological Trends and Treatment Efficacy Within a Cluster-Randomized Trial*. *Open Forum Infectious Diseases* <https://doi.org/10.1093/ofid/ofaa091>
- Vale N, Gouveia MJ, Rinaldi G, Brindley PJ, Gartner F, Correia da Costa JM (2017) Praziquantel for Schistosomiasis: Single-Drug Metabolism Revisited, Mode of Action,

- and Resistance. *Antimicrob Agents Chemother* 61 (5). <https://doi.org/10.1128/AAC.02582-16>
- Wang W, Wang L, Liang Y (2012) Susceptibility or resistance of praziquantel in human schistosomiasis: a review. *Parasitology Research* 111 (5): 1871-1877. <https://doi.org/10.1007/s00436-012-3151-z>
  - WHO (2002) Prevention and control of schistosomiasis and soil-transmitted helminthiasis: Report of a WHO expert committee. World Health Organization
  - WHO (2013) Schistosomiasis: Progress report 2001–2011 and Strategic plan 2012–2020. World Health Organization.
  - WHO (2020) Ending the neglect to attain the Sustainable Development Goals A road map for neglected tropical diseases 2021–2030.
  - Zhang W, Wang C, Zhang X (2019) Mutplot: An easy-to-use online tool for plotting complex mutation data with flexibility. *PloS one*. URL: <https://doi.org/10.1371/journal.pone.0215838>

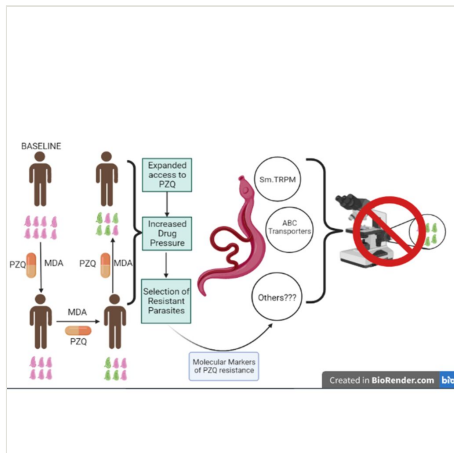


Figure 1.

Graphical Abstract. Adapted from Summers et al. (2022), available under the terms of the [Creative Commons Attribution License 4.0 \(CC BY 4.0\)](https://creativecommons.org/licenses/by/4.0/).

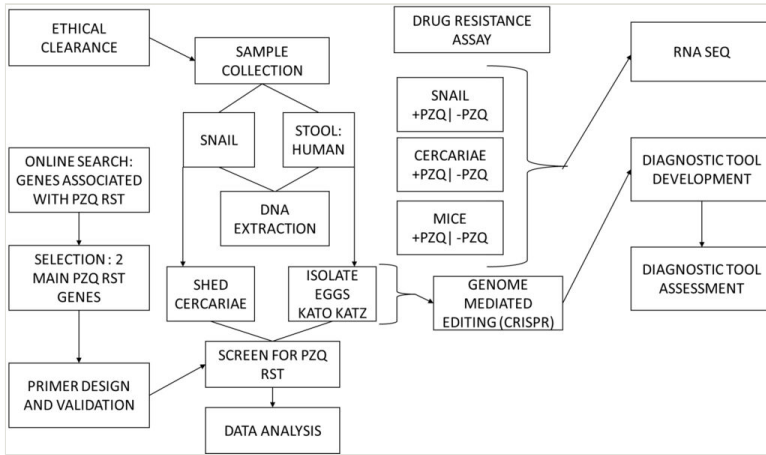


Figure 2.  
Study Design: Workflow of the proposed research.



Table 1.

Online search strategy for genes associated with praziquantel resistance.

Proposed Outcome	Search Items	Total References	Selected References	Final Selected