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Factors influencing the efficacy of praziquantel in a schistosome-exposed population

Grace Zdesenko

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Declaration

I declare that this thesis has been composed solely by myself unless otherwise stated. Where others have contributed to aspects of the work, this is clearly stated by acknowledgement in the accompanying text. This thesis has not been submitted in whole or in part for any other degree or professional qualification. Any contribution from colleagues and the field team, such as gathering participant data, sample collection and processing for storage, as well as parasitology diagnosis for large volumes of samples, are referenced in the text as appropriate. For the work described in **Chapters 4 and 5**, the genomic sequencing of DNA samples was carried out by the Beijing Genomics Institute (BGI, Shenzhen, China). For the collection of samples in **Chapter 5**, I played a large role in the preparation of the fieldwork, including the protocol design and collection of resources, however the samples were collected by a team from the University of Zimbabwe. Further, the mass spectrometry of the dried blood spot extractions in **Chapter 5** was conducted by Glasgow Polyomics (University of Glasgow), however the analysis of the resultant data was my own work. The collection of the Zimbabwean mass drug administration data for **Chapter 6** was also collected by a team from the University of Zimbabwe during the national helminth program and was shared in collaboration with the University of Edinburgh; all other analyses are entirely my work. All publications included in this thesis are my own work and have been explicitly referenced in the text as appropriate. Copies of the publications presented in **Chapters 3 and 4** are included within the thesis as a data chapter, with an introduction, aims/objectives, and a key findings/conclusion section adjoining.

Grace Zdesenko, November 2022

Abstract

Urogenital schistosomiasis, caused by the *Schistosoma haematobium* parasite, is a global cause of morbidity and mortality and affects millions of people each year. The mass drug administration (MDA) of praziquantel (PZQ) is a vital intervention to treat schistosome infections and eliminate schistosomiasis as a public health problem. After decades of use, variable PZQ efficacy and persistent schistosome infections have been reported across multiple schistosome-endemic African countries. However, there is a paucity of information on the factors that influence the efficacy of a PZQ treatment and contribute to the persistence of infection, particularly in schistosome-exposed populations where the drug is commonly used.

To address this, I examined the factors that influence individual responses to PZQ and how these contribute to variable PZQ efficacy. This focused on alterations to PZQ metabolism, which regulates the concentration of the schistosome-killing PZQ, and thus can be a crucial determinant of PZQ efficacy and adverse drug reactions (ADRs). During a review of published studies, I identified several drug and host-related factors, such as drug-drug interactions (DDIs) and the liver's capacity to metabolise PZQ, that influenced the systemic concentrations of PZQ via altered PZQ metabolism, and discussed the resultant impact on PZQ efficacy. This review also highlighted gaps in the research regarding pharmacogenetic (PGx) and metabolomic studies. Consequently, I characterised PGx variations in PZQ-metabolising cytochrome P450 (CYP) enzymes and determined associations between each detected variant and the efficacy of PZQ treatment in *S. haematobium*-infected Zimbabweans. Four single nucleotide polymorphisms (SNPs) across the *CYP1A2*, *CYP2D6* and *CYP3A5* enzymes were significantly associated with PZQ treatment outcome, including genotypes that increased the odds of an individual clearing or not clearing schistosome

infection. A further study using *in vivo* analyte concentrations detected no associations with PZQ efficacy. Yet, there were significant associations between variants in the *CYP1A2* and *CYP2C9* enzymes and *in vivo* analyte concentrations indicative of increased metabolism and decreased PZQ exposure. Both PGx studies provided insight into the drug-gene interactions in schistosome-infected patients during a PZQ treatment and suggested that the PGx impact on PZQ exposure and efficacy may be underestimated in the diverse African populations where PZQ is utilised.

To determine if variable PZQ efficacy and persistent schistosome infections occurred during MDAs in Zimbabwe, I identified persistent hotspots of *S. haematobium* infection prevalence (PPHS) and hotspots of decreasing efficacy of PZQ (EPHS). Further, the risk factors of hotspot emergence were evaluated, and EPHS were not identified as a primary cause for PPHS based on these analyses. Initial infection intensity was significantly higher in PPHS than in responder districts, providing valuable information on the possibility of early identification of persistent schistosome infections to improve on current control strategies. However, there was no clear predictor of EPHS occurrence.

Overall, this thesis highlighted key factors that influence an individual's response to a PZQ treatment, including multiple PGx determinants which were previously underreported. Together, this thesis produced significant novel data towards the characterisation of the host factors that contribute towards variable PZQ efficacy, and in the identification of hotspots of persistent infections. Together, these findings will inform policymakers on the factors that influence PZQ efficacy to improve schistosomiasis control and eliminate this disease.

Lay abstract

Schistosomiasis is a neglected tropical disease caused by parasitic worms and is contracted from contact with contaminated freshwater. Urogenital schistosomiasis, a common form of the disease, is caused by the *Schistosoma haematobium* parasite and is prevalent in Sub-Saharan Africa. Globally, control of this disease relies heavily on the drug praziquantel (PZQ) to kill the parasites. However, reports of people not being cured after PZQ treatment have raised concerns about drug efficacy, persistent infections, and the causes behind these. There is a lack of information on the role the host plays in PZQ efficacy, particularly in African populations where the drug is commonly used. Specifically, there have been few studies evaluating how human drug metabolism, which mediates the removal of the schistosome-killing PZQ, impacts the success of a schistosomiasis treatment with PZQ.

Therefore, I examined the factors that influence a person's response to PZQ, and what contributions this has to the success or failure of PZQ treatment. I reviewed published studies that evaluated responses to PZQ and identified several factors that altered PZQ metabolism and influenced PZQ levels in the body. The results indicated that the changes to efficacious PZQ concentrations were due to factors such as drug formulation, the liver's capacity to metabolise PZQ, as well as interactions with co-administered drugs. This review emphasised the lack of available pharmacogenetic (PGx, how a person's genetics affect drug response) data and a scarcity of what the drug is metabolised into for excretion (metabolites).

Thus, I investigated the PGx variations in the drug-metabolising enzymes that could alter host PZQ concentrations and affect PZQ efficacy. I characterised mutations in the PZQ-metabolising enzymes and detected four that were associated with PZQ efficacy, and two that were associated with decreased PZQ exposure.

Using six years of data collected in Zimbabwe during mass PZQ treatment, I identified hotspots of *S. haematobium* infection prevalence and hotspots of decreased PZQ efficacy. The risk factors of hotspot emergence were also evaluated and decreased PZQ efficacy was not determined to be the cause of the hotspots of *S. haematobium* infection prevalence. However, based on these analyses, the initial intensity of schistosome infection was associated with the hotspot occurrence, providing valuable information on the possibility of early hotspot identification to improve control strategies. No risk factors were associated with hotspots of decreased PZQ efficacy.

Overall, this thesis highlighted key factors that influence an individual's response to a PZQ treatment, including multiple PGx determinants, and performed the first identification of hotspots in Zimbabwe. Together, this thesis produced significant novel data that could further inform policymakers and contribute towards the overall elimination of schistosomiasis.

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Abbreviations

Abbreviation	Definition
ADRs	Adverse Drug Reactions
ANOVA	Analysis Of Variance
AUC	Area Under The Concentration-Time Curve
bp	Base Pairs
BWA-mem	Burrows-Wheeler Aligner
C-trap	Curved Linear Trap
CI	Confidence Interval
C_{max}	Peak Plasma Concentration
CR	Cure Rate
CYP	Cytochrome P450
DALYs	Disability-Adjusted Life Years
DBS	Dried Blood Spots
DDIs	Drug-Drug Interactions
DMPK	Drug Metabolism And Pharmacokinetics
DNA	Deoxyribonucleic Acid
EPHS	Hotspots Of Decreased PZQ Efficacy
ERR	Egg Reduction Rate
GLM	General Linear Models
HILIC	Hydrophilic Interaction Liquid Chromatography
HIV	Human Immunodeficiency Virus
HLM	Human Liver Microsomes
IARC	International Agency For Research On Cancer
HRQoL	Health-Related Quality Of Life
INDELS	Insertions And Deletions
IQR	Interquartile Range

LC-MS	Liquid Chromatography-Mass Spectrometry
LD	Linkage Disequilibrium
LMICs	Low-Middle Income Countries
LM-PCR	Ligation-Mediated Polymerase Chain Reaction
MDA	Mass Drug Administration
mL	Millilitres
MAF	Minor Allele Frequency
MOA	Mechanism Of Action
mg	Milligrams
MS	Mass Spectrometry
m/z	Mass-To-Charge Ratio
NCA	Noncompartmental Analysis
NCBI	National Center For Biotechnology Information
NTD	Neglected Tropical Disease
OOB	Out-Of-Bag
OOB-ER	Out-Of-Bag Predictive Error Rate
PC	Principal Components
PCT	Preventative Chemotherapy
PCA	Principal Component Analysis
PD	Pharmacodynamic
PGx	Pharmacogenetic
PHS	Persistent Hotspots
PK	Pharmacokinetic
PKPD	Pharmacokinetic-Pharmacodynamic
PK-PD-PGx	Pharmacogenetic-Pharmacodynamic-Pharmacogenetic
PPHS	Persistent Hotspots Of *S. Haematobium* Prevalence
PZQ	Praziquantel
PSAC	Preschool-Aged Children

QoL	Quality Of Life
RF	Random Forests
rCYPs	Recombinant Cytochrome P450s
rpm	Revolutions Per Minute
RT	Retention Time
SAC	School-Aged Children
STI	Sexually Transmitted Infection
$t_{1/2}$	Half-Life
TB	Tuberculosis
T_{max}	The Time Taken To Reach Maximum Plasma Concentration
µl	Microlitres
WASH	Water, Sanitation, And Hygiene
WPR	World Population Review
WHO	World Health Organization
°C	Degrees Centigrade

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Chapter 1 - Introduction

1.1 Background

Schistosomiasis, also known as bilharzia or snail fever, is a water-borne neglected tropical disease (NTD) caused by parasitic worms of the genus *Schistosoma* (Gryseels *et al.*, 2006, McManus *et al.*, 2018). Annually, schistosomiasis affects millions of people and is a global cause of morbidity and mortality (WHO, 2022a). This disease disproportionally impacts low-middle-income countries (LMICs), with Sub-Saharan Africa accounting for around 93% of the global cases of schistosomiasis (Onasanya *et al.*, 2021). Schistosomiasis is the second most prevalent parasitic disease in Africa, and in these affected populations the health implications of schistosomiasis begin from a very young age (Colley *et al.*, 2014).

In schistosome-endemic areas, the intensity of infection is typically highest in young people aged 6-20 (Woolhouse, 1998). However, each year an estimated fifty million preschool-aged children (PSAC, ≤5 years old) are also infected worldwide (WHO, 2011b, Mutapi, 2015). During childhood, symptoms associated with schistosome infection include poor growth, poor cognitive development, increased susceptibility to infection, and malnutrition (Woolhouse *et al.*, 2000, Mutapi *et al.*, 2011, Stothard *et al.*, 2013). In later life, the symptoms of chronic schistosome infections include infertility, organ damage, pathology-related cancers, and in the most serious of cases, death (King and Dangerfield-Cha, 2008, McManus *et al.*, 2018). Hence, the disease burden can increase with age until natural worm death, acquired immunity or drug intervention ensues (Colley *et al.*, 2014).

The anthelmintic drug of choice to treat schistosomiasis is praziquantel (PZQ) (WHO, 2013a). Preventative chemotherapy (PCT) programs are utilised to treat schistosome-infected individuals who reside in endemic areas to limit the morbidity and transmission of this

disease (WHO, 2022b). The mass drug administration (MDA) of PZQ occurs during these PCT programs, treating and monitoring schistosome infection in school-aged children (SAC, 6-17 years old) as recommended by the WHO (WHO, 2002b). Nevertheless, there are limitations to the MDA of PZQ during PCT programs. Despite decades of use across the world, PZQ is flawed in its treatment of schistosome infections. Firstly, PZQ is only effective against adult schistosomes, thus a single treatment may not be sufficient to cure individuals who also carry juvenile worm burdens (Secor and Montgomery, 2015). Secondly, the side effects and bitter taste of PZQ contribute to reduced compliance during MDAs, decreasing treatment coverage and benefitting transmission (Omedo *et al.*, 2012). Still, there has been no significant success in discovering new or alternative drugs to overcome the global reliance on PZQ (Aruleba *et al.*, 2019). This is not necessarily due to the technical difficulty in the development of a new drug for this NTD, but rather it is an unattractive pursuit from a market perspective due to insufficient financial incentives and low return on investment (Hussaarts *et al.*, 2017).

Further, PSAC, who are at high risk of infection, are excluded from most PCT control programs despite propagating infection and impairing schistosome control (Stothard *et al.*, 2013, Mutapi, 2015). For many decades, the inequity in PCT treatment for PSAC and the lack of a paediatric formulation of PZQ has hindered the control of schistosomiasis (Stothard *et al.*, 2013, Faust *et al.*, 2020). Presently, a mono-enantiomeric paediatric formulation of PZQ has been developed, however based on the timeline of deployment it will not reach endemic countries in Africa until 2025 (Consortium, 2022a, Lo *et al.*, 2022). Thus, regarding current schistosome control, there are imperfections with the current methods. Yet, one of the most concerning aspects of schistosome control is the requirement for continued efficacy of PZQ treatment during PCT. Persistent schistosome infections and decreased PZQ efficacy have been identified in multiple schistosome-endemic countries during the treatment of

schistosome infections (Danso-Appiah and De Vlas, 2002, Crellen *et al.*, 2016, Kabuyaya *et al.*, 2017, Kittur *et al.*, 2020).

It has been highlighted that an investigation of individuals displaying reduced efficacy is required, particularly in populations being treated for schistosomiasis regularly, to determine the factors that are hindering the continuous control of this parasitic disease (Levecke *et al.*, 2020). In Zimbabwe, a Sub-Saharan African country acting as a case study for this thesis, the national helminth control program utilises MDAs of PZQ to control the highly prevalent *S. haematobium* and less prevalent *S. mansoni* (Mduluza *et al.*, 2020). However, due to the barriers to collecting large-scale or long-term pharmacovigilance data in schistosome-infected populations (Kiguba *et al.*), there is a paucity of research and knowledge on the factors influencing the efficacy of PZQ. By obtaining this information, these factors can be targeted and then addressed, to best optimise the efficacy of a PZQ treatment to prevent the debilitating morbidity of this disease.

There have been multiple reported cases of persistent *S. haematobium* infections after MDAs (Sang *et al.*, 2014, Pennance *et al.*, 2016, King *et al.*, 2020), in addition to numerous individuals failing to clear *S. haematobium* infection after PZQ treatment (Herwaldt *et al.*, 1995, N'Goran *et al.*, 2003, Silva *et al.*, 2005, Ojurongbe *et al.*, 2014). Numerous studies have confirmed there has been no widespread drug resistance in *S. haematobium*, despite over thirty years of use, thus drug resistance against PZQ is unlikely to be a key factor in influencing PZQ efficacy (Secor and Montgomery, 2015). Hence, identifying and targeting the other determinants of variable PZQ efficacy and persistent *S. haematobium* infections are critical in achieving the target of eliminating schistosomiasis as a public health problem by 2030 (WHO, 2020a).

In this chapter, I summarise the current knowledge on schistosome infections, the treatment for schistosomiasis, and the known factors that influence the successful treatment of *S.*

haematobium infection with PZQ. The specific aims and outline of this thesis are also included in this chapter.

1.2 Schistosomiasis

Schistosomiasis is contracted via infection with a parasitic trematode worm of the genus *Schistosoma* (Gryseels *et al.*, 2006). There are different species of these parasitic trematode worms, each with different intermediate hosts, as displayed in **Table 1.1**.

Table 1.1: *Schistosoma* parasite species infective to humans, including the intermediate host and geographical distribution of schistosomiasis (Colley *et al.*, 2014, WHO, 2022a).

Disease	Common Name	Parasite Species	Intermediate Snail Host	Endemic Area
Schistosomiasis	Urogenital Schistosomiasis	*Schistosoma haematobium*	*Bulinus*	Africa, Middle East, Corsica (France)
	Intestinal Schistosomiasis	*Schistosoma mansoni*	*Biomphalaria*	Africa, South America, Caribbean, Middle East
		Schistosoma japonicum	*Oncomelania*	China, Indonesia, Philippines
		Schistosoma mekongi	*Tricula*	Southeast Asia
		Schistosoma guineensis/ intercalatum	*Bulinus* and *B. forskalii*	Rainforest areas of Central and West Africa

The two main forms of schistosomiasis that affect humans are urogenital and intestinal disease (Colley *et al.*, 2014). Africa has the highest global burden of schistosomiasis and hosts the two most prevalent *Schistosoma* species: *S. haematobium* and *S. mansoni* (Utzinger *et al.*, 2009). In Zimbabwe, *S. haematobium* has been reported to have a prevalence of 31.7% and a lower prevalence of *S. mansoni* at 4.6% (Mduluza *et al.*, 2020). Hence, this thesis focuses primarily on describing and evaluating the impact of the most prevalent form of schistosomiasis in Zimbabwe, *S. haematobium*, also known as urogenital schistosomiasis.

1.2.1 Life cycle of *S. haematobium*

The transmission of schistosomiasis involves a complex life cycle. A schematic diagram of the life cycle of the *S. haematobium* species targeted for this thesis is displayed in **Figure 1.1**.

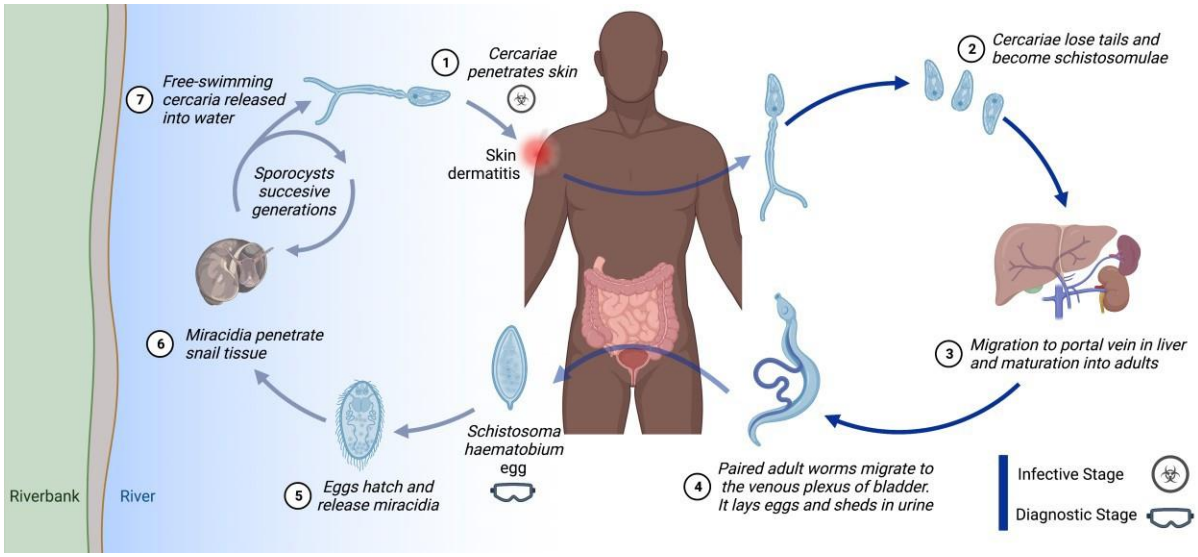

Figure 1.1: The life cycle of the *Schistosoma haematobium* parasite. Based on a figure from CDC https://www.cdc.gov/parasites/schistosomiasis/biology.html [Accessed 27/05/2022].

Initially, transmission to humans occurs after contact with freshwater sites contaminated with schistosome cercariae (**Figure 1.1: [1]**) (Colley *et al.*, 2014). Contact with contaminated freshwater in communities with poor safe water provision can occur via domestic chores (washing), occupational activities, and recreational activities such as playing or cooling off in the river (King and Dangerfield-Cha, 2008). The cercariae are attracted to skin-derived chemicals, water turbulence, shadows, and warmth from the human host (Lichtenbergová and Horák, 2012). Once the cercariae reach the exposed skin, they digest it using enzymes and enter the human host's dermal veins. The cercariae then lose their forked tails and develop an outer coating which resists host immune attack, becoming schistosomulae for migration to

the pulmonary vasculature (**Figure 1.1: [2]**) (Verjee, 2019). The schistosomulae then reach pulmonary capillaries via systemic circulation, maturing into adults in the portal veins of the liver (**Figure 1.1: [3]**). The male and female adult worms then pair and migrate to the venous plexus of the bladder where sexual reproduction occurs (Nelwan, 2019). The female deposits eggs, up to three hundred eggs a day, which are excreted in the urine (Maguire, 2020) (**Figure 1.1: [4]**). Here, the diagnostic phase of urogenital schistosomiasis can occur via microscopy of an infected individual's urine sample (Mott, 1983). Once the urine is deposited by the human host, either by direct contamination or by being carried by the rain, the *S. haematobium* eggs reach freshwater. The eggs then hatch and mature into miracidia (**Figure 1.1: [5]**), which detect and penetrate the intermediate host, the *Bulinus* snail (**Figure 1.1: [6]**) (Colley *et al.*, 2014). At least two successive generations of sporocysts multiply and mature into free-swimming cercariae, which then leave the snail to seek a human host (**Figure 1.1: [7]**); repeating the life cycle of the parasite.

1.2.2 Clinical manifestations and pathology of *S. haematobium* infection

There are numerous clinical manifestations of *S. haematobium*. After the penetration of cercariae, some individuals experience a prickling sensation and may note a rash, commonly termed “swimmer's itch” (McManus *et al.*, 2018). Symptoms of acute schistosomiasis present in patients about 4-8 weeks after initial infection. In contrast, the chronic symptoms of urogenital schistosomiasis can develop and intensify over many years. The clinical symptoms of acute and chronic *S. haematobium* infections have different physical presentations, as presented in **Table 1.2**.

Table 1.2: Physical findings of urogenital schistosomiasis (Ross *et al.*, 2007, Colley *et al.*, 2014, Verjee, 2019).

Classification	Physical Clinical Findings
Acute	Dysuria, haematuria, gastrointestinal symptoms, abdominal pain, fever, fatigue, malaise, myalgia, cough, headache, anorexia, urticarial rashes or respiratory symptoms
Chronic	Growth retardation, anaemia, malnutrition, decreased fitness, abdominal distension, impaired cognition, renal failure, infertility, fibrosis and calcification of the bladder wall, bleeding of the bladder, bacteriuria, and bladder cancer.

The combination of acute urogenital schistosomiasis symptoms such as fever, lethargy, malaise, and myalgia can also be called Katayama syndrome, (Ross *et al.*, 2007). However, Katayama syndrome is rare with *S. haematobium* infections and tends to occur only during heavy infections (≥50 eggs/10mL of urine) (WHO, 2002b, McManus *et al.*, 2018). Diagnosing schistosomiasis using these acute symptoms alone is limited as they are common to a multitude of disorders. In numerous schistosome endemic areas, the clinical symptoms of acute and even chronic schistosome infections can often be confused with malaria or viral infections (Leshem *et al.*, 2008). So, although the clinical presentations of acute schistosome infections described in **Table 1.2** may go unrecognised, the current and future morbidity risk of the patient should not be ignored. Hence, care must be taken to correctly diagnose and treat *S. haematobium* infections, as the chronic symptoms can be severe.

Pathological changes from infection with adult *S. haematobium* worms are dominated by the damaging passage of parasite eggs through the urinary bladder wall, and from eggs that are retained in the bladder tissue and genital organs of the host (Gryseels *et al.*, 2006). During parasite egg excretion, the lumen of the bladder is damaged due to the skimming of the epithelial surface by the egg clusters, resulting in ulceration and bleeding. The uncontrolled and sometimes rapid migration of eggs causes visible hematuria (blood) in the urine of patients (Tzanetou *et al.*, 2007). Furthermore, eggs that remain in the bladder lumen or flow

back to the pulmonary circulation from vesicular vessels via the inferior vena cava can enable the egg to reach other organs in the body (Colley *et al.*, 2014, Verjee, 2019). Prolonged morbidity from shedding *S. haematobium* eggs results in the thickening of the bladder wall or the development of growths due to continuous egg-induced inflammation (Hatz *et al.*, 1990). Conversely, by retaining the parasite eggs, the formation of granulomas, inflammation and fibrosis throughout the surrounding tissues occurs, with the severity of these symptoms correlating with the intensity of schistosome infection (McManus *et al.*, 2018).

Chronic urogenital schistosomiasis can result in severe inflammation and granuloma formation around the opening of the urethra of the bladder. This prevents the host from passing urine and can lead to the swelling of the kidneys and, in some cases, the host can lose the function of their kidneys (Kayange *et al.*, 2015). In women of childbearing age, urogenital schistosomiasis is a common cause of intermenstrual bleeding, ectopic pregnancy, dysmenorrhea, and spontaneous abortion, as well as poor birth outcomes due to placental inflammation and infection (Kjetland *et al.*, 2006). In fact, up to 75% of women affected by *S. haematobium* will have damage to their genital tract, a chronic symptom termed female genital schistosomiasis (FGS) (Lackey and Horrall, 2022). Eggs can be deposited in the cervix during active *S. haematobium* infection, leading to the characteristic FGS cervical lesions and intensive tissue inflammation from liver eggs or clusters of calcified eggs (Leutscher *et al.*, 2000, Randrianasolo *et al.*, 2015). This, in turn, can play a part in increasing human immunodeficiency virus (HIV) transmission and other sexually transmitted infections (STIs) in both sexes due to increased access points for infection transmission (Mbabazi *et al.*, 2011).

Chronic *S. haematobium* infection has been classified by the International Agency for Research on Cancer (IARC) as a carcinogen (CDC, Vennervald and Polman, 2009). After

many years of parasite exposure, the continuous inflammation from schistosome infection is correlated with carcinogenic development from dead calcified eggs forming yellow growths in the bladder mucosa (Burki *et al.*, 1986, Humans, 2012). Hereby, the early diagnosis and treatment of schistosome infection produces better outcomes and reduces the long-term morbidity and health-related problems in patients suffering from *S. haematobium* infection.

1.3 Epidemiology of schistosomiasis

An estimated 700 million people are at risk of contracting schistosomiasis worldwide (Steinmann *et al.*, 2006), and as seen in **Figure 1.2** the majority of those people reside on the African continent (Handzel *et al.*, 2003).

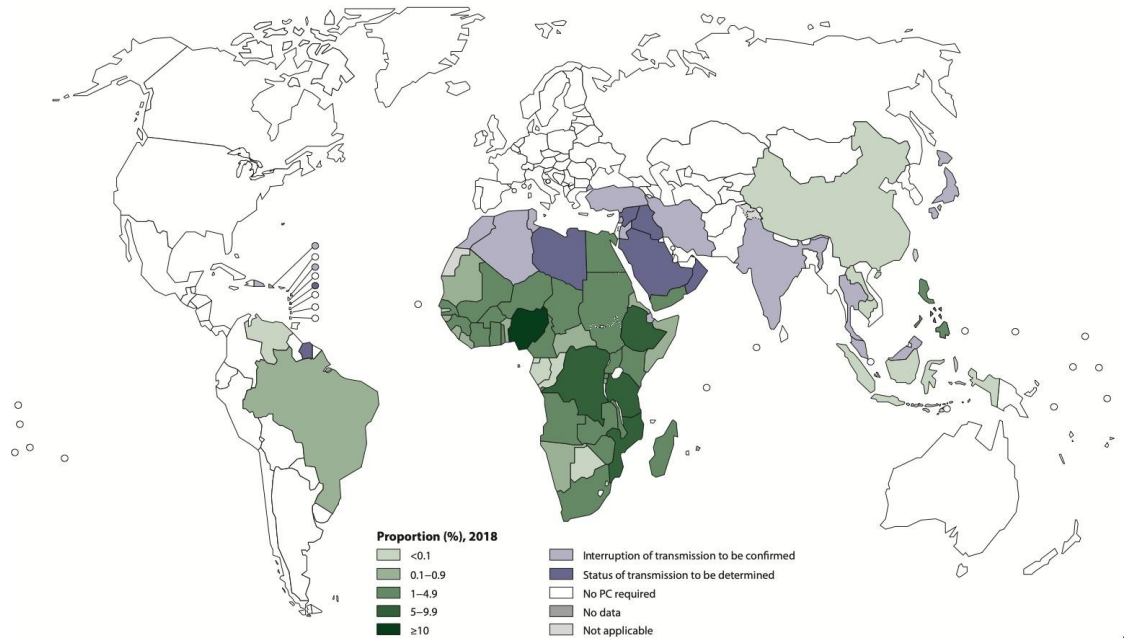

Figure 1.2: Proportion of the global population requiring preventive chemotherapy (PCT) against schistosomiasis in 2018. Sourced from the World Health Organization (WHO) online resource https://cdn.who.int/media/docs/default-source/ntds/schistosomiasis-(bilharzia)/proportion-global-population-requiring-pc-for-sch-2018.pdf?sfvrsn=852fb11b_6 [Accessed 26/05/2022].

Zimbabwe, the case study of this thesis, is a schistosome-endemic country, with infections detected in 91% of districts across the country (Mutsaka-Makuvaza *et al.*, 2019). Zimbabwe is divided into fifty-nine districts, as displayed in **Figure 1.3**, with the prevalence of schistosome infection varying throughout each district in the country.

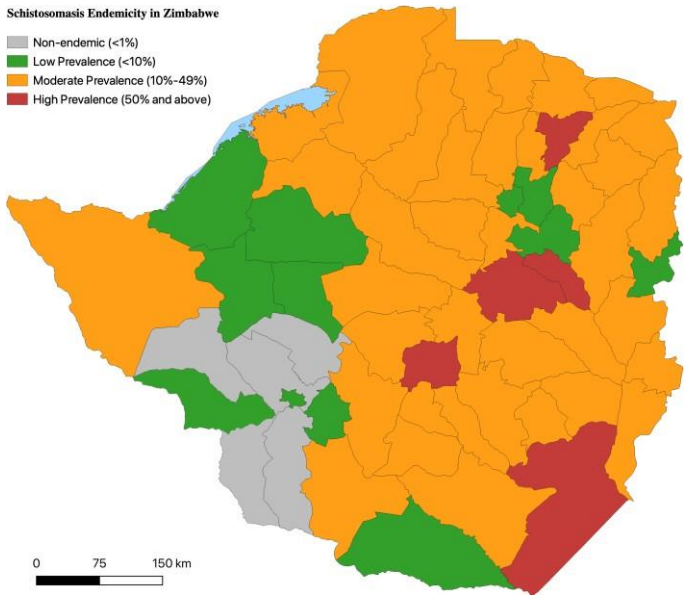

Figure 1.3: Schistosomiasis endemicity in Zimbabwe in 2020. This figure was based on the corresponding image from the Expanded Special Project for Elimination of Neglected Tropical Diseases (ESPEN, 2022) and was created in the QGIS [v.3.22] software.

The WHO categorises areas into the following, based on prevalence: non-endemic ($x \leq 1\%$), low prevalence ($1\% < x \leq 10\%$), moderate prevalence ($50\% \geq x \geq 10\%$) and high prevalence ($x \geq 50\%$) (WHO, 2002b).

The long-term impact of schistosomiasis on the sufferer's health-related quality-of-life (HRQoL) must also be considered across the different populations suffering from schistosomiasis. Mortality is not always the best assessment of the burden of a disease, as is the case with schistosomiasis. The disability-adjusted life year (DALY) described in **Table**

1.3 quantifies the burden of schistosomiasis and measures the gap between current health and ideal health; losing one DALY represents the loss of one year of full health (WHO, 2018).

Table 1.3: Impact of schistosomiasis globally and in Zimbabwe in 2019 (IHME, 2019). Based on recent data from the 'Global Burden of Disease Study' conducted in 2019.

Impact of Schistosomiasis:	DALYs Lost	Number of Mortalities
Globally	1,638,072	11,514
Zimbabwe	9,878	184

DALYs: Disability-Adjusted Life Years.

As of 2019, schistosomiasis was responsible for approximately 11.5 thousand deaths and 1.64 million DALYs globally (IHME, 2019). As this thesis studied Zimbabwean populations, an examination of the impact of schistosomiasis nationally was also extracted in **Table 1.3**. In 2019, it was found that 184 people died from this disease, but there were nearly ten thousand DALYs lost across the schistosome-infected population. This was the second most prevalent death and DALYs of any parasitic disease, only beaten by malaria (IHME, 2019). Schistosomiasis is co-endemic with several other parasites and pathogens in Zimbabwe, including cholera, malaria, rabies, HIV/AIDS, tuberculosis (TB), typhoid, and measles (Missigman, 2017). Thus, the impact of co-morbidities must be considered when assessing the factors that influence the efficacy of PZQ during schistosomiasis treatment as there may be an increased necessity for multiple co-administered medications. So, to prevent the risk of adverse drug reactions (ADRs) or decreased treatment efficacy due to drug-drug interactions (DDIs, **Section 1.10.1**), consideration of patients with multi-morbidity should be taken (Dumbreck *et al.*, 2015).

1.4 Risk factors for schistosome infection

Several factors increase the risk of contracting a schistosome infection. These risks include environmental, biologically-associated and sociocultural factors. Various host-related factors have been significantly associated with an increased risk of schistosome infection, primarily gender and age (Osakunor *et al.*, 2018). A recent systematic review found that due to gender-related roles, males were at higher risk of schistosome exposure from contaminated water sources than females (Ayabina *et al.*, 2021). The increased exposure to contaminated water in males was due to extended time spent fishing or irrigation farming (Tefera *et al.*, 2020). Although females spent time exposed through household chores like laundry, prohibition from other activities due to sociocultural reasons reduced their exposure risks (Sulieman *et al.*, 2017).

Age was also an associated risk with schistosome infection. From a young age, children are involved in activities that expose them to this disease, from accompanying their mothers to the river for household chores as PSAC to playing and assisting their families fetching water as SAC and adolescents; all of which exposes them to the potentially cercariae-contaminated water (Kabuyaya *et al.*, 2017). Hence, as the age of the individual increases, their risk of schistosome infection and increased intensity raises due to their high contact with infected water, until acquired immunity from a history of past infections in adulthood (Woolhouse *et al.*, 1991, Verani *et al.*, 2011). Thus, the significance of these biological factors in the risk of exposure emphasises why it was important to mitigate and control for age and sex, especially when investigating causes of variable PZQ efficacy. As high infection intensity is a common cause of an individual not clearing schistosome infection after a PZQ treatment, if the worm burden is too high, a single dose may not be effective enough to kill the entire schistosome load (Midzi *et al.*, 2008).

Environmental risk factors tend to increase contact with water contaminated with cercaria and increase the risk of contracting schistosomiasis. For instance, the closer the home is to a contaminated water source has been shown to increase a person's risk of schistosome infection (Rudge *et al.*, 2008). Hence, the environmental risks that propagate schistosome infection are a crucial aspect of the investigations in this thesis, particularly those that could be detrimental to the success of PCT programs.

Schistosomiasis disproportionately affects those living in rural and underprivileged areas with limited access to clean water, sanitation, and hygiene (WASH) facilities (Onasanya *et al.*, 2021). WASH interventions are highly effective at decreasing the prevalence of schistosomiasis by reducing the contamination of the environment with schistosome eggs (Esrey *et al.*, 1991, Tanser *et al.*, 2018). So, the implementation of WASH interventions is vital in controlling and reducing the risk of contracting schistosomiasis (Chimbari *et al.*, 2003). Yet, low levels of education on schistosomiasis transmission have been shown to deter the success of preventative WASH practices (Omedo *et al.*, 2014). A recent study in Zimbabwe surveyed over one hundred households to determine the social, cultural, and behavioural influences on schistosome infection (Lampard-Scotford *et al.*, 2022). The study showed that although 60% of the community had access to toilets, 36% of children and 16% of adults did not use them and practised open defecation. This stresses the importance of health education and awareness programs that target both adults and children in the community to further promote behavioural changes such as decreasing freshwater contamination and increasing toilet use (Gyapong *et al.*, 2010). Improving WASH infrastructure and behaviours in Zimbabwe could massively impact the prevention, control, and elimination of schistosomiasis, as well as other NTDs (Waite *et al.*, 2017).

Lastly, the risks of climate change create a vast problem in controlling the spread of schistosomiasis in the future, in which higher temperatures and altered seasonal rainfall patterns will favour the survival of the intermediate host snail and propagate the parasites' development (De Leo *et al.*, 2020). The change in weather systems could also impact schistosome transmission, such as the recent cyclone in Zimbabwe, which could potentially establish new snail populations in previously non-endemic areas, increasing the risk of schistosome infections to residents of these areas (Zheng *et al.*, 2002, Adekiya *et al.*, 2019). Thus, the implementation of snail control, a critical factor of the WHO 2021–2030 roadmap (WHO, 2020a), would be essential to minimise the number of intermediate snail hosts contaminating freshwater sources. Individuals will also be affected by increased temperatures, as this will increase the use of contaminated water sources for recreational use, increase swimming and impact the duration and frequency of exposure (Rudge *et al.*, 2008). The key risk factors are described in **Figure 1.4** below.

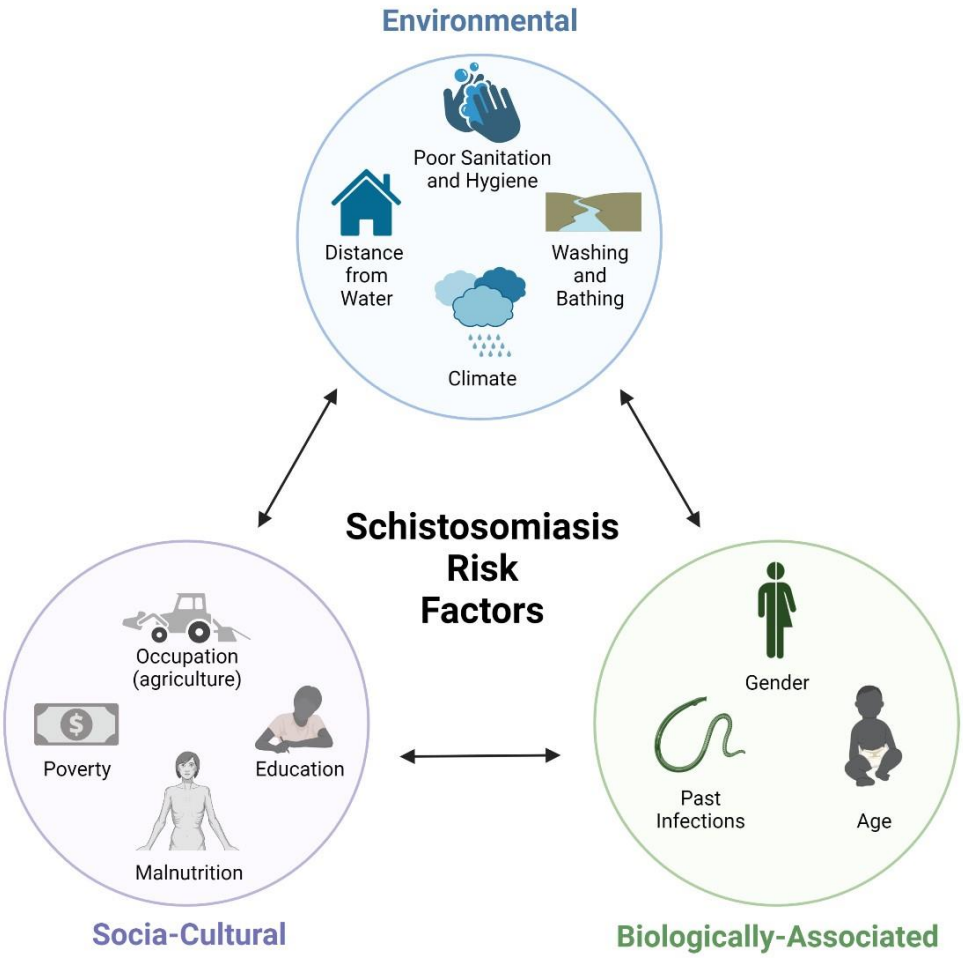

Figure 1.4: A description of the risk factors for contracting schistosomiasis (Bruun and Aagaard-Hansen, 2008, Kabuyaya *et al.*, 2018b, Osakunor *et al.*, 2018).

1.5 Praziquantel (PZQ)

Globally, the standardized treatment for schistosome infection is the drug PZQ. The drug is extensively used worldwide to treat human schistosomiasis (WHO, 2013c, Zwang and Olliaro, 2014). PZQ is a racemic drug, with the standard dose consisting of a 1:1 mixture of two enantiomers as seen in **Figure 1.5**.

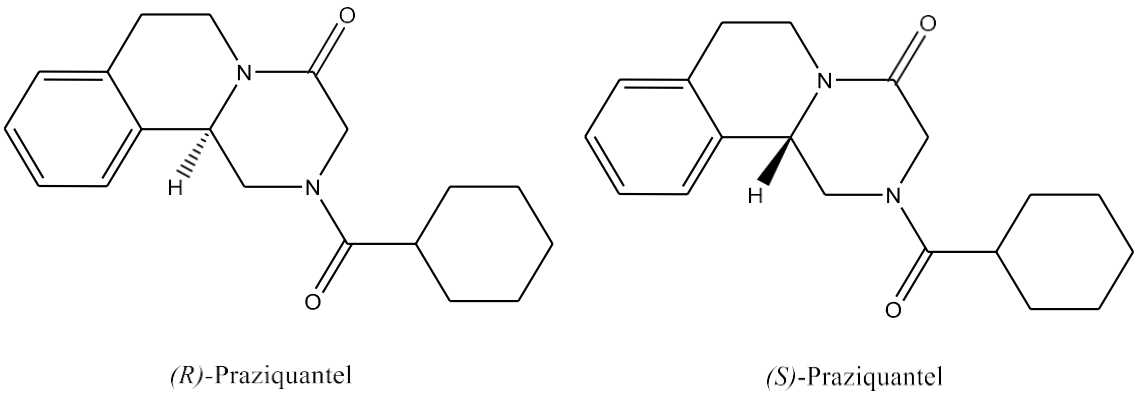

Figure 1.5: The molecular structure of the two praziquantel (PZQ) enantiomers (Zdesenko and Mutapi, 2020).

Only the (R) enantiomer of PZQ (also known as Levo-PZQ or (-)-PZQ) possesses the desired antischistosomal activity of the drug (Meister *et al.*, 2014). The (S) enantiomer of PZQ (also known as Dextro-PZQ or (+)-PZQ) does not have antischistosomal action but has been shown to contribute to some of PZQ's known side effects (Meyer *et al.*, 2009). The side effects of a PZQ treatment are mild in most patients and include headaches, nausea, vomiting, abdominal pain, gastrointestinal problems, joint pain, and muscle pain (Raso *et al.*, 2004, Midzi *et al.*, 2008). Severe neurological symptoms have also been reported, such as dizziness and seizures, however, these have been related to high pre-treatment infection intensity and the release of antigens by the dying schistosomes rather than from a pharmacological effect of PZQ on the patient (Zwang and Olliaro, 2014, McManus *et al.*, 2018).

There is still a lack of clarity surrounding PZQ's antiparasitic mechanism of action (MOA), hence, multiple hypotheses regarding PZQ's MOA have been developed. The predominant theory suggests that PZQ's MOA arises from (R)-PZQ disrupting the schistosome's calcium ion homeostasis, which then causes uncontrolled muscle contraction and eventual worm death (Doenhoff *et al.*, 2008, Thomas and Timson, 2018). In spite of its undefined MOA, PZQ remains the primary drug treatment for this disease. However, most importantly, PZQ

cannot be used to treat early-stage schistosome infections as it only targets adult schistosomes (Gönnert and Andrews, 1977). PZQ's lack of action against juvenile schistosomes, which will then later mature and continue to deposit eggs in subsequent weeks, hampers schistosomiasis control efforts (Ross *et al.*, 2015). Despite this, PZQ is generally well- tolerated in patients of all ages and with different *Schistosoma* parasite species (Cioli and Pica-Mattoccia, 2003) and has been continually used to treat schistosomiasis for decades (Fenwick, 2015).

1.6 Schistosomiasis control

1.6.1 Mass drug administrations (MDAs)

The implementation of PZQ MDAs has been described as an essential public health intervention to treat those exposed to schistosome infection and control infection through successive treatments (WHO, 2002b), with an estimated 200 million people treated worldwide each year (WHO, 2013c). MDAs deliver PZQ to SAC and high-risk adults exposed to *Schistosoma*, without an individual diagnosis, to prevent future morbidity in endemic countries (Webster *et al.*, 2014, Lo *et al.*, 2022). The current WHO treatment strategy recommends that all SAC and high-risk adults whose occupations involve contact with infected water in heavily endemic areas are treated annually during MDAs. Those who live in moderately endemic areas are then treated biennially, and in areas of low endemicity, it is reduced to triennially (Crompton, 2006, WHO, 2011a). A comprehensive study of the success of the 2012-2017 Zimbabwean control program found that after six rounds of PZQ MDAs, the national prevalence of *S. haematobium* had reduced to 1.56% from an initial prevalence of 31.7% (Mduluza *et al.*, 2020).

Yet, MDAs do not currently include one very vulnerable age group: PSAC. Recently, the WHO recommended that, based on evidence from numerous schistosome endemic countries, PSAC should be included in the MDA programs (WHO, 2011b). Despite the WHO recommendations, the strategy to access PSAC has not yet been implemented in MDA, resulting in a "treatment gap" and health inequity (Stothard *et al.*, 2013, Faust *et al.*, 2020).

As of 2022, a paediatric formulation was developed (Consortium, 2022a). This paediatric formulation contains only the active (R)-PZQ enantiomer, creating a smaller-sized tablet, and removing the bitter taste of the (S)-PZQ, therefore it is ideal for younger schistosome-infected patients This new treatment option for PSAC (paediatric-PZQ) has been shown to decrease transmission upon the inclusion into MDA program, as well as reduce the coverage required for SAC and adults (Kura *et al.*, 2022). However, the current timeline for paediatric-PZQ to be available for distribution in schistosome-endemic African countries is estimated to be 2025 (Consortium, 2022b). Meanwhile, while endemic countries wait to roll out paediatric- PZQ, PSAC contract schistosome infections as young as six months old (Woolhouse *et al.*, 2000, Kemal *et al.*, 2019). Currently, PSAC remain reservoirs of infection transmission, damaging the overall control efforts of MDAs (Lelo *et al.*, 2014).

1.7 Concerns regarding praziquantel (PZQ) efficacy

Although PZQ has been successfully used in the treatment of schistosomiasis for many decades, some individuals remain infected after multiple treatments (Lamberton *et al.*, 2010). Generally, responses to a PZQ treatment will vary among different individuals and populations due to a multitude of factors, including demography, treatment regimens, and poorly-understood inter-individual differences (Walker *et al.*, 2022). To understand deviations in the therapeutic efficacy of PZQ, two epidemiological measures are commonly

used (Bajiro *et al.*, 2016): i) the cure rate (CR), which compares the proportion of those who were schistosome egg-positive pre-PZQ treatment who become schistosome egg-negative post-PZQ treatment, or ii) the egg reduction rate (ERR), which compares the reduction in the mean number of schistosome eggs excreted from pre-PZQ treatment to post-PZQ treatment (Zwang and Olliaro, 2014). To date, numerous studies have reported low CRs and ERRs, with many attributing these cases of reduced PZQ efficacy to a high pre-treatment parasite burden (Tchuenté *et al.*, 2004, Alonso *et al.*, 2006, Midzi *et al.*, 2008, Barakat and Morshedy, 2010). Thus, if infection intensity is very high, a single PZQ dose may not be effective enough to kill the entire schistosome load (Midzi *et al.*, 2008). However, studies which have accounted for high levels of infection have still observed low CRs and ERRs (Danso-Appiah and De Vlas, 2002, Kabuyaya *et al.*, 2017), questioning the other causes of decreased PZQ efficacy. So far, widespread resistance has not been verified with minimal cases of *S. mansoni* showing decreased sensitivity to PZQ (Ismail *et al.*, 1996, William and Botros, 2004, Summers *et al.*, 2022) and no studies indicating resistance to PZQ in *S. haematobium* (Vale *et al.*, 2017).

The advantages of curing most of the schistosome-infected population to reduce morbidity takes precedence in endemic countries and is prioritised over precisely targeting individuals who fail to clear schistosome infection with standard PZQ treatment (World Health Organization 2020b). The implications of having a small proportion of individuals who show reduced PZQ efficacy regarding the long-term sustainability of PCT programs are still unknown and certainly warrant close longitudinal monitoring. (Moser *et al.*, 2020). There is an ongoing important debate on how to address these outliers in the context of PZQ efficacy, however, it has been highlighted that an investigation of non-responsive individuals is required (Moser et al., 2020, Levecke *et al.*, 2020). In fact, many MDA programs now require the monitoring of PZQ efficacy, particularly in repeatedly PZQ-treated populations

that are experiencing variable PZQ efficacy. However, without a comprehensive evaluation of the factors that affect the efficacy of PZQ treatment in the relevant schistosome-affected populations, it's hard to target the cause of reduced PZQ efficacy.

Due to the scarcity of published information investigating the causes of variable PZQ efficacy, there is an ongoing need to monitor the efficacy of PZQ in schistosome-infected populations to identify the determinants and prevent future cases of decreased PZQ efficacy (Webster *et al.*, 2014, Walker *et al.*, 2022).

1.8 Hotspots of schistosome infection

Areas with persistent infections that do not decline in schistosome prevalence or intensity after multiple MDAs are termed persistent hotspots (PHS) of schistosome infection (Kittur *et al.*, 2017, Pennance *et al.*, 2022). To date, PHS of schistosome infection have been identified in multiple schistosome-endemic African countries, despite MDA interventions (Pennance *et al.*, 2016, Kittur *et al.*, 2020). In these evaluations, when assessing PHS the differences between an area of persistent infections (hotspot) and responder areas (those that completely clear all schistosome infections) are investigated. Thus far, there is no consensus on the definition of a biological 'hotspot', although most definitions tend to assess a failure to change in infection prevalence and/or high intensity of infection over time (Pennance *et al.*, 2022).

Persistent schistosome infections may be caused by reduced PZQ efficacy, however other risk factors must also be considered (Pennance *et al.*, 2022). PHS can stem from factors such as increased transmission, treatment regimen and contact with water (Pennance *et al.*, 2016, Kittur *et al.*, 2020). Identifying and targeting the determinants of PHS, as well as

investigating whether decreased PZQ efficacy is responsible for PHS emergence, is critical to efficiently control schistosomiasis through PCT programs (Secor and Montgomery, 2015).

Recently, the COVID-19 pandemic halted MDA control programs globally, and there is uncertainty about the future implications of these disruptions on the elimination of schistosomiasis (Oyeyemi *et al.*, 2020, Kura *et al.*, 2021). In response, the WHO has set a new 2021 NTD roadmap to eliminate this disease as a public health problem by 2030, with the timeline presented in **Figure 1.6** (WHO, 2020a, Kura *et al.*, 2022).

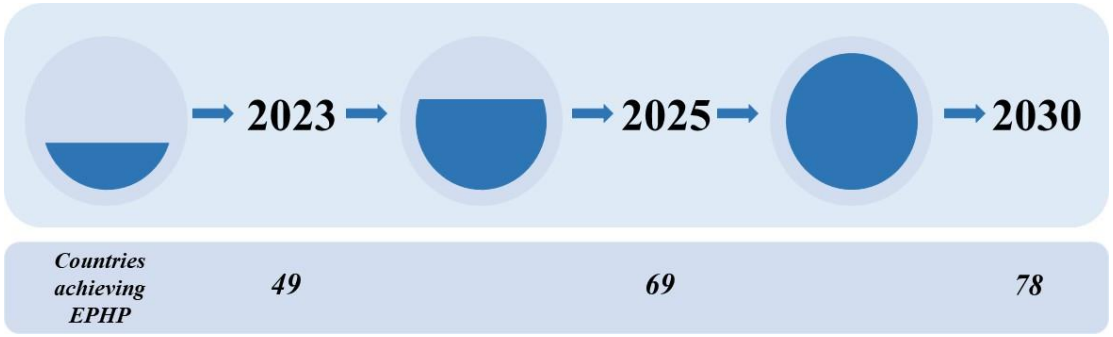

Figure 1.6: The timeline of success to eliminate schistosomiasis as a public health problem (EPHP) as described by the world health organization (WHO).

As MDA programs are generally well accepted among children, parents, teachers, health workers and members of the community (Fleming *et al.*, 2009) their implementation remains a technically feasible and cost-effective intervention (Lo *et al.*, 2016), and the re-introduction of these programs would be favourable to regain control of schistosome infections. Consequently, to aid future control programs there is an urgent need to collect data and develop tools that can be used to identify and predict PHS caused by exacerbated transmission factors or differential PZQ efficacy. Identifying PHS of schistosome infection will play an important role in the planning, allocation, and implementation of resources to eliminate schistosomiasis (Lessler *et al.*, 2017). To date, no study has so far assessed the

possibility of PHS of schistosome infection or decreased PZQ efficacy in Zimbabwe during the MDA control efforts.

1.9 Drug metabolism and pharmacokinetics (DMPK)

Drug metabolism and pharmacokinetics (DMPK) encompasses the evaluation of a drug's metabolic properties and is key to the development, use, and safety of a drug (Zhang and Tang, 2018). The identification of the enzymes involved in the metabolism of a drug, as well as the characterisation of the structure and quantity of the metabolites generated is a crucial part of DMPK analysis (Wen and Zhu, 2015). This information can be used to predict the effects of genetic and environmental factors on the efficacy of the drug and can provide measurable pharmacokinetic (PK) parameters to identify the impact of these factors on active drug concentrations (Williams and Ette, 2000).

1.9.1 Pharmacokinetics (PK)

PK describes the effect of the body on a drug and encompasses the absorption, distribution, metabolism, and elimination (ADME) descriptors (Doogue and Polasek, 2013, Cyprotex, 2015). Initially, absorption describes how an orally ingested xenobiotic drug passes through the permeable barriers of the body (Dressman and Lennernäs, 2000). Commonly, to measure a drug's absorption (Lipinski, 2004), the bioavailability, which describes the fraction of administered drug that enters the systemic circulation (Rowland and Tozer, 2011), the peak plasma concentration (C_{max}), and the time taken to reach maximum plasma concentration (T_{max}) are all assessed. The distribution then evaluates how the drug is dispersed around the body and what tissues it accumulates in. This is commonly assessed using mass spectrometry (MS), examining plasma, tissues and urine to assert where the drug or its metabolic products (metabolites) are located (Vrbanac and Slauter, 2013). Then, metabolism describes the

mechanism of breaking down the drug via chemical reactions called biotransformations, which occur in drug-metabolising enzymes (Hoffmann *et al.*, 2014). This process enzymatically alters the drug molecule, often to a more polar metabolite, to aid excretion from the body (Gibson, 2001). Following this, elimination describes the route in which the drug and the resultant drug metabolites leave the body. These excretion products can also be detected using MS (**Section 1.9.4**). Still, interindividual variability can alter the ADME parameters, as described in **Table 1.4**.

Table 1.4: Examples of the implications of interindividual variability on ADME parameters (Rowland and Tozer, 2011, Tibbitts *et al.*, 2016, Barreto *et al.*, 2021).
ADME: absorption, distribution, metabolism, and elimination.

Parameter	Interindividual Variability	Implications
Absorption	Gut wall function	Malabsorption of drug leading to low bioavailability
Distribution	The size and composition of different organs and fat content	A highly lipid-soluble drug may distribute more into the adipose tissue of individuals with high body fat and become less available to the eliminating organs. Thus, the half-life of the drug may be higher in females compared to males.
Metabolism	Changed expression or activity of drug-metabolising enzymes and other transporters, or co-administration of other medications resulting in drug-drug interactions	Altered concentrations of the active drug can result in sub-therapeutic or toxic concentrations.
Excretion	Impaired renal function	Results in drug accumulation and potential toxicity

This variability can create differences in the concentrations of a drug and its clinical efficacy. The measures to assess PK can be represented in a concentration-time curve, commonly called a PK profile. Furthermore, the PK profiles obtained during PK studies are commonly conducted in healthy adults and can differ from the target populations, such as children or under-investigated ethnicities (Yewale and Dharmapalan, 2012). Overall, by evaluating the factors that influence PK, the impact on a drug's ability to achieve a therapeutic

concentration and even the risk of ADRs can be predicted and, if necessary, avoided (Hedaya, 2012).

1.9.2 Drug metabolism

As briefly described above, drug metabolism describes the chemical alteration of a drug to create a compound that's more easily excreted by the body; a metabolite (Bachmann, 2009). These metabolic biotransformations are primarily mediated by the liver and are divided into phases, termed phase I and phase II (Omiecinski *et al.*, 2010). Phase I reactions involve the oxidation, reduction, and hydrolysis of the parent drug into a more polar metabolite (Lu and Xue, 2019). Phase II reactions attach a conjugate to the parent drug or its metabolite, resulting in a glucuronidation, acylation, sulphate, or glycine addition (Lu and Xue, 2019). The resultant product from either phase I or II metabolism will either be an inactive drug metabolite, which is now ready for excretion, or a pharmacologically active metabolite called a prodrug (Obach, 2013). In some cases, phase I biotransformations can yield a metabolite even more toxic than the parent drug (Guengerich, 1992). In the case of this thesis, I will focus on the enzymes that mediate phase I reactions and the resultant metabolites, as there has been minimal evidence to show a significant phase II contribution to PZQ metabolism (Wang *et al.*, 2014b).

1.9.2.1 Drug-metabolising enzymes

The cytochrome P450 (CYP) enzymes are primarily responsible for phase I drug metabolism, with other hepatic enzymes such as flavin-containing monooxygenases and epoxide hydrolase playing a lesser role (Iyer and Sinz, 1999). The CYPs can influence the bioavailability and toxicity of a drug, with approximately half of the most-prescribed drugs metabolised through the CYPs (Saravanakumar *et al.*, 2019). The CYP enzymes are divided

by a family nomenclature. When the sequence has > 40% homology, the family is separated by an initial number, for instance, *CYP1*, *CYP2*, and *CYP3* (Nelson *et al.*, 1996). Then, sub-divisions of these families indicate > 60% homology, for instance, *CYP1A, CYP1B*, and *CYP1C* (Saha, 2018). The final notation is the number labelling the individual gene itself, for instance, *CYP3A4* (Martignoni, 2006). Due to this diversification of families, human CYP genes are highly polymorphic and there are more than 350 functionally different CYP alleles (Ingelman-Sundberg *et al.*, 2007).

1.9.3 Drug metabolism and pharmacokinetics (DMPK) of praziquantel (PZQ)

Although the use of PZQ in the treatment of schistosome infections has been successful for decades, the PK evaluations of the drug are still in their infancy, with few studies on the PK and pharmacodynamics (PD) of PZQ in humans (Olliaro *et al.*, 2014). In fact, for most NTDs, adequate PK studies to optimise drug treatments for these diseases are still required (Verrest and Dorlo, 2017). A recent review found that ten of the seventeen NTDs were found to have no or very few published PK evaluations, in addition to 94% of identified PK studies having a sample population of fewer than fifty individuals, and only 11% of studies including paediatric patients (Verrest and Dorlo, 2017). Furthermore, these studies are not generally applied in disease-affected populations, thus there is a clear lack of knowledge regarding the tailoring and improvement of drug therapies for NTDs.

PZQ was first developed for veterinary use due to its broad anthelminthic activity against parasitic trematodes and cestodes (Andrews *et al.*, 1983). The move to a human treatment was established during the 1970s when its curative efficacy was confirmed, and motions to progress this drug to the international market were pushed by the WHO owing to its global health benefits (Reich and Govindaraj, 1998). Since then, there has been no significant success in the discovery of new or alternative drugs to overcome the global reliance on PZQ

(Aruleba *et al.*, 2019). So far, the published ADME data on PZQ includes a variety of *in vitro* and *in vivo* studies, with the general ADME parameters of PZQ summarised in **Figure 1.7** below.

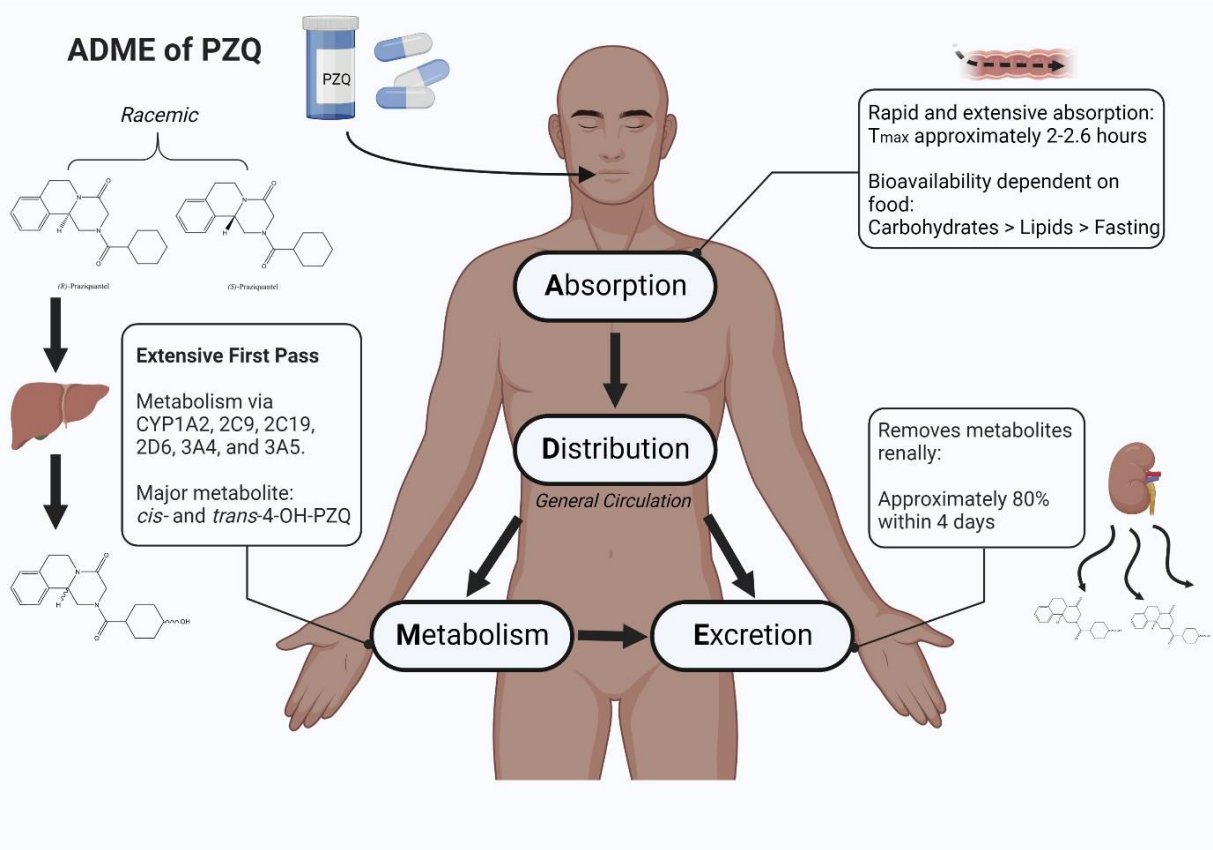

Figure 1.7: Diagram depicting the principal ADME properties of praziquantel (PZQ). The acronyms displayed in the figure represent; T_{max}: Time to reach peak plasma concentration following drug administration, and CYP: Cytochrome P450 enzymes. (Zwang and Olliaro, 2014, da Silva *et al.*, 2017).

The rapid absorption of PZQ occurs approximately 2-2.6h (T_{max}) after oral administration with general systemic circulation of parent PZQ (Olliaro *et al.*, 2014). The metabolism of PZQ occurs in the liver to form PZQ metabolites, with only a small quantity of parent PZQ excreted in the urine (Gandhi and Elshaboury, 2020). Using *in vitro* human liver microsomes (HLM) and recombinant CYPs (rCYPs), PZQ is reported to be metabolised by multiple

CYPs. The contributions of these CYPs to the metabolism of PZQ are estimated as *CYP1A2* (39%), *CYP2C9* (<10%), *CYP2C19* (14%) *CYP2D6* (<10%) and *CYP3A4/5* (30%) (Masimirembwa, 2013, Wang *et al.*, 2014b, Bonate *et al.*, 2018, Kapungu *et al.*, 2020). The *CYP1A2, CYP2C19*, and *CYP3A4* have been shown to exhibit different catalytic activity regarding the two isomers of PZQ (Kapungu *et al.*, 2020). The *CYP1A2* and *CYP2C19* mainly metabolised the (R)-PZQ, and *CYP2C19* and *CYP3A4* mainly metabolised (S)-PZQ.

Many assessments of PZQ's DMPK properties have been performed in rodent models, as is common among DMPK investigations. Yet, assessing the CYP metabolism of PZQ in rodent models is limited as a representation of human schistosomiasis infection, due to their size and lifespan, as it is impossible to reproduce the low infection morbidity of human infection (Farah *et al.*, 2001). So, in addition to the anatomical and immunological differences in the species, these problems make extrapolation from rodents to humans challenging (Fallon, 2000), and is something to always consider when evaluating PZQ metabolism in a rodent model. To remove PZQ from the body, it is converted into xenobiotic metabolites, which have a half-life ($t_{1/2}$) of approximately 4-5 hours and are primarily excreted in urine (Conlon and Ellis, 1985).

The important questions that need to be answered regarding the impact of DMPK on the overall efficacy of PZQ treatment are:

I. What enzymes are involved in PZQ metabolism based on previous studies?
II. Could alterations in these metabolic pathways result in a lack of PZQ efficacy?
III. Are there any detectable PZQ metabolites? If so, are their concentrations altered upon changes to the metabolic pathways?

Therefore, to answer these questions, sufficient background information on the known DMPK of PZQ is required.

1.10 Causes of variability in drug metabolism and its effect on drug efficacy

An individual's response to drug treatment can be influenced by several factors, including age, sex, weight, diet, comorbidities, environmental factors (pollutants, season, location), disease state, treatment compliance, drug-drug interactions (DDIs), and genetics (Rowland and Tozer, 2011, Haga, 2017). Primarily, this thesis focuses on the factors that influence an individual's drug metabolism, and how this impacts the eventual efficacy of the drug. Causes of variability in drug metabolism can stem from the inhibition/induction or genetic polymorphisms of the drug-metabolising enzymes. Subsequently, a drug can exhibit decreased efficacy due to rapid metabolism of the drug-metabolising enzyme via induction, or increased toxicity due to decreased metabolism due to drug-metabolising enzyme inhibition (Ogu and Maxa, 2000). The variability and impact of altered drug metabolism on drug efficacy are generally attributed to changes in the active plasma concentration (exposure) of the drug. This section will further discuss the common causes of variation in drug efficacy that will be relevant for the investigations conducted during this thesis.

1.10.1 Drug-drug interactions (DDIs)

Evaluating DDIs is important when developing and using a drug in areas of high comorbidity, as is common in many Africa countries, as prospective patients may be on multiple medications (Fenwick and Webster, 2006). The CYPs, which are a focus of the investigations in this thesis, are a key site for DDIs (Blower *et al.*, 2005). Thus, any drugs co-administered with PZQ could be inhibitors or inducers of the PZQ-metabolising CYPs (Zhang and Tang, 2018). This is significant for this thesis, as schistosomiasis is co-endemic

with multiple other parasites and pathogens (**Section 1.3**). When a co-administered drug induces a CYP isoform, it can result in increased CYP metabolism. An example of induction due to DDIs was observed in a study co-dosing carbamazepine (the treatment for post-herpetic neuralgia) and indinavir (anti-retroviral therapy). Carbamazepine acted as a potent *CYP3A4* inducer (Hugen *et al.*, 2000) and resulted in a failed anti-retroviral treatment due to an insufficient indinavir plasma concentration. Regarding CYP induction in this thesis, the increased activity of the CYP enzymes could decrease active PZQ concentrations (Cascorbi, 2012).

Conversely, if a co-administered drug inhibits PZQ-metabolising CYP binding sites, it can no longer be metabolised and eliminated via this pathway, resulting in decreased metabolism and increased drug concentrations. An incident of dangerous drug accumulation was observed upon the combination of terfenadine (an antihistamine) with ketoconazole (an antifungal), as fatal ventricular arrhythmias were reported in patients (Lin and Lu, 1998). Terfenadine is metabolised by *CYP3A4* (Yun *et al.*, 1993) and ketoconazole inhibits *CYP3A4* (Greenblatt *et al.*, 2011), thus the excessive increase in terfenadine plasma concentrations due to the inhibition of its CYP metabolic pathway resulted in ADRs (Honig *et al.*, 1993). PZQ accumulation in the body could potentially cause ADRs or severe side effects (Jeffrey D Lewis, 2006). Regarding CYP inhibition in this thesis, DDIs within the PZQ-metabolising CYP pathways could result in the accumulation of PZQ potentially increasing the overall efficacy of PZQ, but the accumulation of (S)-PZQ may risk the associated ADRs (**Section 1.5**) (Mnkugwe *et al.*, 2021a). Overall, failure to recognise the importance of DDIs can lead to under/overdosing, adverse effects, and even fatalities (Beijnen and Schellens, 2004).

1.10.2 Pharmacogenetics (PGx)

Pharmacogenetics (PGx) refers to the study of genetic variations which cause variability in an individual's drug response (Roses, 2000). It has been estimated that around 25% of all drug treatments are influenced by a PGx variant (Ingelman-Sundberg, 2004), hence PGx is an important factor in the resultant efficacy of drug treatment. Thus far, genetic variations in the enzymes that mediate phase I drug metabolism are the most studied areas of PGx investigations (Drew, 2016). Genetic polymorphisms in the CYP enzymes, key mediators of drug metabolism (**Section 1.9.2**), are responsible for the variation in drug metabolism in numerous drug efficacy and toxicity studies (Evans and Johnson, 2001, McLeod and Evans, 2001). These variations are primarily due to single nucleotide polymorphisms (SNPs) of the CYPs, with numerous reported allelic variants already characterised, and whose variations in the gene sequence and subsequent protein structure can lead to an increased or decreased pharmacological effect (Buzkova *et al.*, 2006, Fujikura *et al.*, 2015). This is particularly relevant for this thesis, as an increased or decreased pharmacological effect due to PGx variations could alter the efficacy of PZQ. Hence, an investigation into the PGx variations that can result in altered enzyme function is highly important, particularly as this is an under-represented field of study in PZQ research (Mduluza and Mutapi, 2017, Zdesenko and Mutapi, 2020). PGx variants can alter the metabolic phenotype of the individual possessing an alternate genotype indicative of altered CYP activity, like those described in **Table 1.5**.

Table 1.5: Metabolic phenotypes resulting from an alteration to the gene sequence from single nucleotide polymorphisms (SNPs). Phenotype classification is based on the Clinical Pharmacogenetics Implementation Consortium (CPIC) (Caudle *et al.*, 2017, Gaedigk *et al.*, 2017).

Phenotype	Acronym	Enzyme Function	Example
Poor Metaboliser	PM	A combination of decreased or no enzyme function	*CYP3A5*3* (Kuehl *et al.*, 2001, Sanghavi *et al.*, 2017)
Intermediate metaboliser	IM	A combination of normal and decreased enzyme function	*CYP2D6*41* (Molden and Jukić, 2021)
Normal metaboliser	NM	Unchanged enzyme function	*CYP2C9*9* (Matimba *et al.*, 2009)
Rapid or ultra-rapid metaboliser	UM	Increased enzyme function	*CYP2C19*17* (Sim *et al.*, 2006, Gawrońska-Szklarz *et al.*, 2012)

The use of this genotypic information in PGx investigations can therefore provide reasoning behind treatment successes and failures and guide appropriate dosing. For example, those with rapid or ultra-rapid metabolising (UM) phenotypes with increased CYP enzyme function may convert the drug into inactive metabolites, preventing the desired therapeutic effect. Conversely, those with a poor metabolising (PM) phenotype may be at risk of ADRs from high concentrations of the drug or risk ineffective treatment if the administered treatment is a prodrug (Gearry and Barclay, 2005, Ingelman-Sundberg *et al.*, 2007, Tornio and Backman, 2018). Concerning the administration of drug treatment, it is important to recognise the role that PGx factors play in an individual's response to that treatment, and how an alteration to the 'normal' dose may be required as further depicted in **Figure 1.8**.

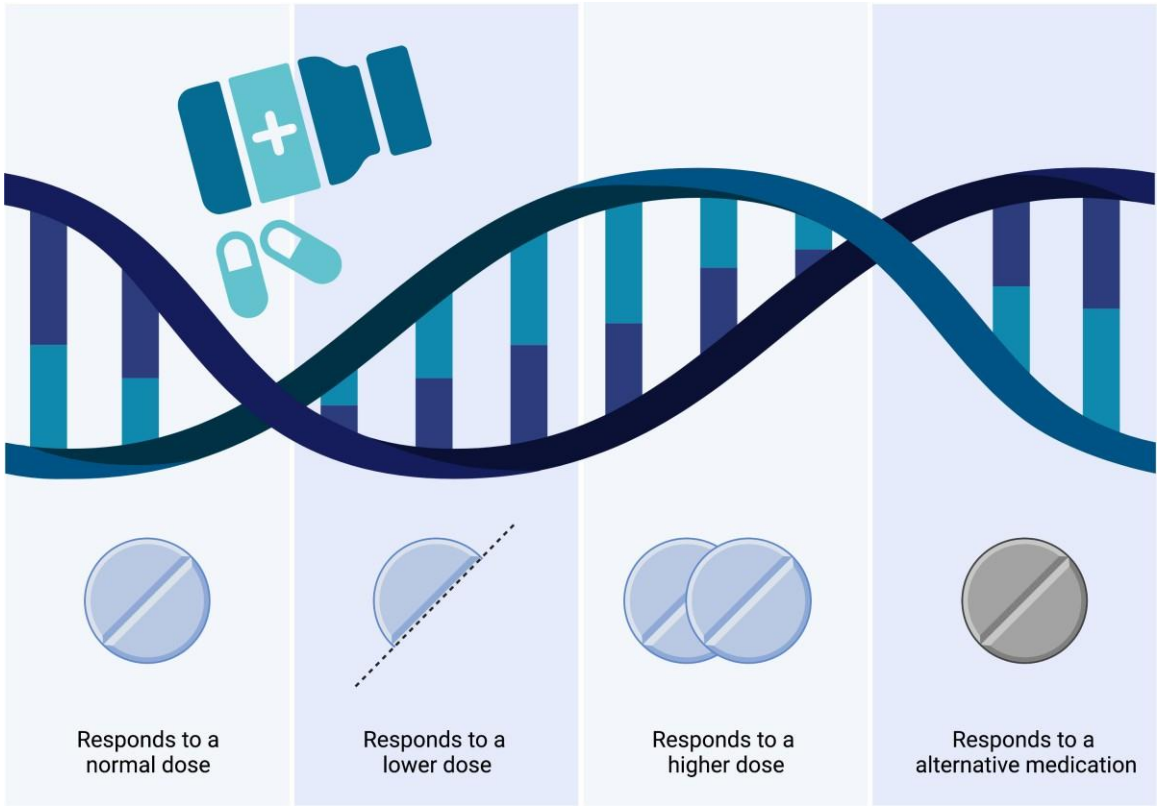

Figure 1.8: Description of the impact pharmacogenetics (PGx) changes can have on an individual's response to drug treatment. The genetic mutations in the drug-metabolising enzymes can result in the patient requiring a lower dose, higher dose, or an alternative treatment.

PGx has already shown success in guiding individualised treatments, a prominent example of which is the tailored usage of the drug warfarin. Warfarin is an anticoagulant widely used for the prevention of strokes and heart attacks and is highly susceptible to genetic variations in the *CYP2C9* enzyme (Dean, 2012). *CYP2C9* metabolises the more potent (S)-warfarin isomer (Baker and Johnson, 2016), and genetic variations such as *CYP2C9*2* and *CYP2C9*3* pose a risk to the patient that carries them. These variants characterise decreased *CYP2C9* metabolism (PM phenotype), leading to higher (S)-warfarin concentrations, and can increase the risk of bleeding and ADRs (Jorgensen *et al.*, 2012). Thus, depending on a patient's PGx profile, reduced warfarin dosages are then given to those with *CYP2C9*2* and *CYP2C9*3*

genotypes. An investigation of active drug concentrations and the metabolic products of the drug can provide further information on a patient's drug response (Linder *et al.*, 1997). For instance, the monitoring of the concentration of thiopurine drug (e.g., azathioprine and 6-mercaptopurine) metabolites during the treatment of inflammatory bowel disease (Gearry and Barclay, 2005, Askanase *et al.*, 2009) can distinguish individuals who are deficient in the thiopurine methyltransferase enzyme that mediates drug metabolism (Baker, 2003). Overall, the tailoring of drug therapies is not a new concept. However, the emergence of the 'precision medicine' approach is hindered by a lack of awareness by clinicians, the high cost to determine genetic information, the rarity of unusual drug responses, and the possibility that multiple PGx factors contribute to altered drug response that cannot be easily targeted (Ingelman-Sundberg, 2001).

1.10.2.1 Pharmacogenetics (PGx) in Africa

PGx is of growing importance in Africa as a tool to identify responders to drug treatment, reduce ADRs, and optimise drug dosing; all of which could revolutionise the treatment of diseases on the continent (Radouani *et al.*, 2020). Not only will PGx benefit the patient with more precise medication, but it can save African healthcare systems the costs of inadequate treatment, overdosing, or the need to treat ADRs (Radouani *et al.*, 2020). Yet, PGx studies in African populations are scarce, despite having a greater genetic diversity than other continental populations (Mpye *et al.*, 2017). This diversity is caused by the bottlenecking of genes after the 'out of Africa' dispersal when individuals and thus their genes spread away from Africa throughout the world (Tucci and Akey, 2019). Hence, African populations possess various unique genetic markers vital to therapeutic efficacy research that could not be evaluated in other populations across the world. PGx studies in the relevant African populations are imperative, as the high genetic diversity means that the distribution of CYP

alleles differs substantially from other global populations (Mpye *et al.*, 2017). This is a key complication of optimising drugs for a different population to which the drug will be used e.g., drugs that are developed in Europe which are then used in African populations (Masimirembwa and Hasler, 1997). It is estimated that Africa carries approximately 25% of the global burden of disease, and with increased disease burden there are amplified requirements for drug treatments to be used in African populations (Murray *et al.*, 2012). Thus, the ability to predict drug efficacy in African populations using PGx research may be able to prevent the waste of resources due to ineffective treatment doses, overdosing, and incidences of ADRs.

Yet, a recent review revealed that only fifteen drugs have been clinically evaluated for PGx implications in African populations, despite the 54 diverse countries and 1.2 billion people on the African continent (Radouani *et al.*, 2020). The studies identified by Radouani *et al.* focused on drugs used by Sub-Saharan African populations, but this highlighted that the research was targeted at malaria, HIV, and TB, but not on schistosomiasis on which this thesis is centred. Initiatives to conduct PGx research and increase genomic capabilities and awareness are already being addressed in projects such as H3Africa ('Accessing Biospecimens from the H3Africa Consortium,' 2017). Still, even with advances in genomic technologies, the use of PGx in Africa is limited. Implementing PGx testing poses a massive financial burden on already challenged public healthcare systems, with policymakers and government departments requiring larger bodies of evidence to demonstrate the cost-effectiveness of large-scale PGx testing (Tata *et al.*, 2020). Furthermore, resources are usually directed toward urgent public healthcare issues, for instance, the current setbacks in the elimination of schistosomiasis created by the COVID-19 pandemic due to the pause in the MDAs (Oyeyemi *et al.*, 2020) and so the need for PGx testing is not as prominent.

Further, large areas of the continent are resource-limited regarding the ability to identify new genetic variants and conduct pharmacogenetic-pharmacodynamic-pharmacogenetic (PK-PD-PGx) drug efficacy studies (Wickremsinhe *et al.*, 2013, Tata *et al.*, 2020, Lee *et al.*, 2021, Maggadani *et al.*, 2021). The ability to collect a time-course of blood samples to obtain the drug concentration-time curve using mass spectrometry (MS), relate it to drug efficacy, and perform sequencing to obtain the PGx data is a costly process, therefore it is pivotal that convenient PK-PD-PGx testing methods are encouraged.

Nevertheless, the importance of PGx testing has already been highlighted in the precision medicine approach to HIV patients across Africa. A reduced dose of efavirenz has been given to patients in Sub-Saharan Africa with genetic variations in the *CYP2B6* gene, whose decreased enzyme function risks ADRs without a dose reduction (Matimba *et al.*, 2016). Furthermore, the cost of genotyping and sequencing is gradually decreasing (Radouani *et al.*, 2020), allowing unique African PGx markers, such as the *CYP2D6*17*, to be characterised and reported in African populations (Masimirembwa *et al.*, 1996). However, there is still a need for greater sources of information on common, rare and novel variants in African populations to further investigate causes of variable drug efficacy not observed in European populations.

1.10.2.2 Pharmacogenetics (PGx) in Zimbabwe

The lack of PGx in relevant Zimbabwean populations is highlighted by the genetic differences compared to European populations (Masimirembwa and Hasler, 1997, Matimba *et al.*, 2009). Consequently, it is not relevant to base the development of a drug for use in a country like Zimbabwe with a diversely different genetic background from European data (Tata *et al.*, 2020). To date, the lack of clinically relevant data in Zimbabwean populations regarding CYP gene polymorphisms has resulted in missed opportunities to prevent variable

drug efficacy and ADRs (Zhou *et al.*, 2021a). For example, a study characterising the distribution of the *CYP2B6*6* variant was linked to the need for a reduced efavirenz dose in patients from Zimbabwe in 2007 (Nyakutira *et al.*, 2008), yet no actionable changes were carried out. This finding was mostly ignored until 2014, when additional studies prompted the reduction of efavirenz dosage globally upon further characterisation of this genetic polymorphism, as previously discussed in **Section 1.10.2.1**. Yet, progress to accept PGx testing has already been actioned in Zimbabwe via the development of an open-array chip to assess clinically relevant PGx variants of drugs commonly used in this population (Mbavha *et al.*, 2022). Further, although there is limited awareness of PGx in Zimbabwe, a study by Muzoriana *et al.* found that pharmacists in Zimbabwe had a positive attitude towards the use of PGx, however, there was a lack of PGx education during their training (Muzoriana *et al.*, 2017).

1.10.2.3 Pharmacogenetics (PGx) of PZQ

Unlike other continents where the prevalence of infectious diseases and the use of older drugs such as PZQ have become a rarity, that is not the case for many areas of Africa. Yet, studies are scarce in people of African ancestry for both old and new drugs, despite African populations carrying a high burden of ADRs (Ampadu *et al.*, 2016, Radouani *et al.*, 2020). The high burden of ADRs may be due to a lack of evaluations of the PGx of drugs used on the continent, and the high levels of genetic diversity in the populations they are utilised. Hence, the African continent and its diverse populations are likely to benefit the most from PGx studies to further investigate the efficacy of its commonly used drugs like PZQ. Currently, the characterisation of the PGx associated with PZQ metabolism has been lacking (Mduluza and Mutapi, 2017). Especially, regarding the clinically relevant CYP variants that metabolise PZQ in the African populations where the drug is most commonly distributed

(Zhou *et al.*, 2017). It is important to clarify whether PGx factors contribute to variable PZQ efficacy and if this indirectly contributes to the long-term morbidity of schistosomiasis infection (Mawa *et al.*, 2021). So far, only one PGx study has focused on PZQ during the treatment of schistosomiasis, despite the disease afflicting over 207 million in Sub-Saharan Africa every year (Oyeyemi *et al.*, 2020, Mnkugwe *et al.*, 2021a).

1.11 Thesis Aims

The research questions and the aims of this thesis, were as follows:

1. **What are the known causes of variable PZQ efficacy?** To identify the known determinants of variable PZQ exposure, and how this influences the efficacy of PZQ, in addition to highlighting the gaps in the research.
2. **What influence does PGx have on the efficacy of PZQ treatment in Zimbabwe?** To characterise and relate the PGx variations in the CYP enzymes involved in PZQ metabolism to the efficacy of a PZQ treatment in a *S. haematobium*-exposed Zimbabwean population. The frequencies of the PGx variants were also compared to the expected frequencies in European and other African studies.
3. **Is there a PGx effect that can be detected *in vivo* using PZQ and its metabolites, and does this relate to the efficacy of PZQ treatment?** To characterise and relate the PGx variations in the CYP enzymes involved in PZQ metabolism to the *in vivo* concentrations of PZQ and its metabolites and the efficacy of PZQ treatment in a *S. haematobium*-exposed Zimbabwean population. The frequencies of the PGx variants were also compared to the expected frequencies in European and other African studies.
4. **Are there hotspots of persistent schistosome infections and variable PZQ efficacy in Zimbabwe?** To determine whether hotspots of persistent *S. haematobium* infection and hotspots of decreasing PZQ efficacy occurred during six years of MDAs in Zimbabwe. The identification of the drivers of hotspot occurrence was also performed to determine the risk factors for hotspot occurrence.

1.12 Thesis Outline

This thesis investigated the factors influencing the efficacy of PZQ in schistosome-exposed populations to fulfil the thesis aims.

In **Chapter 1**, the current knowledge on schistosome infections and its primary drug treatment PZQ is summarised, as well as the known determinants of altered PZQ efficacy.

Chapter 2 summarises the principles of the fieldwork and experimental methods, in addition to the rationale of the statistical methods utilised to answer the thesis-specific research questions.

Chapter 3 investigates factors that alter the levels of PZQ in circulation that may potentially contribute to variable PZQ efficacy. This chapter also highlights gaps in the research that are required to further understand and characterise variable PZQ efficacy during a schistosomiasis treatment. This chapter comprises an introduction, the aims/objectives, a published paper, the key findings, and a conclusion.

Chapter 4 identifies PGx variants involved in PZQ metabolism, as well as PGx variants significantly associated with PZQ efficacy. This chapter comprises an introduction, the aims/objectives, a published paper, the key findings, and a conclusion.

Chapter 5 detects PGx variants that significantly alter the concentration of PZQ and its metabolites, and relates this to the efficacy of a PZQ treatment. This chapter builds on the assessment of the genetic associations found in **Chapter 4** and further describes the drug-gene interactions during a PZQ treatment for *S. haematobium* infection in Zimbabwe.

Chapter 6 identifies hotspots of persistent infection and decreased PZQ efficacy in Zimbabwe following multiple rounds of MDAs, including an analysis of the risk factors of hotspot occurrence.

To conclude, **Chapter 7** summarises and discusses the principal findings of this study in broader terms. Furthermore, this chapter presents suggestions for further research in this field, and how the findings of this thesis could be developed to improve the success of a PZQ treatment for schistosome infection.

Chapter 2 - Methods

2.1 Background

The methods used throughout this thesis were selected to identify factors that influence the efficacy of PZQ. In this chapter, the broader fieldwork, laboratory, and sample processing methods are described, as well as descriptions of the statistical methods applied to answer the thesis-specific aims. Thus, only the scientific rationale and the general methodology applicable to multiple chapters are described here, with the study-specific details and applications of these techniques described separately in **Chapters 3, 4, 5,** and **6**.

2.2 Field studies

2.2.1 Ethical approval

Specific details on the ethical approval of each study are described in the respective chapter. Generally, for fieldwork data collected in Zimbabwe, ethical approval was sought and granted by the Medical Research Council of Zimbabwe and the University of Zimbabwe Institutional Review Board. The Provincial Medical Director provided permission for the research to be conducted in the required province of Zimbabwe, and local permission was obtained from community leaders and representatives. The aims and procedures of the study were explained to the participant or their parent/guardian in their local language, Shona, before enrolment. Written consent was then obtained upon enrolment from the participant or their parent/guardian. As study involvement was voluntary, the participant was free to withdraw from the study at any time.

2.2.2 Study population and site

The study sites, and thus the corresponding study populations, analysed in **Chapters 4, 5 and 6** of this thesis were from multiple districts in Zimbabwe. **Figure 2.1** indicates the districts of Zimbabwe in which the study sites and populations were situated.

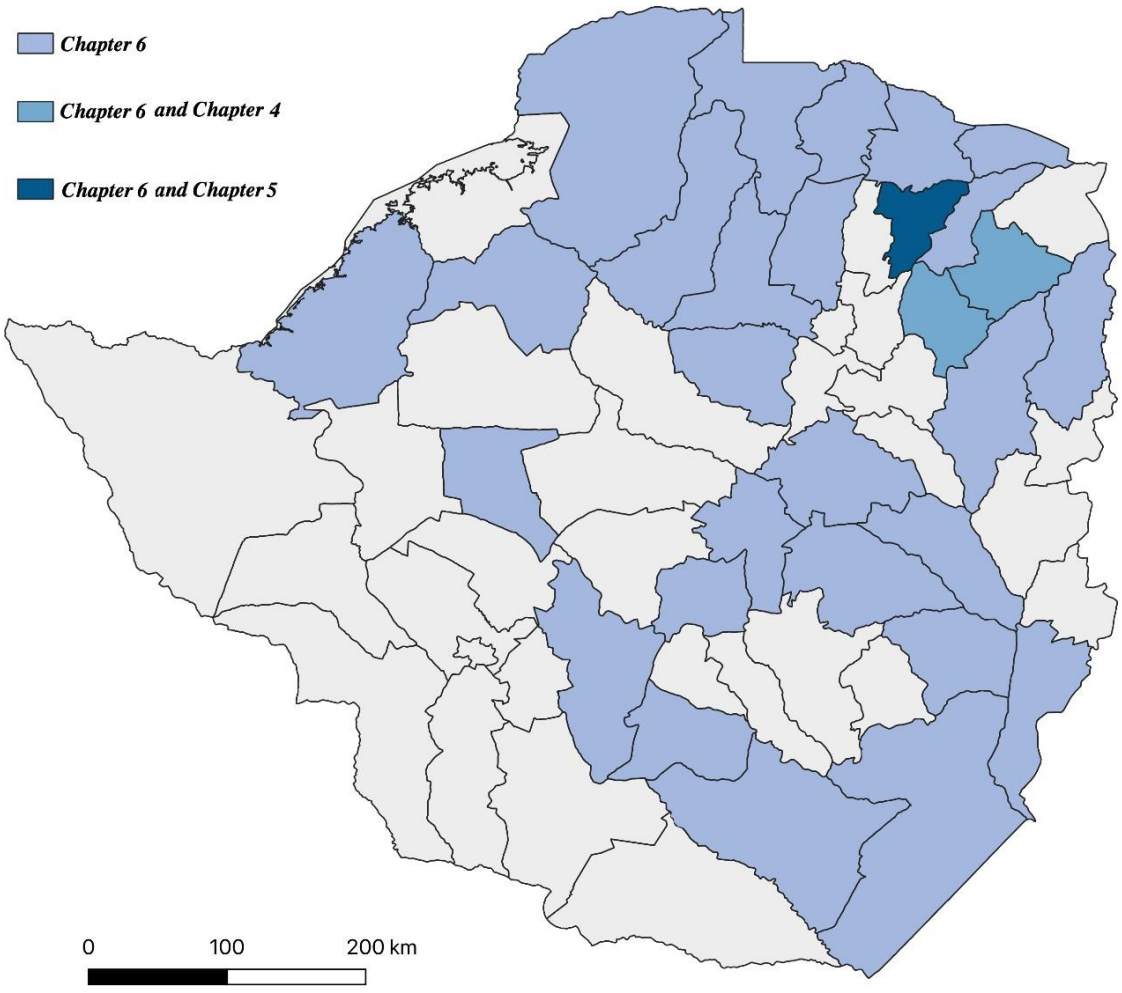

Figure 2.1: Map of Zimbabwe indicating the locations of the study sites based on their district. The districts of Zimbabwe are colour coded by chapter.

Further details of each site and the relevant metadata of the corresponding study population will be presented in each chapter.

2.2.3 Parasitological diagnosis

For the current study, the diagnosis of schistosome infection was performed by trained field technicians from the University of Zimbabwe. Urine (*S. haematobium*) and stool (*S. mansoni)* samples were obtained to conduct the parasitological diagnosis, with each sample collected from the participant between 10am and 2pm. This is the time-period when peak egg excretion is expected, and was deemed most representative of the intensity of schistosome infection (Engels *et al.*, 1996).

For *S. haematobium* diagnosis, the urine samples were examined microscopically using the standard urine filtration procedure (Jordan and Webbe, 1969). The procedure requires approximately 50mL of urine collected from at least two separate urine samples, with each sample taken daily from the participant for three consecutive days. The urines were collected over three consecutive days to improve the sensitivity of schistosome diagnosis due to natural variations in daily egg excretion, and so will average out any anomalous egg excretions (Engels *et al.*, 1996). The urine was thoroughly mixed and 10mL was removed and filtered through a nitrocellulose membrane which captured the parasite eggs. The trapped eggs were stained with iodine and enumerated under a light microscope. The participant's egg count was then documented as the number of eggs per 10mL urine. Overall, the final arithmetic mean egg count per 10mL urine was calculated as an average of all the urine samples over three consecutive days. Throughout this thesis, this was described as *S. haematobium* mean egg count or as *S. haematobium* infection intensity. The *S. haematobium* infection intensity was classified into light (<50 eggs/10mL of urine) and heavy (≥50 eggs/10mL of urine), as defined by the WHO (WHO, 2022b).

Detection of *S. mansoni* was also conducted during these studies. For *S. mansoni* diagnosis, stool samples are surveyed for infection using the Kato-Katz thick smear procedure (Katz *et*

al., 1972). The samples were collected on a single day from each participant. The stool samples were first filtered to remove large particles and were placed on duplicate slides prepared using standard 41.7 mg templates stained with glycerol–malachite green. This aided in the identification of the parasite eggs as the stain targets the stool components but does not penetrate parasite eggs, allowing for easy enumeration (Ash *et al.*, 1994). Under a light microscope, the participant's egg count per slide was counted and documented as the number of eggs per gram of stool, as per WHO guidelines (WHO, 2002b). For both schistosome species, individuals were positive for infection if at least one schistosome egg was detected in their urine or stool samples.

2.2.4 Praziquantel (PZQ) treatment

The treatment for schistosomiasis, as recommended by the WHO, is a 40mg/kg dose of PZQ administered orally using 600mg PZQ tablets (WHO, 2002b, WHO, 2020a). Studies have suggested that higher doses of PZQ could be used to increase the efficacy of a single dose, with a 60mg/kg dose found to have the highest cure rate (CR) and egg reduction rate (ERR) of a study evaluating three different dosages (20mg/kg, 40mg/kg and 60mg/kg) (Coulibaly *et al.*, 2017). However, recent reports have shown that the efficacy of PZQ at 60mg/kg was comparable to 40mg/kg, with a greater number of side effects and no significant advantages over the standard 40mg/kg treatment option (Olliaro *et al.*, 2011, Kabuyaya *et al.*, 2020). Further, although PZQ doses of 20-30mg/kg were found to have similar ERR to standard PZQ treatment, the 40mg/kg treatments had marginally higher CRs and thus are still the recommended dose (Kramer *et al.*, 2014).

Typically, the 40mg/kg PZQ treatment is tailored to the child using a dose pole, as seen in **Figure 2.2,** estimating the number of PZQ tablets needed for treatment according to an individual's height (Montresor *et al.*, 2005).

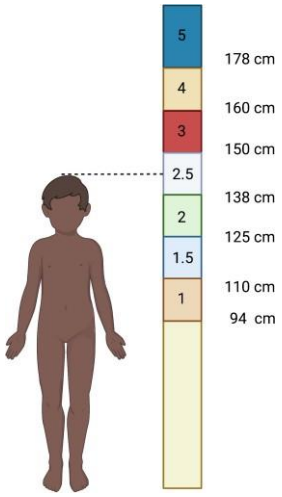

Figure 2.2: Dose pole used to estimate dosage of praziquantel (PZQ) for the treatment of schistosome infection.

The height-based dose-pole is used as a surrogate for weight as scales aren't always available in resource-poor settings (Hall *et al.*, 1999), for instance, rural Zimbabwe where this study focuses. Modifications have been made to extend the dose pole used for school-aged children (SAC) and adults for use in preschool-aged children (PSAC) (Sousa-Figueiredo *et al.*, 2012). The pole leans vertically against a wall, and the corresponding number on the dose pole indicates the number of 600mg PZQ tablets. For example, based on the height of the child in **Figure 2.2**, they require 2.5x600mg PZQ tablets to reach the recommended 40mg/kg dose. A review of the dose pole strategy found that 98% of 9,354 individuals treated using this method achieved an acceptably efficacious PZQ dosage between 30-60mg/kg (Montresor *et al.*, 2005).

To reduce the bitter taste and side effects of PZQ, the treatment was administered as a crushed tablet with bread and juice, as recommended by the WHO (Mutapi *et al.*, 2011, WHO, 2011a). Participants were observed for at least an hour by field clinicians to ensure compliance, record side effects, and determine if the drug treatment was lost through

vomiting. This is a particularly important factor to consider during the studies conducted throughout this thesis, as the loss of the full dose of the drug may play a crucial role in the efficacy of PZQ treatment.

Concerning these studies, in **Chapters 4 and 5**, PZQ was only given to patients who were confirmed to be schistosome egg-positive. During the mass drug administration (MDA) evaluations in **Chapter 6**, the PZQ treatment was tailored to the individual using a dose pole regardless of infection status. The conditions of PZQ treatment for each study population are further described in the relevant chapters.

2.2.5 Blood sampling and storage

To obtain the blood samples required to conduct **Thesis Aim 2 and 3**, approximately 5mL of venous blood was collected from each participant in an EDTA vacutainer tube (BD Vacutainer®) by local nurses in Zimbabwe. Five aliquots of 1mL were made for each blood sample. The samples were stored at 4°C for a maximum of 4 hours and were transferred to a −80°C freezer in the laboratory of the University of Zimbabwe. For each participant, one 1mL whole blood sample was then cold-chain shipped to the University of Edinburgh for long-term storage at −80°C.

2.3 Measures of infection and efficacy

2.3.1 Measure of schistosome infection

During MDAs, the PZQ treatment regime of a schistosome-endemic site is reliant on the baseline prevalence of that area (**Section 1.3**). The equation used to calculate the prevalence of schistosomiasis is displayed in **Equation [I]** below.

Equation [I]

$$\text{Prevalence (\%)} = \left(\frac{\text{Number of individuals positive for schistosome infection}}{\text{Total number of individuals sampled}}\right) \text{ x } 100$$

Assessing the prevalence of *S. haematobium*-infection was important in determining the success and failure of a MDA in the community during **Chapter 6**.

2.3.2 Measures of praziquantel (PZQ) efficacy

The extent of parasite exposure to PZQ is vital for efficacious schistosomiasis treatment. An infected individual must sustain PZQ concentrations above 1μM (312 ng/mL) for at least six hours for schistosome death to occur (Nleya *et al.*, 2019). Therefore, for a successful PZQ treatment to occur, the infected individual must maintain lethal systemic schistosome concentrations and not metabolise the active parent PZQ too quickly. Vice versa, maintaining PZQ concentrations for too long could increase the risk of adverse drug reactions (ADRs), so the individual must clear the drug via metabolism in the appropriate amount of time to avoid this. Here, to evaluate the factors that influence the efficacy of PZQ treatment in schistosome-infected populations, PZQ efficacy was measured based on either epidemiological measures or *in vivo* dose-related descriptive measures. Therefore, although the PZQ efficacy measures assessed in this thesis depend on the outcome being analysed, it will always be described in terms of alterations to lethal PZQ concentrations to increase or decrease the efficacy of a PZQ treatment.

2.3.2.1 Therapeutic efficacy

The therapeutic efficacy of a PZQ treatment was determined by two epidemiological measures, the CR and the ERR (Bajiro *et al.*, 2016). As both measures are commonly

reported and are discussed interchangeably concerning PZQ efficacy, both measures will be described and highlighted throughout these studies.

The CR was calculated using **Equation [II]** below.

Equation [II]

$$\text{CR}\,(\%) = \left(\frac{\text{Number of individuals positive at baseline,but negative post}-\text{PZQ treatment}}{\text{Number of individuals positive at baseline}}\right) \text{x } 100$$

The ERR was calculated using **Equation [III]** below.

Equation [III]

$$\text{ERR}\ (\%) = \left(1 - \left(\frac{\text{arithmetic mean egg counts at post}-\text{PZQ treatment}}{\text{arithmetic mean egg counts at baseline}}\right)\right)\ \text{x } 100$$

The CR expresses the proportion of individuals who are negative post-PZQ treatment, reporting the impact of individual non-responders more than an overview of a population. Conversely, when analysing the ERR of a population, ERR measures the effect of a PZQ treatment on all the infected individuals, ignoring specific individual variability as it is a ratio between all the pre- and post-treatment egg counts (Olliaro *et al.*, 2015). Thus, the variability in CR tends to be higher than in ERR, as shown by a recent review of PZQ efficacy studies (Fukushige *et al.*, 2021). The WHO recommended using ERR in preference to CR as a measure of efficacy, particularly when assessing PCT effectiveness as it is more representative of the morbidity of schistosome infection (Montresor, 2011, WHO, 2013a).

ERR can also be used to evaluate PZQ efficacy on an individual basis, as utilised in these studies, assessing the ratio between the pre- and post-treatment egg counts of each participant. This measure can then indicate whether the individual participant clears (100% ERR) or does not clear (<100% ERR) schistosome infection. Throughout these studies, the

description of the therapeutic efficacy of PZQ treatment was described in terms of its clinical and infection outcome, as presented in **Table 2.1**.

Table 2.1: Definitions of the therapeutic efficacy of a praziquantel (PZQ) treatment used throughout this thesis.

	Definitions of Post-PZQ Treatment Outcomes	
Infection Outcome	Cleared Infection (100% ERR)	Not Cleared Infection (< 100% ERR)
Treatment Outcome	Successful	Unsuccessful

The definitions in **Table 2.1** are utilised when it was not possible to obtain *in vivo* measures, such as the concentrations of parent PZQ and its metabolites (Chin and Lee, 2008), to describe the therapeutic efficacy during certain studies in this thesis. So, when describing decreased PZQ efficacy in an individual in terms of therapeutic efficacy, it will also be described as a lack of sustained exposure to PZQ to kill the schistosome load based on whether the individual did not clear infection or vice versa. For example, if treatment was unsuccessful, it was assumed that the systemic levels of PZQ were too low to successfully kill the schistosome load, therefore the participant did not clear infection (<100% ERR) based on the WHO recommended measure of PZQ efficacy.

2.3.2.2 Pharmacokinetic-pharmacodynamic (PKPD) efficacy

Defining the efficacy of PZQ treatment using pharmacokinetic-pharmacodynamic (PKPD) analysis relates the *in vivo* drug concentrations (PK measure) relative to the therapeutic efficacy (PD outcome) (Negus and Banks, 2018). The PK measure quantified the active concentrations of PZQ and its metabolites, and the PD outcome involved calculating whether the individual was successfully (100% ERR) or unsuccessfully (<100% ERR) treated. By relating the concentrations of PZQ and its metabolites to the outcome of PZQ treatment, the

PKPD approach assessed if the schistosomes were sufficiently exposed to the drug and whether there were any detectable alterations to the rate of PZQ metabolism.

2.4 Pharmacogenetic (PGx) methods

Two chapters in this study used analytical methods to generate PGx data, as further described in **Chapters 4 and 5**. Any chapter-specific details are presented in the corresponding chapters, but the general methods that apply to both chapters are detailed below.

2.4.1 Genomic sequencing

2.4.1.1 DNA extraction and storage

Genomic DNA was extracted from the blood samples using QIAGEN QIAamp DNA MicroKit (Qiagen, GmbH, Germany). This kit was selected as it facilitates the use of small quantities of whole blood (1–100µL) as the sample volumes available for these studies were limited. The blood sample was pipetted into a 1.5mL Eppendorf tube, and 10µL of buffer ATL was added. 10µL of proteinase K and 100µl of buffer AL were added to the Eppendorf and vortexed for 15 seconds. These were incubated at 56°C for 10 minutes, with each sample vortexed at 5 minutes to increase DNA yield. The tubes were centrifuged to remove drops from inside the lid, and 50µL of ethanol (90-100%) was added and vortexed for 15 seconds to thoroughly mix. These were then incubated for a further 3 minutes and centrifuged again to remove drops from the lid. The entire lysate was removed from the Eppendorf into a QIAamp MinElute column in a fresh 2mL collection tube, without wetting the rim. The lid was closed and centrifuged at 8000rpm for 1 minute. The column was then placed in a clean 2mL collection tube, discarding the previous tube with the flow-through waste. 500µL of buffer AW1 was added, without wetting the rim, and centrifuged at 8000rpm for 1 minute. The column was then placed in another clean 2mL collection tube, discarding the previous tube

with the flow-through waste. This step is then repeated with buffer AW2, discarding the tube again with the flow-through waste. In a clean 2mL collection tube, the column was then centrifuged at 14000rpm for 3 minutes to dry the membrane. The column is then placed in a fresh 1.5mL Eppendorf tube, discarding the previous 2mL tube. 50µL of buffer AE was applied to the membrane of the column and left to incubate at room temperature for 5 minutes. Then the tube was centrifuged at 14000rpm for 1 minute. The resultant elution was quality-checked to ensure there was sufficient DNA for sequencing per the Beijing Genomics Institute (BGI, Shenzhen, China) requirements. For example, the average DNA recovery in **Chapter 5** was 1.1µg for the extractions performed, above the 1µg threshold for BGI. The DNA samples were stored at the University of Edinburgh at -20°C until shipped on dry ice for library preparation and genomic sequencing by BGI.

2.4.1.2 Library preparation and sequencing

Two techniques were used in this thesis for the library preparation and sequencing of genomic DNA. The workflow of the genomics sequencing conducted is briefly described in **Figure 2.3**.

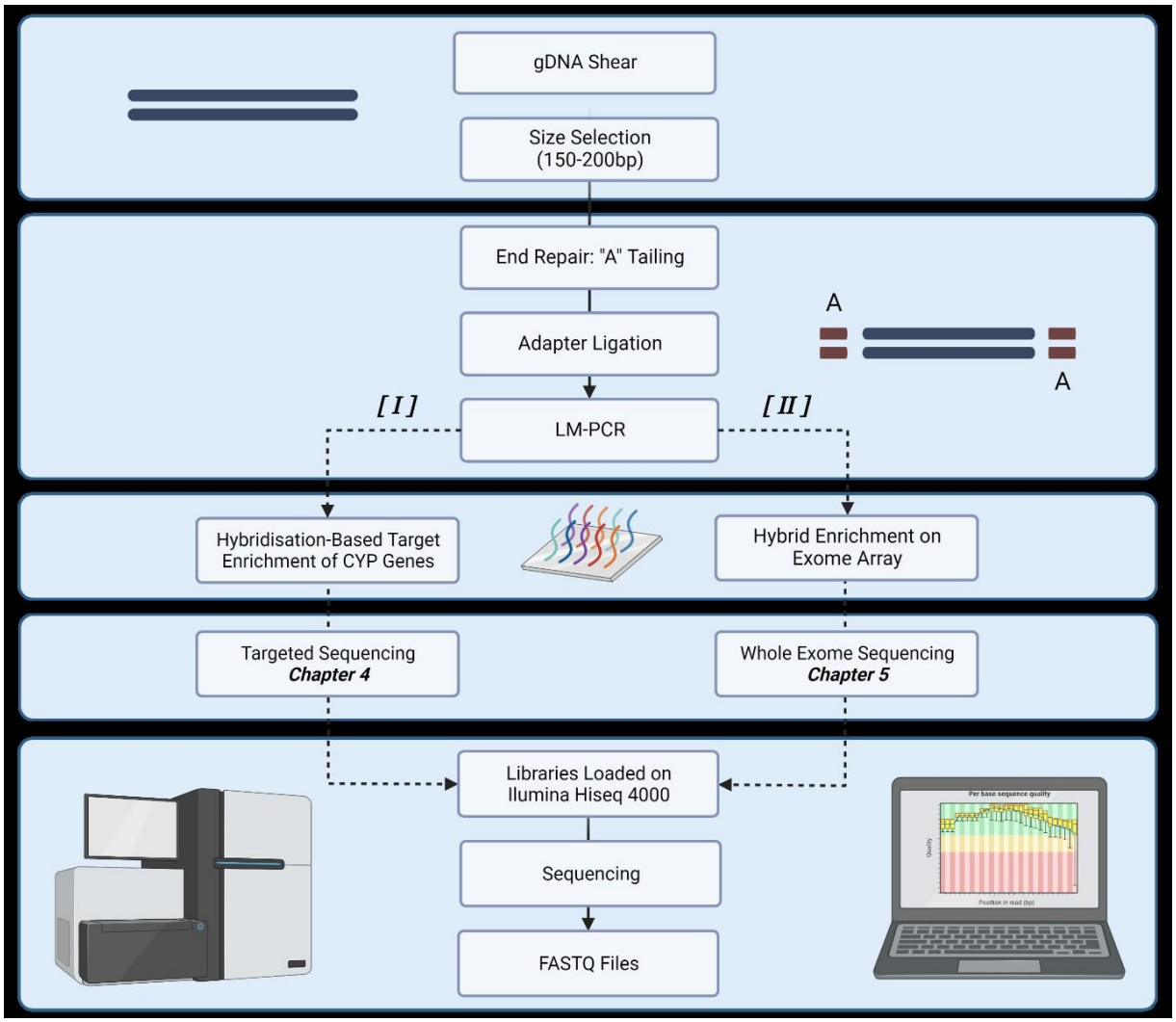

Figure 2.3: The general workflow of the genomic sequencing conducted in this study.

Targeted genomic sequencing was utilised in **Chapter 4** of this study, and was selected as it provides a high sequencing depth of the target regions (here the cytochrome P450 (CYP) genes) for a reduced cost and data burden (Bewicke-Copley *et al.*, 2019). Whole exome sequencing (WES) was utilised in **Chapter 5**, however it was primarily selected as the best cost and data-burden alternative to targeted genomic sequencing, which was unavailable due to supply-chain issues and time constraints. WES is a valuable tool as it sequences the exome, which is the protein-coding section of the human genome (Saarela and Kettunen, 2017), and is a useful tool in many PGx studies to characterise a range of ADME variations (van der Lee *et al.*, 2020, Silgado-Guzmán *et al.*, 2022). The general workflow of both these

sequencing methods began with the quantification of the DNA samples using the Qubit fluorometer (ThermoFisher Scientific, New Territories, Hong Kong) and the NanoDrop™ spectrophotometer (ThermoFisher Scientific, New Territories, Hong Kong). The integrity and purity of DNA were assessed by a 1% agarose gel electrophoresis. Qualified DNA was randomly fragmented into 150bp-250bp fragments by Covaris technology (Covaris, Woburn, USA) and end repair of DNA fragments was performed with an "A" base added to the 3'-end of each strand. Adapters were then ligated to both ends of the end-repaired/dA-tailed DNA fragments. Hybridisation was performed using a ligation-mediated polymerase chain reaction (LM-PCR) via two enrichment methods, as depicted in **Figure 2.3** and described in brief below:

(I) Hybridisation-based target enrichment and selective amplification of the size-selected DNA fragments were performed for the target CYP gene regions. Further details of this method are described in **Chapter 4**.

(II) The size-selected DNA fragments were hybridised to the exome array for enrichment. Non-hybridised fragments which did not adhere to the array were then washed out and captured fragments were amplified. Further details of this method are described in **Chapter 5.**

Each captured library was loaded on the Illumina Hiseq 4000 platform (Illumina, San Diego, USA) and the raw image files were processed by DNBseq base-calling software with default parameters to produce the nucleotide sequences. The sequence data of each individual was generated as paired-end reads and stored in FASTQ format (a text-based format for biological sequence outputs). The FASTQ files used in these analyses are deposited on the Sequence Read Archive (SRA) of NCBI.

2.4.2 Bioinformatic pipeline

Bioinformatic analyses of genomic data relies on a series of transformations, resulting in a pipeline to process the sequence and metadata (Leipzig, 2016). Pipelines tend to be built for the type of data being analysed (e.g. targeted sequenced data) to detect the desired mutations of a specific disease or phenotype (Causey *et al.*, 2018). A general bioinformatic pipeline is presented in **Figure 2.4** below.

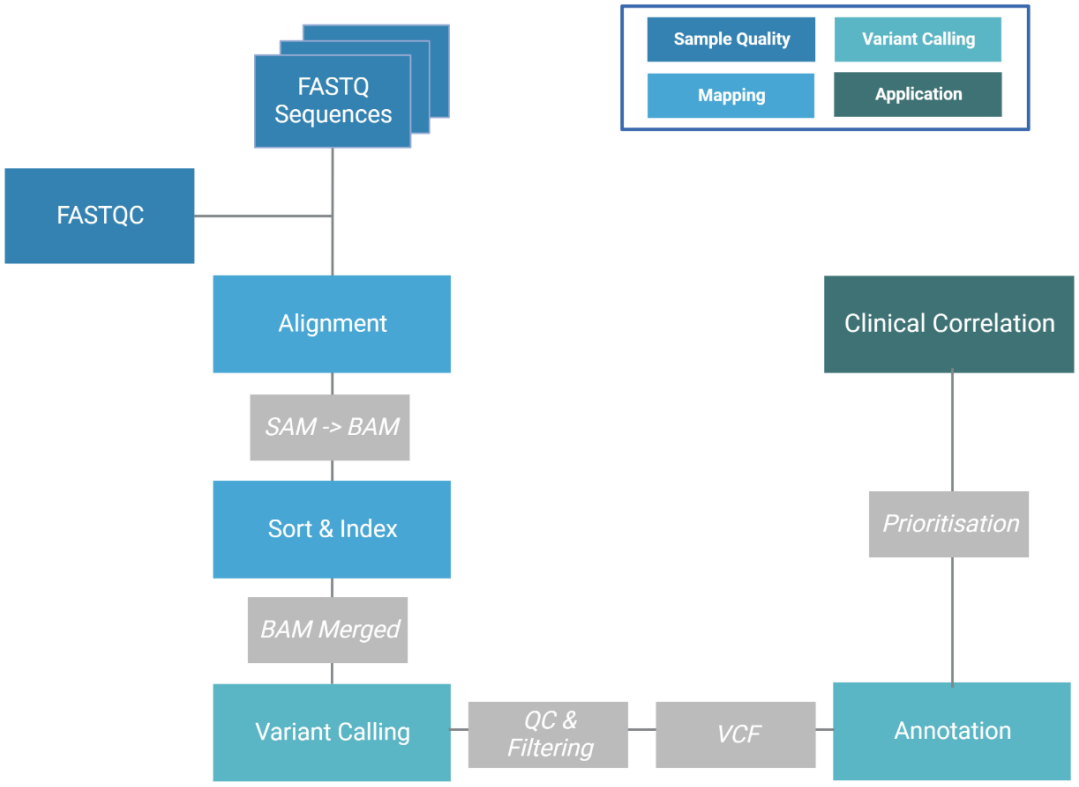

Figure 2.4: A general bioinformatic pipeline (Leipzig, 2016, Causey *et al.*, 2018, Marshall *et al.*, 2020).

Firstly, the raw FASTQ format sequences are checked using FASTQC v0.11.9 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). The output of the FASTQC analysis indicates whether the raw FASTQ sequences are of good quality and provides the number of read pairs generated per sample. For instance, in the sequencing run in **Figure 2.3**

(I), the FASTQ sequences were deemed of good quality and the number of reads per sample ranged from 79,338 to 1,415,332. The raw FASTQ sequences obtained from human samples are mapped against the reference FASTA file using Burrows-Wheeler Aligner (BWA-mem) (Li and Durbin, 2009, Causey *et al.*, 2018). The reference FASTA file comprised of nucleotide sequences, encoded as A, G, C or T, representing a target region or gene obtained from the RefSeqGene database (https://www.ncbi.nlm.nih.gov/refseq/rsg/) via the National Center for Biotechnology Information (NCBI). The alignment to the raw reads produces individual SAM files, which are transformed into a BAM file using a bioinformatic tool like samtools (Li *et al.*, 2009). The BAM files are then sorted, indexed, and merged for joint variant calling, which increased the sensitivity of variant calling in low-coverage regions (DePristo *et al.*, 2011). Variant calling tools are then utilised to obtain the variants data, as well as perform quality control (QC) and hard filtering to ensure coverage and remove low-quality calls (Li, 2011, Krusche *et al.*, 2019). The resultant VCF file is annotated according to the reference human genome assembly using reputable genomic databases such as dbSNP (Sherry *et al.*, 2001), Ensembl (Hubbard *et al.*, 2007), or Gnomad (Karczewski *et al.*, 2020). This is an important annotation as the minor allele frequency (MAF) in ethnicities across the globe is reported via these databases, thus comparisons of the study populations could be performed using this information. Furthermore, concerning the PGx evaluations throughout **Chapters 4 and 5**, CYP variants that have been reported to have important PGx effects were annotated using the recommended PGx databases: The Human Cytochrome P450 Allele Nomenclature Database (PharmVar) (Gaedigk *et al.*) and SNPedia (Cariaso and Lennon, 2012). Following these annotations, the prioritisation of the clinically relevant variants occurred and the relevant clinical meta-data (age, sex, drug concentration etc.) is analysed (Marshall *et al.*, 2020). A bioinformatic pipeline following these guidelines was developed for both **Chapters 4 and 5**, with further details in each.

2.4.3 Variant analysis

2.4.3.1 Models of genetic associations

Each SNP's minor allele *a* and major allele *A* were represented as a contingency table of PZQ treatment outcome (e.g., cleared, not cleared) according to genetic models described by Clarke *et al.* (Clarke *et al.*, 2011) using the PLINK software (Purcell *et al.*, 2007). The description of each genetic model including the genotypes assessed, the degrees of freedom and the test code in PLINK is described in **Table 2.2**.

Table 2.2: Model of genetic association used in the case-control analysis of pharmacogenetic (PGx) data, and the description of the contingency tables used to calculate this.

Model of Association	Genotype Assessed	Contingency Table Description	Degrees of Freedom	PLINK Test
Genotypic	*a/a vs A/a vs A/A*	2 x 3 table of N case-controls by each genotype	2	GENO
Allelic	*a vs A*	2 x 2 table of 2N case-controls by allele count	1	ALLELIC
Dominant	*a/a vs [A/a + A/A]*	2 x 2 table of N case-controls by dominant genotype model	1	DOM
Recessive	*A/A vs [a/A + A/A]*	2 x 2 table of N case-controls by recessive genotype model	1	REC
Cochran-Armitage trend *(additive)*	*a/a vs A/a vs A/A*	2 x 3 table of N case-controls by each genotype, with each allele increasing in importance	1	TREND

a/a: the homozygous reference genotype. A/a: is the heterozygous genotype. A/A: homozygous for the variant genotype.

These models include the genotypic, allelic, dominant, recessive and Cochran-Armitage trend (also known as an additive association (Cochran, 1954, Armitage, 1955)).

2.4.3.2 Hardy–Weinberg equilibrium (HWE)

Variants with deviations from the Hardy–Weinberg equilibrium (HWE) P-value can be indicative of genotyping or genotype-calling errors, population homogeneity, or a true

genetic association with disease risk (Marees *et al.*, 2018). The HWE statistic is calculated using an exact test. The threshold for HWE is generally set at $P < 1x10^{-4}$ (Wigginton *et al.*, 2005, Meyer, 2020), yet due to the problems described above, stringent HWE thresholds are not always recommended and therefore were evaluated and discussed in each relevant chapter.

2.4.3.3 Linkage disequilibrium (LD) and haplotype analysis

It is important to perform conditional and haplotype linkage disequilibrium (LD) analyses to assess whether the single nucleotide polymorphisms (SNPs) detected are independently associated with an outcome, such as PZQ efficacy, with no secondary associations (Schaid *et al.*, 2002). Calculating the extent of LD can account for correlations between nearby variants on the same chromosome that is associated with a specific population more often than if they were unlinked (Holloway and Prescott, 2017). For example, the SNP most strongly associated with an outcome or disease phenotype may be marking a region of LD that contains one or more genes in which the causal polymorphism lies. The calculation of LD was performed using PLINK (Purcell *et al.*, 2007), in combination with the previous genetic associations described in **Section 2.4.3.1**. Haplotype analysis of the SNPs in LD can indicate where there is statistical evidence of strong co-inheritance, and whether there is an association between the haplotype and disease phenotype under investigation (D'Amelio *et al.*, 2012). Haplotype blocks are defined based on the Gabriel method (Gabriel *et al.*, 2002) and are determined using the Haploview software (http://broad.mit.edu/mpg/haploview, Version 4.1) (Barrett *et al.*, 2005). This software is compatible with the output of the genetic analyses performed using PLINK and was therefore deemed the best method of haplotype analysis.

2.4.3.4 Tests of combinative genetic associations

The association between SNP and phenotype may provide an insight into one locus associated with a human trait, however it is becoming increasingly important to evaluate the combinatorial effects of SNPs (Wei *et al.*, 2014). Despite the several types of software capable of detecting interactions between SNPs related to a phenotype (Purcell *et al.*, 2007, Calle *et al.*, 2010, Wan *et al.*, 2010), these methods have two major limitations: statistical validity and SNP combination size (Terada *et al.*, 2016). The Limitless Arity Multiple-testing Procedure (LAMP) provided a method of detecting statistically significant higher-order interactions in combination with the commonly used SNP association tool PLINK (Terada *et al.*, 2016). LAMPLINK used a multiple testing procedure similar to Bonferroni correction, the difference being the division of given tests into two categories: testable and untestable (Terada *et al.*, 2016). Testable ones can cause a false positive result, while untestable ones have no possibility of doing so. Therefore, LAMPLINK only counts the number of testable combinations in the Bonferroni factor and reduces the correction factor. For example, the Bonferroni factor is 1.61×10^{7} when the maximum combined size is limited to five, while the correction factor in LAMPLINK is 2.39×10^{3}. Overall, the application of LAMPLINK in the genomic analysis may uncover synergistic effects of SNPs associated with diseases that may otherwise have been missed.

2.4.4 Prediction models

2.4.4.1 Transcriptional prediction

Across the 208 ADME genes, there have been more than 69,000 single nucleotide variants reported, indicating the complex PGx variability of the human genome (Ingelman-Sundberg *et al.*, 2018). Due to this variability, the need to translate the PGx data

into clinically relevant recommendations is key (Lauschke and Ingelman-Sundberg, 2016). However, there is an absence of experimental examinations due to the massive quantity of investigations required, thus computational predictions are routinely used to predict the functional impact of the PGx variants (Zhou *et al.*, 2018). Prediction models can vary based on the variant type to be analysed. The most commonly investigated variants possess an amino acid substitution and are predicted using the well-defined SIFT tool (Ng and Henikoff, 2003) to investigate the impact of a PGx variant on the gene. Recently, progress has been made to predict the functional impact of non-coding variants that affect splice sites, promoters, or enhancers, as they are increasingly recognised as a contributor to inter-individual variability during PGx analysis (Zhou *et al.*, 2018). Multiple tools have been developed to include these non-coding variants (Zhou and Zhao, 2018), however the GWAVA tool was selected as it was the most widely available tool and performed well in predicting the function effects of non-coding variants (Ritchie *et al.*, 2014). Overall, to fully capture the functional impact of both the coding and non-coding variants, a combination of computation tools was utilised, and each was described in detail in the relevant chapters.

2.4.4.2 Random Forest (RF)

Random forests (RFs) are a promising method of correlating multiple SNPs with an outcome (Brieuc *et al.*, 2018). RFs classify SNPs by importance to discover which ones are most predictive of the desired outcome (Breiman, 2001). The RF algorithm enables the identification of SNPs that explain phenotypic variation collectively that may not have displayed significant changes using univariate analysis of genotypes or MAF. Building the RF begins via a series of classification trees which randomly sample a subset of the participants to form an out-of-bag [OOB] data set. From the OOB, the RF randomly selects and searches for the best SNP predictive of an outcome. The optimal SNP then becomes the first node in the tree and the RF

continues to randomly select a subset of SNPs at each node and partition the data until a full tree has grown. This aims to reduce the variance and bias within and between the trees, improving the RF's predictive power (Goldstein *et al.*, 2011).

The samples not included in the OOB dataset are then classified using the tree, and the misclassification of those individuals provides an estimate of the OOB predictive error rate (OOB-ER) of the RF (Brieuc *et al.*, 2018). Increasing the number of trees and variables at each split will improve the accuracy of RF until the OOB-ER reaches a plateau (Goldstein *et al.*, 2010). The importance of each SNP (titled 'MeanDecreaseAccuracy') is then calculated based on OOB data, testing the prediction accuracy from the original OOB sample with another OOB sample where the genotype SNP was permuted rather than known (Naderi *et al.*, 2016). The importance value of each SNP was then averaged across trees and analysed to ultimately determine those that best explain variation in the outcome. A RF model was built during the PGx studies to predict the importance of each SNP on the outcome of a PZQ treatment and to further add to the discussion of the univariate associations found.

2.5 Pharmacokinetic (PK) methods

2.5.1 Microsampling

PK studies typically measure the concentration of a drug over time using easily obtainable bodily fluids (e.g., blood, plasma, or urine). These fluids are known to contain the desired drug and allow the creation of a PK profile to compare individuals or populations via measurable values such as the C_{max}, T_{max}, or the AUC (Urso *et al.*, 2002, Batchelor and Marriott, 2015). However, creating a PK profile can span many hours and requires multiple blood samples at numerous timepoints (Choi *et al.*, 2013). An individual must undergo an intensive sampling regimen to collect numerous blood samples, which is a highly invasive,

inconvenient and time-consuming procedure (Maggadani *et al.*, 2021). The collection would normally occur via venepuncture, a vastly uncomfortable process that would not be suitable in sensitive populations, particularly in children, as well as the limitations on blood volume and the pain inflicted upon the child during the sampling (Altamimi *et al.*, 2016). Additionally, because of the long time-course of multiple venepuncture blood samples, PK studies require a large number of resources in a clinical setting, something that is not optimal in a limited resource setting (Verrest and Dorlo, 2017). Furthermore, when assessing PK in sensitive populations, such as children and metabolically impaired individuals, the necessity for minimally invasive sampling processes that can increase patient compliance is essential (Batchelor and Marriott, 2015). Consequently, there is a need for alternative sampling techniques that can minimize the invasiveness of the blood sampling procedure and allow PK studies to be conducted in clinically neglected areas.

2.5.1.1 Dried blood spots (DBS)

Microsampling via dried blood spots (DBS) has been utilised for over 50 years for the metabolic screening of diseases and marker metabolites (Henion *et al.*, 2013). These DBS are extracted using a simple finger prick, rather than an intrusive large blood sample, and involves placing droplets of capillary blood on filter paper cards that are then dried at room temperature (Mei *et al.*, 2001). The DBS technique has been growing in popularity as a PK sampling tool for its many advantages, including increased compliance due to the less invasive procedure, the removal of cold chain storage and transport, the lack of necessity of a hospital or clinical environment, and the suitability for low resource settings such as in rural Africa (Edelbroek *et al.*, 2009). An issue with the DBS technique is the hematocrit bias as it can alter the recovered concentrations of the analytes due to its influence on blood viscosity and the spread of the blood spot before drying (Xu *et al.*, 2013). However, previous DBS

studies on PZQ found hematocrit bias to be minimal (Meister *et al.*, 2016b). The general workflow of the DBS extraction is displayed in **Figure 2.5**.

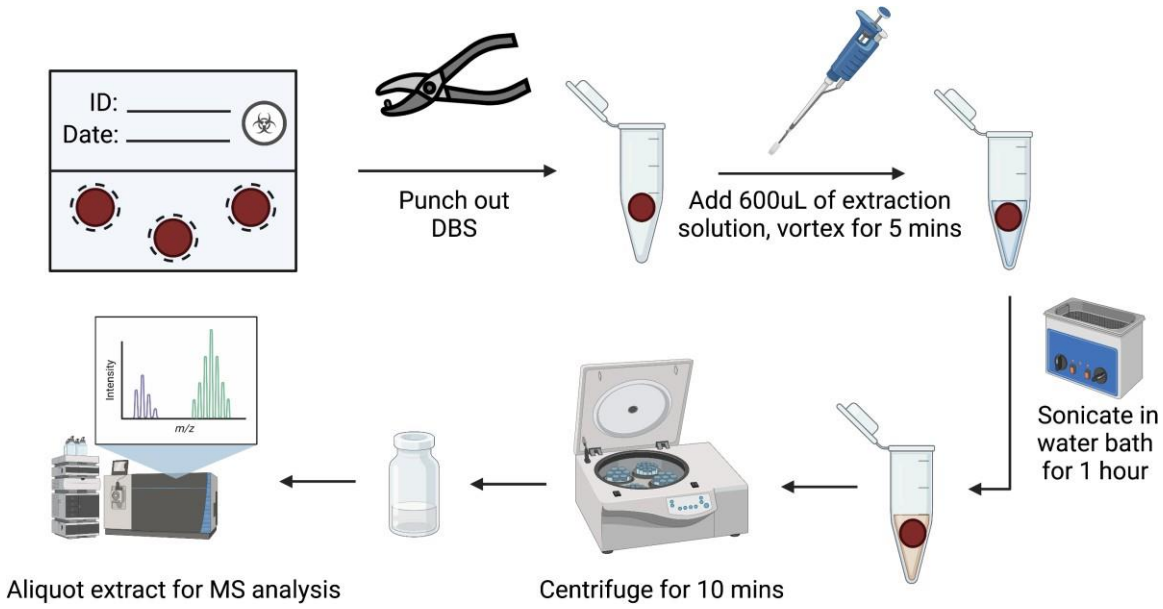

Figure 2.5: Dried blood spot (DBS) extraction workflow. MS: Mass Spectrometry.

The method of sample preparation for the PK analysis of PZQ and its metabolites from the DBS cards was based on a previously published protocol (Meister *et al.*, 2016b), and is further described and developed in **Chapter 5**. The simplicity of the DBS was a crucial technique for assessing the PK of PZQ in the populations it is commonly used: rural Zimbabwe. This technique allowed a dose-response relationship of PZQ to be characterised in the Zimbabwean population, particularly in sensitive populations such as children, without high costs and complicated tools to assess *in vivo* treatment efficacy (Henion *et al.*, 2013).

2.5.2 Mass spectrometry (MS)

MS is a key analytical technique that is applied in DMPK studies to detect and assess the concentration of a drug and its metabolites. Due to the low sample requirements, sensitivity and speed, liquid chromatography-mass spectrometry (LC-MS) has become a vital tool in

determining the PK parameters of drugs (Cuyckens, 2019). The benefit of using MS is that it separates and identifies an analyte of interest, for instance PZQ, from the remaining matrix based on its mass-to-charge ratio (m/z) (Dillen *et al.*, 2012). Further use of MS in DMPK studies is to aid in the understanding of a drug's metabolic products. Metabolite identification has become crucial to understanding the metabolic fate of drug candidates and their efficacy (Wen and Zhu, 2015), hence MS will also be used to detect the PK concentration profile of known PZQ metabolites. Regarding the metabolites analysed, the MS peak and MS transition identification of PZQ and its metabolites of interest has already been performed (Wang *et al.*, 2014b), and the relevant information for the recovery of these analytes is further described in **Chapter 5**.

2.5.2.1 Instrumentation

A Q Exactive was utilised in this thesis, with the general process described below. Firstly, the atmospheric pressure ionization (API) source forms the gas phase of the sample ions and serves as the interface between LC and the MS. The ion optics focuses the ions produced in the API source and transmits the ions through a 90° arc to the quadrupole to remove the neutral gas jet and solvent droplets. The quadrupole determines the range of *m/z* transmitted, hence only ions of a certain range of *m/z* are maintained within bounded oscillations as their velocity carries them through the mass filter. The sample ions then pass through the curved linear trap (C-trap) into the HCD collision cell, which is tuned for transmission and ejection to or from the C-Trap. The fragment spectra are generated in the HCD cell and detected in the Orbitrap mass analyser. The Q Exactive produces a fragmentation pattern comparable to the pattern of typical triple quadrupole spectra (CP, 2022). Details of the MS conditions utilised in this study are available in **Chapter 5**.

2.5.3 Noncompartmental analysis (NCA)

Noncompartmental analysis (NCA) provides PK information for a drug and is utilised in every stage of clinical drug development (Noe, 2020). NCA is a key tool in assessing the efficacy of a drug as it uses the concentration-time curve produced from the *in vivo* PK data and does not rely upon the assumptions about body compartments (model-independent) (Teuscher, 2011). NCA derives the PK parameters of target analytes and evaluates the relationship between drug exposure and efficacy using the PK profile. The NCA parameters most commonly identified include the i) maximum concentration (C_{max}) ii) the time of maximum concentration (T_{max}), and ii) the area under the concentration-time curve (AUC) (Kim *et al.*, 2018). The AUC is commonly used to assess exposure to the drug and will hence be referred to as such throughout this study (Scheff *et al.*, 2011). Each of these parameters were evaluated and discussed, when available, in relation to PZQ efficacy.

2.6 Statistics and data visualisation

The data obtained in these studies were analysed using SPSS statistical software (IBM, version 23), Minitab Statistical Software 20, R software (www.bioconductor.org; www.r-project.org) (R Development Core Team 2011), and PLINK (Purcell *et al.*, 2007), unless otherwise stated. Data were visualised using GraphPad Prism version 7.02 or 8.2.0 (GraphPad Software, Inc.) and R software (www.bioconductor.org; www.r-project.org) (R Development Core Team 2011) or via the Biorender software (BioRender.com), with the spatial data and GPS coordinates visualised using QGIS Version [3.22.2]. This section outlines the various statistical tests used to investigate the different hypotheses throughout this thesis. The use of these statistical techniques, while also controlling for confounding effects, can limit the natural heterogeneity observed in human studies, and allowed the identification of true associations

between selected factors and altered efficacy (Wang *et al.*, 2004). Precise details on the use of these tests and the outcome are detailed in the relevant chapters.

2.6.1 Univariate analyses

For each of the statistical analyses, whether the data followed a normal distribution (e.g., Gaussian distribution or a bell-shaped curve) decided whether parametric or non-parametric univariate tests were suitable. For all statistical analyses, a P-value ≤ 0.05 was considered significant.

2.6.1.1 Parametric tests

When normality was detected, parametric tests were selected and included the following:

I. A paired two-way Student's t-test was used to assess the mean difference between two sets of observations.
II. An unpaired Student's t-test was used to compare the mean of two independent groups to determine if there was a significant difference between the two groups.
III. General linear models (GLMs) were used to investigate the relationship between predictor variables, both categorical and continuous, to an outcome. This included linear regression models and analysis of variance (ANOVA).
IV. Coefficient of Determination (R^2) was used to measure the percentage variation of the independent variable that contributes to the variation of the dependent variable.
V. Descriptive statistics were used for exploratory data analysis to provide summaries on measures of central tendencies (e.g., mean), along with measures of dispersion including the 95% confidence interval (CI).

2.6.1.2 Non-parametric tests

When the data was discrete, categorical, unknown, or did not meet any parametric assumptions, non-parametric tests were selected. The non-parametric tests used in this thesis are as followed:

I. Kruskal–Wallis ANOVA was used to compare whether two or more groups of independent observations were statistically different from each other based on a continuous or ordinal dependent variable.
II. Chi-square ($\chi 2$) tests were used to determine associations between independent categorical variables, testing if there was a statistically significant difference between the expected and observed frequencies using a contingency table. When sample sizes were less than five, a Fisher's exact test was used.
III. McNemar's test was used to determine differences in paired categorical data.
IV. The Mann-Whitney U-test was used to compare differences between two independent groups concerning a continuous variable.
V. Descriptive statistics were used for exploratory data analyses to provide summaries on measures of central tendencies (e.g., median), along with measures of dispersion including the range, interquartile range (IQR) and measures of dispersion including the 95% CI. Categorical data were summarised as absolute numbers and percentages.
VI. The generalised linear model is an extension of the GLM and was used to model categorical or dependent variables against independent variables. These variables do not meet normality assumptions, an example of which includes a binary logistic regression.

2.6.2 Multivariate analyses

To determine if more than one dependent or response variable was related, correlated, or required consideration together, multivariate analyses were used.

2.6.2.1 Principal component analysis (PCA)

Principal component analysis (PCA) was used to assess the variation in datasets, and to identify if there were any clustering or grouping trends. PCA is a data reduction technique to reduce the dimensionality of the dataset and summarise the underlying variabilities of the dependent variables. This is accomplished through linear combinations of these variables called principal components (PC), which each explain a percentage variation of the dataset that is fewer in dimension than the initial data (Jolliffe and Cadima, 2016). Ellipses can be added to PCA dimensions to represent the CI at 95% and highlight the basic clustering/separation of similar/dissimilar samples.

Chapter 3 - Drug metabolism and pharmacokinetics of praziquantel: A review of variable drug exposure during schistosomiasis treatment in human hosts and experimental models

3.1 Introduction

The control of schistosomiasis is heavily reliant on praziquantel (PZQ) to treat the millions of people, the majority of which reside in Africa, who contract schistosome infections each year (Handzel *et al.*, 2003, Steinmann *et al.*, 2006). Yet, several studies in schistosome-endemic African countries have reported cases of decreased PZQ efficacy, observing low cure rates (CRs) and egg reduction rates (ERRs) in populations treated with PZQ (**Section 1.7**). Many of these studies have attributed these cases of decreased PZQ efficacy to a high pre-treatment parasite burden (Tchuenté *et al.*, 2004, Alonso *et al.*, 2006, Midzi *et al.*, 2008, Barakat and Morshedy, 2010), however others have raised concerns about the possibility of drug resistance amongst the parasites. So far, widespread PZQ resistance has not been proven, and studies accounting for pre-treatment infection intensity have still reported variable PZQ efficacy after a standard treatment for schistosomiasis (Danso-Appiah and De Vlas, 2002, Kabuyaya *et al.*, 2017). Hence, what other factors are influencing an individual's response to PZQ treatment and the eventual PZQ efficacy? This question provides the foundation for the investigations in this chapter.

Generally, an individual's response to drug treatment can be impacted by numerous host, environmental, and drug-related factors (**Section 1.10**). However, one of the most crucial characteristics of efficacious drug treatment is sufficient systemic exposure to the active drug.

Factors that alter drug metabolism can modify the systemic concentrations of the active drug (exposure), thus they are key mediators of the overall success of treatment (**Section 1.9**). So far, there is limited data on the impact of altering the drug metabolism and pharmacokinetics (DMPK) of PZQ (**Section 1.9.3**), despite it being the backbone of schistosome control (Olliaro *et al.*, 2014). The cytochrome P450 (CYP) enzymes mediate PZQ metabolism and consequently influence the concentration of the schistosome-killing PZQ (Nleya *et al.*, 2019) (**Section 1.9.3**). Therefore, alterations to the CYP metabolism can influence PZQ efficacy (Zanger and Schwab, 2013, Zhang and Tang, 2018). The CYPs are an important site for drug-drug interactions (DDIs) (**Section 1.10.1**) and pharmacogenetic (PGx) polymorphisms (**Section 1.10.2**), yet information is scarce regarding their impact on PZQ efficacy. By evaluating the role of DDIs and PGx on a CYP's ability to metabolise PZQ, the impact of these factors on PZQ exposure can be characterised.

Consequently, this chapter will focus on the factors that influence PZQ metabolism, the effect this has on the levels of PZQ in circulation, and how alterations in the schistosome-killing systemic PZQ concentrations can impact the eventual efficacy of PZQ. These results will provide insight into potential causes of the reports of low CRs and ERRs across Africa, and characterise predictors of variable PZQ exposure.

3.2 Aims and objectives

I. Determine the DMPK factors that influence PZQ exposure and the efficacy of the drug through an analysis of published studies.

II. Identify any known DDIs that influence PZQ exposure, and potentially PZQ efficacy, through an analysis of published studies.

III. Identify PGx variations that alter the activity of the PZQ-metabolising CYPs, and potentially influence PZQ efficacy, through an analysis of published studies.

IV. Identify the gaps in the research on factors that cause variable exposure of PZQ and could alter PZQ efficacy.

3.3 Published work

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Grace Zdesenko and Francisca Mutapi

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REVIEW

Drug metabolism and pharmacokinetics of praziquantel: A review of variable drug exposure during schistosomiasis treatment in human hosts and experimental models

Grace Zdesenko[1,2]*, **Francisca Mutapi**[1,2]

1 Institute of Immunology & Infection Research, University of Edinburgh, Ashworth Laboratories, King's Buildings, Edinburgh, United Kingdom, **2** NIHR Global Health Research Unit Tackling Infections to Benefit Africa (TIBA), University of Edinburgh, Ashworth Laboratories, King's Buildings, Edinburgh, United Kingdom

* g.zdesenko@sms.ed.ac.uk

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Abstract

Schistosomiasis control is heavily reliant on the drug praziquantel (PZQ), which is used as preventive chemotherapy as part of national helminth control strategies. Given the heavy reliance on PZQ for mass drug administration, there has been considerable research on the potential of parasites developing resistance to the drug, resulting in decreased drug efficacy. However, there have been comparatively fewer studies of other factors that can potentially alter PZQ efficacy. Here, we investigate whether host PZQ metabolism contributes towards variable cure rates. We evaluate factors that can influence the metabolism of PZQ and the resultant effect on the efficacy of PZQ treatment to determine factors that potentially influence an individual's response to the drug. The literature search was directed at published studies from three online databases: Web of Science, PubMed, and EMBASE. The search terms for the review comprised of ([praziquantel OR PZQ] AND [schistosom* OR bilharzia] AND [pharmaco*]) and included studies evaluating PZQ metabolism. Publications were categorised into pharmacokinetics, drug–drug interactions, pharmacogenetics, and metabolite analysis. Forty publications describing human and experimental studies fitted the inclusion criteria and were subjected to data extraction and analysis. The analyses showed that variable exposure to PZQ was associated with alterations in the liver's capacity to metabolise PZQ and observed drug–drug interactions. Other factors influencing the efficacy of PZQ were brand, formulation, and co-administered food. Although some work has been performed on metabolite identification, there was minimal information on PZQ's metabolic pathway, and no pharmacogenetics studies were identified. The study indicated that in both human and experimental studies alterations in the liver's capacity to metabolise PZQ as well as drug–drug interactions affected systemic levels of PZQ that could result in variable cure rates. The study confirmed previous findings of higher antischistosomal activity of (R)-PZQ enantiomer when administered alone compared to the racemate at the same dose as well as improved efficacy when the drug is administered with food. The study also highlighted

study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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the need for more comprehensive studies of the PZQ metabolic pathway and PZQ pharmacogenetic studies in humans.

Author summary

Schistosomiasis is a neglected tropical disease caused by parasitic worms, and its control is heavily reliant on the drug praziquantel (PZQ). Reports of individuals not being cured of schistosomiasis after a treatment have raised concerns about drug efficacy and the potential for the development of drug resistance. Many factors can be responsible for reduced efficacy including host, parasite, and drug–related factors. We investigated the potential effect of varying systemic levels of PZQ on cure rates. We reviewed studies on PZQ metabolism identifying factors that may influence PZQ systemic levels, including the drug administered, host liver health, and genetic polymorphisms of liver enzymes involved in PZQ metabolism. The results indicated that varying levels of PZQ were due to the drug formulation and differences in the liver's capacity to metabolise PZQ as well as drug–drug interactions. The study also highlighted the need for further studies of the effect of human genetics on PZQ's metabolic pathway, and the PZQ metabolites produced.

Introduction

Schistosomiasis is a prominent public health problem [1], with the majority of affected people residing in Africa [2]. Praziquantel (PZQ) is the drug of choice to treat schistosomiasis and is widely used in preventive chemotherapy (PCT) programs (as defined by WHO) [3] across Africa to treat intestinal and urogenital schistosomiasis infections caused by *Schistosoma mansoni* and *S. haematobium* parasites, respectively [4]. Mass drug administration (MDA) of PZQ in PCT to treat schistosome infection and reduce associated morbidity has been a success, with an estimated 235 million people treated with PZQ in 2018 alone [5]. PZQ itself is a racemic drug, with the standard dose consisting of a 1:1 mixture of two enantiomers (see Fig 1).

Only the (R) enantiomer (also known as Levo-PZQ, L-PZQ, or (-)-PZQ) of PZQ has antischistosomal activity; in contrast, the (S) enantiomer (also known as Dextro-PZQ, D-PZQ or (+)-PZQ) does not have antischistosomal action but contributes to some of PZQ's known side effects [6]. PZQ is well-tolerated and effective in patients of all ages with different clinical forms of schistosomiasis [7] and has been used to treat schistosomiasis since the 1980s [8]. The precise mechanism of PZQ's antiparasitic action remains poorly described. Studies suggest that its action arises from the (R)-PZQ enantiomer disrupting the schistosome calcium ion homeostasis causing uncontrolled muscle contraction and death in adult worms [9, 10]. PZQ targets only adult schistosomes; therefore, the drug is not effective against the larval stages infections [11].

The efficacy of PZQ treatment is determined by the cure rate (CR), which compares the number of egg-positive individuals pre-PZQ treatment who become negative for schistosomiasis post-PZQ treatment as well as by the egg reduction rate (ERR), determined by the reduction in mean number of eggs excreted in urine or stool (depending on the schistosome species) from pre-PZQ to post-PZQ treatment [12, 13]. Low PZQ cure rates have been reported by some studies, with many attributing this to a high pretreatment parasite burden [14–17]. However, variable efficacy of PZQ has also been observed in other studies, even when accounting for the level of pretreatment infection [18, 19], suggesting that other factors must be influencing the drug's efficacy. For example, low cure rates and the reduced efficacy of PZQ

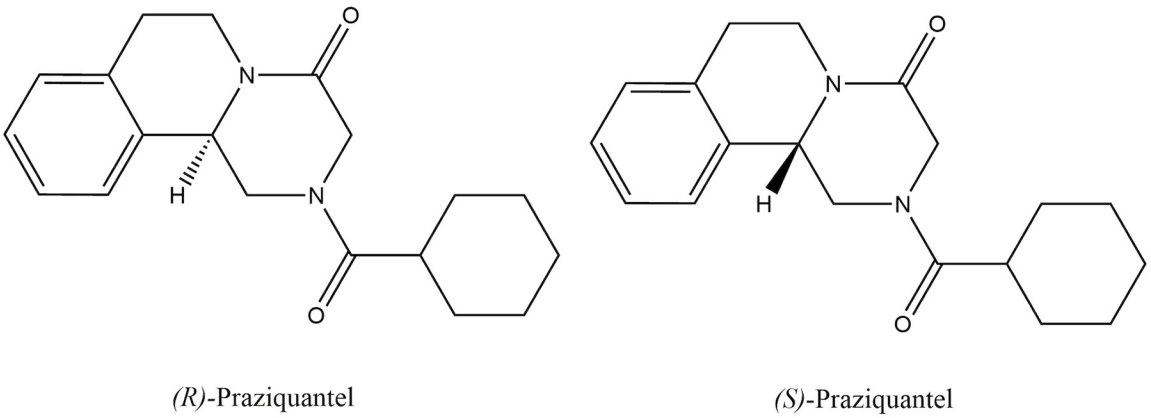

Fig 1. The molecular structure of the two PZQ enantiomers. The (R)-praziquantel has the hydrogen atom (H) pointing down from the chiral center. The (S)-praziquantel has the hydrogen atom (H) pointing up from the chiral center. PZQ, praziquantel.

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treatment have been attributed to patients harbouring schistosomes in different developmental stages and the decreased sensitivity of schistosomes to PZQ treatment [20–23]. Investigations into cases of laboratory and field PZQ resistance indicated that reduced efficacy of drug treatment due to decreased sensitivity and resistance was rare [24, 25]; hence, parasite sensitivity cannot account for all incidences of treatment failure. So, why are these low cure rates occurring? This study investigates whether the level of PZQ in systemic circulation is contributing towards low cure rates, as systemic PZQ may not be exceeding the lethal schistosome concentration. In this study, we will focus on three factors that can influence the amount of PZQ in systemic circulation, and, thus, affect the efficacy of PZQ treatment: PZQ pharmacokinetics (PK), pharmacogenetics, and drug–drug interactions.

The term "PK" describes movement of a drug, encompassing the absorption, distribution, metabolism, and elimination (ADME) parameters of that drug [26]. Metabolism describes the mechanism of breaking down the drug compound and is commonly analysed via biotransformations of the xenobiotic by drug-metabolising enzymes [27]. The Cytochrome P450 (CYP) enzymes mediate the metabolism of PZQ, specifically the following enzymes: CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4, and CYP3A5 [28]. The term pharmacogenetics refers to the variability in an individual's response to a drug due to genetic variations. Genetic polymorphisms in CYPs have linked interindividual variation to metabolism in numerous drug efficacy and toxicity studies [29]. These variations are primarily due to single nucleotide polymorphisms (SNPs) of the CYPs, and these can lead to an increased or decreased pharmacokinetic effect [30]. The distribution of CYP alleles differs substantially between populations, emphasising the need for optimising drugs for the population in which the drug will be used, e.g. the efficacy of drugs tested in Europe may not have the same efficacy in African populations [31]. Furthermore, analysing pharmacogenetic differences in the metabolic (drug metabolism) products can provide additional information on a patient's drug response [32].

The CYPs are also an important site for drug–drug interactions (DDIs) [33]. PZQ is metabolized by multiple CYPs, and so DDIs within these CYP pathways could result in the formation and accumulation of metabolic by-products or a reduction of the drug's therapeutic effect [34]. The bioavailability of a drug can be altered by DDIs via competition for protein-binding sites on the CYPs, affecting the overall efficacy of a drug. DDIs can induce the CYPs (increased metabolism), increasing the activity of the CYP enzymes and decreasing the overall bioavailability of the active drug [35]. Vice versa, if a coadministered drug inhibits the PZQ's

enzymatic binding sites, it can no longer be metabolised and eliminated via this pathway (decreased metabolism), and drug accumulation could result in toxic levels in the body [36]. Evaluating DDIs is important when developing and using any drug treatment, especially when regarding comorbidity, as patients may be on multiple medications [37]. Overall, evaluating whether pharmacogenetics factors and DDIs influence a CYP's ability to metabolise PZQ will indicate the role of altered metabolism in variable schistosomiasis CR.

Methods

Literature search strategy

The search strategy was guided by the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines [38], and the studies included in this review were published prior to May 12, 2019. The search used three online databases: Web of Science, PubMed, and EMBASE. The search terms for the review comprised of ([praziquantel OR PZQ] AND [schistosom* OR bilharzia] AND [pharmaco*]) and included animal and experimental models that evaluated PZQ metabolism. As the main burden of schistosomiasis in sub-Saharan Africa is attributed to two species of schistosomes [4], only studies on *S. haematobium* and *S. mansoni* were included in this review. The citations were compiled in EndNote X8 and duplications were identified and removed.

Literature screening and inclusion and exclusion criteria

The titles and abstracts of the Endnote Library were screened, and any that met the inclusion criteria were put aside for a review of the full text. If the abstract was not available or it was unclear whether the study fully met the inclusion criteria, it was selected for full evaluation. After a full evaluation of each text, the articles included in this review were required to fit into one of the following topics: PK, pharmacogenetics, drug–drug interactions, or metabolites analysis. Selection criteria within each category were based on whether (1) the article was available as full journal text and in English, (2) the units of the numerical pharmacokinetic parameters were clearly defined, (3) the article included human and animal *in vivo*/*in vitro* models for human extrapolation, and (4) models were either a healthy control or infected with *S. mansoni* or *S. haematobium*. If a paper could not be located through an online repository, it was requested from the British Library. Any papers that could not be obtained from these sources were excluded.

Data extraction and data analysis

Pharmacokinetic data, including the dose of PZQ and any "Drug B" measured (racemic or enantiomeric PZQ), the model, *Schistosoma* infection status, the number of subjects (N), the numerical pharmacokinetic parameter and its units, and any external test conditions, such as fasting or fed states, were documented in an Excel spreadsheet. To extract comparable data from each study, it was concluded that the *in vivo* pharmacokinetic parameters to quantify drug-concentration-time relationships would be as follows: area under the plasma-concentration curve (AUC), peak plasma concentration (C_{max}), time to reach peak plasma concentration following drug administration (T_{max}), and drug-elimination half-life ($t_{1/2}$) [39]. Data points were extracted, grouped together by parameter, and converted into standard SI units. If no units were quoted, the data point was excluded from the data tables. Where the published data was presented in a graph, Data Thief III software was used to extract the numerical values. The data points were analysed to detect trends affecting PZQ metabolism between studies, creating new combined graphical results. These graphs were then analysed in the results to assess

whether the combined data reflected the hypothesis of this review. Only limited statistical analysis was possible due to sparsity of data and heterogeneity of populations used.

To evaluate the efficacy of PZQ without pharmacokinetic values, the worm burden reduction (WBR) in the case of experimental models, ERR, and CR were also collected. For pharmacogenetic factors, the population ethnicity of each human study was recorded when provided in the source publication as genetic factors can determine response to a drug [40]. By linking the individuals in the studies by ethnicity, it implies a common genetic ancestral background that could influence a therapeutic response to a drug [41]. For the papers that depicted PZQ metabolite analysis, the structural identity of each metabolite was created using ChemDraw Prime v16.0, and any information about its metabolic pathway was reported.

Results

The search yielded a combined total of 873 studies and, after the removal of duplicates, 425 titles and abstracts were screened, resulting in 95 publications for a full-text review. There were 55 excluded publications, leaving 40 publications for data extraction and analysis (S1 Table). The results of this systematic search are displayed in a flow chart (S1 Fig).

PK and PZQ efficacy

In the PK category of this review, data points extracted from multiple papers describing healthy and infected human and animal models provided an overview of the published PK and efficacy of PZQ treatment. The animal models obtained from the search strategy were exclusively rodents, and included mice, rats, and hamsters. The human studies included adults (16 years or older), school-aged children (SAC) (6 to 15 years), and preschool-aged children (PSAC) (5 years or younger). An evaluation of these as separate study groups was made based on the model, either rodent or human, and parameters measured.

Experimental studies: PK and PZQ efficacy

The relative exposure of PZQ in the reviewed rodent models was compared using $t_{1/2}$, T_{max}, C_{max}, and AUC values (refer to S2 Table). The effects of different PZQ doses, brands, and infection status on the exposure parameters (C_{max} and AUC) of PZQ in various rodent models were described in six studies [42–47].

Hepatic CYP metabolism. On average, based on the PK data extracted from these papers, the general trend of the compiled exposure parameters was that with increasing PZQ dose, the C_{max} and AUC also increased. Upon closer inspection, there were some deviations from this trend with a common factor; it appeared that the healthy rodent models tended to have a lower exposure to PZQ than the infected rodents. A study by Botros and colleagues [42] in healthy and *S. mansoni*–infected mice showed that in the infected mice there was a significant difference in maximum concentration of PZQ in systemic circulation, approximately triple that found in healthy mice. The increased exposure was attributed to the reduced liver capacity of infected rodents, resulting in decreased metabolism of parent PZQ and, therefore, longer exposure in the circulation. Botros and colleagues [43] and Kokwaro and colleagues [44] conducted further analysis of the overall decreased hepatic CYP activity and the resultant alteration of PZQ's PK parameters due to schistosomiasis, once again with significant differences between *S. mansoni*–infected and uninfected mice detected. The relative $t_{1/2}$, T_{max}, C_{max}, and AUC values were increased proportionally to the degree of decreased CYP activity, an influential factor if this effect is also observed in humans. In fact, due to decreased hepatic CYP activity, severe side effects were observed by Gotardo and colleagues [45] from toxicity caused by the higher C_{max} and AUC values in infected mice treated with 400 mg/kg PZQ.

PZQ brands. Additional factors which may have contributed to variable PZQ exposure were investigated by comparing the parasitic efficacy of the different brands of PZQ available. To compare efficacy, two parameters were measured: (1) WBR, as the World Health Organization describes a reduction in adult worm counts as a measure of the efficacy of an anthelmintic in experimental models [48], and (2) the percentage inhibition of hepatic CYP450 activities as a result of *S. mansoni* infection, observing the extent of CYP450 inhibition when compared to healthy mice. The results in S3 Table are adapted from Botros and colleagues [42], with the highest WBR and lowest CYP450 inhibition was achieved with pure PZQ, with Distocide and Biltricide showing comparable efficacy. Interestingly, Bilharzid, brand T3A, and Epiquantel had a significantly lower WBR than the other brands analysed, yet had significantly higher CYP450 inhibition. In comparison to pure PZQ, Distocide and Biltricide, the Bilharzid, brand T3A, and Epiquantel were not aiding the recovery of the CYP450 activity from schistosomiasis infection as well as the other brands examined.

PZQ formulations. Further studies focused on the PZQ compound itself as a topic of discussion, with Zhang and colleagues [46] and Meister and colleagues [49] showing the pharmacological differences between the (R) and (S)-PZQ enantiomers, confirming the antischistosomal activity of (R)-PZQ. A comparison of the activity of racemic PZQ (rac-PZQ) compared to each PZQ enantiomer in mouse models can be seen in Fig 2 (data in S4 Table).

This is consistent with (R)-PZQ being the pharmacologically active enantiomer, with a significantly higher WBR than the (S)-PZQ and a higher WBR than the current standard rac-PZQ treatment at the same dose [49, 50]. One contradictory piece of data extracted during this review was from Tanaka and colleagues [50], in which the (S)-PZQ had a higher WBR than rac-PZQ and (R)-PZQ at 50 mg/kg of PZQ, yet this could be due to interanimal variability as the study was only conducted in seven mice. Overall, this discrepancy in the activity of the enantiomers was only observed in one paper and was not seen in any of the human studies.

A PZQ formulation (Polymorph B) was tested in mice by Lombardo and colleagues [47] as a new treatment option in comparison to the current PZQ tablet. Polymorph B aimed to increase efficacy and improve the PK parameters of the current PZQ formulation, which has low bioavailability and low water solubility, by enhancing PZQ's solubility and dissolution. The crystalline formulation created from grinding rac-PZQ showed improved physical properties, particularly increasing PZQ chemical stability and doubling water solubility. Yet, the PK parameters of both enantiomers of Polymorph B had a lower exposure profile compared to a reference PZQ, indicating lower bioavailability of the new drug formulation compared to the current standard PZQ. This was most prominently visualised in the AUC value, which was approximately 40% lower for Polymorph B than reference PZQ.

Human studies: PK and PZQ efficacy

As with the rodent models, the relative exposure of PZQ was compared using $t_{1/2}$, T_{max}, C_{max}, and AUC values, with 13 papers containing human PK data sets [51–63].

PZQ formulations. As seen in the rodent models, data in human studies also showed a significant difference in antischistosomal activity between the two PZQ enantiomers. Four papers [51–54] (S5 Table) measured the PK of each PZQ enantiomer after a racemic dose in humans. The results were compiled and, as Fig 3 depicts, there were clear differences in enantiomer exposure between the biologically active (R)-PZQ and the (S)-PZQ.

Despite (R)-PZQ being the desired circulating pharmacoactive substance, the results extracted during this review in healthy normal volunteers (HNV) showed that the exposure parameters of the (R)-PZQ enantiomer were significantly lower than the (S)-PZQ. Therefore, the C_{max} and the AUC of the active enantiomer are lower than the inactive enantiomer at the

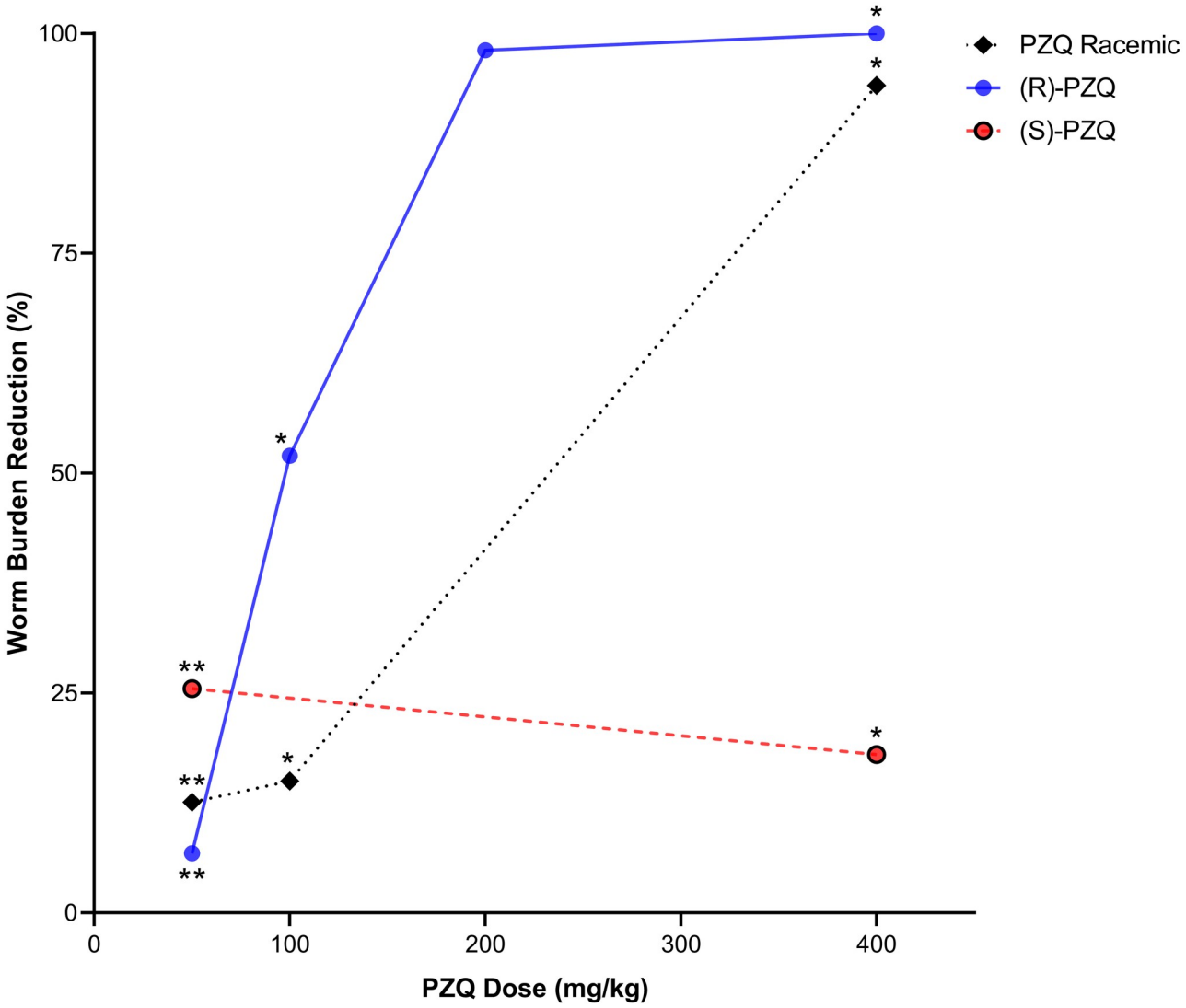

Fig 2. Comparison of the percentage WBR in an infected mouse model when dosing racemic PZQ or a single enantiomer. The data for this graph were extracted from two papers in this review and evaluated to assess the WBR-dose response [49, 50]. The relationship between WBR and drug formulation was found to be significantly different ($^{*}P < 0.01$, $^{**}P < 0.001$) for each PZQ dose based on the results of the independent samples Kruskal-Wallis Test. PZQ, praziquantel; WBR, worm burden reduction.

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same dose (Fig 3), so, when PZQ is dosed as a racemic tablet, there are significantly lower proportions of the desired enantiomer entering the circulation. This is the opposite of the desired action and demonstrates that a dose with a rac-PZQ tablet is not reflective of the dose of active (R)-PZQ.

Two studies in this review evaluated the PK of a small orally dispersible tablet (ODT) formulation that would allow for fast dispersion and an acceptable taste [51, 52]. The relative bioavailability of a rac-PZQ ODT formulation was compared with the single active enantiomer (R)-PZQ ODT ((R)-ODT), to determine if there were pharmacokinetic differences between ODTs and the current rac-PZQ tablet. When dosed at 40 mg/kg, the rac-PZQ reference is 50:50 of (R)-PZQ to (S)-PZQ; therefore, it was expected that when the (R)-ODT was dosed at 20 mg/kg, it would be approximately equal to that of the 40 mg/kg of rac-PZQ or the PZQ

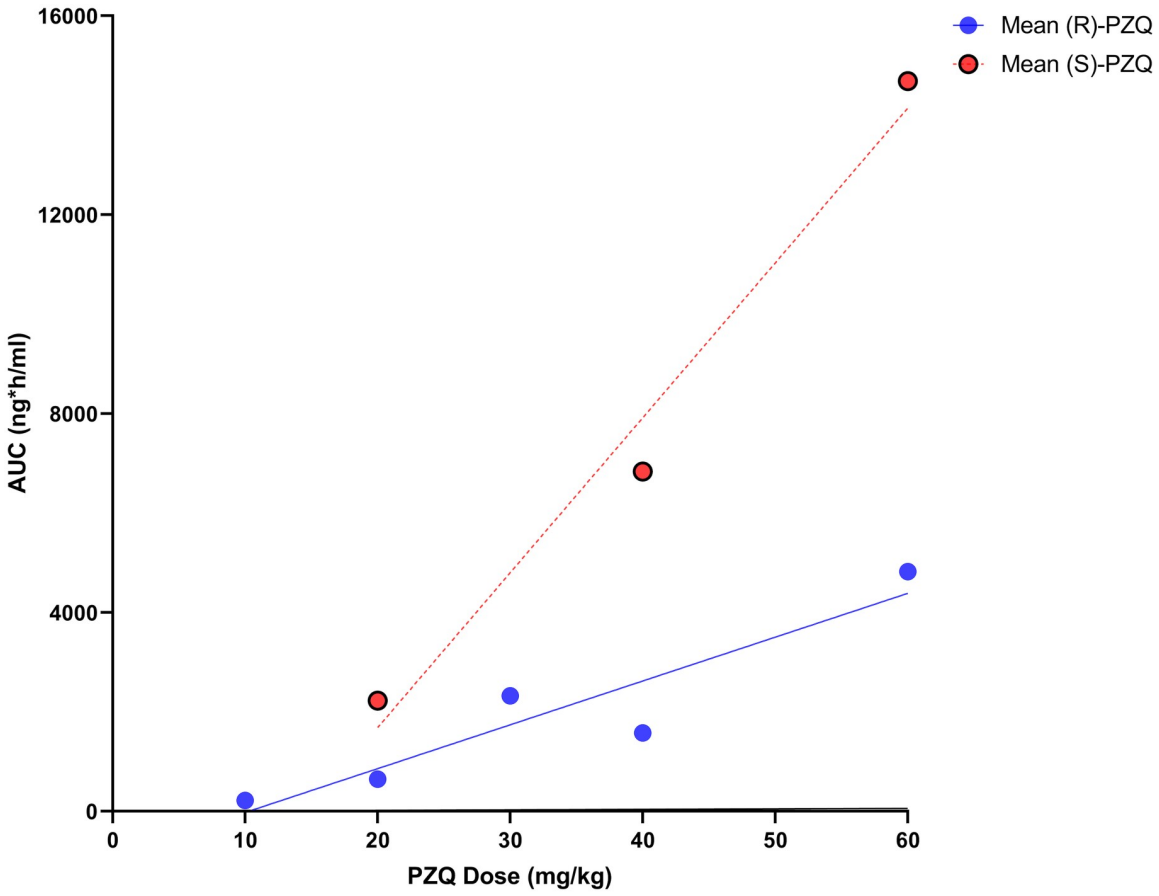

Fig 3. The mean AUC values extracted from the included studies against the PZQ dose of each of the PZQ enantiomers in HNV. The PZQ enantiomers were measured after a racemic PZQ dose or the (R)-PZQ dosed alone. These data were extracted from multiple papers in this review, separated by PZQ enantiomer, and the AUC was averaged for each dose and plotted on a graph for analysis [51–54]. Using a linear regression model, the difference in AUC between (R)-PZQ and (S)-PZQ in humans was found to be statistically significant ($P < 0.05$). AUC, area under the curve; HNV, healthy normal volunteers; PZQ, praziquantel.

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reference. However, this did not appear to be the case as the 20 mg/kg dose of (R)-ODT was only around 40% of the PZQ reference.

Hepatic CYP metabolism. The activity of the drug-metabolising CYPs during PZQ metabolism and the resultant variable exposure of the drug was assessed by multiple studies. With regard to hepatic CYP activity, el Guiniady and colleagues [55] investigated the potential decrease in CYP metabolism in *S. mansoni*–infected adult patients with either liver cirrhosis or splenomegaly (data in S6 Table), building on the rodent study by Kokwaro and colleagues [44]. The study concluded that, in general, the C_{max}, T_{max}, and AUC were significantly higher in the liver-impaired patients compared to the controls. This was determined to be due to the delay in elimination of PZQ to its metabolites as a result of decreased CYP function [55]. In humans, as the severity of cirrhosis increased, the patients had an elevated C_{max} and AUC in comparison to HNV and nonimpaired *S. mansoni* patients at the same PZQ dose.

Concerning the hepatic metabolism of PZQ in children, Kovac and colleagues [56] investigated the PK parameters of SAC and PSAC regarding age-related metabolism and the level of CYP maturity in children. Data points from three papers [56–58] were obtained (S7 Table), including the $t_{1/2}$, T_{max}, C_{max}, and AUC. The increase in AUC with increased dose for SAC and PSAC infected with *S. mansoni* and *S. haematobium* were compared in Fig 4, measuring

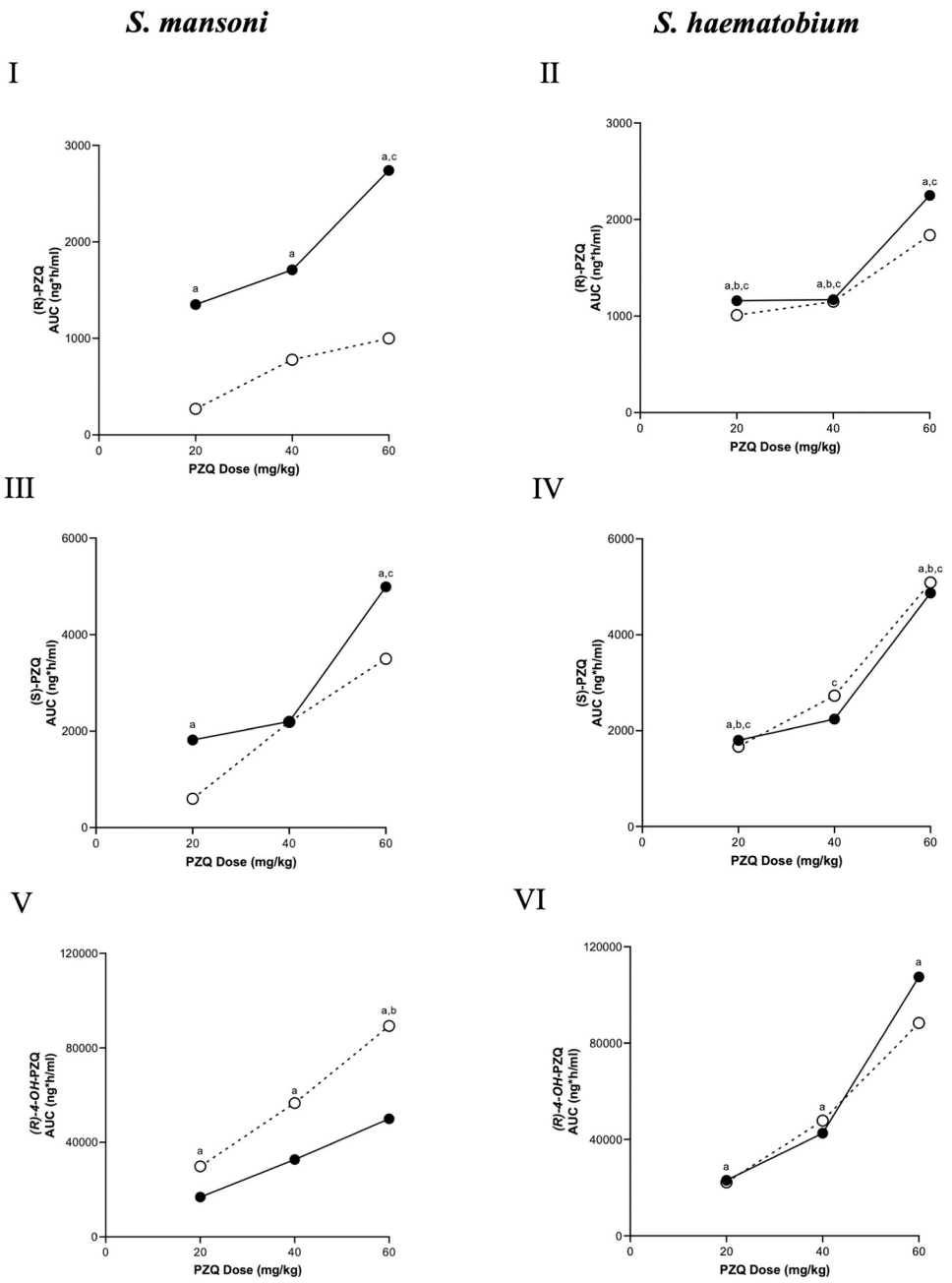

Fig 4. The mean AUC values extracted from the included studies against PZQ dose of (R)-PZQ [I, II], (S)-PZQ [III, IV], and the major metabolite (R)-trans-4-OH-PZQ [V, VI] at 20, 40, and 60 mg/kg. The data are extracted from Kovac and colleagues [56], which investigated *S. mansoni* and *S. haematobium* infected PSAC (●) and SAC (○) treated with PZQ. The AUC values were then plotted against the dose of PZQ administered for further analysis. $P < 0.05$ was considered statistically significant; a: significant difference between SAC and PSAC for the same dose and analyte, b: significant difference between *S. haematobium* and *S. mansoni* for the same dose, age group, and analyte, and c: significant difference between (R)-PZQ and (S)-PZQ for the same dose, age group, and species. AUC, area under the curve; PSAC, preschool-aged children; PZQ, praziquantel; SAC, school-aged children.

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the (R)-PZQ (Fig 4[I, II]), (S)-PZQ (Fig 4[II, IV]), and the major metabolite *(R)-4-OH*-PZQ (Fig 4[V, VI]).

There was a significant difference between the AUC values of the infected *S. mansoni* SAC and PSAC at the same dose and analyte, with the exception of (S)-PZQ at 40 mg/kg. Additionally, the AUC of (R)-PZQ and (S)-PZQ in *S. mansoni* SAC was found to be significantly different at the same dose but only at 60 mg/kg for PSAC. Generally, the AUC values of the infected *S. mansoni* PSAC were higher than the infected *S. mansoni* SAC for both the PZQ enantiomers (Fig 4[I, III]) [56], suggesting that once dosed with PZQ, PSAC metabolised the drug more slowly. This is in accordance with the age-related model by Bonate and colleagues [52], which stated that due to the decreased CYP activity of the PSAC, the drug remains in circulation longer, resulting in a higher AUC. Conversely, the AUC for the *(R)-4-OH*-PZQ metabolite was higher in SAC because they had potentially metabolised the drug more rapidly than PSAC (due to increased CYP activity), leading to a higher circulating level of the metabolite (Fig 4 [V]). Regarding the exposure in *S. haematobium*-infected children, there was no significant difference between exposure levels in *S. haematobium*-infected PSAC and SAC (Fig 4[II, IV, VI]).

PZQ dosing. In infected children, PZQ is delivered at a dose extrapolated from adult tolerance studies. Bustinduy and colleagues [58] and Bonate and colleagues [52] both described the limitations of current treatment models and further investigated whether the dosing regime was contributing to low CR in children (S8 Table). Both studies highlighted that the extrapolation of infected African children from healthy European adult volunteers is misjudged, and the resultant model cannot predict the differing bioavailability in each population. The resultant extrapolation model concluded that the standard method was not predictive of treatment success and that further studies are required to optimise PZQ treatment, looking at the effect of higher dosage, sex, weight, and PZQ enantiomeric activity [58]. Additionally, Bonate and colleagues [52] described the potential of using a model that includes the degree of maturation of the CYP isoforms involved in PZQ metabolism, and aimed to use this model to predict PZQ exposure (AUC) based on age.

Impact of food. There are additional factors which need to be considered that could affect PZQ efficacy, specifically factors not attributed to the host biology. The varying fasting and fed state of multiple studies were found to contribute to variable exposure of PZQ, noting that the bioavailability of PZQ increases with the administration of food [64]. Mandour and colleagues [63] confirmed that, after oral administration in HNV, the bioavailability is reliant on food intake, with PZQ clearance affected by content of the diet. A high oil diet enhanced PZQ absorption, and a high carbohydrate diet appeared to inhibit CYP activities due to an accumulation of metabolites, preventing further metabolism and allowing PZQ to remain in systemic circulation longer.

Drug–drug interactions

There is a paucity of information on DDIs with PZQ, with only 17 published papers on the topic. Of these studies, many evaluated the use of DDIs to lower PZQ dosing regimens for a combined drug treatment. To define the effect of each drug combination, an effect-based strategy was introduced, which followed the principle that if one component alters the ability of PZQ to reach the necessary lethal schistosome concentration via alterations to PZQ metabolism, then this action was designated a pharmacological effect [65]. Therefore, this review used a pharmacokinetic-based assessment of DDIs based on the alteration of CYP activities and potentially the overall efficacy of PZQ treatment [66]. This "bioavailability model" focused on the change in the AUC due to these drug combination effects to aid further discussion of

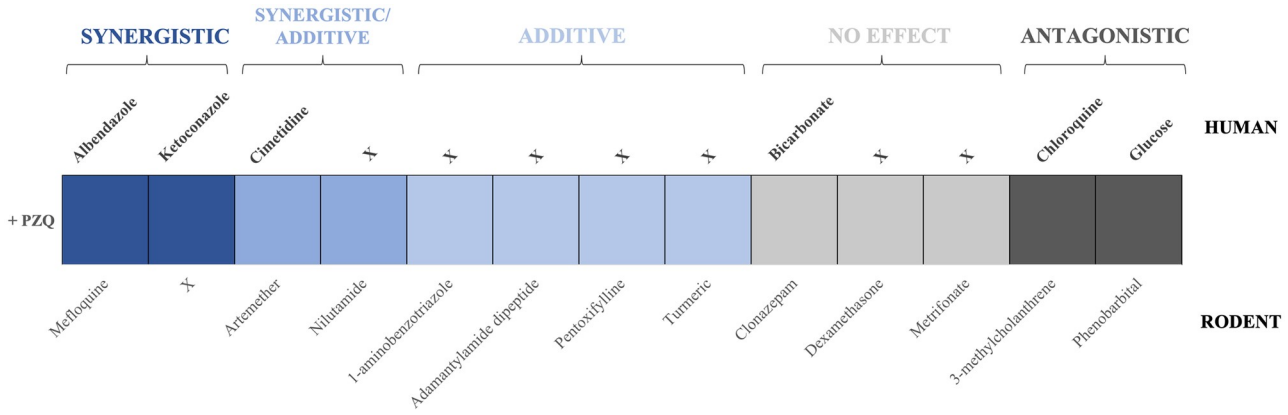

Fig 5. The DDIs identified by the review, and the resultant effect on PZQ exposure. The *in vivo* model and "Drug B" are listed as well as the overall effect of the drug combination on exposure of PZQ. The effect of the drug combination on PZQ efficacy in rodents was calculated based on the percentage change in WBR of each drug alone in comparison to the combined action (S9 Table) [73–83]. The effect of the drug combination on PZQ efficacy in humans was calculated based on the percentage change in AUC of each drug alone in comparison to the combined action (S10 Table) [54, 59, 84–86]. AUC, area under the curve; DDI, drug–drug interaction; PZQ, praziquantel; WBR, worm burden reduction.

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potential DDIs that could alter PZQ efficacy [67, 68]. In combination with the descriptive results of each DDI by the papers reviewed, this allowed a comparison of the potential combined effect of the two drugs (E_{PZQ+B}) to the effects of its individual components (E_{PZQ} and E_B) [69]. The effect of the DDI is expressed as (1) a synergistic effect: the combined therapy has a greater therapeutic effect than each drug alone following the principle $E_{PZQ}+E_B \ll E_{PZQ+B}$, where $E_{PZQ+B} >$ 20% change in AUC compared with PZQ alone; (2) an additive effect: similar to each drug used individually, no significant increase in activity $E_{PZQ}+E_B \approx E_{PZQ+B}$; (3) an antagonistic effect: interactions that could decrease therapeutic efficacy, in which $E_{PZQ+B} <$ -20% change in AUC compared with PZQ alone, or (4) no effect to treatment, these results were not outside the threshold of -20% $\leq E_{PZQ+B} \geq$ 20% change in AUC compared with PZQ alone [70–72]. The PZQ drug combinations obtained in this review are listed in Fig 5, depicting the result of the drug on PZQ action as described by the published DDI studies.

Experimental studies: drug–drug interactions

Synergistic and additive drug–drug interactions. To reduce the dose of PZQ while still having an efficacious effect, synergistic activity using the antimalarial mefloquine was explored in rodents by El-Lakkany and colleagues [73]. The WBR of PZQ + mefloquine was over double the value of PZQ alone, indicating a synergistic effect that maintained lethal schistosome PZQ concentrations and that could be applicable to humans treated with both drugs. Furthermore, when PZQ combined with another antimalarial, artemether, synergistic and additive effects were observed in separate rodent studies, even when PZQ was reduced to around a quarter of its recommended dose. A PZQ + artemether study by Utzinger and colleagues [74] concluded that the lower doses used in the combined treatment was safer and more efficacious (with over double the WBR) than PZQ alone and was suggested as a basis for a human clinical trial [74, 75].

Another study aimed to decrease the dose of PZQ, with Keiser and colleagues [76] combining nilutamide with PZQ to increase WBR compared to PZQ alone. It appeared that at low doses the combination had an additive effect on PZQ, with nilutamide obtaining a higher WBR alone. Interestingly, a synergistic effect was seen with PZQ + nilutamide

(100 mg/kg + 200 mg/kg) dose, with a 67% increase in the WBR than PZQ alone at that same dose, something that was not observed for nilutamide below 200 mg/kg.

Further additive effects were evaluated by El-Lakkany and colleagues [77] and Botros and colleagues [78], using pentoxifylline and adamantylamide dipeptide in combination with PZQ (S11 Table). Both combinations used a subcurative dose of PZQ in the DDI to evaluate the enhancement of each drug on PZQ's therapeutic effect, with both combinations showing comparable results to the full dose of PZQ [77, 78]. Abla and colleagues [79] co-dosed PZQ with 1-aminobenzotriazole, a pan-CYP inhibitor, and predicted a reduction in PZQ clearance and in turn increase C_{max} and AUC of the pharmacoactive (R)-PZQ in plasma. Yet, in *S. mansoni*–infected mice, 1-aminobenzotriazole was found to have only a small impact on the WBR, only increasing it by approximately 25%, reaching similar levels to PZQ alone (additive) taking into account interanimal variability. This was similar to the combination of turmeric and PZQ [80], in which a significant additive effect was observed, with around a 24% increase in WBR.

Antagonistic drug–drug interactions. Masimirembwa [81] and colleagues reported two antagonistic effects with PZQ in rats, as combinations with both phenobarbital and 3-methylcholanthrene decreased the C_{max} and AUC of PZQ, with phenobarbital decreasing PZQ exposure greater than 3-methylcholanthrene.

No effect. It is also important to analyse the combinations that have no effect on PZQ efficacy and can, therefore, be confidently co-administered without affecting PZQ's systemic concentration. Araujo and colleagues [82] and Ebeid and colleagues [83] investigated the combination of PZQ with clonazepam and metrifonate. Both studies concluded that there was no beneficial synergistic or additive action with the combined treatments of PZQ and that any antischistosomal action originated from PZQ alone and not a DDI. Furthermore, dexamethasone, a multiple CYP inducer, was expected to antagonistically decrease PZQ exposure and decrease PZQ efficacy due to an increase in the CYPs available to metabolise PZQ. Contrary to these predictions by Abla and colleagues [79], dexamethasone decreased plasma exposure of (R)-PZQ by approximately 10 fold but did not affect overall PZQ efficacy in rodents.

Human studies: drug–drug interactions

Five studies [54, 59, 84–86] describing human DDIs and the resultant variable drug exposure after oral administration of drug and food combinations were examined. The drug combinations and the percentage change in AUC from PZQ are shown in Fig 6.

Synergistic DDIs. Nleya and colleagues [84] evaluated the effect of combining PZQ with ketoconazole, as ketoconazole is a prominent inhibitor of the CYP3A4/5 isoforms which are known metabolic pathways of PZQ [88]. There was a 75% increase in the relative bioavailability of PZQ (measured by the change in the AUC) when co-dosed with ketoconazole, with the C_{max} increasing by 96% in response to the DDI. To measure the extent of the synergistic effect, the study was dosed at one-half the recommended value for a PZQ treatment (20 mg/kg PZQ + 200mgs ketoconazole). Despite this, 9 of 29 individuals reached the 1μM therapeutic threshold required to kill adult schistosomes, compared to only 2 of 29 when PZQ was given alone.

PZQ + cimetidine were evaluated by Jung and colleagues [59], with the potential to simultaneously treat schistosomiasis and neurocysticercosis, building on a study by Metwally and colleagues [85] that noted elevated PZQ sera concentrations with this DDI. Compared to PZQ alone, the plasma levels of PZQ more than doubled during combined drug administration, with a C_{max} greater than 400 ng/ml even after 12 hours, suggesting a synergistic effect.

Lima and colleagues [54] treated HNV with a combination of PZQ and albendazole (anthelmintic used for treating soil-transmitted helminths), with the aim of improving the therapeutic efficacy of both drugs by increasing plasma concentrations of the active forms of both drugs.

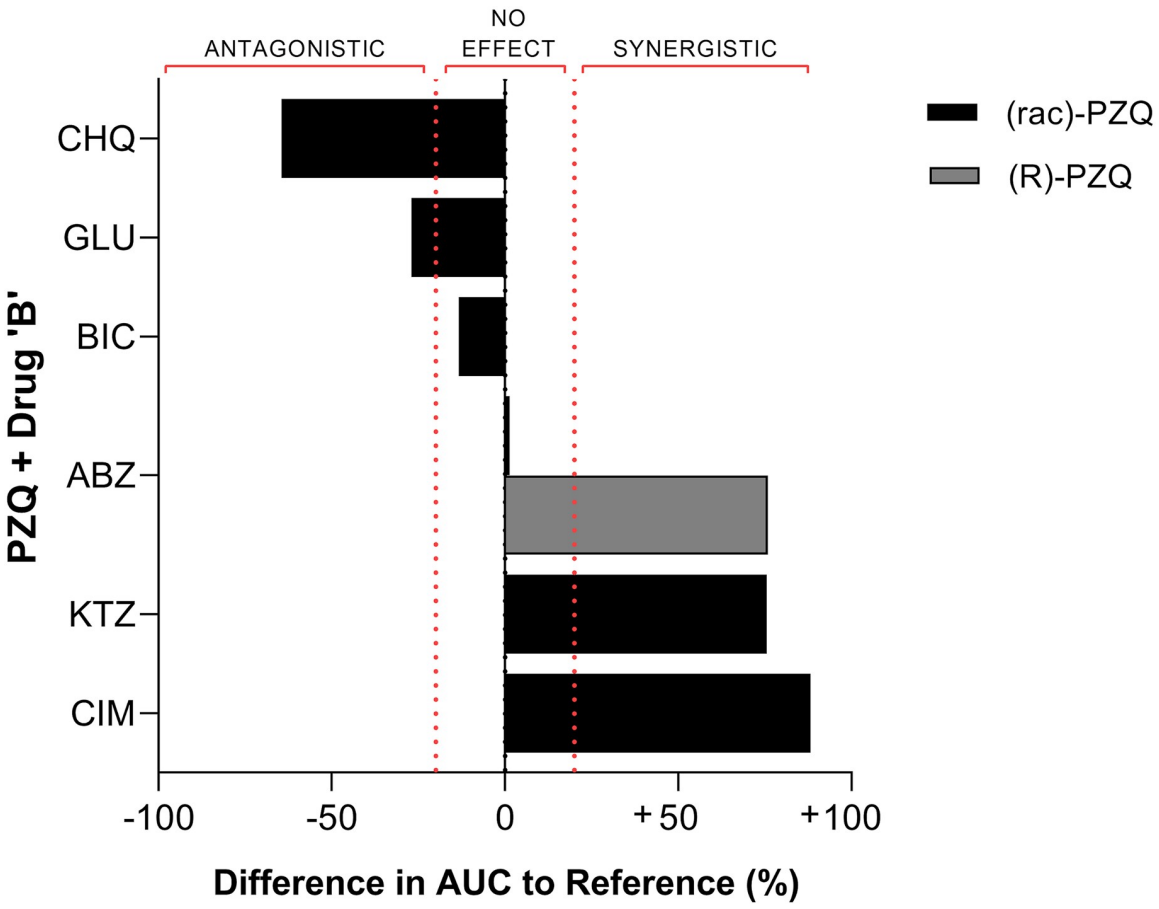

Fig 6. A summary of the human DDIs. Each bar represents the percentage change in AUC during the drug combination in comparison to PZQ alone. The effect of the drug combination is listed above the bar chart, with ±20% representing the boundaries of no effect due to interindividual variation [87]. The data were extracted from five papers [54, 59, 84–86], compiled in S10 Table, analysed to create percentage change in AUC and then plotted to aid further analysis. ABZ, albendazole; AUC, area under the curve; BIC, bicarbonate; CHQ, chloroquine; CIM, cimetidine; DDI, drug–drug interaction; GLUC, glucose; KTZ, ketoconazole; PZQ, praziquantel.

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This conclusion can be visualised in Fig 6, in which the combination of PZQ + albendazole appears to have no effect on the AUC of rac-PZQ. Opposing this, Lima and colleagues [54] showed that PZQ increased the C_{max} of the active albendazole metabolite, and, in turn, albendazole increased the AUC of the pharmacologically active (R)-PZQ: a synergistic effect. In Fig 6, the AUC of (R)-PZQ increased by 76%, which would not be apparent if only the rac-PZQ was measured.

Antagonistic drug–drug interactions. Masimirembwa and colleagues [86] investigated the antagonistic effect of the CYP inhibitor chloroquine in HNV, with chloroquine expected to increase the bioavailability of PZQ due to its action as a CYP3A4 inhibitor. In contrast, it was found to decrease the C_{max} and AUC of PZQ, decreasing overall exposure, with 50% of individuals studied not reaching the lethal schistosome plasma concentration of 1μM. Masimirembwa and colleagues [86] determined this effect was not due to alterations in CYP activities but, rather, an alternate mechanism of chloroquine.

An antagonistic effect was also observed by Metwally and colleagues [85], when PZQ was co-dosed with glucose. However, as depicted in Fig 6, the percentage change of the AUC for

the combination compared to PZQ alone was −27%, which was just outside of the 20% "no effect" threshold [87]. Therefore, although this has been noted as an antagonistic effect with glucose, it may be due to interindividual variation slightly skewing the results because there were only 20 participants in this study, and there may be no effect regarding this interaction.

No effect. Although the combination of bicarbonate with PZQ did reduce the AUC of PZQ [85], it was not outside the 20% threshold (see Fig 6). Similar to glucose, this combination is not likely to have an effect on the efficacy of PZQ and is most likely due to the mean calculation of the AUC and the interindividual variation in the metabolism of the participants in the study.

Pharmacogenetics

Human studies: pharmacogenetics. The search did not yield any studies targeting the pharmacogenetics of PZQ metabolism. However, the data extracted from the included studies contained numerous examples of metabolic variability in the human studies. Although there were no specific studies on pharmacogenetics, the reasoning behind the variable drug exposure and efficacy of PZQ was postulated by many authors to be attributed to interindividual variation and host genetic factors [43, 45, 55–59, 61–63, 75, 85, 86]. The majority of the included studies did not evaluate this hypothesis further but merely stated that CYP polymorphisms and CYP-related maturity may be causing variation in PZQ PK parameters and bioavailability. The ethnicities of the individuals included in the human studies in this review were also recorded. Of the 17 human studies, 17.6% studied individuals from Europe, 17.6% from South America, and 64.7% from Africa. The ethnicities of the study populations included in this review were recorded to aid further discussion.

Metabolite analysis of praziquantel

Seventeen metabolites were elucidated from four papers [28, 53, 84, 89], and their structures are displayed in Fig 7. PZQ and its enantiomeric metabolites, including their CYP pathway and structure, were evaluated by multiple *in vivo* and *in vitro* techniques. Using human plasma, human urine, human and mouse liver microsomes, and human recombinant enzymes, there were 8 distinguishable mono-oxidised metabolites, 2 dehydrogenated mono-oxidised metabolites, 3 di-oxidised metabolites, and 4 glucuronide metabolites detected.

A study by Nleya and colleagues [84] depicted the metabolic pathway of PZQ to its main metabolite; 4-OH-PZQ (Fig 7[II]). This study showed that when PZQ was concomitantly administered with CYP3A4/5 inhibitor ketoconazole, significant changes in the level of metabolites in circulation were observed. The AUC of the main 4-OH-PZQ metabolites were increased by 57% (*cis*-) and 67% (*trans*-) when co-dosed with ketoconazole, with 30 times more *trans* isomer than the *cis* as seen in Fig 8. The higher levels of the *trans*- isomer stems from the favourable cyclohexane ring conformation placing the bulky OH substituent equatorial to prevent steric hindrance compared to the axial (*cis*-) conformation [90].

Discussion and conclusion

Heterogeneity in PZQ drug efficacy has been reported in multiple studies [18, 19]. Given the concern about the development of drug resistance in schistosome parasites, there has been considerable research on the potential for parasites developing resistance. However, it is critical to investigate the impact of nonparasite related factors that give rise to variability in PZQ drug efficacy. The majority of studies determined this to be due to interindividual variation and host genetic factors with no further analysis [43, 45, 55–59, 61–63, 75, 85, 86]. Here, we determined whether variable cure rates in humans could be attributed to fluctuating levels of

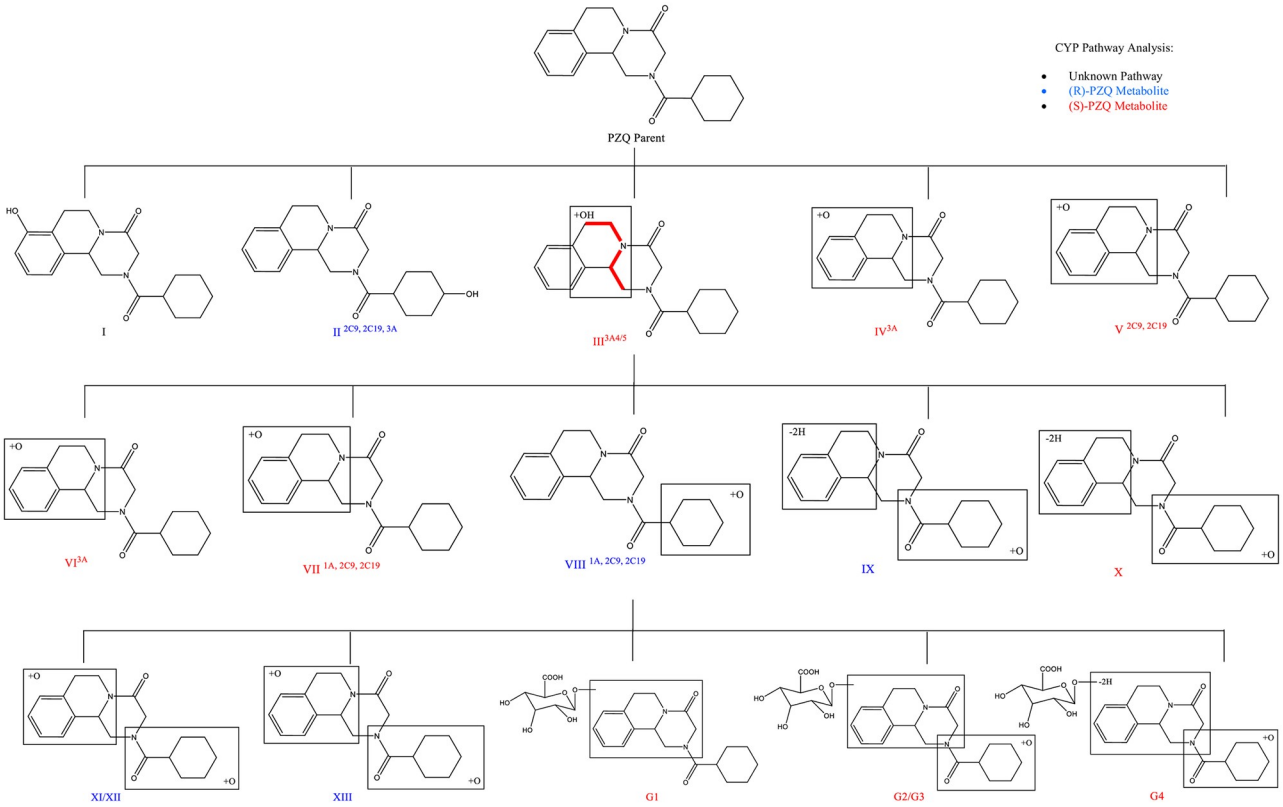

Fig 7. A comprehensive map of PZQ metabolites extracted and combined from studies that focused on the metabolite analysis of PZQ. I: 8-OH-PZQ [89], II: 4-OH-PZQ [28, 53], III: X-OH-PZQ [84], IV: O-PZQ [28], V:O-PZQ [28], VI: O-PZQ [28], VII: O-PZQ [28], VIII: O-PZQ [28], IX: (-2H)-O-PZQ [28], X: (-2H)-O-PZQ [28], XI/XII: O_2-PZQ [28], XIII: O_2-PZQ [28], G1: Gluc-PZQ [28], G2/G3: Gluc-O-PZQ [28], G4: (-2H)-Gluc-O-PZQ [28]. The chemical structures and metabolite map were created using ChemDraw Prime v16.0. Gluc, glucuronide; PZQ, praziquantel.

https://doi.org/10.1371/journal.pntd.0008649.g007

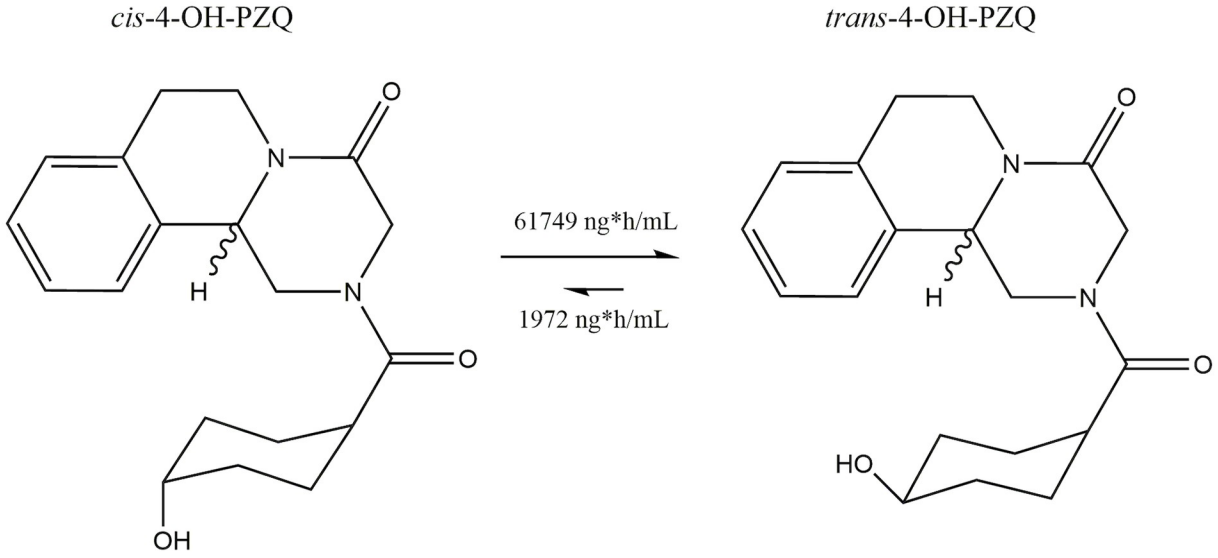

Fig 8. The favourable formation of *trans*-4-OH-PZQ compared to the *cis*-4-OH-PZQ isomer created from pharmacokinetic data by Nleya and colleagues [84]. The equatorial position points upwards and the axial position points downwards. The chemical structures were created using ChemDraw Prime v16.0. PZQ, praziquantel.

https://doi.org/10.1371/journal.pntd.0008649.g008

PZQ in systemic circulation and explored the factors that influence an individual's response to PZQ treatment, focusing on the metabolism of the drug and the resultant efficacy of treatment.

Multiple studies reviewed confirmed (R)-PZQ as the pharmacologically active enantiomer and that the exposure of the desired (R)-PZQ enantiomer was significantly lower than the (S)-PZQ when dosed as rac-PZQ [46, 49–54]. The reasoning behind the chiral differences in the elimination of each enantiomer has been postulated to be due the variations in their affinity in binding to the active site of the CYPs [28]. If the affinity of (R)-PZQ for CYP1A2 is higher than (S)-PZQ, then the metabolism of the active (R)-PZQ to its major metabolite (as described by Nleya and colleagues [84]) would be greater than metabolism of (S)-PZQ, resulting in a more rapid decrease in (R)-PZQ plasma exposure than for (S)-PZQ. However, as affinity to CYP1A2 has not yet been evaluated, the metabolic differences between (*R*)-PZQ and (*S*)-PZQ are still unclear. These studies highlight the benefits of treatment with the (R)-PZQ enantiomer alone in comparison to using the racemate and supporting the development of new formulations with the active enantiomer, as is the case for the ODT paediatric formulations of PZQ currently undergoing clinical trials [91]. In this case, the ODT formulation increased the AUC of the desired (R) enantiomer for the rac-ODT and (R)-ODT in comparison to the reference rac-PZQ, but it had a nonproportional PK profile [51, 52]. This limited the interpretation of each PZQ enantiomers PK profile in the ODT and emphasises the need for an (R)-ODT dose-finding study in PSAC to fully define the PK in paediatric patients and find the correct dosing regimen.

In experimental models, host schistosome infection status was an important factor influencing PZQ's pharmacokinetic parameters, with PZQ's exposure parameters in infected animals higher on average than in uninfected animals [42–45]. The studies proposed that *S. mansoni* infection interferes with liver function, preventing PZQ metabolism. However, this does not easily translate to human hosts. Instead variable exposure in human hosts tended to be associated with alterations in the liver's capacity to metabolise PZQ due to other factors, as observed in studies in which liver disease impaired CYP function, leading to increased levels of PZQ in systemic circulation. In patients suffering from liver cirrhosis and also treated with PZQ, the C_{max} and AUC increased with the severity of cirrhosis in comparison to nonimpaired *S. mansoni*–infected and healthy patients at the same PZQ dose [55]. The increase in exposure parameters is contrary to the study performed in rats by Kokwaro and colleagues [44] and further highlights the limitations of experimental models in capturing human dynamics.

In human studies, age-related maturation of hepatic CYPs has been postulated, suggesting that the hepatic CYP pathways are not fully matured in children and affect drug AUC [52, 58]. The effect of age-related metabolism was investigated in a study of *S. mansoni*–infected children in which the AUC values of the PSAC were higher than the SAC for both the PZQ enantiomers in the circulation, yet the AUC for the *(R)-4-OH*-PZQ metabolite was higher in SAC [56]. This suggested PSAC could be metabolising the drug more slowly than the SAC, with higher concentrations of the parent drug found in PSAC and higher concentrations of metabolite found in SAC. This is consistent with higher exposure to active PZQ in the PSAC, resulting in higher cure rates compared to SAC. This is contrary to a study performed by Coulibaly and colleagues in which the cure rates in PSAC were lower at 40 and 60 mg/kg than in the SAC. This concurred with the data from this review at 60 mg/kg, as the SAC had a 5% higher cure rate than PSAC. However, Coulibaly and colleagues did suggest that during the study the crushing of the tablets may have altered the PK of PZQ, which may account for the difference in efficacy [92]. This age-related effect has not yet been observed in *S. haematobium*. Ofori-Adjei and colleagues [57] reported no differences in the PK parameters between uninfected

and *S. haematobium* infected SAC, concluding that *S. haematobium* infection does not influence PZQ metabolism due to alterations to hepatic function [56].

Non–host-related factors also affected exposure and efficacy of PZQ treatment, and these included PZQ brands and the coadministration of food or other drugs. Simultaneous administration of PZQ and food can alter the PK as food can delay absorption, affect stomach pH, alter blood flow, or interact with the PZQ itself [93]. One of the initial PZQ tolerance studies in humans provided the adult volunteers with a standardised meal for continuity in the study [61], but during PCT programs, this is not particularly representative, as diet can vastly differ. Fed groups have a lower clearance of PZQ due to altered absorption, highlighting the importance of attaining a fed state during treatment and in human PK studies to enhance success of exposure to the anthelmintic [63]. Of the different brands of PZQ on the market, Distocide and Biltricide were found to be more comparable in terms of exposure to pure PZQ [42]. Even so, the PZQ brands evaluated showed a decreased AUC, C_{max}, and $t_{1/2}$ and, therefore, decreased efficacy and drug exposure in comparison to pure PZQ, indicating that treatment failures may be due to variable exposure of PZQ arising from the quality of the PZQ formulation.

A host attribute that can also affect drug metabolism is genetic polymorphism of liver enzymes involved in drug metabolism, i.e., pharmacogenetics. While CYP pharmacogenetic studies have been conducted in different ethnic populations [94], there are relatively few studies on PZQ metabolism. [94]. In recent publications, trials of more efficacious formulations and age-dosing models were being performed in European populations instead of the majority drug target population in Africa [52, 61]. Although 90% of schistosomiasis infected people live in Africa there have been no studies of PZQ population genetics in Africa [95, 96], highlighting the need for pharmacogenetic studies on the clinically relevant CYP variants in target populations [94]. For example, CYP2D6 is a metabolic route of PZQ; therefore, the presence of CYP2D6*17, which is unique to populations of African origin [97], exemplifies the need for its impact on PZQ metabolism and overall treatment efficacy to be evaluated. Nleya and colleagues [84] have provided evidence CYP1A2 may be the metabolic route of PZQ to its main metabolite in Zimbabwean volunteers. As a recent study reported that Zimbabwean children in schistosomiasis-endemic areas exhibited decreased CYP1A2 activity, and, as PZQ is metabolised via this pathway, this could also have detrimental implications on bioavailability [45].

In order to translate molecular findings into drug metabolism and predict efficacy, there is a need to characterise the PZQ metabolites, their quantities and their effect. Wang and colleagues [28] have performed the most comprehensive evaluation of PZQ's metabolites, albeit in experimental models, which identified phase I and phase II metabolites using both *in vivo* and *in vitro* methods. Fifteen metabolites were structurally identified from urine and faeces of mice 24 hours after PZQ dosing. The *in vitro* incubations using human liver microsomes (HLM) and human recombinant enzymes confirmed the metabolic activity of CYP1A2, CYP2C9, 2C19, 2D6, and 3A4/5, with metabolic products identified via all of these pathways. However, the *in vivo* results from the experimental studies were not identical to the human *in vitro* HLM, with 3 dioxidised metabolites not detected in HLM. The Zimbabwean study, one of the few human studies, also characterised the metabolic pathway of PZQ to its main metabolite: 4-OH-PZQ [84]. The combination of PZQ with CYP3A4/5 inhibitor ketoconazole resulted in significant changes in the AUC of the main 4-OH-PZQ metabolites, with increases of 57% (*cis*-) and 67% (*trans*-) when co-dosed with ketoconazole. The overall increase in the AUC of both 4-OH-PZQ metabolites revealed that when the CYP3A4/5 pathway was inhibited, there was a greater exposure of the main metabolite in the circulation. This indicates that CYP3A4/5 is not the metabolic route of 4-OH-PZQ and suggests that other CYP pathways, primarily CYP1A2 or CYP2C19, are instead involved in the principal elimination of the active

parent drug. So what metabolite is the CYP3A4/5 pathway producing? The study reported a novel metabolite, X-OH-PZQ, which was suppressed upon administration of ketoconazole with PZQ, and therefore appeared to be dependent on CYP3A4/5 for its formation. The X-OH-PZQ levels were reduced by approximately 57%, providing categorical evidence that this novel metabolite is produced via the CYP3A4/5 pathway. Other studies have discussed the use of CYP3A inhibitors to reduce the conversion of PZQ to its main metabolite (Fig 7[II]) [53]; however, based on the results from this study, it appears that the introduction of a CYP1A2/CYP2C19 inhibitor to PZQ may result in a greater exposure of active parent drug. If the 4-OH-PZQ metabolic pathway is inhibited, then a reduction in the elimination of PZQ could occur and result in higher circulating concentration of the parent drug enhancing therapeutic efficacy. The exact structure of X-OH-PZQ was, at this time, not yet fully determined; to achieve this, the exact nature of the hydroxylation biotransformation must be evaluated. The intricacies of metabolite identification is potentially one of the factors contributing to the limited number of results addressing this topic, as Schepmann and colleagues [89] demonstrated, dedicating an entire paper to elucidating the structure of one phase I metabolite: 8-OH-PZQ (Fig 7[I]). By obtaining further information on PZQ's metabolites via the detection and quantification of each metabolite, they could be compared between individuals for variability. As individual genetic variation in the CYP enzymes could affect PZQ metabolite concentration in systemic circulation, there is the potential to use the metabolite itself as genetic marker without the need for sequencing.

As schistosomiasis is coendemic with several other parasites and pathogens, affected populations can be subjected to drug coadministration, giving rise to DDIs. Malaria is one such coendemic parasite, and coadministering the antimalarials mefloquine and artemether with PZQ was investigated in experimental models, building on work from an *in vitro* experiment by Keiser and colleagues [98]. The coadministration of reduced doses of PZQ + mefloquine and PZQ + artemether showed enhanced pharmacological activity and efficacy over PZQ alone [73–75]. Other coendemic infections include intestinal helminths, and helminth control programs often coadminister PZQ and albendazole or mebendazole [99]. In humans, the combination of the anthelmintic albendazole + PZQ resulted in an increase in the plasma concentrations of the active enantiomers of both drugs [54]. Previous reports presented contradictory data regarding the DDIs between PZQ and albendazole, concluding that coadministration of PZQ + albendazole does not alter PZQ PK [100, 101]. However, Lima and colleagues [54] demonstrated a synergistic interaction upon this combination with the AUC of the (R)-PZQ increasing by 76%, which would mean an increase in the exposure of the active drug to the schistosomes. This interaction was determined to be due to the albendazole inhibiting CYP1A and CYP3A pathways, indicating the (R)-PZQ cannot be metabolised and remains in systemic circulation longer, leading to higher (R)-PZQ plasma concentrations. Nonetheless, even with the advantage of increased PZQ exposure, the increased plasma concentrations of both active drugs risks unknown adverse effects without further investigation [54]. This beneficial combination is not the case with chloroquine. This antimalarial drug, when coadministered with PZQ, decreased the AUC of PZQ by approximately 64% compared to PZQ alone [86] and may create too low a systemic concentration of PZQ to be lethal to schistosomes. This antagonistic effect on PZQ metabolism was deemed not to be due to CYP alterations, highlighting the need for further investigations into this DDI mechanism.

Other drugs affecting CYP activity have also been shown to synergistically affect PZQ plasma availability. For example, cimetidine more than doubled the plasma levels of PZQ during combined drug administration than PZQ alone. Cimetidine nonselectively inhibits CYP1A2, 2C9, 2D6, and 3A4 [102], all of which are metabolic pathways of PZQ. A nilutamide + PZQ combination was also found to be synergistic in rodent infections, significantly

increasing WBR by 67% compared with PZQ alone [76]. In humans, coadministering PZQ + ketoconazole can increase the duration of active PZQ exposure [84] by inhibiting the CYP3A4/5 pathway to reduce clearance of PZQ by 30% and enhance parasite exposure to PZQ [84]. The AUC value at 20 mg/kg PZQ when coadministered with ketoconazole was comparable with a study using 40 mg/kg PZQ alone [63]. Despite this, upon a linear extrapolation of the level of PZQ exposure from this combination, it predicted only a 37.5% reduction in PZQ dose. Therefore, regardless of this synergistic activity, there would not be a significant reduction of the current tablet size with this DDI and, so, would not be a better alternative than the development of a new formulation [84].

There is definite need for more studies of PZQ metabolism in humans, specifically in affected populations. The majority of studies reviewed here were experimental models of schistosomiasis, and findings from these cannot always be easily translated or extrapolated to human hosts, particularly the PZQ metabolite characterisation studies. Although animal studies are useful as models of potential DDIs, some enzymes that are orthologous to the CYPs are not representative of human responses [43]. A methodological limitation of this review was the measurement used. One of the main measures of exposure used to compare drug exposure in this review is the AUC; however, the absolute bioavailability is generally a more representative parameter. It is calculated using the AUC of the intravenous route and the AUC of the IV dose [103], but this data was not available for the majority of included PK studies. The quantity and type of data available in the original publications also limited the conclusions in this review, particularly when data were presented as the mean values postanalysis and not the raw data; therefore, no further statistical analysis was possible. Additionally, we could not identify clearly established drug-specific thresholds beyond which reductions in AUC might lead to alterations in drug effect. The use of the 20% threshold in change in AUC was a representative value to identify important changes in systemic bioavailability [87]. While the lack of defined thresholds may hinder the clinical interpretations in this review, there was no definitive method to determine the DDIs from the data available. Therefore, the DDI effects described here were based on efficacy of PZQ treatment stemming from exposure to the active drug and are not necessarily the confirmation of a mechanistic interaction. Also, the low number of published studies that were obtained for each topic using the search criteria highlighted the gap in the knowledge in PZQ metabolism during schistosomiasis treatment. When the search criteria used in this review was applied to tuberculosis, there was approximately eight times the number of search results (S12 Table). This increase in results could be due to the number of different drugs used in tuberculosis, in comparison to the one drug used for schistosomiasis; nonetheless, this shows the difference in the quantity of published research for schistosomiasis in comparison to a disease that can occur in every part of the world.

Overall, schistosomiasis control is predominantly reliant on a single drug, PZQ, for treating millions of people. Variable cure rates from the drug raise concerns about the possibility of the development of drug resistance amongst the parasites; therefore, there is a need to determine the sources of heterogeneity in cure rates and determine the relative contribution of host-related factors. Our review has shown that several such factors can result in variable levels of PZQ in systemic circulation that potentially contribute to these low cure rates. These include drug formulation (enantiomers) and brand, the health of the host' s liver, host age, coadministered drugs, and host genetics. There is need for more of these studies in affected human populations, especially in Africa, where host and parasite attributes are studied simultaneously, to fully understand the sources of heterogeneity in PZQ efficacy.

Supporting information

S1 Fig. Flow chart guides by the PRISMA for a systematic review, including the records excluded based on the specific criteria. PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analysis.
(TIFF)

S1 Table. Included studies obtained using the inclusion and exclusion criteria of this review, separated into topics of focus.
(PDF)

S2 Table. Comparison of different brands of PZQ on the PK parameters in a rodent model. PK, pharmacokinetic; PZQ, praziquantel.
(PDF)

S3 Table. Comparison of different brands of PZQ on efficacy of treatment in an infected murine model. PZQ, praziquantel.
(PDF)

S4 Table. Comparison of the WBR in an infected murine model when dosing racemic PZQ, or a single enantiomer. PZQ, praziquantel; WBR, worm burden reduction.
(PDF)

S5 Table. Pharmacokinetic parameters of PZQ enantiomers and its major metabolites in HNV after administration of racemic PZQ, or the single PZQ enantiomer itself. HNV, healthy normal volunteers; PZQ, praziquantel.
(PDF)

S6 Table. Pharmacokinetic parameters in human models after administration of racemic PZQ; includes P and HNV. HNV, healthy normal volunteers; P, infected patients; PZQ, praziquantel.
(PDF)

S7 Table. Pharmacokinetic parameters of PZQ, PZQ enantiomers, and the major metabolite in HSAC, infected SAC, and infected PSAC after the administration of racemic PZQ. HSAC, healthy school age children; PSAC, preschool-aged children; PZQ, praziquantel; SAC, school-aged children.
(PDF)

S8 Table. Efficacy parameters of racemic PZQ treatment on infected PSAC and SAC, and the effect of the different brands of PZQ available. PSAC, preschool-aged children; PZQ, praziquantel; SAC, school-aged children.
(PDF)

S9 Table. The comparison of dosing racemic PZQ alone, or with drug "B" on PZQ's anti-schistosomal efficacy in rodent models. PZQ, praziquantel.
(PDF)

S10 Table. The pharmacokinetic parameters of PZQ, its enantiomers, and the major metabolite in HNV after the administration of racemic PZQ with drug "B" to evaluate potential DDIs. DDI, drug–drug interactions; HNV, healthy normal volunteers; PZQ, praziquantel.
(PDF)

S11 Table. The comparison of racemic PZQ alone, and with drug "B" in reducing hepatic granuloma diameter in murine models. PZQ, praziquantel.
(PDF)

S12 Table. Comparison of the results of this systematic review in comparison to tuberculosis.
(PDF)

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3.4 Key findings and conclusions

This chapter contains a comprehensive review and analysis of the DMPK factors that impact the exposure of PZQ during a schistosomiasis treatment. The literature search identified forty publications using three online databases (**Paper 1: S1 Fig**) that satisfied the designated inclusion criteria (**Paper 1: Literature screening and inclusion and exclusion criteria**). Alterations in the liver's capacity to metabolise PZQ were identified as an important determinant of PZQ efficacy, preventing PZQ metabolism and significantly increasing the concentration of PZQ in the systemic circulation. These alterations included liver disease, infection status, *Schistosoma* species and age-related CYP activity (**Paper 1: Hepatic CYP metabolism**). Together, these findings stressed the importance of investigating the factors that impact liver-mediated drug metabolism.

Age-related maturation of hepatic CYPs significantly influenced the metabolism of PZQ between school-aged children (SAC) and preschool-aged children (PSAC), based on the results of three publications, altering the concentrations of PZQ and its metabolites (Ofori-Adjei *et al.*, 1988, Bustinduy *et al.*, 2016, Kovac *et al.*, 2018a). Data from the SAC and PSAC in these three publications were extracted and compared during this analysis (**Paper 1: S7 Table**), with contrasting results. Specifically, data extracted from a study by Kovac and colleagues was plotted (**Paper 1: Fig 4**) to visualize the differences in exposure between the age groups. A significant difference in PZQ exposure based on age group was only detected in patients with *S. mansoni* infections, with PSAC found to have significantly higher concentrations of parent PZQ compared to SAC (Kovac *et al.*, 2018a). This supported the hypothesis of decreased drug metabolism in younger children due to differentiation in their liver capacity and highlighted an influential factor to be aware of when evaluating causes of variable PZQ efficacy. However, in a study by Coulibaly and colleagues,

the CRs and PZQ concentrations were lower in PSAC than in SAC. Despite these contrasting results, due to the recent development of the new paediatric formulation (Consortium, 2020, Consortium, 2022a), the issue of variable PZQ exposure in PSAC due to age-related metabolism upon the standard racemic PZQ treatment should not be an influential factor in the future in the efficacy of a PZQ treatment for schistosomiasis. Interestingly, there was no significant difference between SAC and PSAC with *S. haematobium* infections. This was postulated to be due to the pathology of infection as, unlike *S. mansoni* which commonly inflames and enlarges the liver (Danso-Appiah *et al.*, 2013), *S. haematobium* is not as commonly associated with hepatosplenomegaly (Wilson *et al.*, 2011). However, the overall impact of *S. mansoni*-related liver morbidity on PZQ PK parameters is essentially unknown and warrants further attention (Summers *et al.*, 2022).

Based on the results of this study, there was an absence of PGx studies assessing the impact of host genetics on PZQ efficacy. Despite the clinical relevance of PGx studies (**Section 1.10.2**), no published studies on PGx of PZQ were identified in the literature search. PGx studies are vital to characterize host-genetic determinants of PZQ metabolism that decrease active PZQ concentrations and reduce PZQ efficacy. The necessity for PGx research concerning schistosomiasis treatment is therefore important, not just globally, but in Africa where 93% of schistosome-infected people reside (Onasanya *et al.*, 2021). African populations are extremely genetically diverse (Mpye *et al.*, 2017), so it is necessary to determine whether PGx changes are preventing an individual from clearing schistosome infection in populations where PZQ is most commonly used. Currently, there have been unique African PGx variants, such as the *CYP2D6*17*, reported in African populations that can alter drug concentrations (Masimirembwa *et al.*, 1996). Therefore, the lack of published information on the PGx of PZQ, particularly in schistosome-endemic African populations where PZQ treatment is given on mass, is a significant gap in the research that requires

exploration as it may be a substantial contributing factor to the variable efficacy of PZQ. The alterations to CYP metabolism detected in this study, whether, disease-related or genetic, emphasise the importance of fully characterizing the factors that alter DMPK properties of PZQ and the impact on the eventual efficacy of a PZQ treatment.

Although there were many factors identified that influenced the concentration of active PZQ in circulation via altered PZQ metabolism and hence on PZQ efficacy, the analysis of PZQ's metabolites was sparse. Metabolite concentrations are commonly used to compare the metabolic stability of a drug, but can also be used to evaluate changes in CYP metabolism between individuals. For example, you can determine ultra-rapid metabolisers (UM) via the large quantities of drug metabolite compared to normal metabolisers, and thereby the determination of PZQ metabolites is important in assessing interindividual variability of a PZQ treatment. From the results of this study, seventeen PZQ metabolites had been documented, yet very few had been confirmed in humans (**Paper 1: Metabolite analysis of praziquantel**). This was a key finding as no study *in vivo* or *in vitro* had comprehensively produced an overall map of the biotransformations of PZQ and all of its metabolites, hence a map of all reported PZQ metabolites was compiled for this chapter using the results of this search (**Paper 1: Fig 7**). Furthermore, the 4-OH-PZQ metabolite has been reported to have some antischistosomal activity (Kovac *et al.*, 2017), thus assessing metabolites concerning PZQ efficacy requires further investigation. The paucity of metabolite studies detected in this chapter emphasised the need to analyse PZQ metabolism and the products of those pathways in detail in humans, particularly in African populations where PZQ is primarily administered.

Based on the results of this chapter, there were also multiple non–host-related factors that were found to affect the exposure and efficacy of a PZQ treatment. For instance, individuals who were administered food before PZQ was administered were reported to have a higher

concentration of active PZQ due to increased absorption, increasing PZQ exposure, and stressing the significance of attaining a fed state during PZQ treatment. If unfed, the individual increased their risk of treatment failure due to decreased exposure to PZQ, and therefore fed-state was identified as a potential contributing factor toward decreased PZQ efficacy (Mandour *et al.*, 1990). Different brands of PZQ were also shown to decrease treatment efficacy and PZQ exposure in comparison to pure PZQ (Botros *et al.*, 2011), indicating that treatment failures could arise from the quality of the PZQ formulation administered. Thus, when considering influential factors of reduced efficacy, the drug brand itself may be a contributing factor.

Furthermore, a combined analysis of data extracted from four studies revealed that the exposure of the pharmacoactive (R)-PZQ enantiomer was significantly lower than the inactive (S)-PZQ (**Paper 1: Fig 3**). This was a key finding to highlight, as a patient dosed with a racemic PZQ tablet had lower exposure to the desired (R)-PZQ enantiomer compared to the pharmacologically inactive (S)-PZQ. This analysis further highlighted the benefits of a single (R)-PZQ enantiomer treatment in comparison to the current racemic PZQ treatment, supporting the development of new formulations of only (R)-PZQ (Bagchus *et al.*, 2019) and the rollout of the enantiomeric paediatric formulation (Consortium, 2022b) to prevent unsuccessful PZQ treatment due to decreased exposure of the schistosome-killing enantiomer.

Finally, as previously discussed (**Section 1.10.1**), schistosomiasis is co-endemic with several other parasites and pathogens (Fenwick and Webster, 2006). This presents the issue of co-administered medications and therefore potential DDIs, which can alter active PZQ concentrations due to inhibition and induction of the CYP enzymes (Blower *et al.*, 2005, Zhang and Tang, 2018). Before this analysis, no study had collected concise information on

the DDIs that may impact the efficacy of a PZQ treatment. From the seventeen published studies assessing the DDIs with PZQ, multiple co-administered drugs were found to modify the systemic concentrations of PZQ compared to PZQ alone (**Paper 1: Drug-drug interactions**). Only five of these studies were conducted in humans, revealing three synergistic drugs, two antagonistic drugs and one drug that did not affect PZQ exposure. Of all the DDIs assessed regarding their influence on PZQ exposure and thus PZQ efficacy, the antimalarial chloroquine was of particular note. Chloroquine was found to act antagonistically with PZQ, decreasing the exposure of the schistosome-killing parent PZQ compared to PZQ alone (**Paper 1: Fig 6**). The mechanism of this antagonistic interaction is unknown, as there is uncertainty whether it acts as an inducer for the CYP enzymes that metabolise PZQ (Rezaee *et al.*, 2021). Yet, many other drugs have noted decreased plasma concentrations upon co-administration with chloroquine (Seideman *et al.*, 1994, Munera *et al.*, 1997, Ilo *et al.*, 2006, Ilo *et al.*, 2008). As malaria is a common co-endemic parasite in areas treated for schistosomiasis, DDIs via this combination must be acknowledged as an influential factor of note when assessing the causes of reduced PZQ efficacy.

To conclude, this chapter addressed **Thesis Aim 1** (**Section 1.11**) and provided a comprehensive review and analysis of the non-parasite factors affecting the exposure and efficacy of PZQ, the drug of choice for schistosome control. From the results of this study, it was clear that several such DMPK factors can result in variable levels of PZQ in circulation and potentially contribute to the reported cases of decreased PZQ efficacy. These include drug formulation (enantiomers), PZQ brand, differences in the liver's capacity to metabolise PZQ, host age, co-administered drugs, and host genetics; all of which have been shown in the literature to alter drug exposure. Most of the studies reviewed here were conducted in experimental models of schistosomiasis, however these findings cannot be easily translated or extrapolated to humans (Fallon, 2000). Similarly, the low number of published studies that

were obtained during the systematic search accentuated the scarcity of knowledge of the DMPK factors that could influence the exposure of active PZQ and therefore the efficacy of PZQ treatment for schistosomiasis. Henceforth, there is a need to further investigate the factors that influence the DMPK of PZQ in humans, specifically in affected populations, to characterize determinants of PZQ efficacy. This study also highlighted the urgent need to characterise the PGx of PZQ metabolism in the schistosome-exposed African populations to better understand the relative contributions of host genetics to variable PZQ efficacy, especially as the African population are so genetically diverse compared to other global populations. By identifying determinants of heterogeneity in PZQ efficacy, these factors can be monitored and controlled to achieve the best outcomes for schistosome-infected individuals during a PZQ treatment. Overall, this chapter successfully evaluated the known DMPK factors that can lead to variable levels of systemic PZQ, and in turn, discussed the resultant effect on the efficacy of PZQ treatment.

Chapter 4 - Pharmacogenetics of praziquantel metabolism in a *Schistosoma haematobium* exposed Zimbabwean population

4.1 Introduction

Pharmacogenetic (PGx) studies investigate the role of host-genetic variations in drug response and predominantly focus on single nucleotide polymorphisms (SNPs) in the drug-metabolising enzymes (Drew, 2016) (**Section 1.10.2**). The most common drug-metabolising enzymes are the cytochrome P450s (CYPs), hence PGx studies tend to concentrate on identifying mutant CYP variants whose altered function influences the efficacy of an administered drug (Evans and Johnson, 2001, McLeod and Evans, 2001). For example, if a PGx variant increases the activity of the CYP, there will be increased drug clearance and decreased drug exposure, and the patient may then fail to achieve treatment efficacy (Gearry and Barclay, 2005). Conversely, PGx variants that decrease CYP activity may contribute towards higher-than-expected drug concentrations, potentially enhancing treatment efficacy but also risking adverse drug reactions (ADRs) due to high concentrations of the drug (Ahmad *et al.*, 2018).

Praziquantel (PZQ) is vital in the treatment of schistosomiasis (**Section 1.5**), and the control of schistosome infections in endemic countries is reliant on this singular drug treatment (**Section 1.6**) (Ross *et al.*, 2002, WHO, 2013c). However, the growing requirement for PZQ across the world has raised concerns regarding the risk of drug resistance (Landouré *et al.*, 2012), although so far reports of parasite resistance are rare (Cupit and Cunningham, 2015, Vale *et al.*, 2017). Instead, factors such as increased transmission and additional infections

with juvenile worms have been theorised to be responsible for cases of reduced PZQ efficacy rather than widespread drug resistance (Midzi *et al.*, 2008). Nevertheless, reports of decreased PZQ efficacy (Danso-Appiah and De Vlas, 2002, Kabuyaya *et al.*, 2017) and low levels of persistent infections across multiple African countries (Pennance *et al.*, 2016, Kittur *et al.*, 2020) warrant further investigation. This includes Zimbabwe, where this chapter centres (Midzi *et al.*, 2008), thus the causes of variable PZQ efficacy require evaluation.

In **Chapter 3** of this thesis, several factors were found to affect PZQ metabolism and contribute to the variable exposure and efficacy of PZQ, however there were clear gaps in the knowledge on PGx during PZQ efficacy. At the start of this PhD project, there were no published studies evaluating the impact of host genetics on PZQ metabolism, despite multiple studies in **Chapter 3** highlighting this as the potential leading cause for variable PZQ efficacy and variable systemic PZQ concentrations (Zdesenko and Mutapi, 2020). Currently, only one study (published in 2021) has assessed the effect of PGx variations during a PZQ treatment. This study was conducted in a Tanzanian population and confirmed a PGx variant in *CYP2C19* decreased active PZQ concentrations (Mnkugwe *et al.*, 2021a). This showed, for the first time, that PGx changes can influence the concentration of the schistosome-killing PZQ.

In this chapter, the impact of PGx on PZQ efficacy will be described using the following hypotheses to identify if PGx information can be used to identify treatment failures and improve treatment efficacy. First, if a PGx change results in increased CYP activity, then PZQ may be metabolised rapidly to its metabolites, and systemic PZQ concentrations will be reduced compared to a normal metaboliser. This could result in systemic PZQ concentrations that do not reach or exceed the levels required to clear schistosome infection, potentially resulting in an unsuccessful treatment. Second, if a PGx change decreases or inactivates a

CYPs activity, the systemic concentrations of PZQ may be higher due to decreased PZQ metabolism. This may result in the individual with this PGx change sustaining PZQ concentrations and increasing their odds of clearing schistosome infection (Zimmermann *et al.*, 2007). However, with too high PZQ concentrations there could be an increased risk of ADRs. **Figure 4.1** depicts the potential implications of different PGx variants on the function of the PZQ-metabolising CYP enzymes and highlights the potential consequence on the efficacy of a PZQ treatment.

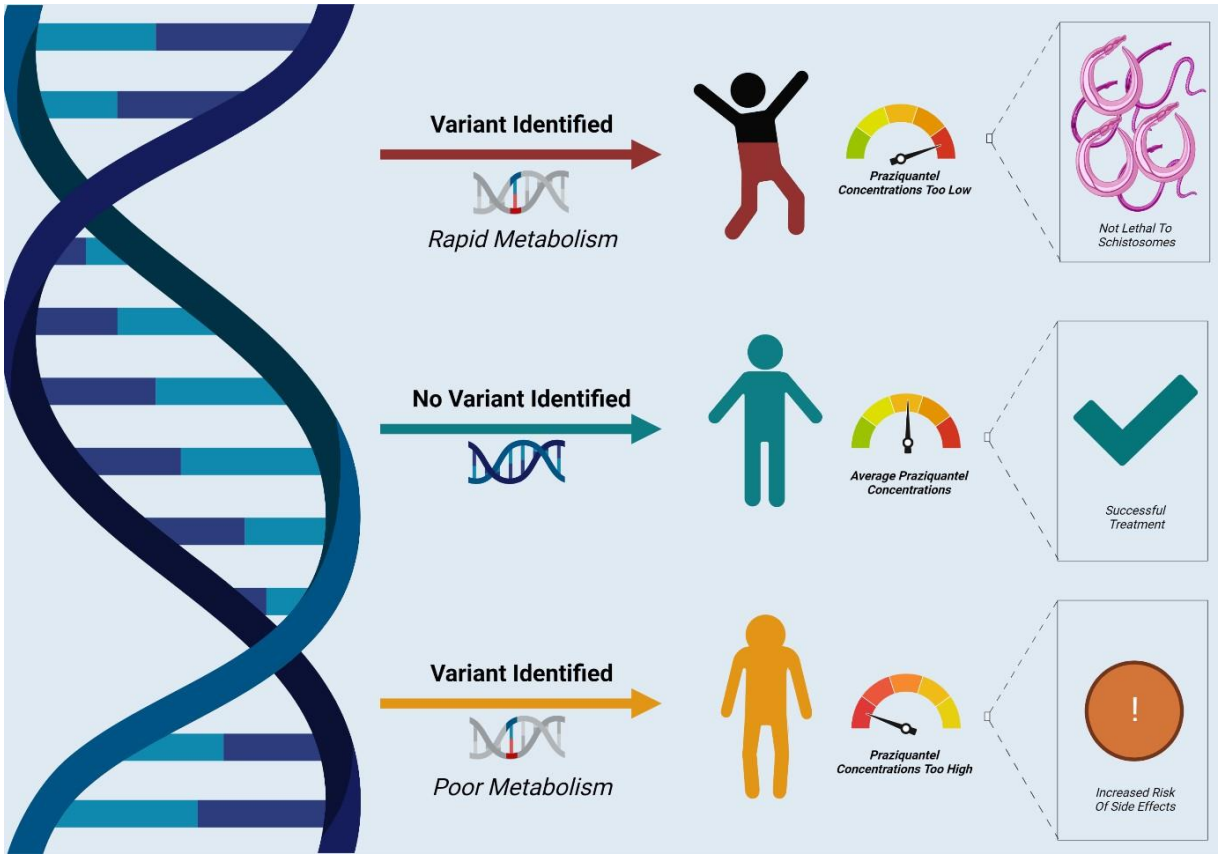

Figure 4.1: Potential implications of genetic variants, specifically single nucleotide polymorphisms (SNPs), on the metabolism of the cytochrome P450 (CYP) enzymes during a schistosomiasis treatment with praziquantel (PZQ).

In this study, the efficacy of PZQ treatment was defined using egg reduction rate (ERR), as recommended by the WHO (WHO, 2013a), to determine whether an individual cleared

(100% ERR) or did not clear (<100% ERR) schistosome infection. The *CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4*, and *CYP3A5* enzymes have been identified to have some involvement in PZQ metabolism, in both *in vitro* and *in vivo* studies (Bonate *et al.*, 2018, Kapungu *et al.*, 2020), as identified in **Chapter 3**. Thus, to determine whether PGx variants influenced the efficacy of a PZQ treatment in Zimbabwe, this chapter investigated these six PZQ-metabolising CYPs and examined if PGx changes impacted the efficacy of a PZQ treatment in Zimbabweans suffering from *S. haematobium* infections.

4.2 Aims and objectives

The aims and objectives of the study were:

I. Detect SNPs in the CYP enzymes involved in PZQ metabolism in a *S. haematobium*-exposed Zimbabwean population using targeted genomic sequencing.

II. Compare the frequency of the PGx variants of these CYPs in a *S. haematobium*-exposed Zimbabwean population with the frequencies in European and other African studies using genetic databases.

III. Relate each CYP variant to the efficacy of PZQ using clearly defined binary treatment outcomes (cleared infection: 100% ERR, did not clear infection: <100% ERR).

IV. Predict the outcome of a PZQ treatment, based on the SNPs detected, using a random forest (RF) model.

4.3 Published Work

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Grace Zdesenko, Takafira Mduluza, Francisca Mutapi

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Pharmacogenetics of Praziquantel Metabolism: Evaluating the Cytochrome P450 Genes of Zimbabwean Patients During a Schistosomiasis Treatment

Grace Zdesenko[1,2]*, *Takafira Mduluza*[2,3] *and Francisca Mutapi*[1,2]

[1]Ashworth Laboratories, Institute of Immunology and Infection Research, University of Edinburgh, Edinburgh, United Kingdom, [2]Ashworth Laboratories, NIHR Global Health Research Unit Tackling Infections to Benefit Africa (TIBA), University of Edinburgh, Edinburgh, United Kingdom, [3]Department of Biochemistry, University of Zimbabwe, Harare, Zimbabwe

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***Correspondence:**
Grace Zdesenko
g.zdesenko@sms.ed.ac.uk

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Schistosomiasis is a parasitic disease infecting over 236 million people annually, with the majority affected residing on the African continent. Control of this disease is reliant on the drug praziquantel (PZQ), with treatment success dependent on an individual reaching PZQ concentrations lethal to schistosomes. Despite the complete reliance on PZQ to treat schistosomiasis in Africa, the characterization of the pharmacogenetics associated with PZQ metabolism in African populations has been sparse. We aimed to characterize genetic variation in the drug-metabolising cytochrome P450 enzymes (CYPs) and determine the association between each variant and the efficacy of PZQ treatment in Zimbabwean patients exposed to *Schistosoma haematobium* infection. Genomic DNA from blood samples of 114 case-control Zimbabweans infected with schistosomes were sequenced using the *CYP1A2*, *CYP2C9*, *CYP2C19*, *CYP2D6*, *CYP3A4*, and *CYP3A5* genes as targets. Bioinformatic tools were used to identify and predict functional effects of detected single nucleotide polymorphisms (SNPs). A random forest (RF) model was then used to assess SNPs most predictive of PZQ efficacy, with a misclassification rate of 29%. SNPs were detected across all six genes, with 70 SNPs identified and multiple functional changes to the CYP enzymes predicted. Only four SNPs were significantly associated with PZQ efficacy using χ^2 tests, with rs951840747 (OR: 3.61, $p = 0.01$) in the *CYP1A2* gene having the highest odds of an individual possessing this SNP clearing infection, and rs6976017 (OR: 2.19, $p = 0.045$) of *CYP3A5* determined to be the most predictive of PZQ efficacy *via* the RF. Only the rs28371702 (CC) genotype (OR: 2.36, $p = 0.024$) of *CYP2D6* was significantly associated with an unsuccessful PZQ treatment. This study adds to the genomic characterization of the diverse populations in Africa and identifies variants relevant to other pharmacogenetic studies crucial for the development and usage of drugs in these populations.

Keywords: praziquantel, PZQ, schistosomiasis, drug metabolism, pharmacogenetics, cytochrome P450, single nucleotide polymorphisms, SNP

1 INTRODUCTION

Schistosomiasis is an ever prominent public health problem in Africa (Ross et al., 2002), with the majority of individuals affected on the continent suffering from *S. haematobium* and *S. mansoni* infections (Utzinger et al., 2009). Mass drug administration for the control of schistosome infections utilizes praziquantel (PZQ) as the drug of choice to reduce morbidity (Daumerie and Savioli, 2013). PZQ is racemic, with the (R)-PZQ enantiomer possessing antischistosomal activity and (S)-PZQ contributing to PZQ's known side effects (Meyer et al., 2009). The efficacy of PZQ treatment is determined by two outcomes: egg reduction rate (ERR), determined by the reduction in mean number of eggs excreted in urine or stool (depending on the schistosome species) from pre-PZQ to post-PZQ treatment, and the cure rate (CR) which gives the proportion of egg-positive individuals pre-PZQ treatment who become negative for schistosomiasis post-PZQ treatment (Zwang and Olliaro, 2014). Our previous study showed that various factors, including PZQ metabolism, contribute towards variable CRs (Zdesenko and Mutapi, 2020). Like several African countries, Zimbabwe has been administering PZQ as part of a national helminth control program for over 10 years. We have previously reported that the national program has significantly reduced infection prevalence (Mduluza et al., 2020), however there have been multiple reports of hotspots of schistosomiasis infection across other areas of Africa (Kittur et al., 2017; Kittur et al., 2019). Consequently, it is critical to understand the reasons for the persistence of infection, especially if the implementation of this knowledge can aid in improving control of this disease. In this study, we highlight the paucity of pharmacogenetic studies in African populations, focusing on Zimbabwe, where PZQ is heavily used. Such studies are important to inform on drug failures (Drew, 2016) and contribute towards the global efforts to eliminate schistosomiasis, a goal recently highlighted in the new WHO NTD roadmap (WHO, 2020).

Drug metabolism is mediated by the cytochrome P450 (CYP) enzymes, and genetic polymorphisms in CYPs have already been linked to inter-individual variation in drug metabolism in numerous drug efficacy and toxicity studies (Evans and Relling, 1999). Due to random mutation, meiotic recombination, and genetic drift, the African continent has greater genetic diversity than any other continental population (Tishkoff et al., 2009; Rajman et al., 2017). Nonetheless, African populations are the least studied in terms of pharmacogenetics for the majority of drug treatments (Radouani et al., 2020). Therefore, the lack of characterization of the pharmacogenetics associated with PZQ metabolism needs to be addressed (Mduluza and Mutapi, 2017). The distribution of CYP variants differs substantially between populations, thus pharmacogenetic studies conducted in European populations are not always representative of other ethnicities, including the more genetically diverse African populations (Masimirembwa and Hasler, 1997). Therefore, by characterising single nucleotide polymorphisms (SNPs) in the CYP enzymes that are key in mediating PZQ metabolism we have the potential to inform on associations of SNPs with an individual's treatment response (Evans and Johnson, 2001; McLeod and Evans, 2001). SNPs that potentially decrease or inactivate the CYP enzyme may reduce the metabolism of active PZQ to its inactive metabolites, sustaining a lethal PZQ concentration to the schistosomes, and increasing the likelihood of clearing infection (Zimmermann et al., 2007; Nleya et al., 2019). Conversely, an increased rate of PZQ metabolism may result in systemic PZQ concentrations that do not reach or exceed the lethal levels required to clear schistosome infection.

To date, six CYPs*: CYP1A2*, *CYP2C9*, *CYP2C19*, *CYP2D6*, *CYP3A4*, and *CYP3A5* have been identified to be involved in the PZQ metabolism pathways (Bonate et al., 2018; Kapungu et al., 2020), as well as being responsible for metabolising 90% of market drugs (Slaughter and Edwards, 1995; Wilkinson, 2005). Each CYP has varying contributions to PZQ metabolism, and the conclusive pathway does not always include CYP2D6. However, as the impact of CYP2D6 in African populations is scarce we included this enzyme to provide valuable information for, not just this study, but other pharmacogenetic analyses. Thus, in this study we focused on the genetic variants of these CYPs and their association with PZQ clearance of *S. haematobium* infections in Zimbabweans resident in a schistosome endemic area. We aimed to characterize the SNPs present in these six CYPs, their frequency in the Zimbabwean population, and be the first to assess their association with PZQ efficacy in this genetically diverse population.

2 MATERIALS AND METHODS

2.1 Study Design, Demographic Characteristics, and Measures of Praziquantel Efficacy

The case-control study aimed to investigate whether 1) there was genetic variation in the CYP450 enzymes involved in PZQ metabolism, 2) determine if there was an association between each variant and the efficacy of PZQ treatment in a Zimbabwean population exposed to *S. haematobium* infection, and 3) predict an individual's PZQ treatment outcome based on all the SNPs detected. The samples used in this study were part of a larger study on the immunological effects of schistosomiasis conducted in the Mashonaland East province, which has a *S. haematobium* prevalence of 30.4% (Mduluza et al., 2020). Samples included in the current study were from two districts in this province, Mutoko and Murewa, and details of their parasitology and blood sampling have been previously published (Osakunor et al., 2020). This case-control study was designed in the context of a pharmacogenetic evaluation, in which the subjects are divided into those with a positive response to PZQ who cleared schistosome infection, and those with negative or no response who did not clear schistosome infection. These groups then constitute as cases and controls that could be related to the treatment phenotype (Weiss et al., 2001). As this is a novel and exploratory study there were no baseline studies to inform sample size calculations, but samples were selected to ensure matching on treatment outcome, sex, and age. This matching was essential in

the study to reduce the impact of factors known to affect the efficacy of treatment (e.g., host pre-treatment infection intensity, all individuals fed prior to treatment), minimizing all known heterogeneities that would affect the drug efficacy. Furthermore, as confirmed by the initial health assessment, none of the participants were on concomitant drug treatments to remove the risk of drug-drug interactions.

The characteristics and matching of the 114 participants selected for this study are described in **Supplementary Table S1**. All participants were positive for *S. haematobium* infection and were treated at baseline with PZQ. These individuals were then followed up 6 weeks later to obtain the post-treatment efficacy outcome. The efficacy of PZQ treatment was determined by the ERR, determined by the reduction in mean number of eggs excreted in urine from pre-PZQ to post-PZQ treatment (Zwang and Olliaro, 2014). At the 6-weeks efficacy check, the subjects were divided into either: 1) negative and cleared schistosome infection (n = 57, 100% ERR), indicative of a successful treatment, or 2) still positive for schistosomes and did not clear infection (n = 57, <100% ERR), indicative of an unsuccessful treatment; controlling each sample for age, sex, and initial egg burden. To ensure treatment compliance each individual was checked by a health worker to confirm they had swallowed the tablet.

2.2 DNA Extraction and Target Sequencing

Genomic DNA was extracted from the blood samples using QIAamp DNA MicroKit (Qiagen, GmbH, Germany), according to manufacturer's protocol. The DNA samples were shipped on dry ice for library preparation and targeted metagenomic sequencing to BGI (Beijing Genomics Institute, Shenzhen, China). Briefly, DNA samples were quantified using the Qubit fluorometer (ThermoFisher Scientific, New Territories, Hong Kong) and the NanoDrop™ spectrophotometer (ThermoFisher Scientific, New Territories, Hong Kong). The integrity and purity of DNA was assessed by a 1% agarose gel electrophoresis. Qualified DNA was randomly fragmented by Covaris technology (150 bp–250 bp fragments) (Covaris, Woburn, United States) and end repair of DNA fragments was performed with an "A" base added to 3′-end of each strand. Adapters were then ligated to both ends of the end repaired/dA tailed DNA fragments. A hybridisation-based target enrichment and selective amplification of the size-selected DNA fragments was performed using ligation-mediated PCR for the target CYP gene regions. PCR products were purified with the AxyPrep Mag PCR clean up Kit (Axygen Scientific, Taipei, Taiwan). Adapter-ligated DNA fragments were separated by electrophoresis through a 2% agarose gel to recover the target fragments, purified using the QIAquick Gel Extraction kit (Qiagen, GmbH, Germany), and circularised to produce DNA nanoballs. Targeted enrichment of the *CYP1A2*, *CYP2C9*, *CYP2C19*, *CYP2D6*, *CYP3A4*, and *CYP3A5* genes was performed using a custom Agilent SureSelect Target Enrichment Kit (Agilent Technologies, Santa Clara, United States) according to the manufacturer's protocol. Sequencing probes for the six targeted genes were custom designed using Agilent's SureDesign tool (www.agilent.com/genomics/suredesign) to include all exons, introns, intergenic, and promoter regions according to the tools reference databases. After initial amplification of the target regions, different pairs of index primers were added to each sample in a second thermocycling step with barcode recognition. This produced a library of DNA amplicons representing individuals for sequencing and allowed multiple libraries to be pooled together to be sequenced in the same run. Each captured library was loaded on the Illumina Hiseq 4000 platform (Illumina, San Diego, United States) and the raw image files were processed by DNBseq base calling software with default parameters. The sequence data of each individual was generated as paired-end reads and stored in FASTQ format.

2.3 Bioinformatic Analysis

Variant Call Format (VCF) file from the raw sequence data was produced according to **Supplementary Procedure S1**, with alignment ensuring SNP regions of interest were covered [as selected from a pharmacogenetic review of African populations (Zhou et al., 2017)], in addition to the full genes including exon/intron boundaries and flanking regions. Each SNP was determined to have an average coverage of >87X per sample. Each SNP was assigned an ID and designated as either: 1) reported CYP allele, 2) reported rs-code, or 3) a novel SNP. The SNPs were designated a novel ID if no corresponding SNP position was found in dbSNP (Sherry et al., 2001) or Ensembl (Hubbard et al., 2007), according to the human genome assembly GRCh38.

2.4 Variant Analysis and Transcriptional Prediction

The SNPs were assigned an official allele nomenclature using The Human Cytochrome P450 Allele Nomenclature Database (PharmVar) (Gaedigk et al., 2018) and SNPedia (Cariaso and Lennon, 2012), with the remaining variants designated an rs-number using dbSNP (Sherry et al., 2001) and Gnomad (https://gnomad.broadinstitute.org/). The nucleotide changes and the amino acid change of each SNP was obtained using Mutalyzer (Wildeman et al., 2008) and Ensembl Variant Effect Predictor (Hubbard et al., 2007), respectively. A predicted effect on protein function of missense variants was obtained using the SIFT tool (Ng and Henikoff, 2003). SIFT predictions are only shown for complete Ensembl proteins. For non-coding variations, the Genome-Wide Annotation of Variants (GWAVA) (Ritchie et al., 2014) scoring tool was used to predict the functional impact of the SNP. SNPs with a MAF <5% were removed from subsequent analysis but were reported in **Supplementary Table S2** due to lack of power to detect an association in this sample size.

2.5 Statistical Analysis

Univariate association of SNP to PZQ efficacy was performed using PLINK (Purcell et al., 2007) to visualize and interpret results. Each SNP's minor allele *a* and major allele *A* was represented as a contingency table of PZQ treatment outcome by either genotype count (*aa*, *Aa*, and *AA*) or allele count (*a* and

TABLE 1 | Characteristics and minor allele frequency (MAF) of the 70 single nucleotide polymorphisms (SNPs) detected in the Zimbabwean subjects. Each SNP had a MAF > 5%, and the predicted and reported functional changes of each cytochrome P450 (CYP) were described.

DNA strand	Enzyme	Identifier	Allele	MAF (%)				Chromosome position	Nucleotide change	Amino acid change	SIFT/ GWAVA prediction	Reported enzyme activity	Variant consequence	Region
				EXP	AFR	ZIM	EUR							
1	*CYP1A2*	rs2069514	*CYP1A2*1C*	29.39	31.32	—	1.99*	74745879	G > A	—	-/DEL	Decreased (Nakajima et al., 1999)	Upstream Gene	—
		rs762551	*CYP1A2*1F*	53.51	56.2	57	67.99**	74749576	C > A	—	-/DEL	Increased (Lu et al., 2020)	Intron	I1
		rs2069526	*CYP1A2*1K*	11.84	12.33	—	2.39*	74749000	T > G	—	-/TOL	Decreased (Al-Ahmad et al., 2017)	Intron	I1
		NOVEL_74753482	Novel-1	12.72	—	—	—	74753482	G > A	—	-/TOL	—	Intron	I6
		NOVEL_74753485	Novel-2	86.84	—	—	—	74753485	G > C	—	-/TOL	—	Intron	I6
		NOVEL_74753512	Novel-3	13.16	—	—	—	74753512	C > T	—	-/TOL	—	Intron	I6
		NOVEL_74753515	Novel-4	7.02	—	—	—	74753515	G > A	—	-/TOL	—	Intron	I6
		NOVEL_74747828	Novel-5	8.33	—	—	—	74747828	C > T	—	-/DEL	—	Upstream Gene	-
		NOVEL_74747713	Novel-6	6.14	—	—	—	74747713	A > G	—	-/DEL	—	Upstream Gene	-
		NOVEL_74747716	Novel-7	7.46	—	—	—	74747716	C > T	—	-/DEL	—	Upstream Gene	-
		NOVEL_74747757	Novel-8	10.09	—	—	—	74747757	G > A	—	-/DEL	—	Upstream Gene	-
		rs1022705765	rs1022705765	10.53	0*	—	—	74753493	G > A	—	-/TOL	—	Intron	I6
		rs1450415112	rs1450415112	9.65	0*	—	—	74753511	A > G	—	-/TOL	—	Intron	I6
		rs951840747	rs951840747	10.09	0*	—	—	74753480	C > T	—	-/TOL	—	Intron	I6
	CYP2C9	rs2256871	*CYP2C9*9*	14.04	8.17	13	0.1*	94949217	A > G	His251Arg	DEL/DEL	Normal (Matimba et al., 2009)	Missense	E5
		NOVEL_94956743	Novel-9	7.46	-	—	—	94956743	T > C	—	-/TOL	—	Intron	I5
		NOVEL_94977838	Novel-10	6.14	-	—	—	94977838	C > T	—	-/TOL	—	Intron	I6
		rs2017319	rs2017319	13.60	12.03	10	0.2*	94988878	C > T	Ala441 (3D)	-/TOL	—	Synonymous	E9
		rs75541073	rs75541073	16.23	19.14	—	0.1*	94948233	G > A	—	-/TOL	—	Intron	I4
		rs9332127	rs9332127	14.47	15.81	—	0.1*	94947714	G > C	—	-/TOL	Decreased (Wang et al., 2008; Gu et al., 2010)	Intron	I3
		rs9332232	rs9332232	13.60	11.95	11	0.2*	94986275	T > C	—	-/TOL	—	Intron	I8
		rs9332241	rs9332241	7.02	3.78	5	0*	94989116	C > T	—	-/TOL	—	3′ UTR	E9
	CYP2C19	rs4244285	*CYP2C19*2*	16.23	17.02	15	14.51	94781859	G > A	Pro218	-/TOL	Decreased (Dandara et al., 2001)	Synonymous	E5
		rs12248560	*CYP2C19*17*	15.79	23.52	—	22.37	94761900	C > T	—	-/DEL	Increased (Sim et al., 2006; Gawrońska-Szklarz et al., 2012)	Upstream Gene	-
		rs7902257	*CYP2C19*27*	13.60	8.25	—	0.1*	94761665	G > A	—	-/-	Uncertain (Helsby and Burns, 2012)	Upstream Gene	-
		NOVEL_94779010	Novel-11	6.58	—	—	—	94779010	G > A	—	-/TOL	—	Intron	I3
		rs17879992	rs17879992	11.84	9.76	—	6.96	94775871	T > C	—	-/TOL	—	Intron	I3
		rs17884938	rs17884938	9.21	5.82	—	0.2*	94780934	T > A	—	-/TOL	—	Intron	I4
		rs17885567	rs17885567	5.70	5.60	10	0*	94850227	C > T	—	-/TOL	—	Intron	I8
		rs4917623	rs4917623	10.96	19.82	22**	50.8**	94849811	T > C	—	-/TOL	—	Intron	I7
		rs4986894	rs4986894	14.91	16.26	15	14.51	94762608	T > C	—	-/DEL	—		-

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TABLE 1 | (*Continued*) Characteristics and minor allele frequency (MAF) of the 70 single nucleotide polymorphisms (SNPs) detected in the Zimbabwean subjects. Each SNP had a MAF > 5%, and the predicted and reported functional changes of each cytochrome P450 (CYP) were described.

DNA strand	Enzyme	Identifier	Allele	MAF (%)				Chromosome position	Nucleotide change	Amino acid change	SIFT/ GWAVA prediction	Reported enzyme activity	Variant consequence	Region
				EXP	AFR	ZIM	EUR							
													Upstream Gene	
		rs76267522	rs76267522	5.26	2.34	—	0.1*	94780959	T > C	—	-/TOL	—	Intron	I4
−1	*CYP2D6*	rs28371706	*CYP2D6*17*	16.67	21.80	20	0.2*	42129770	G > A	Thr107Ile	TOL/-	Decreased (Masimirembwa et al., 1996)	Missense	E2
		rs17002853	rs17002853	5.70	0*	—	0.1*	42128325	A > G	Leu231Pro	DEL/-	—	Missense	E5
		rs28371702	rs28371702	75.88	77.08	29*	54.77*	42129950	A > C	—	-/-	Normal/Decreased (Zhang et al., 2021)	Intron	I1
	CYP3A4	NOVEL_99761942	Novel-12	7.89	—	—	—	99761942	T > C	—	-/TOL	—	Intron	I11
		NOVEL_99764101	Novel-13	7.89	—	—	—	99764101	T > C	—	-/TOL	—	Intron	I9
		NOVEL_99766252	Novel-14	5.26	—	—	—	99766252	C > T	—	-/TOL	—	Intron	I10
		NOVEL_99764034	Novel-15	16.23	—	—	—	99764034	C > T	—	-/TOL	—	Intron	I11
		NOVEL_99764099	Novel-16	8.33	—	—	—	99764099	C > T	—	-/TOL	—	Intron	I12
		NOVEL_99762108	Novel-17	16.23	—	—	—	99762108	T > C	Ile396Val	TOL/DEL	—	Missense	E11
		NOVEL_99762115	Novel-18	7.46	—	—	—	99762115	C > T	Val393	-/TOL	—	Synonymous	E11
		NOVEL_99762116	Novel-19	7.46	—	—	—	99762116	A > G	Val393Ala	TOL/DEL	—	Missense	E11
		NOVEL_99784449	Novel-20	15.35	—	—	—	99784449	A > G	—	-/DEL	—	Upstream Gene	—
		NOVEL_99784471	Novel-21	11.40	—	—	—	99784471	T > A	—	-/DEL	—	Upstream Gene	—
		rs1006181087	rs1006181087	8.33	0*	—	—	99762118	C > T	Val392	-/TOL	—	Synonymous	E11
		rs12721622	rs12721622	9.65	4.39	—	0*	99768236	A > T	—	-/TOL	—	Intron	I7
		rs1479820461	rs1479820461	99.12	0*	—	0*	99783699	G > C	—	-/DEL	—	ntron	I1
		rs2687110	rs2687110	62.28	59.23	—	99.7**	99773128	A > T	—	-/TOL	-	Intron	I3
		rs2687116	rs2687116	23.25	27.91	—	97.02**	99768320	C > A	—	-/TOL	Decreased (Panczyk, 2014)	Intron	I7
		rs28988583	rs28988583	11.84	8.70	—	2.09*	99769086	A > G	—	-/TOL	—	Intron	I6
		rs3735451	rs3735451	82.02	81.39	—	9.94*	99758352	T > C	—	-/TOL	—	Intron	I13
		rs746971934	rs746971934	8.33	0*	—	—	99762120	C > A	Val392Leu	DEL/DEL	—	Missense	E11
		rs778270963	rs778270963	15.79	0*	—	—	99762101	C > A	Ser398Ile	TOL/-	—	Missense	E11
		rs915268104	rs915268104	6.58	0*	—	—	99783718	C > T	—	-/DEL	—	Intron	I1
	CYP3A5	rs776746	*CYP3A5*3*	15.79	18	77.6**	94.33**	99672916	T > C	—	-/-	Inactive (Kuehl et al., 2001; Sanghavi et al., 2017)	Intron	I3
		rs10264272	*CYP3A5*6*	15.35	15.43	22	0.3*	99665212	C > T	Lys208	-/DEL	Inactive (Kuehl et al., 2001; Sanghavi et al., 2017)	Synonymous	E7
		NOVEL_99672254	Novel-22	15.79	—	—	—	99672254	G > A	—	-/TOL	—	Intron	I4
		NOVEL_99672211	Novel-23	97.81	—	—	—	99672211	G > C	—	-/TOL	—	Intron	I4
		NOVEL_99672251	Novel-24	5.26	—	—	—	99672251	C > T	—	-/TOL	—	Intron	I4
		NOVEL_99672285	Novel-25	15.35	—	—	—	99672285	C > T	—	-/TOL	—	Intron	I4
		NOVEL_99666556	Novel-26	6.14	—	—	—	99666556	A > G	—	-/DEL	—	Intron	I6
		rs1039108105	rs1039108105	9.21	0*	—	0*	99656082	G > C	—	-/TOL	—	Intron	I10
		rs1458424958	rs1458424958	5.26	0*	—	0*	99673203	A > G	—	-/TOL	—	Intron	I3
		rs1462057054	rs1462057054	16.67	0*	—	0*	99672249	A > G	—	-/TOL	—	Intron	I4

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A)

TABLE 1 | (*Continued*) Characteristics and minor allele frequency (MAF) of the 70 single nucleotide polymorphisms (SNPs) detected in the Zimbabwean subjects. Each SNP had a MAF > 5%, and the predicted and reported functional changes of each cytochrome P450 (CYP) were described.

DNA strand	Enzyme	Identifier	Allele	MAF (%)				Chromosome position	Nucleotide change	Amino acid change	SIFT/ GWAVA prediction	Reported enzyme activity	Variant consequence	Region
				EXP	AFR	ZIM	EUR							
		rs2040992	rs2040992	100	99.92	—	98.61	99664949	G > A	—	-/DEL	—	Intron	I7
		rs41303322	rs41303322	10.53	11.88	—	0*	99664999	T > C	—	-/TOL	—	Intron	I7
		rs4646453	rs4646453	9.21	8.77	—	1.89*	99662739	C > A	—	-/TOL	—	Intron	I13
		rs6976017	rs6976017	20.18	13.99	—	3.08*	99652376	G > A	—	-/TOL	—	Intron	I11
		rs8175345	rs8175345	9.65	8.09	—	0*	99672695	G > A	—	-/DEL	—	Intron	I3

** and ** represents a significantly higher or lower difference in the minor allele frequency (MAF) in this Zimbabwean (ZIM) study population compared to the reported MAF in the African (AFR)/European (EUR) population in the 1,000 Genomes Project using Pearson's chi-square tests.*

SIFT, sorting Intolerant From Tolerant; GWAVA, Genome-Wide Annotation of Variants; DEL, Deleterious effect on protein function; TOL, Tolerated effect on protein function. Region described by: Exon (E) and Intron (I). CYP, Cytochrome P450 enzyme.

according to genetic models described by Clarke et al. (2011). PLINK assessed these genetic models using χ^2 tests, with the χ^2_{Yates} calculated to assess possible type I error for further discussion. χ^2 tests were used to assess significant differences between the MAF of the SNPs against three populations; the African and European populations as reported by 1,000 Genomes Project (Auton et al., 2015), and the Zimbabwean population based on a limited number of studies conducted (Matimba et al., 2009; Dandara et al., 2004; Dandara et al., 2001; Matimba et al., 2008; Masimirembwa et al., 1995; Roy et al., 2005) using IBM SPSS Statistics v.25 (https://www.ibm.com/uk-en/analytics/spss-statistics-software). The 1,000 Genomes Project was selected as a comparison as it contained the most representative database of eastern African populations and was therefore the most relevant to this study. Quantile plots were used to discern whether the *p*-values followed the null distribution, with the observed $-\log_{10}$ (*p*-values) of each SNP plotted against expected $-\log_{10}$ (*p*-values) (**Supplementary Figure S1**). The ORs were presented with a 95% CI and all statistical significance was set at $p \leq 0.05$. LAMPLINK was used to detect statistically significant combinatorial interactions between ≥2 SNPs using the procedure set out by Terada et al. (2016). Linkage disequilibrium (LD) and Hardy–Weinberg equilibrium (HWE) between pairs of SNPs on the same chromosome were assessed using the Haploview software (http://broad.mit.edu/mpg/haploview, Version 4.1) (Barrett et al., 2005). As deviations from HWE may be indicative of genotyping or genotype-calling errors, SNPs with HWE ($P < 1 \times 10^{-4}$) were excluded from analysis (Marees et al., 2018). The LD test statistics and the strength of LD was designated according to **Supplementary Table S3**. To assess non-random associations between SNPs in LD, the presence of haplotypes was evaluated in Haploview, with blocks indicating where there was statistical evidence of strong co-inheritance. Haplotype blocks were defined based on the Gabriel method (upper 95% CI of the D′ value is ≥0.98, and the lower 95% CI is ≥0.7; MAF, >5%) (Gabriel et al., 2002). The random forest (RF) model was built a series of classification trees by randomly sampling a subset of the subjects to form an out-of-bag (OOB) data set. The RF then randomly selects and searches, building a tree for the best SNP predictive of a PZQ treatment outcome. Further information regarding the development of the model is described in **Supplementary Figure S2**.

3 RESULTS

3.1 Detected Single Nucleotide Polymorphisms

3.1.1 Minor Allele Frequency

A total of 70 Single Nucleotide Polymorphisms (SNPs) with an allele frequency in the population >5% were identified, with the Minor Allele Frequency (MAF) and variant characteristics of each SNP displayed in **Table 1**, including the predicted or reported functional change of each SNP to its respective CYP450 enzyme. The allele frequencies of each SNP (**Table 1**; Column EXP) were in HWE, with 10 common CYP alleles, 34 rs-number SNPs, and 26 novel SNPs to be evaluated. Comparisons

between the MAF of the different populations showed that 17.1% of detected SNPs were found at a significantly higher frequency in this Zimbabwean study population than expected in an African population. These significantly different SNPs were not detected in the African populations at all (MAF = 0%), yet this study detected those same SNPs with a frequency as high as 99.1% in comparison, exemplifying the scarcity of relevant frequency data in Zimbabwe. Furthermore, although the SNPs detected for genetic evaluation are described as the minor allele, 4.3% of variants in this study had a MAF greater than 90%. This included rs2040992 of *CYP3A5*, which was detected as a completely homozygous SNP in this population but was not significantly different from the frequency reported in African populations. Yet, 4.3% of detected SNPs were also found to be significantly different to other genetic studies conducted in Zimbabwe, with 2.8% of detected SNPs significantly lower in the study population compared to the reported frequency in Zimbabwe. This included the *CYP3A5*3* polymorphism in which this study detected a significantly lower frequency than in European populations and other Zimbabwean studies reported, with a −78.5% and −61.8% decrease, respectively. The significantly lower MAF detected in this study compared to other Zimbabwean study populations only occurred for one additional SNP: rs4917623 of *CYP2C19*. For the remaining common CYP alleles, there were no significant differences in MAF detected in this study between either the African or Zimbabwean populations.

Interestingly, for the allele frequencies detected in the Zimbabweans in this study, only 52.9% had been previously detected in a European population. In fact, 87% of comparable alleles in this Zimbabwean population had significantly different frequencies than in their corresponding European populations. Regarding comparisons of this study population to European populations, of the 10 common CYP alleles identified in this study, 60% were found at a significantly higher percentage in the Zimbabwean study population than expected based on European populations. These CYP alleles included the *CYP1A2*1C* and *CYP1A2*1K* variants which have been reported to decrease CYP function yet were found at a +27.4% and +9.45% high frequency than observed in Europeans. If present in the Zimbabwean population at a significantly higher frequency than what is presented by European studies, these two variants could decrease CYP metabolism which could lead to higher exposure of an administered drug in Zimbabweans and impact the success and safety of a drug treatment.

3.1.2 Predicted Functional Consequences

Alterations to the nucleotide sequence by SNPs can impact the resultant protein translation. These changes can affect protein function and therefore impact a drug's metabolism, including the compromise and enhancement of protein function, or having no effect at all. In this study, there were 7 missense, 5 synonymous, 47 intronic, one 3′ UTR, and 10 upstream gene variants detected, with each classification impacting the consequent DNA sequence differently. In this study, 3 missense variants were predicted by SIFT to be a deleterious change, and therefore possibly damaging to the function of their respective CYP enzyme. These potentially deleterious variants included *CYP2C9*9* (His251Arg) of the *CYP2C9* gene, rs17002853 (Leu231Pro) in *CYP2D6* gene, and rs746971934 (Val392Leu) in the *CYP3A4* gene. These SNPs alterations damaged the translated protein, which could eventually affect each enzyme's metabolising capacity. Conversely, a further 4 missense variants were predicted to result in a tolerated amino acid change, with no alteration to protein function and overall capacity of the enzyme. These tolerated changes included the *CYP2D6*17* polymorphism, in which SIFT predicted Thr107Ile to have no impact *CYP2D6* function, as well as Novel-17 (Ile396Val), Novel-19 (Val393Ala), and rs778270963 (Ser398Ile) of the *CYP3A4* enzyme. However, as the SIFT predictor tool is not always definitive of resultant protein function, the GWAVA prediction was also reported. For the novel missense findings described above, GWAVA predicted they may be deleterious to *CYP3A4* function, rather than tolerated as SIFT described.

For the synonymous SNPs detected that do not change the encoding amino-acid, two SNPs were already well reported to impact CYP function; the *CYP2C19*2* and *CYP3A5*6* alleles. These SNPs are reported to decrease or inactivate their respective CYPs function, however only the *CYP3A5*6* was predicted to be deleterious by GWAVA. The remaining 3 synonymous SNPs were predicted to be tolerated, with no protein translation to alter the function of the CYPs, including one novel SNP: Novel-19 (Val393) of *CYP3A4*. From the remaining intronic and upstream gene variants whose functional effect could not be predicted by SIFT, the GWAVA algorithm was used. GWAVA predicted that a further 15 SNPs possibly damaged their respective CYP function, of which three were reported CYP alleles (*CYP1A2*1C*, *CYP1A2*1F*, and *CYP2C19*17*) whose alterations to CYP function are already well described. A further seven possibly damaging SNPs were novel discoveries (Novel-5, Novel-6, Novel-7, Novel-8, Novel-21, Novel-20, and Novel-26) and five were reported variants (rs1479820461, rs2040992, rs4986894, rs8175345, and rs915268104), yet no published studies in relation to their impact on CYP function have been conducted.

3.2 Single Nucleotide Polymorphisms Associations With Praziquantel Efficacy

Tests of association between SNP and PZQ efficacy was assessed using multiple genetic models. Independent association of the 70 SNPs with PZQ efficacy can be found in **Supplementary Table S4**, and a quantile plot of the genotypic associations was produced (**Supplementary Figure S1**). This indicates little evidence of an overall systematic bias yet displays a slight deviation from the null hypothesis ($y = x$), suggesting a small number of SNPs possessed stronger associations with PZQ efficacy than expected by chance as indicated by the slight upward curvature near the tail of the distribution. The association to PZQ efficacy was driven by SNPs in the *CYP1A2*, *CYP2D6*, and *CYP3A5* genes, with the *p*-values of SNPs above the threshold for significance labelled in **Figure 1** to ascertain significant SNP deviations.

To further determine the mechanism behind the SNP deviations from the null hypothesis, hetero/homozygous genotypes were tested for an association with PZQ efficacy,

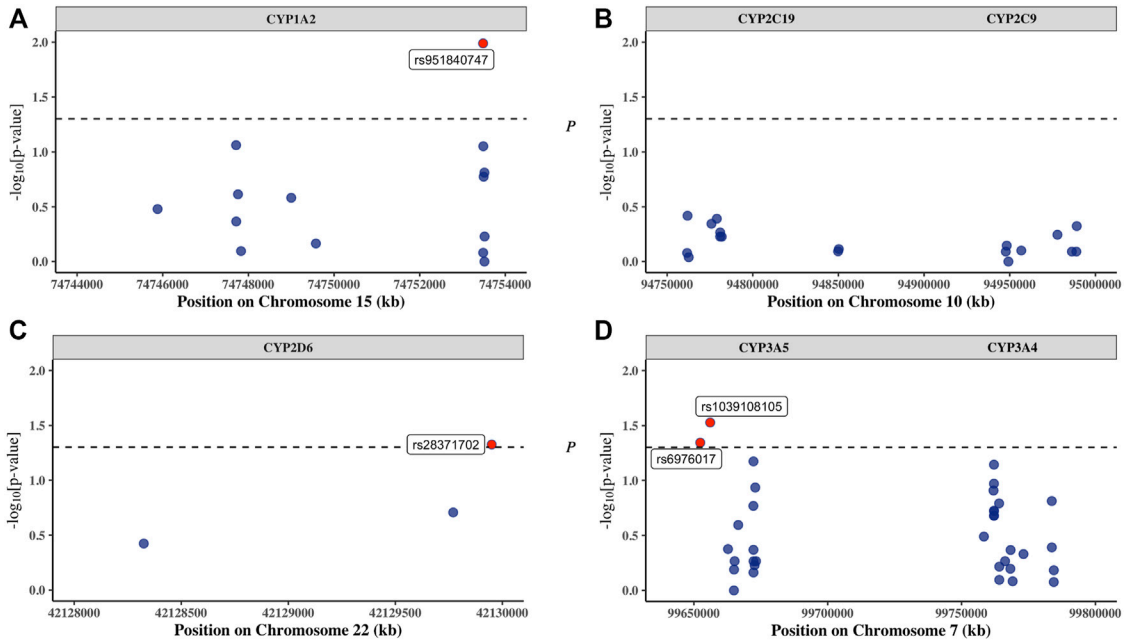

FIGURE 1 | The position of each single nucleotide polymorphism (SNP) on their respective chromosome versus –log10 (*p*-value) is displayed for **(A)** *CYP1A2*, **(B)** *CYP2C9/19*, **(C)** *CYP2D6*, and **(D)** *CYP3A4/5*. The threshold for significance was set at $p = 0.05$ (black dotted line), with SNPs significantly associated with PZQ efficacy (red dots) labelled with their SNP identification.

with the results of each SNPs significance in **Supplementary Table S5**. Overall, 4 SNPs were found to have a significant association with PZQ efficacy (**Table 2**), with multiple genetic models conducted to best determine the genetic mechanism behind the association. To aid further discussion of the discovery of significantly associated SNPs, results of χ^2_{Yates} test representing an adjusted *p*-value accounting for a continuity correction can be seen in **Supplementary Table S6**.

Each SNPs respective odds ratio (OR) towards PZQ efficacy was also calculated. The OR of all SNPs were log-transformed to normalize their distribution and are displayed in **Supplementary Figures S3, S4**, with the SNPs presented to either increase odds of clearing or not clearing infection across all six genes. Of the 4 SNPs significantly associated with PZQ efficacy, the OR of an individual clearing or not clearing infection based on the presence of the SNP is visualised in **Figure 2**.

The rs951840747 (CT) ($p = 0.01$) of *CYP1A2* was significantly associated with PZQ efficacy and had the highest OR, implying that individuals possessing this genotype had a 3.61 (95% CI: 1.30, 10) increase in odds of clearing infection compared to those without this genotype. This was closely followed by the rs1039108105 (GC) genotype ($p = 0.03$) of *CYP3A5*, which also increased the odds of clearing infection with the presence of this SNP by 3.04 (95% CI: 1.08, 8.51). Both rs951840747 and rs1039108105 were only detected as a heterozygous genotype, therefore the interpretation of the dominant, recessive, and Cochran-Armitage trend models was not relevant. A similar effect was observed with rs6976017 ($p = 0.045$) of *CYP3A5*, whose significance via the genotypic association model arose from the rs6976017 (GA) heterozygous genotype, with individuals possessing this genotype over double the odds (OR: 2.19, 95% CI: 1.0, 4.81) of clearing infection compared to those with alternate genotypes at this site. The rs6976017 (AA) homozygous genotype of *CYP3A5* was not found to be significantly associated with PZQ efficacy ($p = 0.079$), which may be attributed to the smaller sample size of the rs6976017 (AA) in comparison. As the rs6976017 (AA) genotype was not a significant finding, the insignificant associations of the allelic, dominant, trend and recessive models were not evaluated with regards to the significance of the presence of rs6976017 (GA) in individuals who cleared infection due to the combinative analysis. Of the 4 SNPs found to be significant, genotypic analysis of rs28371702 of *CYP2D6* was the closest to the significance threshold ($p = 0.047$), yet both the (AC) and (CC) genotype of rs28371702 had a significant association when individually associated with PZQ efficacy. From the analysis of rs28371702 genotype frequencies it appears that the two genotypes had different affinities towards treatment outcome (**Figure 3**).

The rs28371702 (AC) of *CYP2D6* was significantly associated with increased odds of an individual clearing infection (OR: 2.58, 95% CI: 1.20, 5.55) and the homozygous rs28371702 (CC) was significantly associated with increased odds of an individual not clearing infection (OR: 2.36, 95% CI: 1.11, 5.04). The results of basic associations using a recessive model support these findings, resulting in a significant difference in those individuals who did not clear infection with the rs28371702 (CC) genotype compared to rs28371702 (AA + AC). Due to the conflicting PZQ efficacy per genotype the results of the dominant, allelic, and Cochran-Armitage trend models, which all imply that with an

TABLE 2 | The single nucleotide polymorphisms (SNPs) found to be significant associated with an individual clearing infection *via* basic association analysis. Association tests were used to determine the genotype significantly associated with PZQ efficacy using PLINK; these are listed below each genetic model. The frequency of the allele/genotype for each model is listed, with the frequency separated by treatment outcome.

Enzyme	SNP	MAF (%)		Genotypic association						Allelic association				Cochran-armitage trend				Dominant model				Recessive model			
				P			Genotype	*AA vs. aA vs. aa*			χ2 test		*A vs. a*		T^2 test		*A vs. a*		χ2 test		aA + AA vs. aa		χ2 test		AA vs. aa + aA	
		NC	*C*	*DF*	FE	χ^2		*P*	*NC*	*C*	*DF*	*P*	NC	C	*DF*	*P*	NC	C	DF	P	NC	C	DF	P	NC	C
CYP1A2	rs9518407 47	2.632	7.456	1	**0.018**	**0.010**	***CT***	**0.010***	0/6/51	0/17/40	1	**0.016***	6/108	17/97	1	**0.010**	6/108	17/97	1	0.010*	6/51	17/40	1	NA	0/57	0/57
							TT	NA																		
CYP2D6	rs28371702	40.351	35.326	2	**0.031**	**0.047**	***AC***	**0.014***	37/18/2	25/31/1		0.089	92/22	81/33		0.061	92/22	81/33		0.559	55/2	56/1		0.024*	37/20	25/32
							CC	**0.024***																		
CYP3A5	rs10399108105	2.632	6.579	1	0.052	**0.030**	***GC***	**0.030**	0/6/51	0/15/42		**0.039**	6/108	15/99		**0.030**	6/108	15/99		0.030	6/51	15/42		NA	0/57	0/57
							CC	NA																		
	rs6976017	9.211	10.965	2	**0.041**	**0.045**	***GA***	**0.050**	3/15/39	0/25/32		0.509	21/93	25/89		0.489	21/93	25/89		0.176	18/39	25/32		0.079	3/54	0/57
							AA	0.079																		

*Bold indicates the SNP genotype that were determined to be significantly associated with PZQ efficacy. The significance threshold is a p ≤ 0.05, with the * indicating a $P_{adj} \leq 0.05$ obtained from χ^2_{Yates} test representing an adjusted p-value accounting for a continuity correction.*

FE, Fishers Exact Test; CYP, Cytochrome P450 enzyme; NC, Not cleared group; C, Cleared group.

increasing number of alleles there was an increased risk of being significantly associated with the same treatment outcome, were not significant ($0.5 < p < 0.1$).

3.3 Combinative Analysis of Single Nucleotide Polymorphisms Associated With Praziquantel Efficacy

3.3.1 Linkage Disequilibrium and LAMPLINK Analysis

We performed conditional and haplotype Linkage Disequilibrium (LD) analyses to assess whether SNPs detected in this study, particularly those SNPs significantly associated with PZQ efficacy, were independent with no secondary associations. LD analysis for each chromosome can be seen in **Supplementary Figure S5**, and 24 SNP pairs had evidence of LD. Of specific interest was rs6976017 of *CYP3A5*, found to be significantly associated with PZQ efficacy, which had strong evidence of LD with Novel-14 (**Supplementary Table S7**) and a high LOD of 6.24 and D' value of 1. This suggested a strong chance that these SNPs are co-inherited, yet the low correlation value (r^2 = 0.22) and an OR of 0.687 towards clearing infection casts doubt on this association in relation to predicting PZQ efficacy. This indicates co-inheritance but no ability to predict PZQ efficacy based on the shared haplotype in the Zimbabwean subjects, supporting the independence of rs6976017 associations. No other SNPs significantly associated with PZQ efficacy were found to be in LD, indicating single-variant associations rather than a combinative effect of other loci. Haplotype analysis found three haplotype blocks across chromosome 7 and chromosome 10, with the LD statistics of each block displayed in **Supplementary Table S8**. The frequencies of each haplotype block were analysed for significant differences in PZQ efficacy (**Supplementary Table S9**), with no haplotype blocks significantly associated. Additionally, no significant SNP combinations were detected using LAMPLINK analysis, with 411 testable combinations assessed and an adjusted significance level of $p = 1.22 \times 10^{-4}$.

3.3.2 Random Forest Model

To determine which SNPs were most predictive of PZQ efficacy, we assessed SNP importance in determining an individual's treatment outcome, and whether this could be correctly predicted and classified using a Random Forest (RF) model. RFs allow the assessment of the multiple SNPs in an individual, not their overall frequencies in the whole population, and produced the importance of each SNP based on all the SNPs included. The summary of each RF model and the resulting misclassification rate can be seen in **Supplementary Figure S6**. The RF model with the lowest error rate was selected, with an overall misclassification rate of 29% (**Supplementary Table S10**), indicating a misclassification rate below what would be expected by chance. The most important SNPs with regards to predicting PZQ efficacy are presented in **Supplementary Figure S6A**. To evaluate if the SNPs significantly associated with PZQ efficacy *via* the univariate analysis were the strongest predictors when combined with other SNPs in this model, the RF importance vs. significance was plotted. **Supplementary Figure S6B** shows the normalised genotypic χ^2

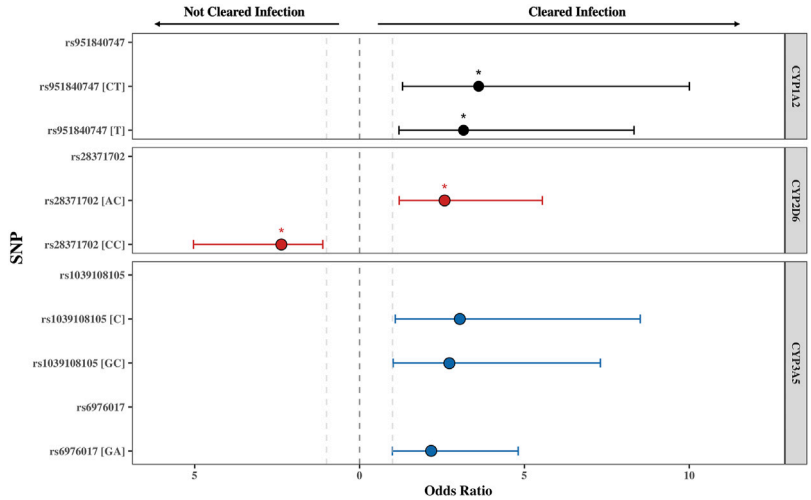

FIGURE 2 | The odds ratio (OR) of the single nucleotide polymorphisms (SNPs) found to be significantly associated with PZQ efficacy for *CYP1A2, CYP2D6*, and *CYP3A5*. The threshold for significance was set at $p \leq 0.05$, with the * indicating a $P_{adj} \leq 0.05$ obtained from χ^2_{Yates} test representing an adjusted *p*-value accounting for a continuity correction. All SNPs displayed were below this threshold. The grey lines represent OR = 1, representing no impact of the SNP on PZQ efficacy. The error bars illustrate the 95% confidence interval. The genotype or allele of each SNP is displayed in parentheses, illustrating whether the OR represents the allelic, heterozygous genotype, or homozygous genotype for the alternate allele.

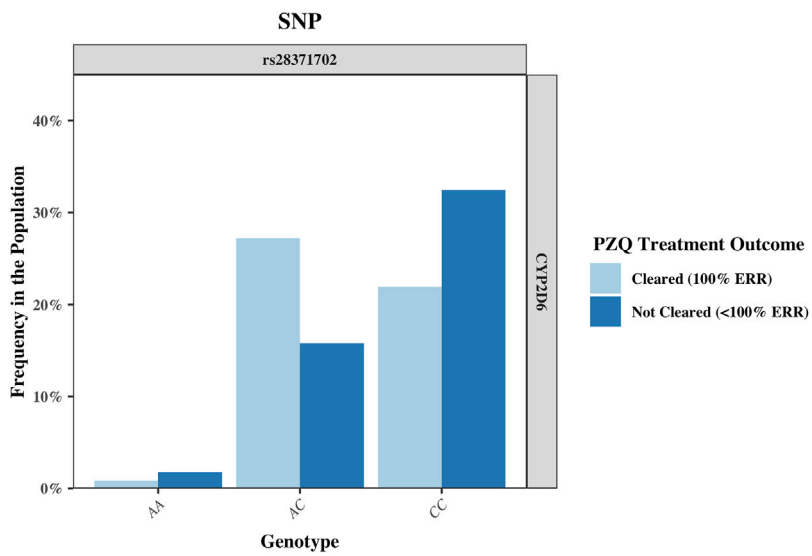

FIGURE 3 | The frequency of the single nucleotide polymorphism (SNP) rs28371702 in the CYP2D6 gene, separated by genotype and sub-divided by PZQ treatment outcome. The genotype for the rs28371702 is as follows: AA (reference allele), AC (heterozygous for alternate allele), and CC (homozygous for alternate allele). PZQ: Praziquantel, ERR: Egg reduction rate.

test of association of SNPs plotted against their permutation importance values from the RF model. There was a consensus between the strength of association and the permutation importance values, with all SNPs significantly associated with PZQ efficacy in the top five most important predictors. Interestingly, rs1022705765, an intron variant located in the *CYP1A2* gene, was not significantly associated with PZQ efficacy but was designated as the second most important predictor. Furthermore, multiple novel SNPs were deemed to be predictive of PZQ efficacy, with upstream *CYP1A2* variant Novel-8 ranking highest in importance at sixth, and the missense *CYP3A4* variant Novel-17 ranking seventh.

4 DISCUSSION AND CONCLUSION

To date, there have been minimal studies investigating pharmacogenetics in African populations, especially

considering the numerous drugs used on the continent (Radouani et al., 2020). Pharmacogenetics is of growing importance in Africa, with more drugs marketed for use in local populations due to the high disease burden and the increasing management of co-infections requiring concurrent medications. Yet, pharmacogenetic studies in African populations are less abundant than others around the world, as highlighted by a recent review that discovered that only 15 drugs have been clinically evaluated for pharmacogenetic implications in African populations over the vast continent of 54 countries and 1 billion people (Radouani et al., 2020). These studies focused on drugs used in the management of the high disease burden faced by sub-Saharan African populations, focusing on malaria, HIV, and Tuberculosis. Since this review was published, only one study has been conducted that focused on the pharmacogenetics of PZQ and the treatment of schistosomiasis, despite this disease being the second most important parasitic infection on the continent afflicting over 207 million in sub-Saharan Africa (Oyeyemi et al., 2020). The recent paper by Mnkugwe et al. (2021) has presented the first example of pharmacogenetic influences on PZQ concentrations in patients in Tanzania, with *CYP2C19*2/*3* genotypes confirmed to increase active PZQ concentrations compared to the *CYP2C19*1* and *CYP2C19*17*. This finding, and the fact that several studies have reported hotspots of persistent schistosome infections with CR lower than 100% across Africa (Sang et al., 2014; Wiegand et al., 2017; Kittur et al., 2019; Knopp et al., 2019; Mawa et al., 2021) only further emphasises the need for research, and particularly pharmacogenetic research, to characterise the determinants of treatment efficacy. As variability in PZQ efficacy has been attributed to multiple host, parasite, and environmental factors, we and others have highlighted the scarcity of studies documenting the impact of PZQ pharmacogenetics in African populations on treatment success (Mduluza and Mutapi, 2017; Zdesenko and Mutapi, 2020). We postulate that some decreased CR can be attributed to the pharmacogenetics of the people treated with PZQ. Consequently, this study aimed to identify polymorphisms in the genes encoding the CYP450 enzymes involved in PZQ metabolism in Zimbabwean participants exposed to *S. haematobium* infection and determined if there was an association with PZQ efficacy.

We identified 70 SNPs across the six CYP450 genes (*CYP1A2*, *CYP2C9*, *CYP2C19*, *CYP2D6*, *CYP3A4*, and *CYP3A5*) and evaluated their minor allele frequency (MAF). Of the detected SNPs, 14.3% were well documented polymorphic CYP alleles and 48.5% had been previously reported on genetic databases but not evaluated in terms of drug metabolism. In fact, 27.2% of SNPs in this Zimbabwean population were not consistent with the MAF previously reported in Africans, with 4.3% also significantly differing from other Zimbabwean frequency studies. This may appear to be a large portion of the SNPs identified, yet these deviations are entirely feasible as the African continent has greater genetic diversity than any other continental population. Furthermore, the 1,000 Genomes study reports the average MAF across the entire continent and therefore is not always representative of the genetic heterogeneity in Zimbabwe (Tishkoff et al., 2009; Rajman et al., 2017). This is especially pivotal when regarding comparisons to MAF in Europeans, as the protocols developed for drug treatments in Europeans e.g., dosage, do not automatically apply to diverse African populations. This study emphasizes the problem in this approach as 87% of SNPs detected here occurred at significantly different frequencies in Africans compared to Europeans, highlighting further problems of standardised drug usage in Zimbabwe based on European studies without the full knowledge of the pharmacogenetic implications. Furthermore, 37.1% of SNPs analysed in this study were novel, and their discovery provides additional insight into the genetic diversity of Zimbabweans and the scarcity of representative genomic information.

To gather further information on the clinical relevance of these SNPs, we used computational tools to predict if these variants shift the metabolic capacity of each enzyme and whether this elicited variability in the efficacy of a PZQ treatment. We identified multiple missense, synonymous, intron and upstream genetic variants that could be damaging to CYP function, but three detected CYP alleles challenged the accuracy of these predictions. Firstly, the *CYP2D6*17* polymorphism was predicted to be a tolerated change, with Thr107Ile having no impact on *CYP2D6* function. Yet, *CYP2D6*17* is reported to be one of the most functionally important SNP in African populations, resulting in a decrease in *CYP2D6* function (Dandara et al., 2001). This was also the case for the synonymous variant *CYP2C19*2*, which is reported to be the most frequent *CYP2C19* defect worldwide, reducing the enzymes activity *via* an aberrant splice site (Dandara et al., 2001). Yet again, this change was predicted to be a tolerated change in the Zimbabwean population. Conversely, *CYP2C9*9* was predicted to be a deleterious variant, yet *CYP2C9*9* has been reported as having no translated impact on drug clearance when investigated *in vivo* (Matimba et al., 2009). These discrepancies in the ability to computationally predict functional effect without an *in vivo* analysis of the SNP phenotype highlights the need for further investigation into the *in vivo* activities of the SNPs detected in this study. This is particularly relevant for the novel SNPs detected in this study. Novel SNPs were found in five of the six CYP450 enzymes evaluated in this study, but two prominent novel missense SNPs are of particular interest due to their amino acid changes; Novel-17 and Novel-19 of *CYP3A4*. Novel-19 was of particular interest as the alteration of the Val393 amino acid in the binding pocket of the *CYP3A4* enzyme (Lill et al., 2006) may have implications on the function of the enzyme. These novel missense changes further emphasise the need for more definitive studies on the functional impact of the novel SNPs.

Multiple SNPs detected in this study had already been reported to be significantly associated with the success or failure of a drug treatment, specifically the *CYP1A2*1C* (Nakajima et al., 1999), *CYP1A2*1F* (Lu et al., 2020), *CYP1A2*1K* (Al-Ahmad et al., 2017) of *CYP1A2*, rs9332127 of *CYP2C9* (Chen et al., 2014), *CYP2C19*2* (Dandara et al., 2001) and *CYP2C19*17* (Sim et al., 2006; Gawrońska-Szklarz et al., 2012) of *CYP2C19*, *CYP2D6*17* (Masimirembwa et al., 1996) and rs28371702 (Zhang et al., 2021) of *CYP2D6*, *CYP3A5*3*,

*CYP3A5*6* (Kuehl et al., 2001; Sanghavi et al., 2017), rs4646453 (Wang et al., 2019), and rs6976017 (Jorgensen et al., 2009) of *CYP3A5*. Considering the additional predicted functional effects described in this study, we subsequently determined whether there was an association between each SNP and the efficacy of a PZQ treatment; whether the individual cleared or did not clear infection. Therefore, by testing the statistical association of each SNP to the treatment outcome, we also investigated whether the presence of a SNP could be used to predict treatment success or failure. Our data revealed that four SNPs were significantly associated with PZQ efficacy, with the results driven by SNPs in the *CYP1A2*, *CYP2D6*, and *CYP3A5* genes. From *in vitro* assessments of PZQ metabolism, contributions of these CYPs to the metabolism of PZQ are estimated as *CYP1A2* (39%), *CYP2D6* (<10%), and *CYP3A4/5* (30%) (Masimirembwa et al., 2013). Three of the four SNPs associated with PZQ efficacy were found to significantly increase the odds of an individual clearing infection, including the rs951840747 of *CYP1A2*, and rs1039108105 and rs6976017 of *CYP3A5*. The SNP with the highest OR was rs951840747 (CT) (p = 0.01) of *CYP1A2*, signifying that individuals possessing this genotype had a 3.61 increase in odds of clearing infection. The rs951840747 is located on intron 6 of the *CYP1A2* gene and has not been previously reported in the literature in relation to drug metabolism. Conceivably, this functional effect may be a similar mechanism to *CYP1A2*1K*, also a *CYP1A2* intron SNP, whose presence results in decreased enzyme activity and increased drug concentrations; which for rs951840747 would result in sustained lethal schistosome levels and could contribute to an increased odds of clearing infection (Zimmermann et al., 2007). This is a particularly relevant finding as Nleya and colleagues have provided evidence in Zimbabwean volunteers that *CYP1A2* is the predominant excretion route of PZQ to its main metabolite (Nleya et al., 2019). Therefore, although an individual with this SNP had a significantly increased odds of clearing infection, further pharmacokinetic evaluation is required to confirm if this intron variant contributes towards decreased metabolism of PZQ *via CYP1A2*, preventing the removal of the active parent drug from circulation to its main metabolite and increasing success and efficacy of PZQ treatment.

Decreased metabolism of PZQ may also be representative of the functional effect of the rs1039108105 and rs6976017 variants in the *CYP3A5* gene, which both significantly increased the odds of an individual clearing infection and thus the efficacy of PZQ. *CYP3A4/5* also mediates the biotransformation of PZQ (Wang et al., 2014), so any detrimental alterations to its function can impair metabolism, maintaining exposure of the active drug to the schistosomes and increasing parasite death. There was no literature regarding rs1039108105, but the rs6976017 variant has been sparsely reported. One study significantly associates the SNP with warfarin sensitivity during efficacy studies in a European population (Jorgensen et al., 2009). The pharmacogenetic mechanism described derives from diminished clearance of warfarin, creating increased concentrations than expected from the normal dose. A parallel effect may be occurring with rs6976017 in this study, as there were significantly higher odds of an individual possessing this SNP clearing infection potentially due to increased PZQ concentrations *via* decreased metabolism of *CYP3A5*.

However, the rs28371702 variant of the *CYP2D6* gene had a different affinity to treatment outcome than the other SNPs significantly associated with PZQ efficacy. The homozygous (CC) genotype had over double the odds of an individual with this genotype not clearing infection, whereas the heterozygous (AC) genotype increased the odds of clearing infection by 2.6 times. rs28371702 has previously been reported by to have different *CYP2D6* phenotypes for each genotype, significantly effecting the pharmacokinetics of aripiprazole during a pharmacokinetic-pharmacogenetic study in healthy Chinese subjects (Zhang et al., 2021). However, that study reported the homozygous rs28371702 (CC) genotype was significantly associated with a decreased T_{max} and increased exposure to aripiprazole (indicative of decreased *CYP2D6* function), and the rs28371702 (AC) genotype decreased exposure of aripiprazole (indicative of an ultra-rapid *CYP2D6* function). This contradicts the findings of this study, in which rs28371702 (CC) had significantly more individuals not clearing infection, indicating decreased PZQ exposure and PZQ efficacy. Sustained exposure of the schistosomes to PZQ concentrations above 1 μM for at least 6 h is critical for parasite death the occur (Nleya et al., 2019) and the eventual success of a PZQ treatment, therefore unmaintained lethal PZQ concentrations due to increased *CYP2D6* metabolism will fail to successfully destroy the schistosome load and clear infection. Yet, as *CYP2D6* has a smaller role than other CYPs in the metabolism of PZQ (<10%), the combined influence of SNPs in other CYP pathways with a greater role in PZQ metabolism may be the reasoning behind the discrepancy. Consequently, greater research into the exact mechanism of this SNP is required, particularly with PZQ pharmacokinetic analysis. Overall, for individuals possessing any of the four genotypes that were significantly associated with increased odds of clearing schistosomiasis infection and consequently increased PZQ efficacy, the mechanism potentially stemmed from the decreased function of the *CYP1A2, CYP2D6*, or *CYP3A5* enzymes, permitting increased PZQ concentrations. Increased PZQ concentrations is commonly observed with other CYP polymorphisms, yet high plasma drug exposure may in fact increase the risk of adverse drug reactions (ADRs). PZQ has been known to cause treatment-related side effects including headaches, abdominal pain, and vomiting upon treatment for schistosomiasis (Midzi et al., 2008), and one study concluded that the PZQ-metabolising *CYP3A5*3/*6/*7* variants had a significantly higher number of ADRs than those with no defective alleles (Mnkugwe et al., 2021). However, this study found no association with the CYP3A5 alleles and parent PZQ concentrations, it was postulated that increased concentrations of a metabolite was responsible for these adverse events. Therefore, it must be noted that increased or reduced enzyme activity is not always desirable as it increases the risk of ADRs due to altered drug concentrations (Bijl et al., 2008). Thus, we further highlight the importance of characterising SNPs in the genes involved in

the metabolism of PZQ in Zimbabweans as alterations in PZQ concentrations may be amplifying the detrimental side effects of treatment.

Yet, drug metabolism and variations in drug efficacy can include multiple genes and numerous polymorphisms (Shastry, 2007), consequently assessing the combinative power of the SNPs was important to gain the complete view of a SNPs importance in predicting treatment success. LAMPLINK and haplotype analysis of SNP combinations towards PZQ efficacy yielded no results, so evaluations of the importance of the SNPs as predictors was conducted *via* a RF model. RF are a promising method of associating polygenic SNPs with an outcome (Botta et al., 2014), and by classifying these SNPs by importance it allows us to discover those most predictive of PZQ efficacy and support the univariate results. When comparing the independent statistical associations to the RF importance values, they were generally concordant with the univariate *p*-values regarding associations to PZQ efficacy, supporting both approaches. However, they did not agree perfectly. Overall, rs6976017 of *CYP3A5* was determined to be the most predictive SNP of PZQ efficacy. Although rs951840747 had the lowest *p*-value and highest odds of clearing infection, it was fifth in predicting PZQ efficacy when analysed as a combinative data set. Those SNPs not adhering to the univariate results were considered to be due to between-SNP interactions, as the RF model considers the combined effect of all the other SNPs in the data set. Although, predictive importance can be skewed by a high MAF, as was observed with rs1479820461 and Novel-23, which both have a MAF >90%. Higher MAF tends to be associated with higher SNP importance in other studies (Boulesteix et al., 2012), therefore these SNPs predictive importance was attributed to model preference of SNPs with higher MAF rather than actual predictive effect. In this regard, the second most important predictor for PZQ efficacy rs1022705765, an intron 6 variant in the *CYP1A2* gene, had a MAF of 16%, consequently its predictive importance may arise from a SNP interaction rather than a high MAF. rs951840747 is also an intron 6 variant in the *CYP1A2* gene and was significantly associated with PZQ efficacy. Nevertheless, no LD was detected with rs1022705765 (or with any other SNP pair), suggesting a different combinative effect between the two relating to PZQ efficacy. However, studies assessing the combinative effect of pharmacogenetic SNPs are rare in African populations, with no literature assessing SNP interactions during a PZQ treatment (Bienczak et al., 2016). Generally, mutations in the CYP genes introns and gene-flanking regions are drastically under-reported, yet altered functional activity due to intron mutations have been discovered in the *CYP1A2, CYP2C19*, *CYP2D6*, and *CYP3A5* genes, with approximately 15 alleles identified (Ingelman-Sundberg and Sim, 2010). *CYP3A5*3* in intron 3 (eighth most predictive SNP) results in an inactive *CYP3A5* protein in individuals who carry the homozygous genotype (*3/*3), something that three individuals in this study possessed (Kuehl et al., 2001). All these individuals did not clear infection, however there was no significance due to the small number of homozygous carriers. This predictive importance, in addition to the frequency of unsuccessfully treated homozygous carrier, suggests the SNPs functional implications on PZQ efficacy is larger than expected, but due to the small sample size its effect was not significant *via* statistical associations in this study. Thus, although there were multiple SNPs, novel and otherwise, in the RF model that were not found to be significantly associated to PZQ efficacy, the predictive power of the SNPs presented in this model shows promise in predicting treatment outcome with a 29% misclassification rate. A recent study evaluating the use of RF models on predicting a binary response for medical data sets between the sample sizes of 60–102 showed that the average misclassification rate of this study was within the expected range for human data (25–50%) as produced by Janitza and Hornung (2018). However, the true impact of the independent associations and the combinative effect of these SNPs must be completely evaluated with pharmacokinetic measures, particularly the novel findings, as the association with PZQ efficacy is currently only inferred.

As far as we know, this is the first investigation into the pharmacogenetics of PZQ efficacy in Zimbabwe and the second to be performed in Africa, with no previous studies evaluating the pharmacogenetic impact in relation to the success or failure of a schistosomiasis treatment. Therefore, SNPs detected in this study may influence only part of PZQ pharmacology, with PZQ efficacy potentially compounded by drug-drug interactions, overall health, and other environmental or nongenetic factors (Sorice et al., 2012). Additionally, study limitations such as the relatively small samples size and the uneven distribution of males to females (76: 38) prevents genetic variants found primarily in one sex being significantly detected, as some CYP genes present different expression patterns between genders (Franconi and Campesi, 2014). Likewise, 152 SNPs passed bioinformatic quality control, but due to the lack of power of low frequency SNPs in a small study population MAF <5% were removed from association analysis, as a disease association in one individual would not be possible to confirm. Consequentially, outcome-associated SNPs may be disregarded. Also, although we assessed the CYP2D6 pathway we did not assess deletions or multiplications of the gene, both of which could impact PZQ concentrations and resultant PZQ efficacy. Moreover, as this was a novel and exploratory analysis our study did not apply multiple testing correction in the statistical analyses which may increase the type II error. We applied a Yates correction to examine significant findings and reduce the risk of false positives, but as this continuity correction can be very stringent with smaller sample sizes we only used these results to further support our significant findings for future validation of these findings. Lastly, and the most prominent limitation, is the necessity for a pharmacokinetic-pharmacogenetic approach to gain stronger evidence of the SNP associations to PZQ efficacy. It was beyond the scope of this study to collect the time-course of samples required to assess the plasma levels of PZQ during of the anthelminthic treatment. Additionally, despite the valuable novel information generated and the practical issues of obtaining pharmacogenetic participant data, this study this was only based on a single cohort of predominantly young individuals. This is a limitation to a pharmacogenetic study and restricts the interpretations of the data produced by this analysis. Hence, this

study should be replicated in a second independent Zimbabwean cohort of increased sample size, age range and with the relevant *in vivo* data for further pharmacokinetic analysis to fully capture and inform in the genetic diversity of this country. It is important to stress that the aim of our analysis of these SNPs was not to confirm the metabolising phenotype for these individuals, but to identify if there may be any underlying genetic factors significantly contributing to variable drug efficacy to justify further investigation into the pharmacogenetics of PZQ treatment. The study achieved this aim and future mechanistic studies will be informative of the overall drug-gene interaction.

Overall, while implementing pharmacogenetics poses a massive financial burden on public health-care systems (Tata et al., 2020), eliminating schistosomiasis is reliant in our understanding of these determinants of drug efficacy. African populations possess various unique genetic markers vital to therapeutic drug responses, henceforth the applicability of pharmacogenomics during PZQ treatments will consequently reduce prevalence of treatment failures and ADRs. The SNPs identified in this study are an important source of potentially unique African pharmacogenetic markers, such as the *CYP2D6*17*, which could also be applied to multiple drug efficacy studies. The importance of African genetic data has already been highlighted in the near-personalised management of HIV patients in sub-Saharan Africa, with reduced doses of efavirenz given to patients with genetic variations in the *CYP2B6* gene (Matimba et al., 2016). Therefore, the contribution of studies such as this highlights the impact pharmacogenetics can have on the efficacy of a drug treatment, and further promotes precision public health to enrich data on the genomics of African populations. We believe the data obtained in this study can be applicable to multiple drug efficacy studies, and the Zimbabwean genomic data can contribute to the growing genetic map of the country. We are currently undertaking a more thorough investigation of the pharmacogenetics-pharmacokinetics of PZQ in Zimbabweans during a schistosomiasis treatment to gather more conclusive evidence and validate the causality of associations determined in this study.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number can be found below: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA826901.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Medical Research Council of Zimbabwe (Nos. MRCZ/A/2435 and MRCZ/A/933) and the University of Zimbabwe Institutional Review Board. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

FM conceived the idea and supervised the research. The collection of the blood samples and parasitology data was performed by FM and TM. GZ extracted, compiled, curated, and analysed the genetic data. GZ produced the figures and interpreted the data. FM acquired the funding for this study. GZ prepared the draft manuscript. All authors were involved in review, editing, and approval of the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2022.914372/full#supplementary-material

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4.4 Key findings and conclusions

This chapter comprises the first investigation into the PGx of PZQ efficacy in Zimbabwe, and the second PGx study on PZQ efficacy to be performed in Africa. Using a Zimbabwean population, this study successfully characterised multiple PGx variants that may influence the success or failure of a PZQ treatment. Seventy SNPs were detected across the *CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4,* and *CYP3A5* genes and their minor allele frequency (MAF) was compared to other known populations (**Paper 2: Table 1**). This comparison highlighted that approximately 87% of SNPs detected in this Zimbabwean population occurred at significantly different MAF compared to the expected MAF in a European population (**Paper 2: Section 3.1.1**). This is a key finding as drug efficacy studies are generally developed in healthy European volunteers (Tata *et al.*, 2020), therefore PGx evaluations may not automatically apply to a genetically diverse African population like Zimbabwe (Masimirembwa and Hasler, 1997). Therefore, the current PGx impact on PZQ efficacy may be vastly underestimated. Moreover, 37% of SNPs detected in this study were novel, further highlighting the need to conduct genomic studies in African populations for improving drug use on the continent. This is particularly relevant as 93% of global schistosome infections are found in Sub-Saharan Africa (Onasanya *et al.*, 2021), thus further work to characterise PGx in these populations during a PZQ treatment is required.

This chapter detected four SNPs that were significantly associated with PZQ efficacy (**Paper 2: Table 2**). Firstly, the rs951840747 variant in the *CYP1A2* gene, a SNP previously unreported concerning drug metabolism, was significantly associated with PZQ efficacy. This is an important discovery as a recent study in Zimbabwean volunteers showed that *CYP1A2* is the predominant route of PZQ metabolism to its main metabolite (Nleya *et al.*, 2019). Individuals possessing this SNP were nearly four times more likely to clear their

schistosome infections compared to those who did not possess the rs951840747 variant. This variant's functional effect is hypothesised to be a similar mechanism to *CYP1A2*1K*, a *CYP1A2* intron SNP like rs951840747, which results in decreased enzyme activity and increased drug concentrations (Al-Ahmad *et al.*, 2017). Hence, rs951840747 may act similarly towards the decreased metabolism of PZQ via *CYP1A2*, preventing PZQ metabolism and maintaining exposure of the active drug to the schistosomes, increasing the overall success and efficacy of PZQ treatment. This hypothesis of decreased CYP metabolism of PZQ was also applied to the other PGx findings in this study. Both the rs1039108105 and rs6976017 variants of the *CYP3A5* gene were also found to significantly increase the odds of an individual clearing infection and consequently were associated with increased efficacy of PZQ. Although there were no published studies regarding the rs1039108105 variant, the rs6976017 variant had been previously reported to be significantly associated with the increased warfarin concentrations in a European population (Jorgensen *et al.*, 2009). Individuals possessing the rs6976017 variant were twice as likely to clear schistosome infection. It was concluded this was also potentially due to increased or sustained lethal PZQ concentrations consistent with decreased metabolism of *CYP3A5*, a mechanism which has been observed in other *CYP3A5* PGx variants (Kuehl *et al.*, 2001, Sanghavi *et al.*, 2017). However, the risks of ADRs cannot be ignored. If these variants, which increase the likelihood of clearing schistosome infection, result in increased systemic plasma concentrations of PZQ then further work to ensure ADRs are not a risk is required.

A further key finding was the rs28371702 variant detected in the *CYP2D6* gene, which had a significantly different affinity to PZQ treatment outcome depending on the genotype. The homozygous rs28371702 [CC] genotype was significantly associated with non-clearance of infection, whereas the heterozygous rs28371702 [AC] genotype was found to significantly increase the odds of an individual clearing infection. It was concluded that the increased

likelihood of an unsuccessful PZQ treatment with rs28371702 [CC] stemmed from decreased PZQ exposure and unmaintained lethal PZQ concentrations, therefore the individual possessing this genotype was unable to clear schistosome infection. Other PGx variants have been shown to increase *CYP2D6* activity, decreasing drug exposure (Wang *et al.*, 2014a), thus it is feasible that this variant may be following a similar mechanism. Moreover, the rs28371702 variant has previously been reported to have different *CYP2D6* phenotypes for each genotype, significantly impacting the PK of the drug aripiprazole (Zhang *et al.*, 2021). However, the Zhang *et al.* study contradicted the findings of this chapter, with the rs28371702 [AC] genotype associated with decreased aripiprazole exposure. The current study and the aripiprazole study both had low sample sizes (n=114 and n=140), therefore further research on the function of this CYP variant, its genotype, and its implications on drug exposure is required. Thus, based on the results of this study, patients with PGx variations that were significantly associated with PZQ efficacy (cleared or not cleared infection) may require dose alterations to tailor PZQ treatment to their PGx needs. This in turn could ensure successful PZQ treatment and reduce the risk of ADRs.

As variations in drug efficacy can depend on numerous polymorphisms (Shastry, 2007), a RF model was developed to assess the combined effect of the different SNPs and their importance in predicting PZQ efficacy (**Paper 2: Section 3.3.2**). When compared to the univariate analyses described above, the RF importance values were generally concordant regarding their association with PZQ efficacy. All four SNPs significantly associated with PZQ efficacy were in the top five most predictive of PZQ treatment outcome (cleared or not cleared schistosome infection) using the RF model. Overall, these results indicated the RF model showed promise as a prediction tool for PZQ treatment outcome, and hence PZQ efficacy, based on all the SNPs detected in an individual.

The significant associations between PGx variants and PZQ efficacy detected in this study suggest there is an underestimated PGx influence during a PZQ treatment for schistosomiasis. Therefore, the application of PGx could potentially improve PZQ efficacy in endemic areas that require PZQ, such as in Africa. However, for this to occur, genomic technologies must become more commonplace to bridge the equity gap between richer and poorer countries (Broder *et al.*, 2002), thus utilising PGx to treat a broad patient population may be unfeasible at this time (WHO, 2002a). Yet, PGx research will not only benefit the patient with more precise medication but can prevent the waste of healthcare resources due to ineffective treatment doses, and reduce the costs and incidences of ADRs (Radouani et al., 2020). Additionally, PGx can determine the variations that affect drug efficacy as a whole, not just for PZQ, therefore it may ultimately play an important role in optimising the use of drugs to combat other neglected tropical diseases (NTDs) and infectious diseases of the developing world (Pang, 2003).

To conclude, this chapter addressed **Thesis Aim 2** (**Section 1.11**) and provided the first analysis of the PGx determinants of PZQ efficacy in Zimbabwe. From the results of this study, it was clear that PGx could be a key contributing factor to variable PZQ efficacy. The detection of variants significantly associated with PZQ efficacy exemplifies the importance of conducting PGx studies on PZQ, particularly in African populations where the drug is commonly used. This investigation provides a vital insight into the PGx reasons behind the successes and failures of a PZQ treatment and presented data that, not only applies to schistosomiasis research, but multiple drug efficacy studies. Yet, the findings from this study are limited as additional evaluations with *in vivo* measures must be conducted. Overall, this chapter filled the paucity of knowledge on the PGx variations that impact the efficacy of PZQ treatment and enriched the genomic data of a relatively uncharacterised African population that applies to multiple drug efficacy studies.

Chapter 5 - Pharmacokinetic-pharmacodynamic-pharmacogenetic analysis of praziquantel treatment in a *Schistosoma haematobium* exposed Zimbabwean population

5.1 Introduction

The control of schistosomiasis relies on the mass drug administration (MDA) of the anthelminthic praziquantel (PZQ) (WHO, 2002b) (**Section 1.6**). Despite this intervention, variable PZQ efficacy in the treatment of *S. haematobium* infections has caused concern about the continuing effectiveness of PZQ (Tchuenté *et al.*, 2004, Keiser *et al.*, 2014) (**Section 1.7.2**). Multiple studies have attributed variable PZQ efficacy to variations in the host rather than due to decreased PZQ sensitivity or resistance in the schistosomes (Midzi *et al.*, 2008, Zwang and Olliaro, 2014, Ross *et al.*, 2015). Consequently, there is a need to evaluate the host factors that cause variable PZQ; a point that has been highlighted by multiple studies (Zdesenko and Mutapi, 2020, Fukushige *et al.*, 2021).

Host drug efficacy relies on an understanding of pharmacokinetics (PK), pharmacodynamics (PD), and pharmacogenetics (PGx) (Lam, 2016). PK refers to the processes the body has on the drug, such as host drug metabolism, and PD refers to the drug's effect on the body, such as drug treatment outcomes (Negus and Banks, 2018). To maximise drug efficacy and minimize potential adverse drug reactions (ADRs), the relationship between drug concentration to drug effect is assessed using pharmacokinetic-pharmacodynamic (PKPD) analysis (Derendorf and Meibohm, 1999). Concerning PZQ efficacy during treatment for schistosomiasis, PKPD analysis would evaluate the concentrations of PZQ and its

metabolites to whether an individual cleared schistosome infection, as investigated in previously published studies (Bustinduy *et al.*, 2016, Meister *et al.*, 2016a).

PGx variations in the cytochrome P450 (CYP) enzymes (**Section 1. 10.2**) can alter the concentrations of a drug and its metabolites and can shift the balance between PK and PD to influence drug efficacy or contribute towards ADRs (Maganda *et al.*, 2016). For instance, PGx variants that increase drug metabolism can decrease drug efficacy by decreasing active parent concentrations in systemic circulation and increasing metabolite concentrations (Negus and Banks, 2018). Thus, the inclusion of PGx studies in PKPD analyses could give a more representative picture of host influences on PZQ efficacy. Presently, there is a lack of characterisation of PGx impact on PZQ treatment (Mduluza and Mutapi, 2017, Zdesenko and Mutapi, 2020, Fukushige *et al.*, 2021), despite multiple studies in schistosome-infected patients citing PGx factors as a primary cause of variable PZQ efficacy (Ofori-Adjei *et al.*, 1988, Mandour *et al.*, 1990, Bustinduy *et al.*, 2016, Kovac *et al.*, 2018a).

So far, only one pharmacokinetic-pharmacodynamic-pharmacogenetic (PK-PD-PGx) study has investigated the impact of PGx variations on PZQ concentrations and PZQ efficacy in *S. mansoni* patients (Mnkugwe *et al.*, 2021a). To the best of my knowledge, no study has investigated the effect of PGx variations in *S. haematobium*-infected individuals using *in vivo* concentrations of PZQ and its metabolites, and none have related these findings to PZQ efficacy. Additionally, based on the results of **Chapter 3**, no study has catalogued PZQ's metabolites in humans and related these to PGx variants beyond the major metabolite, 4-OH-PZQ (trans/cis) (Zdesenko and Mutapi, 2020). Understanding the relationship between the PK-PD-PGx could provide insight into whether altered concentrations of PZQ and its metabolites (PK measure) due to PGx variants (PGx) are influencing PZQ efficacy (PD outcome). This chapter aims to investigate the PK-PD-PGx relationship between PZQ and its metabolites in a schistosome-infected population using a fieldwork-applicable microsampling

technique to further characterise the drug-gene interactions during a PZQ treatment for schistosomiasis.

5.2 Methods

5.2.1 Aims and objectives

I. Identify PZQ and its metabolites in a *S. haematobium*-exposed Zimbabwean population using microsampling.

II. Characterise the PK profile of PZQ and its metabolites in a *S. haematobium*-exposed Zimbabwean population using individual and pooled sampling methods.

III. Relate the *in vivo* concentrations of PZQ and its metabolites to PZQ efficacy using population PKPD analysis.

IV. Compare the frequency of the PGx variations in the PZQ-metabolising CYP enzymes in a *S. haematobium*-exposed Zimbabwean population with the frequencies of European and other African studies using genetic databases.

V. Relate CYP genotype to the *in vivo* concentrations of PZQ and its metabolites, as well as to PZQ efficacy using PK-PD-PGx analyses.

5.2.2 Ethical approval

Ethical approval was sought and granted by the Medical Research Council of Zimbabwe (MRCZ/A/2435) and the University of Zimbabwe Institutional Review Board. The Provincial Medical Director provided permission for the research to be conducted in the required province of Zimbabwe, and local permission was obtained from community leaders and representatives. The aims and procedures of the study were explained to each participant or their parent/guardian in their local language, Shona, before enrolment. Written consent was then obtained upon enrolment from the participant or their parent/guardian. As study involvement was voluntary, the participant was free to withdraw from the study at any time.

5.2.3 Study population and inclusion criteria

This study was conducted in November 2019, and participants were recruited from the Mupfure clinic in the Shamva district, one of the seven districts in the Mashonaland Central province of Zimbabwe (see **Chapter 2**). The study participants were selected using the following criteria: i) positive for *S. haematobium*, ii) treated with PZQ, iii) gave a baseline venous blood sample for PGx analysis, iv) provided dried blood spot (DBS) samples for the 0.5-hour, 1.5-hour, 2.5-hour, and 4-hour timepoints post-PZQ administration, and v) had a 3-week parasitological diagnosis to determine the efficacy of PZQ. The efficacy of PZQ treatment was the measurable PD outcome utilised in this study was the egg reduction rate (ERR), determined by the reduction in the mean number of eggs excreted in urine from pre-PZQ to post-PZQ treatment (**Equation III, Section 2.3.2.1**). At the 3-week efficacy check, the subjects were divided into i) negative for schistosome eggs and cleared infection (n=36, 100% ERR), indicative of a successful treatment, or ii) still positive for schistosome eggs and did not clear infection (n=2, <100% ERR), indicative of an unsuccessful treatment. Characteristics, such as age and sex, were confirmed from national ID cards for people aged sixteen and above and from birth certificates for children below this age. The demographics of the participants (n=38) included in this study are displayed below in **Table 5.1**.

Table 5.1: Demographics of the Zimbabwean study population.

Variable		N (%)
Age Category	*SAC (6-17 Years)*	28 (73.7)
	Adults (≥18 Years)	10 (26.3)
Sex	*Male*	24 (63.2)
	Female	14 (36.8)
Baseline *S. haematobium* Intensity	*Light (<50 eggs/10mL)*	31 (81.6)
	Heavy (≥50 eggs/10mL)	7 (18.4)
Infection Status at Efficacy Check	*Cleared (100% ERR)*	36 (94)
	Not Cleared (<100% ERR)	2 (5.3)

SAC: School-Aged Children, ERR: Egg Reduction Rate.

To ensure treatment compliance was not the cause of reduced PZQ efficacy, each participant was checked by a health worker to confirm they had swallowed the PZQ tablet. Multiple confounding factors have been shown to influence PZQ efficacy, including, sex, and initial infection intensity (Zwang and Olliaro, 2017). Therefore, each participant was matched on age category, sex, and initial infection intensity to remove known confounders of drug efficacy. The case-matching based on these criteria can be seen in **Supplementary Table 1 in Appendix 1.** For each aim of the study, the sampling procedure, including the number of participants and the samples utilised, is displayed in **Figure 5.1**.

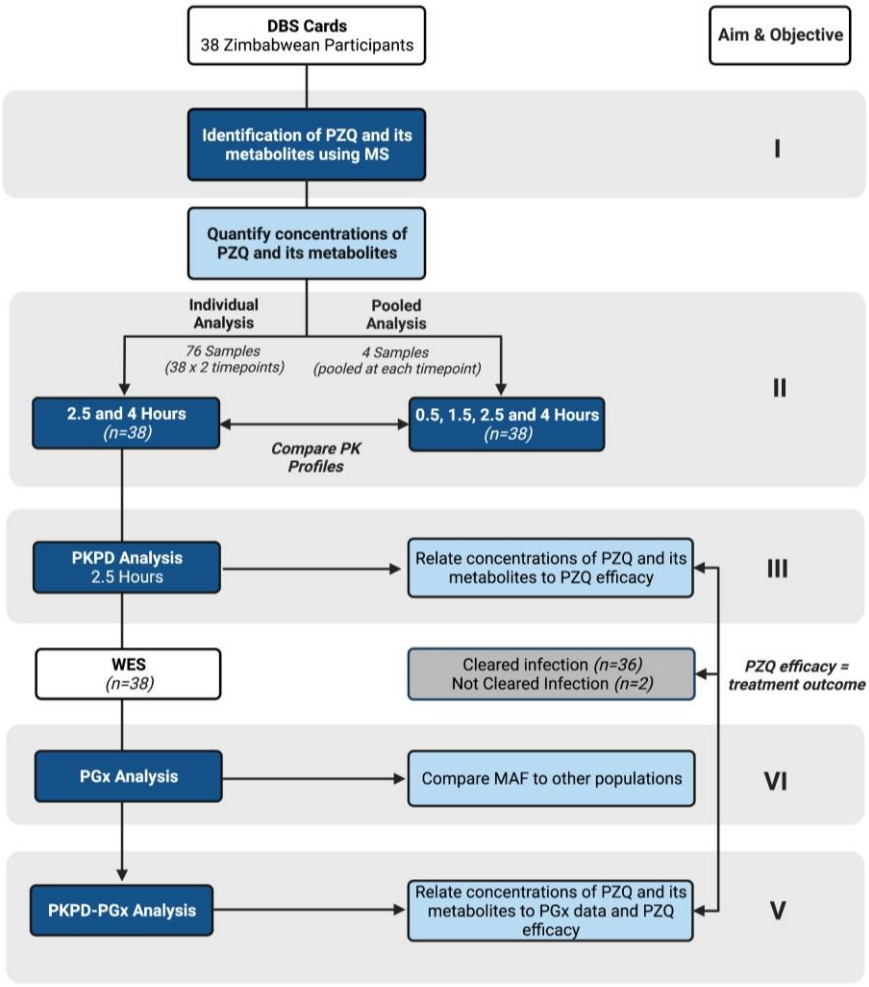


Figure 5.1: A description of the sampling procedure and the number of participants (*n*) for each aim of this study. This includes the key comparisons to be achieved with each aim. Acronyms; DBS: dried blood spots, MAF: minor allele frequency, PD: pharmacodynamics, PGx: pharmacogenetic PK: pharmacokinetic, PK-PD-PGx: pharmacokinetic-pharmacodynamic-pharmacogenetic, WES: whole exome sequencing.

5.2.4 Dried blood spots (DBS)

5.2.4.1 Collection of dried blood spots (DBS)

To obtain capillary blood (±0.1mL) for the DBS, a standard lancet was used to perform a finger prick at 0.5 hours, 1.5 hours, 2.5 hours, and 4 hours after treatment with PZQ. Five drops of blood at each timepoint were transferred onto the DBS card (Whatman™ 903

Protein Saver Card), dried for approximately 1 hour, and then stored in sealed plastic bags with desiccant. The DBS cards were transferred to a -80°C freezer at the University of Zimbabwe until shipped on dry ice to the University of Edinburgh for analysis. The DBS cards were then kept at -20°C on-site until the DBS extraction method was optimised. The general protocol for the collection of the DBS and the extraction method is described in **Chapter 2**.

5.2.4.2 Optimisation of extraction method

To determine PZQ and its metabolites could be detected using DBS, different extraction solvents were tested on Zimbabwean participants from the Mupfure clinic who were treated with PZQ for *S. haematobium* infection who did not possess a 3-week efficacy check (**Supplementary Table 2 in Appendix 1**). These additional samples were not included in the current study but were collected using the same protocol, therefore were valuable for method development to ensure good analyte recovery. Two extraction methods were tested to determine the best solvent conditions to recover PZQ and its metabolites from the DBS cards, as displayed in **Table 5.2.**

Table 5.2: The two extraction solvent conditions tested to determine the best extraction method to recover praziquantel (PZQ) and its metabolites from the dried blood spot (DBS) cards.

Extraction Method (EM)	Extraction Solvent (Ratio)
EMA	ACN:H_2O (4:1)
EMC	chloroform:MeOH:H_2O (1:3:1)

ACN: Acetonitrile, Extraction Method A (EMA), Extraction Method C (EMC), H_2O: Water, MeOH: Methanol.

Each DBS card was defrosted for 30 minutes before extraction. Three circular DBS (12.5mm in diameter) were punched from the DBS filter card for both the 0.5-hour and 4-hour

timepoints and were added to the separate Eppendorf's along with 600uL of either Extraction Method A (EMA) or EMC (**Table 5.2**). Three DBS spots were selected to allow for technical replicates of each timepoint to check the reproducibility between each spot. The samples were thermos-mixed for 5 minutes at 1200rpm and sonicated in an ultrasonic bath for 1 hour at room temperature. The samples were then centrifuged at 2250rpm for 10 minutes at 25ºC. The DBS extracts were then placed in separate autosampler vials for mass spectrometry (MS) analysis.

5.2.4.3 Extraction method selection

PZQ and a hydroxy metabolite (OH-PZQ) were detected, confirming at least one of the extraction methods had successfully extracted the drug and a metabolite from the DBS card. As seen in **Figure 5.2A**, EMA obtained the highest intensity of parent PZQ (active drug) at the 0.5-hour timepoint compared to EMC.

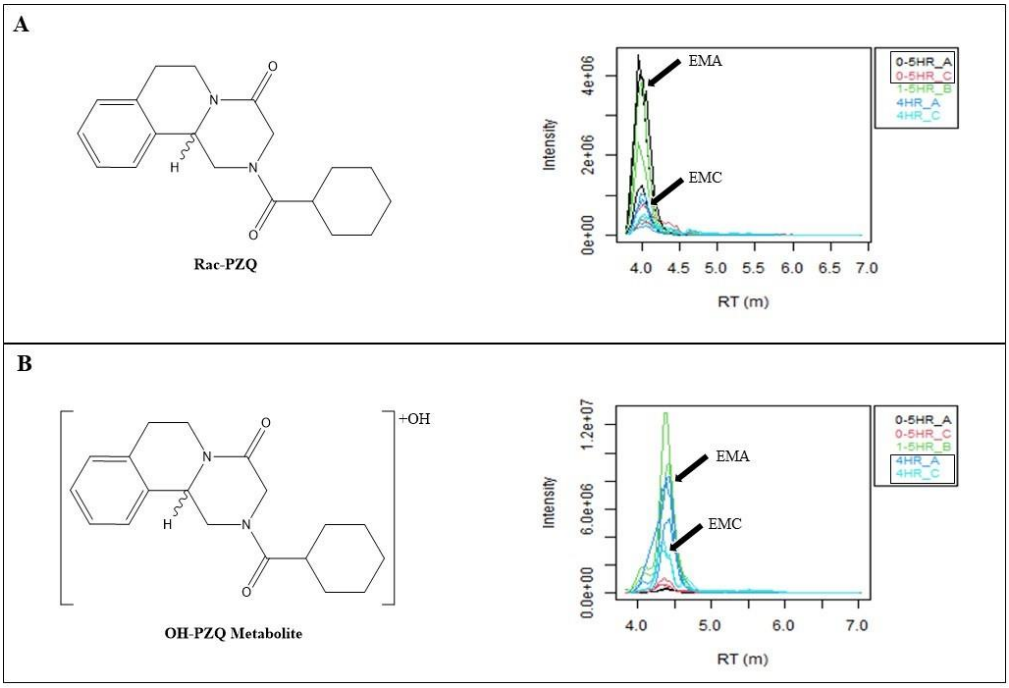


Figure 5.2: Preliminary analysis of the dried blood spot (DBS) extractions and the peak intensity of the response detected. [A] Detection of praziquantel (PZQ) at 0.5 hours using extraction method A (EMA) and extraction method C (EMC). [B] Detection of a mono-oxidised metabolite of PZQ at 4 hours using EMA and EMC. Peak colours key: black=0.5-hour timepoint sample using EMA, red=0.5-hour timepoint sample using EMC, dark blue=4-hour timepoint sample using EMA, light blue=4-hour timepoint sample using EMC, green=1.5-hour timepoint sample using extraction method of Glasgow Polyomics which was not further evaluated.

It was also important to be able to successfully detect a PZQ metabolite at the end of the 4-hour time course to assess the conversion of the active parent PZQ. **Figure 5.2B** shows that the 4-hour timepoint EMA had a higher intensity, hence implied higher recovery, than EMC. Overall, EMA was selected as the optimal extraction method and these solvent conditions were used for the sample preparation of the final Zimbabwean study population to recover PZQ and its metabolites from the DBS card.

5.2.4.4 Final DBS preparation and internal standard (IS) preparation

One circular DBS (12.5mm in diameter) collected at the 0.5-hour timepoint was punched from each participant's DBS filter card and was added into a separate Eppendorf's along with 600uL of ACN:H_2O (4:1) containing 600ng/mL of the internal standard (IS) d11-PZQ (Cayman Chemical). The samples were thermos-mixed for 5 minutes at 1200rpm and sonicated in an ultrasonic bath for 1 hour at room temperature. The samples were then centrifuged at 2250rpm for 10 minutes at 25ºC. The DBS extracts were then placed in separate autosampler vials for MS analysis. This process was repeated for the 1.5-hour, 2.5-hour and 4-hour timepoints, to produce individual samples for each participant. Following these extractions, 100µL of each of the 0.5-hour timepoints was pooled into one autosampler vial to allow for the pooled MS sampling of the entire Zimbabwean study population at this timepoint. This was repeated for the 1.5-hour, 2.5-hour and 4-hour timepoints using DBS in this study, as indicated in **Figure 5.1[II]**.

5.2.5 Pharmacokinetic (PK) methods

5.2.5.1 Analytical methods and quality control (QC)

5.2.5.1.1 Mass spectrometry (MS)

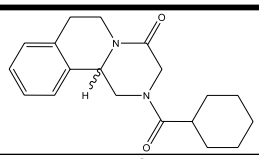
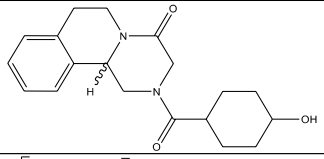
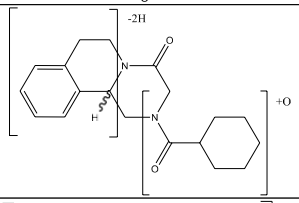
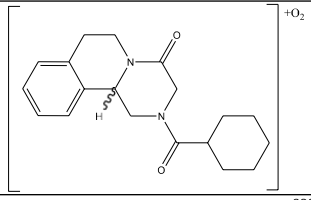
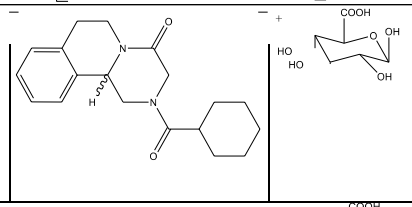
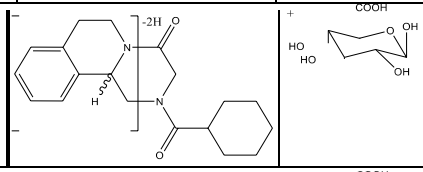
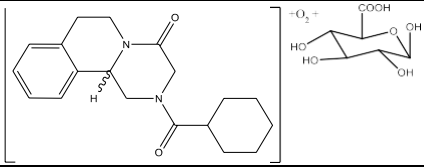
To separate and detect each analyte in this study, hydrophilic interaction liquid chromatography (HILIC) was performed on a Dionex UltiMate 3000 RSLC system (Thermo Fisher Scientific, Hemel Hempstead, United Kingdom) using a ZIC-pHILIC column (150mm × 4.6mm, 5µm column, Merck Sequant, Gillingham, United Kingdom). The column elutes the samples with a linear gradient (20mM ammonium carbonate in H_2O [A] and ACN [B]) over 26 minutes at a flow rate of 0.3mL/min as described in **Supplementary Table 3 in Appendix 1**. The column was maintained at 25°C, and 10µL of each sample was injected

into the LC-MS/MS system for analysis, with samples maintained at 5°C before injection. The general process of MS is described in **Chapter 2**. A Thermo Scientific Q Exactive Orbitrap MS was used for the MS analysis, running in positive/negative switching mode, with the specific setting listed in **Supplementary Table 4 in Appendix 1.** For positive mode ionisation: source voltage +3.8kV, S-Lens RF Level 30.00, S-Lens Voltage -25.00 (V), Skimmer Voltage -15.00 (V), Inject Flatopole Offset -8.00 (V), Bent Flatapole DC -6.00 (V). For negative mode ionisation: source voltage-3.8kV. The raw data was converted from a Thermo-specific file format to the open format mzXML. The unique signals were then extracted using the centwave algorithm and matched across biological replicates based on the mass-to-charge ratio (m/z) and retention time (RT). The grouped peaks were then filtered based on relative standard deviation and combined into a single file. The combined datasets were then filtered on signal-to-noise score, minimum intensity and minimum detections. The final peak set was then gap-filled and converted to text for use with IDEOM v18 (Creek *et al.*, 2012).

5.2.5.1.2 Analyte identification

The detection of PZQ and its metabolites using MS had already been performed (Wang *et al.*, 2014b, Meister *et al.*, 2016b), thus the relevant analyte identifiers were known. The analyte information is described in **Table 5.3** and was utilised to identify PZQ and its metabolites in this study.

Table 5.3: Analyte identification for praziquantel (PZQ) and its metabolites, in addition to the key fragments required to identify the PZQ metabolites. The data were obtained from published studies (Wang *et al.*, 2014b, Meister *et al.*, 2016b).

Analyte	Reported $[M+H]^+$	Exact Mass	Structure	Key Fragment Ions (m/z)	Published Information
PZQ	313.18	312.18		Parent	Well documented.
4-OH-PZQ	329.18	328.18		203 174 146 132	Major human metabolite. Has been detected as *trans*-4-OH-PZQ and *cis*-4-OH-PZQ for both of the (R)/(S)-PZQ enantiomers.
(-2H)-O-PZQ	327.173	326.173		144 130	Detected in the urine and faeces of mice.
O2-PZQ	345.182	344.182		219 201	Detected in the urine and faeces of mice.
O-PZQ-Glucuronide	505.218	504.218		395 329 219	Detected in the urine of mice.
(-2H)-O-PZQ-Glucuronide	503.204	502.204		393 327 217	Detected in the urine of mice
O2-PZQ-Glucuronide	521.215	520.215		393 345 327 217	Detected in the urine of mice.

Acronyms: 4-OH: 4-monohydroxy, (-2H)-O: dehydro-mono-oxidised, O2: di-oxidised, min: minute, m/z: mass-to-charge ratio.

Quality control (QC) of the detected MS peaks was conducted to ensure the capture of the complete peak area, with no overlapping or cut-off peaks. After QC, a calibration curve was plotted using the known standards across the d11-PZQ (IS) concentrations. The d11-PZQ standards were 0.1uM, 1uM, and 10uM, and from the average of a triplicate of each of these

standards, a line of best fit through the origin was plotted (**Supplementary Figure 1 in Appendix 1**). The equation of the line was then used to calculate the concentration (uM) in each sample. Using $y=x+c$, the concentration (uM) was equal to the peak area/6_{x10}^{8}. To ensure this equation had an acceptable accuracy, a comparison of the known d11-PZQ standards versus the concentration extracted from the peak area of MS was also conducted, as seen in **Supplementary Table 5 in Appendix 1.** The recovery of d11-PZQ from the DBS cards was deemed within an acceptable range (>100%). To allow comparisons to other PK studies on PZQ, the concentration of each analyte was converted into units of ng/mL.

5.2.5.2 Mass spectrometry (MS) sampling methods

The PK profiles were plotted as graphs of mean analyte concentration versus time. Two MS sampling methods were analysed to assess the PK of the Zimbabwean study population as described in aim (II) of this chapter. The MS sampling methods were compared to assess the feasibility of characterising the population PK using a limited number of pooled MS samples when resources are limited for PK studies (as discussed in **Section 2.5.2**). Previous evaluations of the PK of PZQ using DBS found the C_{max} (maximum observed concentration) was 2.5 hours, and decreased concentrations of PZQ were observed at the 4 hours, indicating PZQ metabolism (Lima *et al.*, 2009, Kovac *et al.*, 2018a). Thus, the individual MS sampling method only investigated each participant's DBS sample at the 2.5-hour and 4-hour timepoints due to limited costs for MS sampling. This assumed that the C_{max} had not been obtained before 2.5 hours. The PK profile of each analyte using individual MS sampling method was plotted from 0-hour, 2.5-hour and 4-hour data using the 'loess' function in the R environment v3.6.1, and the Data Thief III software then extrapolated the 0.5-hour and 1.5-hour timepoints for each individual for further analysis. The PK profile of each analyte using

the pooled MS sampling method was plotted from the pooled 0.5-hour, 1.5-hour, 2.5-hour and 4-hour timepoints of all the participants.

5.2.5.3 Noncompartmental analysis (NCA)

Noncompartmental analysis (NCA) obtained the PK parameters of each analyte to evaluate the relationship between drug exposure and efficacy. NCA calculated the $C_{max,}$ T_{max} (time of maximum observed concentration) and AUC (area-under-the-curve) parameters of racemic PZQ and its metabolites. The AUC is also commonly used to assess exposure to the drug and will hence be referred to as such throughout this study (Scheff *et al.*, 2011). Specifically, in this study, the $AUC_{(0\text{-last})}$ was reported. This measures the AUC from dosing to the time of the last measured concentration, which is calculated using the linear-up log-down method. This calculates the $AUC_{(0\text{-}4)}$ from the initial time of dosing (t=0 hours) to the time of the last sampling timepoint (t=4 hours). The linear trapezoidal rule was used for the ascending part of the PK curve, while the log trapezoidal rule was used for the descending part of the PK curve (Teuscher, 2011). NCA was conducted using the *NonCompart* and *ncar* packages within the R environment v3.6.1. The NCA results obtained using these packages were comparable to the commercial software WinNonlin® (Kim *et al.*, 2018).

5.2.6 Pharmacogenetic (PGx) methods

5.2.6.1 DNA extraction and sequencing

Genomic DNA was extracted from each participant's whole blood sample using QIAamp DNA MicroKit (Qiagen, GmbH, Germany), according to the manufacturer's protocol described in **Section 2.4.1.1**. The DNA samples were shipped on dry ice for library preparation and whole exome sequencing to BGI (Beijing Genomics Institute, Shenzhen, China). After the quantification of the DNA samples, the integrity and purity of DNA were

assessed by a 1% agarose gel electrophoresis. Qualified DNA was then randomly fragmented and end repair of DNA fragments was performed with an “A” base added to the 3’-end of each strand. Adapters were then ligated to both ends of the end-repaired/dA-tailed DNA fragments. The size-selected DNA fragments were hybridised to an exome array for enrichment. Non-hybridised fragments which did not adhere to the array were then washed out, and captured fragments were amplified. Each captured library was loaded on the Illumina Hiseq 4000 platform (Illumina, San Diego, USA) and the raw image files were processed by DNBseq base calling software with default parameters. The sequence data of each individual was generated as paired-end reads and stored in FASTQ format. The FASTQ files were deposited on the Sequence Read Archive (SRA) of NCBI, with an acquisition number of PRJNA897802.

5.2.6.2 Bioinformatic pipeline

Details on the general process of a bioinformatic pipeline can be seen in **Chapter 2**. Here, the raw reads were initially filtered by BGI, removing the adaptor sequences, contamination, and low-quality reads from the raw reads. If the sequence read i) matched ≥25% of the adapter sequence ii) had bases with a quality value ≤10 that accounted for ≥50% of the entire read, or iii) the N content accounted for ≥0.1%, the entire read was deleted. To filter for clean reads, the Phred threshold score was also set at ≥33. After this, the quality assessed the raw FASTQ format sequences from each sample were further processed using FASTQC v0.11.9 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). The output of the FASTQC analysis showed that the raw FASTQ sequences were of good quality, and the number of read pairs generated per sample ranged from 20,315,195 to 27,956,273 in the sequencing run. Subsequently, the reads had an output quality Phred threshold score of >33 and a read length of 150 bp.

The raw FASTQ sequences were then mapped against the reference FASTA file using Burrows-Wheeler Aligner (BWA-mem) (Li and Durbin, 2009). The FASTA file is available in **Supplementary Data 1 in Appendix 1** and aligns with the variant using the 200 bases on either side of the variant's chromosomal location based on the NCBI RefSeqGene database (https://www.ncbi.nlm.nih.gov/refseq/rsg/). The alignment contained the seventy CYP variants detected in **Chapter 4**, including the novel variants. This targeted PGx variants in the *CYP1A2*, *CYP2C9*, *CYP2C19*, *CYP2D6*, *CYP3A4*, and *CYP3A5* enzymes involved in the metabolism of PZQ (Masimirembwa, 2013, Wang *et al.*, 2014b, Bonate *et al.*, 2018, Kapungu *et al.*, 2020).

The alignment to the raw reads produced individual SAM files, which were then transformed into a BAM file using samtools (Li *et al.*, 2009). These BAM files were then sorted and indexed and merged for joint variant calling. Variant calling was performed using varFilter (vcfutils.pl) and bcftools (Li, 2011) to remove low-quality calls. The joint-variant calling resulted in a VCF file containing the CYP variants, and more specifically, the genotype data of each participant. Coverage of each CYP variant was determined to be >14X, and each variant achieved complete genotyping. Coverage within this range has been previously shown to call variants with high accuracy (Song *et al.*, 2016).

5.2.6.3 Variant identification and analysis

SNPs and INDELS were evaluated. Each variant was assigned an ID and designated as either: i) reported CYP allele, ii) reported rs-code, or iii) a novel ID from **Chapter 4**, according to the human genome assembly GRCh38. The official nomenclature used to identify the CYP alleles was The Human Cytochrome P450 Allele Nomenclature Database (PharmVar) (Gaedigk *et al.*) and SNPedia (Cariaso and Lennon, 2012), with the remaining variants designated with an rs-number or a novel ID (from **Chapter 4**) using dbSNP (Sherry

et al., 2001), Gnomad (Karczewski *et al.*, 2020) and Ensembl (Hubbard *et al.*, 2007). The final VCF file was then assigned the corresponding meta-data (e.g. age) for analysis.

Linkage disequilibrium (LD) and Hardy–Weinberg equilibrium (HWE) between pairs of SNPs on the same chromosome were assessed using the Haploview software (http://broad.mit.edu/mpg/haploview, Version 4.1) (Barrett *et al.*, 2005), with further information on the necessity of these calculations described in **Chapter 2**. The strength of LD was designated based on the criteria in **Supplementary Table 6 in Appendix 1**. The HWE statistic was calculated using an exact test (Wigginton *et al.*, 2005, Meyer, 2020). Due to the low sample size and preliminary nature of this study, no variants were excluded based on HWE to allow for exploratory analysis. To assess non-random associations between SNPs in LD, the presence of haplotypes was also assessed using the Haploview software (http://broad.mit.edu/mpg/haploview, Version 4.1) (Barrett *et al.*, 2005), with blocks indicating where there was statistical evidence of strong co-inheritance. Haplotype blocks were defined using the Gabriel method (upper 95% CI of the D' value is ≥ 0.98, and the lower 95% CI is ≥ 0.7; MAF, >5%) (Gabriel *et al.*, 2002). LAMPLINK software was used to detect statistically significant combinatorial interactions between ≥2 SNPs using the procedure set out by Terada *et al.* (Terada *et al.*, 2016).

5.2.6.4 Prediction models

The nucleotide changes and the amino acid changes of each SNP were obtained using Mutalyzer (Wildeman *et al.*, 2008) and the Ensembl Variant Effect Predictor (Hubbard *et al.*, 2007), respectively. The predicted effect on protein function of missense variants was obtained using the SIFT tool (Ng and Henikoff, 2003). To account for non-coding variations, the GWAVA (Ritchie *et al.*, 2014) scoring tool was also used to predict the functional impact of the variant. To assess whether the detected CYP variants were predictive of PZQ efficacy,

a random forest (RF) model was built. Further information on the use of the RF model in predicting an outcome and how to build a RF is described in **Section 2.4.5.2**. This followed the RF model developed in **Chapter 4** using the R package *randomForest*. For the current study, the dominant genetic model (*a/a vs A/a+A/A*) (**Section 2.4.4.1**) was used to classify the genotypes in the RF to assess whether the presence of at least one minor allele was associated with an individual clearing or not clearing infection (Goldstein *et al.*, 2011). This allowed for a higher-powered model compared to the genotypic model (*a/a vs A/a vs A/A*).

5.2.7 Statistical analysis and data visualisation

Univariate association of CYP genotype to PZQ efficacy was performed using PLINK (Purcell *et al.*, 2007) to visualize and interpret results, using $\chi 2$ or fisher's exact tests to assess each genetic model. Each variant's minor allele *a* and major allele *A* were represented as a contingency table of PZQ treatment outcome by either dominant model count (*a/a, [A/a + A/A]*) or genotype count (*aa, Aa, AA*), according to genetic models described in **Section 2.4.4.1** (Clarke *et al.*, 2011). As defective CYP genotypes tend to occur at lower MAF, participants with heterozygous or homozygous alternate genotypes are generally fewer than the reference genotype. The dominant genotypic model was utilised to increase power, with the *aA+AA* genotypes grouped to investigate the impact of the CYP genotype on the concentration of PZQ and its three detected metabolites. The remaining statistical analyses were computed using IBM SPSS Statistics v.27 (https://www.ibm.com/uk-en/analytics/spss-statistics-software) unless otherwise stated. Fisher's Exact Tests were used to assess significant differences between the MAF of the CYP variant against three populations; the African and European populations as reported by 1000 Genomes Project (Auton *et al.*, 2015), and the Zimbabwean population based on a limited number of studies conducted (Masimirembwa *et al.*, 1995, Dandara *et al.*, 2001, Dandara *et al.*, 2004, Roy *et al.*, 2005, Matimba *et al.*, 2008,

Matimba *et al.*, 2009, Bains *et al.*, 2013, Muyambo *et al.*, 2022, Zdesenko *et al.*, 2022). The 1000 Genomes Project was selected as a comparison as it contained the most representative database of eastern African populations and was, therefore, the most relevant to this study. Only when no MAF was available via these databases, the ALFA Project was utilised (L. Phan and E. Moyer, 2020).

Descriptive statistics include the arithmetic mean ± standard deviation (SD), the median, the first quartile (Q1), Q3, and the interquartile range (IQR). Additionally, the metabolite ratio was calculated using the metabolite concentration/PZQ concentration to compare the relative change in exposure between parent and metabolite. The assessment of PGx changes to the concentration of PZQ and its metabolites, metabolite ratio and AUC with genotype was performed using a one-way analysis of variance (ANOVA). If required, Tukey multiple comparisons of means of the ANOVA results were performed to determine which genotype drove the significance of the ANOVA results. A binary logistic regression was also performed to determine if PZQ exposure (AUC) contributed to an individual clearing *S. haematobium* infection. Principal component analysis (PCA) was performed to test whether within-group dispersion of the concentration of parent PZQ and the three detected metabolites could be attributed to sample-related metadata or genotypic changes. All statistical significance was set at $P \leq 0.05$. Data visualization was processed using R packages within the R environment version 4.0.3 unless otherwise stated.

5.3 Results

5.3.1 Metabolite identification

Seven distinct analytes were identified, including the parent drug PZQ and six metabolite signals. The six metabolites were initially identified using their m/z and RT and were allocated a metabolic description based on previous PZQ metabolite studies (Wang *et al.*, 2014b). The metabolites identified are shown in **Table 5.4** below.

Table 5.4: The analytes detected using dried blood spots (DBS) collected from a *Schistosoma haematobium*-infected Zimbabwean population following a praziquantel (PZQ) treatment.

ID	Metabolic Description	Precursor Ion (m/z)	Retention Time (min)
PZQ	Parent	313.1930	3.71
4-OH-PZQ	Phase I Metabolite	329.1860	4.17
(-2H)-O-PZQ	Phase I Metabolite	327.1710	4.41
O2-PZQ	Phase I Metabolite	345.1820	4.46
O-PZQ-Glucuronide	Phase II Metabolite	505.2130	4.90
(-2H)-O-PZQ-Glucuronide	Phase II Metabolite	503.1800	5.41
O2-PZQ-Glucuronide	Phase II Metabolite	521.1900	6.60

Acronyms: 4-OH: 4-monohydroxy, (-2H)-O: dehydro-mono-oxidised, O2: di-oxidised, min: minute, m/z: mass-to-charge ratio.

The structure of each of the three phase I metabolites and their reported CYP pathways are displayed in **Figure 5.3**.

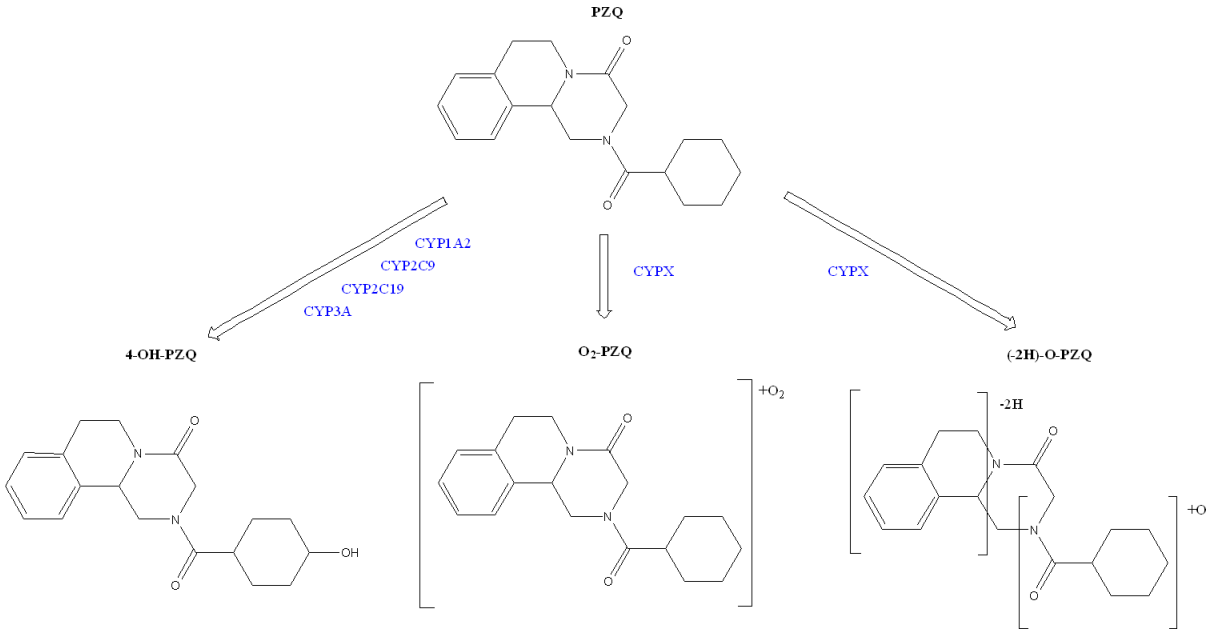


Figure 5.3: The structures of praziquantel (PZQ) and the three detected phase I metabolites, including any known routes of cytochrome P450 (CYP) metabolism (Wang *et al.*, 2014b, Nleya *et al.*, 2019, Kapungu *et al.*, 2020). CYPX represents an unknown pathway of metabolism. Acronyms: 4-OH: 4-monohydroxy, (-2H)-O: dehydro-mono-oxidised, O2: di-oxidised.

The metabolic pathways of the (-2H)-O-PZQ and O2-PZQ metabolites have not been reported in the literature. As this project primarily focuses on phase I drug metabolism (CYP metabolism), the glucuronide metabolites formed via phase II glucuronidation biotransformations were excluded from further analysis.

5.3.2 Population pharmacokinetic (PK) analysis

The quantification of the *in vivo* concentration of PZQ and the three detected metabolites was performed to characterise the PK profile of the Zimbabwean study population.

5.3.2.1 Comparison of mass spectrometry (MS) sampling methods

Two methods (see **Section 5.2.4.2** for further details) were utilised to determine whether the PK profile of this Zimbabwean population could be characterised using fewer pooled MS samples. The PK concentration-time profiles of PZQ and the three detected phase I metabolites in this population were compared in **Figure 5.4** below (PK data available in **Supplementary Table 7 in Appendix 1**), visualising the difference between the pooled MS sampling method and the individual MS sampling method.

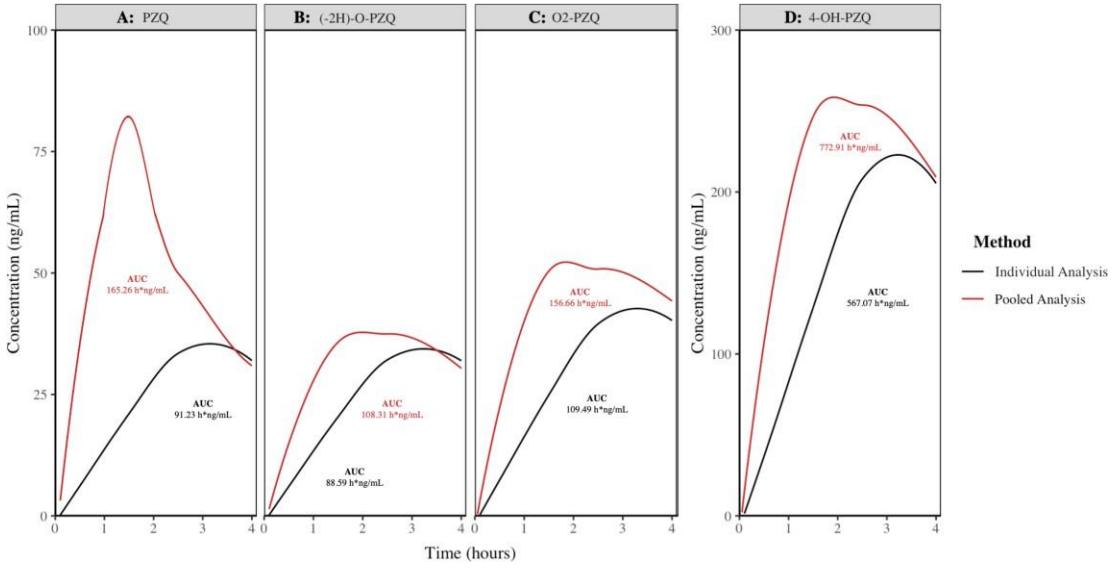


Figure 5.4: Comparison of the pharmacokinetic (PK) profile of the Zimbabwean study population between the pooled and individual analyses. The pooled analysis displays results from the pooled mass spectrometry (MS) samples for each timepoint. The individual analysis was calculated from the mean of each participant's 2.5-hour MS sample and 4-hour MS sample. These were obtained using dried blood spot (DBS) cards. Acronyms: PZQ: praziquantel, 4-OH: 4-monohydroxy, (-2H)-O: dehydro-mono-oxidised, O2: di-oxidised.

NCA investigated the differences in the resultant PK parameters between the pooled MS sampling method and the individual MS sampling method, as shown in **Supplementary**

Table 8 in Appendix 1. The exposure of the drug (AUC), as indicated in **Figure 5.4**, and the remaining NCA parameters were compared between each analyte and method. The C_{max} of PZQ, (-2H)-O-PZQ, O2-PZQ and 4-OH-PZQ using the pooled MS sampling method were 82.2, 38.9, 53.8 and 258.2 ng/mL respectively, at a T_{max} of 1.5-hours. In comparison, the C_{max} ± SD of PZQ, (-2H)-O-PZQ, O2-PZQ, and 4-OH-PZQ using the individual MS sampling method was 33.9 ± 21.1, 32.3 ± 14.8, 39.8 ± 17.5, and 210.1 ± 80.7, ng/mL, respectively, at a T_{max} of 2.5-hours. The AUC of PZQ, (-2H)-O-PZQ, O2-PZQ, and 4-OH-PZQ using the pooled MS sampling method were 165.3, 108.3, 156.7, and 772.9 ng/mL, respectively. In comparison, the mean AUC ± SD of PZQ, (-2H)-O-PZQ, O2-PZQ, and 4-OH-PZQ using the individual MS sampling method was lower at 91.2 ± 47.1, 88.6 ± 36.8, 109.5 ± 43, 567.1 ± 195.6 ng/mL, respectively.

Statistical differences between these two MS sampling methods for both the C_{max} and the AUC of each analyte could not be conducted due to the lack of replicates for the pooled MS samples at each timepoint, preventing the calculation of the SD. Hence, the comparison of these PK profiles was merely for descriptive purposes to compare if the PK profile obtained using pooled samples was comparable to analysing each participant. Thus, only the 2.5-hour and 4-hour timepoints analysed via both MS sampling methods were further analysed, comparing the differences in concentration of each analyte between the MS sampling methods. The concentrations of the pooled MS samples at both the 2.5-hour and 4-hour timepoint lie within the SD of the mean concentration of the individual MS samples, indicating the pooled method was a good representation of the Zimbabwean study population's PK at these timepoints. Hence, the pooled MS sampling method displayed a viable option for characterising the PK profile of a Zimbabwean population using a minimal number of samples, as the timepoints that were analysed by both methods showed similar concentrations of each analyte. Despite these advantages, because of a lack of individual

metadata for the PGx analysis when pooling the MS samples, the remaining experimental investigation of the *in vivo* concentrations of this chapter will only focus on the analysis of the individual MS samples.

5.3.2.2 Mean analyte concentrations of the study population

Using the individual MS sampling method, the C_{max} of the Zimbabwean study population was 2.5-hours. Thus, the 2.5-hour timepoint was selected to capture the PGx effects. The distribution of the concentration of PZQ and the three detected metabolites in the Zimbabwean study population at 2.5-hours is displayed in **Figure 5.5**.

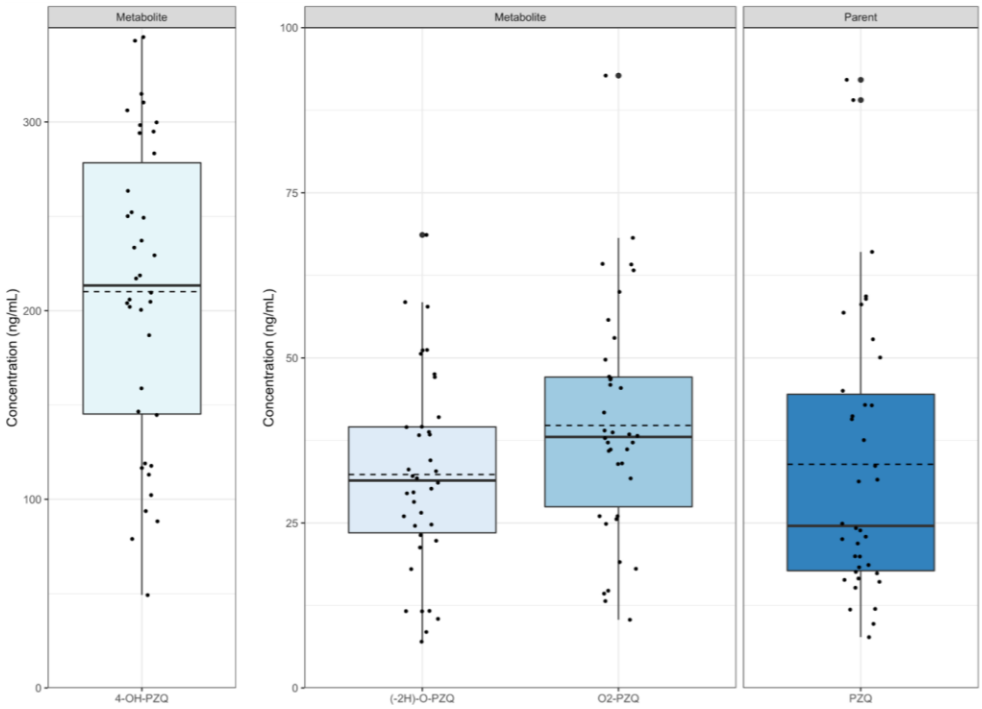


Figure 5.5: Boxplots of the distribution of each detected analyte in the Zimbabwean study population grouped by the parent praziquantel (PZQ) or its metabolites (4-OH-PZQ, (-2H)-O-PZQ, O2-PZQ). This represents the concentrations of each analyte using the 2.5-hour timepoint. The horizontal box lines represent the first quartile, the median, and the third quartile. Whiskers denote the range of points within the first quartile −1.5× the interquartile range and the third quartile +1.5× the interquartile range. The dots represent the individual sample concentrations or AUC. The dotted line represents the arithmetic mean of the sample population. Acronyms: 4-OH: 4-monohydroxy, (-2H)-O: dehydro-mono-oxidised, O2: di-oxidised.

As seen in **Figure 5.5**, the mean concentration at the C_{max} of the three PZQ metabolites in this Zimbabwean population deviated from the median, with a slight skew from the symmetrical distribution to the right for (-2H)-O-PZQ and O2-PZQ, and the left for 4-OH-PZQ. Regarding the mean concentration of parent PZQ, the data was skewed to the right with the mean higher than the median for the Zimbabwean population.

5.3.2.2.1 Effect of metadata on analyte concentration

Matching of the samples selected in this Zimbabwean study population is described in **Section 5.2.2** to remove known confounders of drug efficacy. PCA was performed to assess whether the age category, sex, or baseline infection intensity had any influence on the concentration of each analyte in each participant in this Zimbabwean study population. In the PCA, the concentration profiles did not appear to cluster separately based on the tested metadata, as seen in **Figure 5.6**.

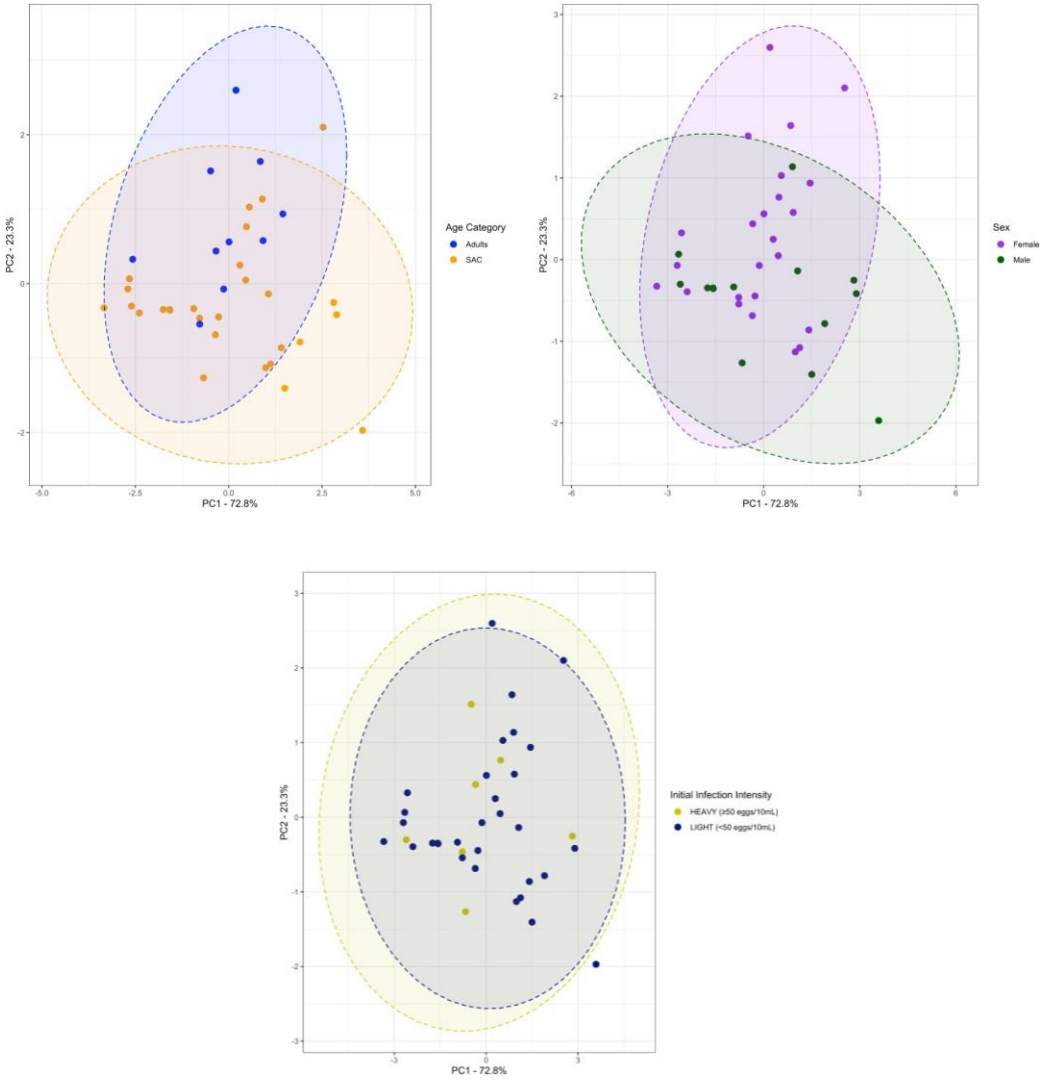


Figure 5.6: Principal component analysis (PCA) plots of the concentrations (ng/mL) of praziquantel (PZQ), 4-OH-PZQ, (-2H)-O-PZQ, and O2-PZQ across the samples,

annotated by age category, sex, and baseline infection intensity. PC1 and PC2 explained 96.1% of the total variation in the concentration of the components.

This analysis indicated that the concentrations of each analyte in each sample were similar to each other based on age category, sex, or baseline infection intensity, indicating that the control matching to limit known factors that influence drug response was successful.

5.3.3 Population pharmacokinetic-pharmacodynamic (PKPD) analysis

To compare the PK experimental data with PZQ efficacy (PKPD analysis), the infection status at three weeks was compared to the *in vivo* concentrations of PZQ, 4-OH-PZQ, (-2H)-O-PZQ, and O2-PZQ. The comparison of the mean concentration of PZQ and the three detected metabolites at each timepoint between the participants who cleared infection versus those who did not clear infection is presented in **Supplementary Table 9 in Appendix 1** and visualised in **Figure 5.7** below.

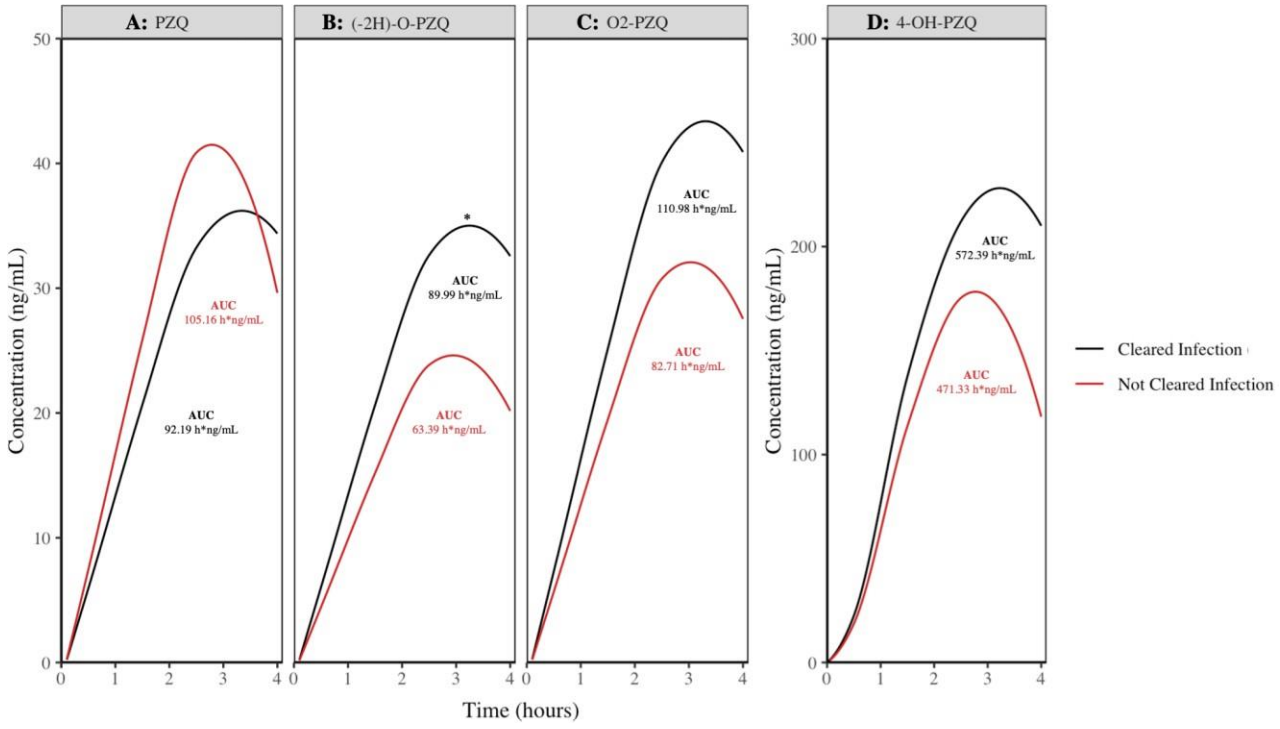


Figure 5.7: Comparison of the mean concentrations of the participants who cleared infection versus the participants who did not clear infection for [A] praziquantel (PZQ), [B] (-2H)-O-PZQ, [C]O2-PZQ, and [D] 4-OH-PZQ. The area-under-the-curve (AUC) values are presented under the relevant curves. * represents a significant difference ($P<0.05$) in concentration between the groups using one-way ANOVA. Acronyms: 4-OH: 4-monohydroxy, (-2H)-O: dehydro-mono-oxidised, O2: di-oxidised.

There were no significant differences between the two groups for the PZQ, 4-OH-PZQ and O2-PZQ mean concentrations. However, there was a significant difference in the concentration of the (-2H)-O-PZQ metabolite between the participants who cleared infection versus those who did not clear infection at 2.5-hours. Generally, the participants who had cleared infection had higher concentrations of the three metabolites, 4-OH-PZQ, (-2H)-O-PZQ and O2-PZQ, for both timepoints analysed by the individual MS sampling (2.5/4-hours). However, the participants who had not cleared infection had higher PZQ concentrations at 2.5-hours compared to those who cleared infection, but a lower PZQ concentration at 4-

hours. Thus, participants who did not clear infection did not sustain PZQ concentrations like participants who were confirmed to have cleared infection, with a decreased mean PZQ concentration of 29.5 ± 34.9 ng/mL compared to those who had cleared infection at 34.4 ± 20.6 ng/mL at 4-hours.

Additionally, there were no significant differences in the mean AUC between the two groups, and the NCA parameters are given in **Supplementary Table 10 in Appendix 1.** A binary logistic regression was also performed to further characterise PKPD associations, with no relationship between AUC and the probability of an individual clearing infection detected (P=0.587). PCA also indicated that the concentration profiles did not appear to cluster separately based on 3-week infection status as seen in **Supplementary Figure 2 in Appendix 1**, indicating that the concentrations of the samples were similar to each other regardless of 3-week infection status.

5.3.4 Pharmacogenetic (PGx) analysis

5.3.4.1 Detected CYP variants

5.3.4.1.1 Minor allele frequency (MAF)

There were eleven detected CYP variants in this Zimbabwean population, with the variant characteristics, including the MAF and the predicted and reported functional change of each variant on its respective CYP enzyme, presented in **Table 5.5**.

There were eight reported CYP alleles, two rs-number SNPs, and one novel SNP (identified in **Chapter 4**) detected, with the MAF ranging from 1.3% to 65.8%. There was no LD or haplotypes detected between the CYP variants (**Supplementary Table 11 in Appendix 1**).

Comparisons between the MAF of this Zimbabwean study population found that 18.2% of detected CYP variants had significantly higher MAF than expected based on current studies in African populations. Additionally, 36.4% were significantly different to other genetic studies in Zimbabwe. Novel-2, *CYP3A5*3*, *CYP1A2*1C* and *CYP2D6*17* were found at a significantly different MAF than other Zimbabwean populations, with a -34.9%, -67.1%, +19.3% and +28.2% change respectively. Importantly, 72.7% had significantly different MAF than European populations. Further, 36% of the detected CYP variants were predicted to have a MAF<1% in European populations but had MAF>10% in the Zimbabwean study population. The *CYP1A2*C, CYP2C9*9, CYP2D6*17*, *CYP3A5*6* and *CYP3A5*7*, all of which have been reported to decrease or inactivate their respective CYPs function, had a significantly higher MAF in this Zimbabwean study population than expected based on MAF in European populations.

Table 5.5: Characteristics and minor allele frequency (MAF) of the 11 cytochrome P450 (CYP) variants in the Zimbabwean participants. The single nucleotide polymorphisms (SNPs) and INDELs were identified, with the predicted and reported functional changes of each CYP described.

DNA Strand	Enzyme	Identifier	Allele	MAF (%)				Chromosome Position	Nucleotide Change	Amino Acid Change	SIFT/GWAVA Prediction	Reported Enzyme Activity	Variant Consequence	Region
				EXP	AFR	ZIM	EUR							
1	*CYP1A2*	rs2069514	*CYP1A2*1C*	48.68	31.32*	29.39*	1.99*	74745879	G>A	-	-/DEL	Decreased (Nakajima *et al.*, 1999)	Upstream Gene	-
		rs762551	*CYP1A2*1F*	50	56.2	57	67.99**	74749576	C>A	-	-/DEL	Increased (Lu *et al.*, 2020)	Intron	I1
		NOVEL_74753485	Novel-2	50	-	86.84**	-	74753485	G>C	-	-/TOL	-	Intron	I6
	CYP2C9	rs2256871	*CYP2C9*9*	14.47	8.17	13	0.1*	94949217	A>G	His251Arg	DEL/DEL	Uncertain (Allabi *et al.*, 2005, Mitchell *et al.*, 2011, Cariaso and Lennon, 2012)	Missense	E5
-1	*CYP2D6*	rs28371706	*CYP2D6*17*	50	21.80*	20*	0.2*	42129770	G>A	Thr107Ile	TOL/DEL	Decreased (Masimirembwa *et al.*, 1996)	Missense	E2
		rs3892097	*CYP2D6*4*	1.32	6.05	2	18.59**	42128945	C>T	-	-/DEL	Inactive (Zhou *et al.*, 2017)	Splice Acceptor	I2
		rs1135840	rs1135840	65.79	62.28	-	56.74	42126611	C>G	Ser486Thr	TOL/DEL	Increased (Allegra *et al.*, 2017)	Missense	E9
		rs16947	rs16947	31.58	33.98	-	31.82	42127941	G>A	Arg296Cys	TOL/DEL	Normal (Zhou *et al.*, 2017)	Missense	E6
	CYP3A5	rs776746	*CYP3A5*3*	10.53	18	77.6**	94.33**	99672916	T>C	-	-/DEL	Inactive (Kuehl *et al.*, 2001, Sanghavi *et al.*, 2017)	Intron	I3
		rs10264272	*CYP3A5*6*	21.05	15.43	22	0.3*	99665212	C>T	Lys208	-/DEL	Inactive (Kuehl *et al.*, 2001, Sanghavi *et al.*, 2017)	Synonymous	E7
		rs41303343	*CYP3A5*7*	11.84	11.8	19	0*	99652770	A>AA	Pro345ProXaa	-/-	Inactive (Lamba *et al.*, 2012)	Frameshift	E11

* and ** represents a significantly higher or lower difference in the minor allele frequency (MAF) in this Zimbabwean (ZIM) study population compared to the reported MAF in the African (AFR)/European (EUR) population in the 1000 Genomes Project/ALFA Project using Fisher's Exact Test. SIFT: Sorting Intolerant From Tolerant, GWAVA: Genome-Wide Annotation of Variants. DEL: Deleterious effect on protein function, TOL: Tolerated effect on protein function. The region is described by: Exon (E) and Intron (I). CYP: Cytochrome P450 enzyme.

5.3.4.1.2 Predicted functional consequences

As described in **Chapter 2**, alterations to the nucleotide sequence can impact the resultant protein translation, affect protein function, and therefore impact a drug's metabolism. Here, four missense, one synonymous, three intronic, one splice acceptor, one frameshift, and one upstream gene variant were detected, with each classification impacting the consequent DNA sequence differently.

Of the four missense variants, only *CYP2C9*9* (His251Arg) of the *CYP2C9* gene was predicted by SIFT to be a deleterious change, and therefore possibly damaging to the function of the *CYP2C9* enzyme and its metabolising capacity. The remaining three missense variants were predicted to be a tolerated amino acid change, with no alteration to protein function and metabolising capacity of the enzyme. These tolerated changes included the *CYP2D6*17* (Thr107Ile), rs1135840 (Ser486Thr), and the rs16947 (Arg296Cys) variants, in which SIFT predicted to have no impact on *CYP2D6* function. However, as the SIFT predictor tool is not always definitive of resultant protein function, for all four missense variants, GWAVA predicted they may be deleterious to their respective CYPs function.

One synonymous *CYP3A5*6* SNP was detected. It was reported to inactivate *CYP3A5's* function and was further predicted to be deleterious by GWAVA. From the remaining intronic, upstream gene and splice acceptor variants whose functional effect could not be predicted by SIFT, the GWAVA algorithm was used. GWAVA predicted that three SNPs possibly damaged their respective CYP function, the *CYP1A2*1C* and *CYP1A2*1F* of the *CYP1A2* gene, and *CYP3A5*3* of the *CYP3A5* gene, all of whose alterations to CYP function are already well described. Only Novel-2 was predicted to be a tolerated change by GWAVA. No computational predictions could be made for the frameshift *CYP3A5*7* variant, however it was reported in the literature to inactivate *CYP3A5* function.

5.3.5 Pharmacokinetic-pharmacodynamic-pharmacogenetic (PK-PD-PGx) analysis

The PGx variations were related to the *in vivo* concentrations obtained for each individual and analyte at the 2.5-hour timepoint.

5.3.5.1 Effect of CYP genotype on *in vivo* analyte concentration

To investigate whether the analyte concentrations of the Zimbabwean study population (**Section 5.3.2.2**) were skewed due to PGx changes, the *in vivo* concentration of PZQ and its metabolites were analysed with the corresponding PGx data. The NCA parameters were also obtained for PZQ and each metabolite, as well as the metabolic ratio between PZQ and its metabolites. Two genetic models were utilised to analyse the relationship between the *in vivo* analyte concentration and CYP genotype (see **Section 5.2.6**).

5.3.5.1.1 Dominant analysis

The mean concentrations of PZQ, 4-OH-PZQ, (-2H)-O-PZQ, and O2-PZQ between the different CYP genotypes is summarised in **Table 5.6**.

Table 5.6: Comparison of the mean concentrations (ng/mL) ± standard deviation (SD) of praziquantel (PZQ), 4-OH-PZQ, (-2H)-O-PZQ, and O2-PZQ and the metabolic ratio (metabolite/PZQ) between the detected CYP genotypes using one-way ANOVA. This analysis was performed using the dominant genotypic model.

				PZQ		4-OH-PZQ				(-2H)-O-PZQ				O2-PZQ			
Enzyme	**Variant**	**Genotype**	**N**	**Mean Concentration (ng/mL) ± SD**	**P-value**	**Mean Concentration (ng/mL) ± SD**	**P-value**	**Ratio ± SD**	**P-value**	**Mean Concentration (ng/mL) ± SD**	**P-value**	**Ratio ± SD**	**P-value**	**Mean Concentration (ng/mL) ± SD**	**P-value**	**Ratio ± SD**	**P-value**
CYP1A2	***CYP1A2*1C***	*GG*	1	58.1 ± -	0.25	306.3 ± -	0.232	5.4 ± -	0.506	39.6 ± -	0.627	0.7 ± -	0.473	45.4 ± -	0.747	0.8 ± -	0.434
		GA & AA	37	33.2 ± 21		207.5 ± 80.2		7.6 ± 3.4		32.1 ± 15		1.2 ± 0.7		39.6 ± 17.7		1.5 ± 0.9	
	CYP1A2*1F	*CC*	9	44.8 ± 24.1	0.074	208.4 ± 73.6	0.944	5.5 ± 2.8	**0.035***	28.8 ± 11.4	0.423	0.8 ± 0.4	**0.031***	33.9 ± 11.3	0.25	0.9 ± 0.6	**0.03***
		CA & AA	29	30.5 ± 19.3		210.6 ± 84		8.1 ± 3.3		33.4 ± 15.8		1.3 ± 0.7		41.6 ± 18.7		1.7 ± 1	
	Novel-2	*GC & CC*	38	33.9 ± 21.1	-	210.1 ± 80.7	-	7.5 ± 3.3	-	32.3 ± 14.8	-	1.2 ± 0.7	-	39.8 ± 17.5	-	1.5 ± 0.9	-
CYP2C9	***CYP2C9*9***	*AA*	29	33.8 ± 22.4	0.991	199.4 ± 81.4	0.098	7.1 ± 3.1	0.244	29.3 ± 12.9	**0.009***	1.1 ± 0.6	0.134	36 ± 14.1	**0.008***	1.4 ± 0.8	0.157
		AG & GG	9	34.1 ± 17.6		244.4 ± 71.9		8.6 ± 3.9		42.1 ± 17.2		1.5 ± 0.9		52 ± 22.2		1.9 ± 1.2	
CYP2D6	***CYP2D6*4***	*CC*	37	33.2 ± 21	0.25	207.5 ± 80.2	0.232	7.6 ± 3.4	0.506	32.1 ± 15	0.627	0.2 ± 0.7	0.473	39.6 ± 17.7	0.747	1.5 ± 0.9	0.434
		CT & TT	1	58.1 ± -		306.3 ± -		5.3 ± -		39.6 ± -		0.7 ± -		45.4 ± -		0.8 ± -	
	CYP2D6*17	*GA & AA*	38	33.9 ± 21.1	-	210.1 ± 80.7	-	7.5 ± 3.3	-	32.3 ± 14.8	-	1.2 ± 0.7	-	39.8 ± 17.5	-	1.5 ± 0.9	-
	rs1135840	*CG & GG*	38	33.9 ± 21.1	-	210.1 ± 80.7	-	7.5 ± 3.3	-	32.3 ± 14.8	-	1.2 ± 0.7	-	39.8 ± 17.5	-	1.5 ± 0.9	-
	rs16947	*GG*	14	36.9 ± 21.1	0.541	196.4 ± 69.5	0.56	7.4 ± 3.6	0.876	34.8 ± 17.2	0.332	1.3 ± 0.8	0.533	41.6 ± 16.1	0.659	1.7 ± 1.2	0.496
		GA & AA	24	32.5 ± 21		210.3 ± 71		7.6 ± 3.3		30.2 ± 11.7		1.1 ± 0.6		39.4 ± 13.9		1.4 ± 0.8	
CYP3A5	***CYP3A5*3***	*TT*	30	33.9 ± 21.6	0.992	211.1 ± 88.5	0.879	7.3 ± 3	0.39	33.3 ± 16	0.439	1.2 ± 0.7	0.778	40.7 ± 18.7	0.524	1.5 ± 1	0.964
		TC & CC	8	33.9 ± 20.7		206.2 ± 43.9		8.4 ± 4.6		28.6 ± 9.5		1.1 ± 0.6		36.2 ± 11.8		1.5 ± 1	
	CYP3A5*6	*CC*	22	30.6 ± 20.5	0.273	193.5 ± 77	0.139	7.6 ± 3.4	0.771	31.4 ± 15.3	0.662	1.3 ± 0.7	0.444	39.7 ± 18.7	0.965	1.7 ± 1	0.295
		CT & TT	16	38.3 ± 21.7		232.9 ± 82.5		7.3 ± 3.3		33.6 ± 14.7		1.1 ± 0.6		39.9 ± 16.1		1.3 ± 0.8	
	CYP3A5*7	*A*	29	34.7 ± 22.2	0.668	208.2 ± 81.3	0.795	7.3 ± 3.3	0.474	32.4 ± 15.1	0.926	1.2 ± 0.7	0.872	40 ± 18	0.898	1.5 ± 1	0.917
		AA	9	31.2 ± 18.1		216.3 ± 83		8.2 ± 3.7		31.9 ± 14.8		1.2 ± 0.7		39.1 ± 16.5		1.6 ± 0.9	

CYP: cytochrome P450, ANOVA: analysis of variance. Bold indicates $P \leq 0.05$.

The PK profile of each CYP variant discussed in this section is visualised in **Supplementary Figure 3 in Appendix 1**. Only three variants analyte concentrations varied with CYP genotype; the *CYP1A2*1C, CYP1A2*1F* and *CYP2C9*9* alleles. The frequency of these variants by genotype in the Zimbabwean study population is presented in **Figure 5.8**.

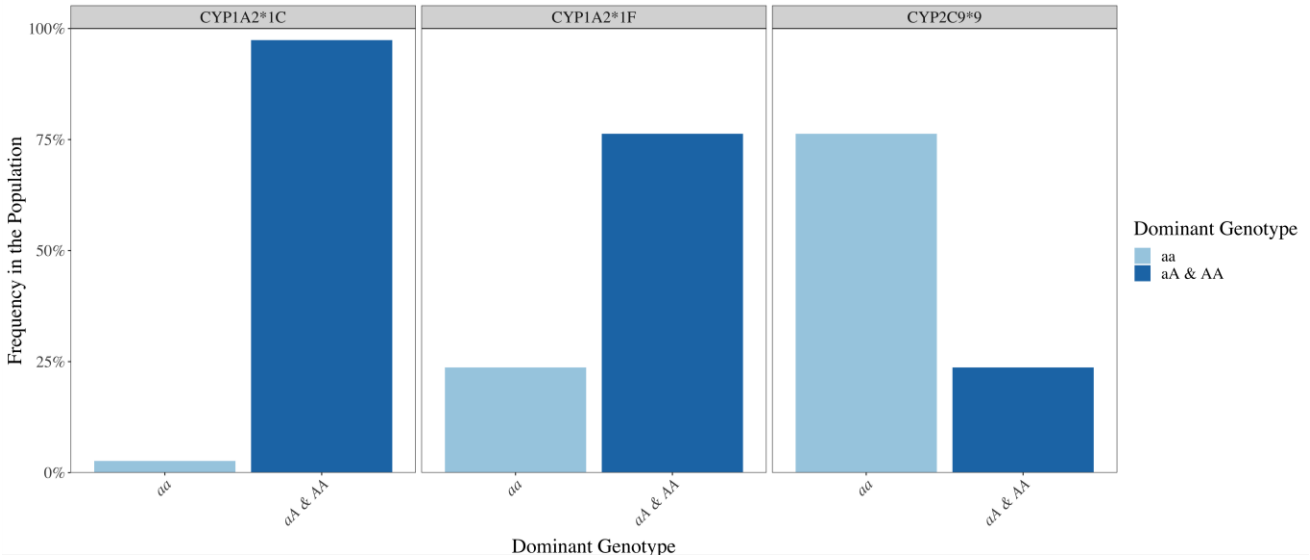


Figure 5.8: Frequency of *CYP1A2*1C, CYP1A2*1F* and *CYP2C9*9* genotypes using the dominant model in the Zimbabwean study population. *aa* represents the reference genotype, and *aA & AA* represent the dominant alternate genotypes.

For the *CYP2C9*9* variant, for both the (-2H)-O-PZQ and O2-PZQ metabolites, participants with *CYP2C9*9* [AG & GG] genotypes had significantly higher concentrations ($P \leq 0.05$) than the homozygous reference *CYP2C9*9* [AA] genotype carriers, as seen in **Figure 5.9A and Figure 5.9B**.

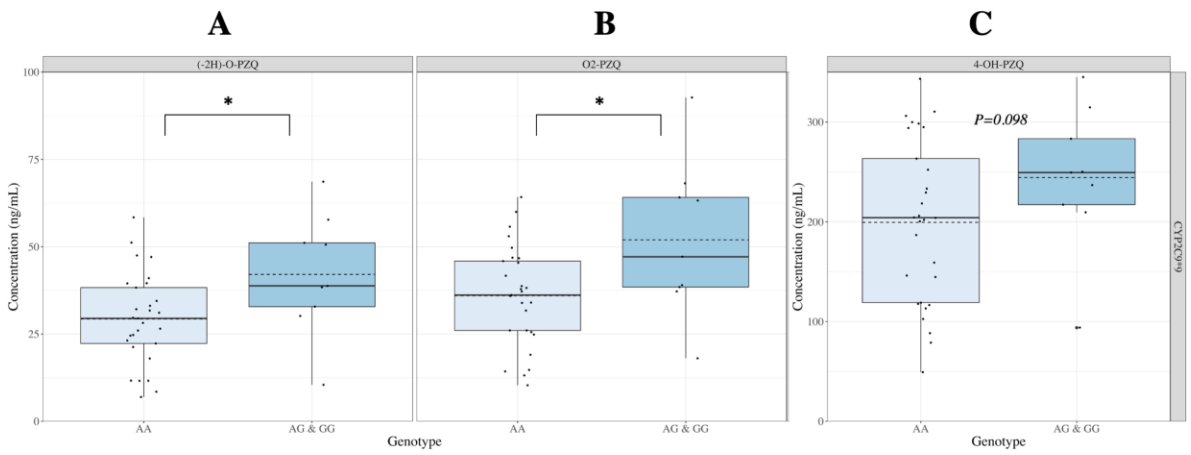


Figure 5.9: Boxplots of the distribution of [A] (-2H)-O-PZQ, [B] O2-PZQ and [C] 4-OH-PZQ concentrations in the Zimbabwean study population, separated by *CYP2C9*9* genotype in the dominant model. The dotted line represents the arithmetic mean of the sample population. The dots represent the individual sample concentrations. * represents significance values of P≤0.05 from the one-way ANOVA calculation. The concentrations of each analyte were calculated using the 2.5-hour timepoint. The horizontal box lines represent the first quartile, the median, and the third quartile. Whiskers denote the range of points within the first quartile −1.5× the interquartile range and the third quartile +1.5× the interquartile range. Acronyms: 4-OH: 4-monohydroxy, (-2H)-O: dehydro-mono-oxidised, O2: di-oxidised.

Although not significant (P=0.098), the mean concentration of the main metabolite 4-OH-PZQ was higher in *CYP2C9*9* [AG & GG] genotypes at 244.4 ± 71.9 ng/mL compared to the wild-type *CYP2C9*9* [AA] genotype at 199.4 ± 81.4 ng/mL. Despite differences in the metabolite concentrations between CYP genotypes, the parent PZQ concentrations did not vary between the *CYP2C9*9* [AA] and *CYP2C9*9* [AG & GG] genotypes (P=0.991), with mean PZQ concentrations of 33.8 ± 22.4 ng/mL and 34.1 ± 17.6 ng/mL, respectively.

The wild-type *CYP1A2*1F* [CC] had a higher mean and median than the *CYP1A2*1F* [CA&AA] genotypes as presented in **Figure 5.10** below, however it was not a significant finding (P=0.074).

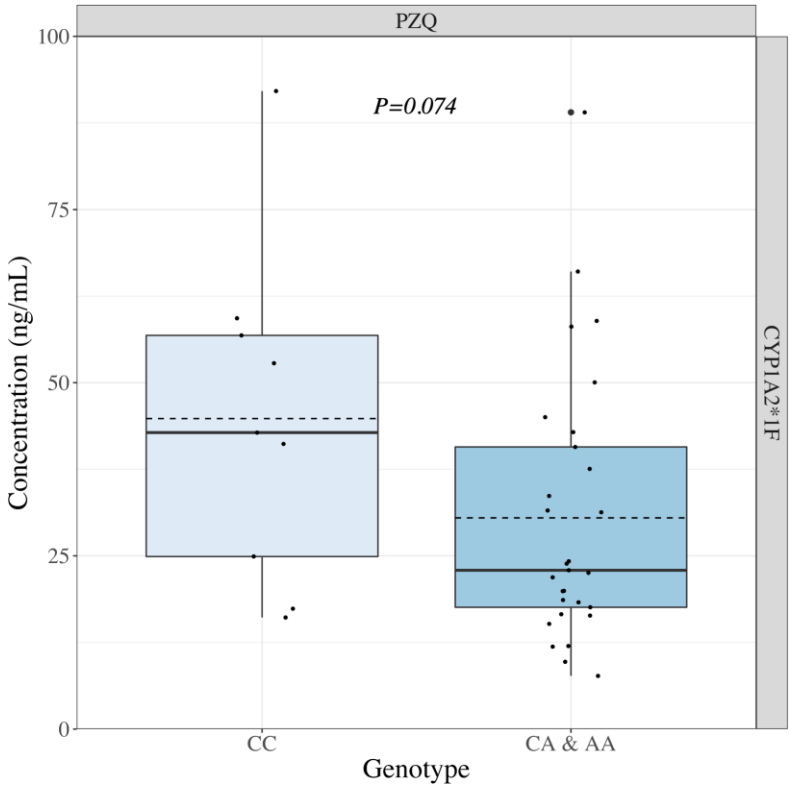


Figure 5.10: Boxplots of the distribution of praziquantel (PZQ) concentration in the Zimbabwean study population, separated by *CYP1A2*1F* genotype in the dominant model. The dotted line represents the arithmetic mean of the sample population. The dots represent the individual sample concentrations. The concentrations of each analyte were calculated using the 2.5-hour timepoint. The P-value was calculated using a one-way ANOVA. The horizontal box lines represent the first quartile, the median, and the third quartile. Whiskers denote the range of points within the first quartile −1.5× the interquartile range and the third quartile +1.5× the interquartile range.

Although there were no significant differences between the *CYP1A2*1C* [GG] reference genotype and the *CYP1A2*1C* [GA &AA] alternate genotypes, the differences in the PK

profiles (**Supplementary Figure 3 in Appendix 1**) and the mean concentrations of each analyte (**Table 5.6**) suggest altered *CYP1A2* activity between the genotypes. However, only one participant had the *CYP1A2*1C* [GG] reference genotype so no associations could be confirmed. PCA was used to determine if the concentration profiles clustered separately based on the dominant genotypes of the CYP variants. Regarding the separation of these concentration profiles by genotypes, there was no distinct clustering by genotype, as seen in **Supplementary Figure 4 in Appendix 1.**

5.3.5.1.2 Genotypic analysis

Comparison of the mean concentrations of PZQ, 4-OH-PZQ, (-2H)-O-PZQ and O2-PZQ between the separate CYP genotypes using the genotypic model are summarised in **Supplementary Table 12 in Appendix 1**. Only three CYP variants did not correlate with the dominant model and required genotypic analysis; *CYP1A2*1F, CYP2C9*9* and rs1135840 of the *CYP2D6* gene. The frequency of these variants by genotype in this Zimbabwean study population is presented in **Figure 5.11**.

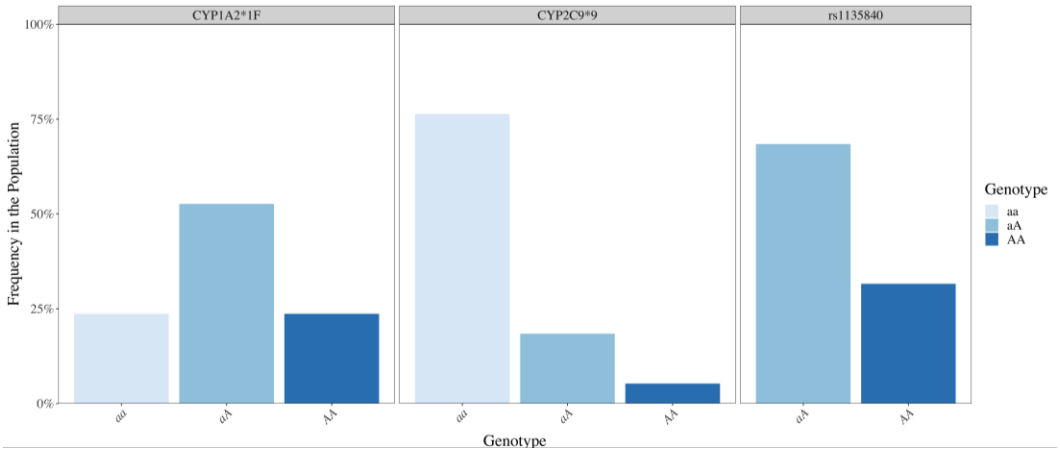


Figure 5.11: Frequency of *CYP1A2*1F*, *CYP2C9*9* and rs1135840 genotypes using the genotypic model in the Zimbabwean study population. *aa* represents the reference genotype, *aA* represents the heterozygous alternate genotype, and *AA* represents the homozygous alternate genotype.

Of these three CYP variants, only the *CYP2C9*9* genotypes had significantly different mean concentrations of the (-2H)-O-PZQ and O2-PZQ metabolites, as visualised in **Figure 5.12A** and **Figure 5.12B** below.

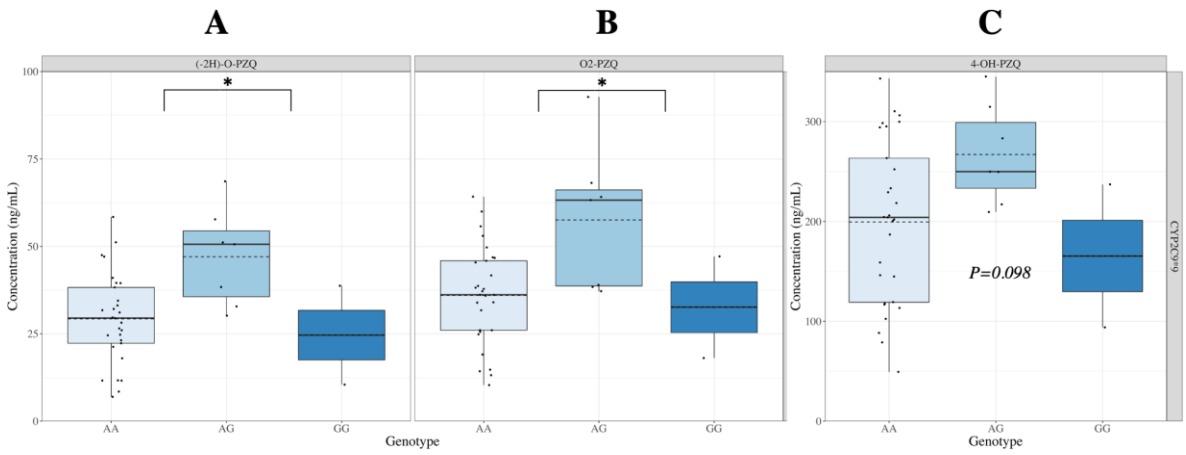


Figure 5.12: Boxplots of the distribution of the [A] (-2H)-O-PZQ, [B] O2-PZQ and [C] 4-OH-PZQ concentrations in the Zimbabwean study population, separated by *CYP2C9*9* genotype. The dotted line represents the arithmetic mean of the sample population. The dots represent the individual sample concentrations. * represents significance values P≤0.05 from the one-way ANOVA calculation. The concentrations of each analyte were calculated using the 2.5-hour timepoint. The horizontal box lines represent the first quartile, the median, and the third quartile. Whiskers denote the range of points within the first quartile −1.5× the interquartile range and the third quartile +1.5× the interquartile range. Acronyms: PZQ: praziquantel, 4-OH: 4-monohydroxy, (-2H)-O: dehydro-mono-oxidised, O2: di-oxidised.

Based on Tukey multiple comparisons of the means of the ANOVA results, the concentrations were significantly higher (P≤0.05) in participants with *CYP2C9*9* [AG] genotype compared to the homozygous reference *CYP2C9*9* [AA] genotype and homozygous alternate *CYP2C9*9* [GG] genotype. The mean concentration of the main

metabolite, 4-OH-PZQ, was also higher in participants with *CYP2C9*9* [AG] genotype compared to the homozygous reference *CYP2C9*9* [AA] genotype and homozygous alternate *CYP2C9*9* [GG] genotype, although it was not significant (P=0.098), as seen in **Figure 5.12C**.

Comparisons of the three *CYP1A2*1F* genotypes with analytes concentrations found no significant associations, although there was a lower mean PZQ concentration for both the heterozygous *CYP1A2*1F* [CA] genotype and homozygous alternate *CYP1A2*1F* [AA] genotype, as presented in **Figure 5.13**, corresponding with dominant model results in **Section 5.3.4.1.1**.

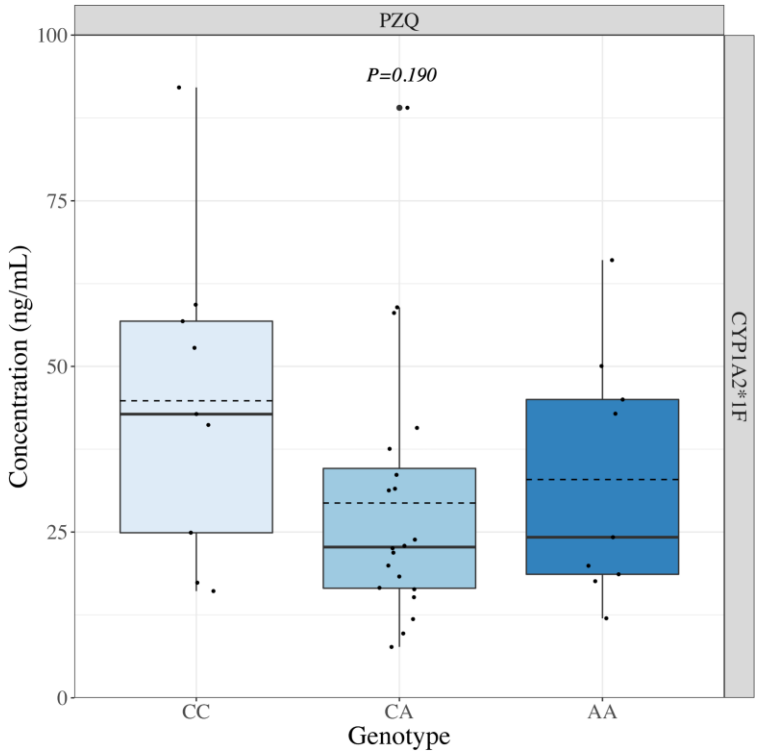


Figure 5.13: Boxplots of the distribution of praziquantel (PZQ) concentration separated by *CYP1A2*1F* genotype in the Zimbabwean study population. The dotted line represents the arithmetic mean of the sample population. The dots represent the individual sample concentrations. The P-value was calculated using a one-way ANOVA. The concentrations of each analyte were calculated using the 2.5-hour timepoint. The horizontal box lines represent

the first quartile, the median, and the third quartile. Whiskers denote the range of points within the first quartile −1.5× the interquartile range and the third quartile +1.5× the interquartile range.

The genotypes of rs1135840 of the *CYP2D6* gene did not have any significant relationship with the concentration of any detected analyte. Regarding the separation of the analytes concentration profiles by CYP variant genotype using PCA, there was no distinct clustering by genotype, as displayed in **Supplementary Figure 5 in Appendix 1.**

5.3.5.2 Effect of CYP genotype on metabolite ratio

5.3.5.2.1 Dominant analysis

The metabolic ratio (metabolite/PZQ) for all three metabolites using the dominant model is shown in **Table 5.6**. The metabolic ratio was significantly higher in *CYP1A2*1F* [CA & AA] carriers compared to the wild-type *CYP1A2*1F* [CC] genotype for all three metabolites, 4-OH-PZQ/PZQ, (-2H)-O-PZQ/PZQ and O2-PZQ/PZQ, as displayed in **Figure 5.14**.

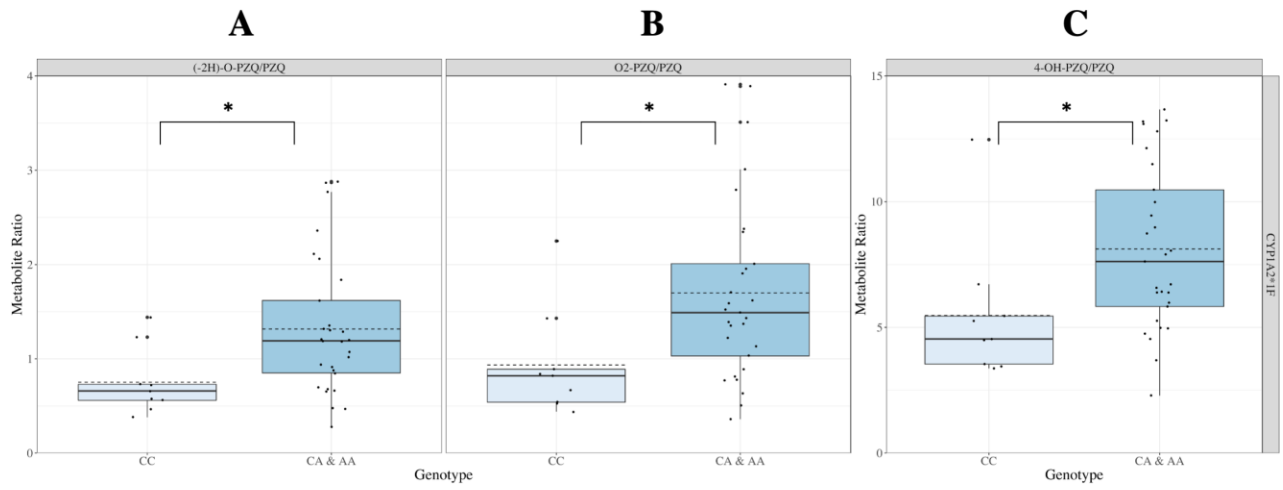


Figure 5.14: Boxplots of the distribution of the metabolite ratio of [A] (-2H)-O-PZQ, [B] O2-PZQ and [C] 4-OH-PZQ in the Zimbabwean study population, separated by *CYP1A2*1F* genotype in the dominant model. The dotted line represents the mean of the sample population. The dots represent the individual sample concentrations. * represent significance values P≤0.05 from the one-way ANOVA calculation. The concentrations of each analyte were calculated using the 2.5-hour timepoint. The horizontal box lines represent the first quartile, the median, and the third quartile. Whiskers denote the range of points within the first quartile −1.5× the interquartile range and the third quartile +1.5× the interquartile range. Acronyms: PZQ: praziquantel, 4-OH: 4-monohydroxy, (-2H)-O: dehydro-mono-oxidised, O2: di-oxidised.

The remaining CYP variants did not have any significant relationship between the CYP genotype and metabolic ratio.

5.3.5.2.2 Genotypic analysis

The metabolic ratio (metabolite/PZQ) for all three metabolites using the genotypic analysis is shown in **Supplementary Table 12 in Appendix 1**. There were no metabolic ratios that were significantly different between the CYP genotypes. However, the ratios for 4-OH-PZQ/PZQ

in *CYP1A2*1F* [CA] and the *CYP1A2*1F* [AA] carriers were 8.5 and 7.3, respectively, and were higher than those with the wild-type *CYP1A2*1F* [CC] genotype at 5.5. This correlated with the general trend of the dominant model results above although, unlike the dominant model, it was not significant via the genotypic model (P=0.076).

5.3.5.3 Effect of CYP genotypes on analyte exposure

5.3.5.3.1 Dominant analysis

The NCA parameters of PZQ and the three detected metabolites using the dominant model are displayed in **Supplementary Table 13 in Appendix 1**. The mean AUC of PZQ in the wild-type *CYP1A2*1F* [CC] genotype was significantly higher than the *CYP1A2*1F* [CA & AA] genotypes (P≤0.05), as shown in **Figure 5.15A**.

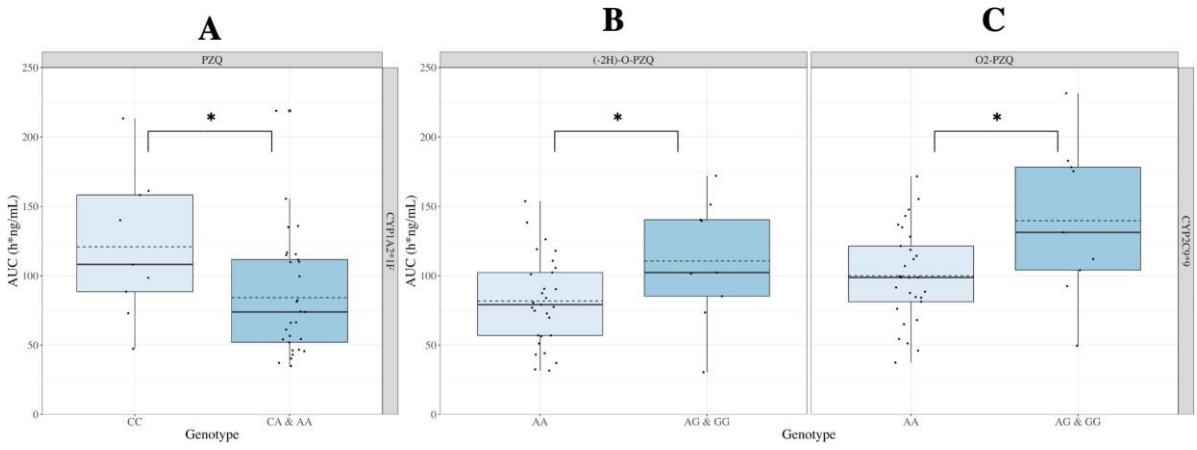


Figure 5.15: Boxplots of the distribution of the area-under-the-curve (AUC) of [A] praziquantel (PZQ), [B] (-2H)-O-PZQ, and [C] O2-PZQ in the Zimbabwean study population, separated by CYP variant and then genotype in the dominant model. The dotted line represents the mean of the sample population. The dots represent the individual sample concentrations. * represent significance values P≤0.05 from the one-way ANOVA calculation. The concentrations of each analyte were calculated using the 2.5-hour timepoint.

The horizontal box lines represent the first quartile, the median, and the third quartile. Whiskers denote the range of points within the first quartile −1.5× the interquartile range and the third quartile +1.5× the interquartile range. Acronyms: 4-OH: 4-monohydroxy, (-2H)-O: dehydro-mono-oxidised, O2: di-oxidised.

For the *CYP2C9*9* variant, the mean AUC of the (-2H)-O-PZQ and O2-PZQ metabolites were significantly higher (P≤0.05) in participants with the *CYP2C9*9* [AG & GG] genotypes compared to the wild-type *CYP2C9*9* [AA] genotype, as shown in **Figure 5.15B** and **Figure 5.15C**. As evaluated in **Section 5.3.4.1** regarding mean PZQ concentration, the *CYP1A2*1C* variant of interest was also of interest regarding PZQ exposure, as shown in **Supplementary Figure 6 in Appendix 1**. For PZQ and all three of its detected metabolites, the *CYP1A2*1C* [GG] reference genotype had a higher mean AUC compared to the *CYP1A2*1C* [GA &AA] alternate genotypes, yet this was not significantly different for any analyte.

5.3.5.3.2 Genotypic analysis

The NCA parameters of PZQ and the three detected metabolites using the genotypic model are displayed in **Supplementary Table 14 in Appendix 1**. Only three CYP variants did not correlate with the dominant model analysis as described in **Section 5.3.5.1.2**. The frequency of the *CYP1A2*1F, CYP2C9*9* and rs1135840 variants by genotype in the Zimbabwean study population were shown in **Figure 5.16**. The *CYP1A2*1F* genotypes had no significant association with analyte exposure, although there was a lower mean AUC for PZQ (**Figure 5.16A**) for both the heterozygous *CYP1A2*1F* [CA] genotype and homozygous alternate *CYP1A2*1F* [AA] genotype which correlated with the dominant results above.

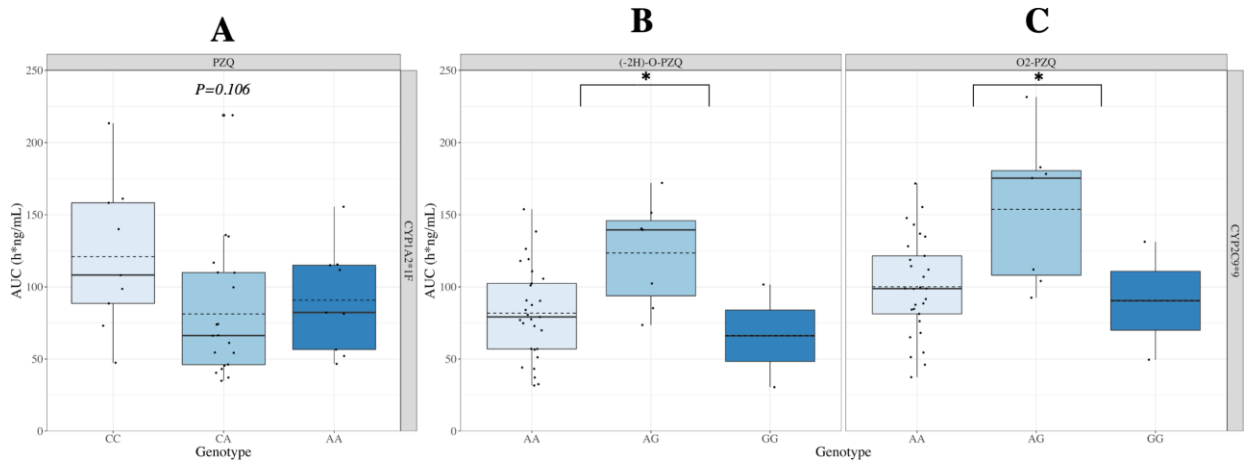


Figure 5.16: Boxplots of the distribution of the area-under-the-curve (AUC) of [A] praziquantel (PZQ), [B] (-2H)-O-PZQ, and [C] O2-PZQ in the Zimbabwean study population, separated by CYP variant and then genotype. The dotted line represents the mean of the sample population. The dots represent the individual sample concentrations. * represents significance values P≤0.05 from the one-way ANOVA calculation. The concentrations of each analyte were calculated using the 2.5-hour timepoint. The horizontal box lines represent the first quartile, the median, and the third quartile. Whiskers denote the range of points within the first quartile −1.5× the interquartile range and the third quartile +1.5× the interquartile range. Acronyms: 4-OH: 4-monohydroxy, (-2H)-O: dehydro-mono-oxidised, O2: di-oxidised.

For the *CYP2C9*9* variant, the mean AUC of both the (-2H)-O-PZQ and O2-PZQ metabolites were significantly higher (P≤0.05) in participants with *CYP2C9*9* [AG] genotype compared to the homozygous reference *CYP2C9*9* [AA] genotype and homozygous alternate *CYP2C9*9* [GG] genotype (**Figure 16B** and **Figure 16C**). Based on Tukey's multiple comparisons of means of the ANOVA results, the mean AUC was significantly higher (P≤0.05) in participants with *CYP2C9*9* [AG]. The genotypes of the

rs1135840 of the *CYP2D6* gene did not have any significant association with the exposure of the analytes.

5.3.5.4 Effect of CYP genotype on PZQ efficacy

The efficacy of PZQ treatment, which was designated as whether the individual had cleared or not cleared infection (see **Section 5.2.2**), was the designated PD outcome of this study. Overall, 94.8% (36/38) of the treated Zimbabwean study population had cleared infection at 3-weeks post-treatment. Fisher's exact tests were performed between each CYP genotype and the 3-week outcome, with no significant associations found for any CYP variant using the dominant (**Supplementary Table 15 in Appendix 1**) or the genotypic (**Supplementary Table 16 in Appendix 1**) model.

5.3.5.5 Predictions of praziquantel (PZQ) efficacy

A RF model was built based on the protocol developed in **Chapter 4** to determine the best CYP variant predictive of PZQ efficacy. The RF model produced an OOB ERR of 5.26%, yet none of the CYP variants had a positive importance on the model. This was postulated to be primarily due to the overall low sample size and lack of participants who had not cleared infection. Hence the RF mode was not utilised any further in this chapter. Also, no significant combinations of PGx variants were associated with PZQ efficacy based on LAMPLINK analysis, with 11 testable combinations assessed and an adjusted significance level of $8.33_{x10^{-3}}$.

5.4 Discussion

The identification of PGx variants involved in drug response is critical for the improvement of healthcare in Africa, as the ability to predict drug efficacy in underreported African populations can prevent the waste of medical resources, treatment failure, and ADRs (Radouani *et al.*, 2020). This chapter investigated the PK-PD-PGx relationships of the antischistosomal drug PZQ, which is utilised across Africa to control schistosome infections (WHO, 2022b). In the current study, the parent PZQ drug, three phase I and three phase II metabolites were identified using a microsampling technique ideal for field-based studies in rural Africa, achieving aim (I) of this chapter. Although the three glucuronide metabolites from the phase II biotransformations were excluded from this analysis, multiple glucuronide metabolites of PZQ have previously been detected in the urine of mice and humans (Meier and Blaschke, 2000, Wang *et al.*, 2014b). So, although not evaluated further in the current study, the detection of low *in vivo* concentrations of the glucuronide metabolites using DBS may indicate a phase II metabolic pathway of PZQ that has not yet been fully evaluated in humans and requires additional *in vivo* investigation.

Three phase I metabolites, including the main metabolite 4-OH-PZQ, and the (-2H)-O-PZQ and O2-PZQ metabolites were detected. The structure of 4-OH-PZQ, the major metabolite of PZQ, and its metabolism via the *CYP1A2, CYP2C9* and *CYP2C19* enzymes have been well-characterised in the literature during *in vivo* animal and human studies (Meier and Blaschke, 2000, Wang *et al.*, 2014b, Kovac *et al.*, 2018a, Nleya *et al.*, 2019, Kapungu *et al.*, 2020), hence it is usually the only metabolite investigated during PKPD studies of PZQ (Kovac *et al.*, 2018a, Kapungu *et al.*, 2020, Mnkugwe *et al.*, 2021a). However, the evaluation of the *in vivo* concentrations and the metabolic pathway of the (-2H)-O-PZQ and O2-PZQ metabolites has not been performed in humans. The O2-PZQ metabolite has been detected in the

urine/faeces of mice and healthy human volunteers, but no further PKPD analysis or CYP evaluation was conducted (Meier and Blaschke, 2000, Wang *et al.*, 2014b). The O2-PZQ is postulated to be a derivative of a mono-oxidised PZQ metabolite based on the MS/MS spectra, thus may therefore also be metabolised by a similar *CYP3A* pathway (Wang *et al.*, 2014b). Moreover, the (-2H)-O-PZQ had only been detected in the urine/faeces of mice and had never been detected before in humans, thus was a novel *in vivo* finding, adding evidence to the metabolic pathway of PZQ in humans (Meier and Blaschke, 2000, Wang *et al.*, 2014b). Despite the limited knowledge of this metabolite, the (-2H)-O-PZQ is postulated to be a further product of the 4-OH-PZQ metabolite based on the MS/MS spectra (Wang *et al.*, 2014b). Thus, the CYP pathway to produce this metabolite may be similar to that of the major PZQ metabolite (*CYP1A2/2C9/2C19*). The identification of both these metabolites in humans, and their further characterisation may be useful in monitoring PGx changes in the enzymes they are produced by to further characterise the PGx impact on PZQ exposure.

A population PK profile was also obtained, quantifying the *in vivo* concentrations of each analyte using DBS. However, to achieve aim (II) of this chapter, the *in vivo* concentrations of PZQ and its metabolites were analysed using two methods of MS sampling: a pooling of the Zimbabwean study population at each timepoint, in addition to the individual sampling of each participant at the 2.5-hour and 4-hour timepoints (see **Section 5.2.2** for further details). The PK concentration-time curves of the three PZQ metabolites were similar between the two MS methods. The PK of the metabolites followed the same general curve to a C_{max} at 2.5-hours and then decrease at 4-hours for excretion. This was expected, as the 2.5-hour timepoint was selected for individual MS sampling based on previous human studies PZQ from DBS (Kovac *et al.*, 2018a). Yet, the C_{max} of PZQ using the pooled MS samples was at the 1.5-hour timepoint rather than at 2.5-hours, resulting in different PK profile pre-2.5 hours. Thus, if this study was to be repeated, the individual MS sampling would have been

performed at the 1.5-hour and 4-hour timepoints to properly capture the C_{max} and the correct PK profile of this Zimbabwean study population. Despite this, the *in vivo* analyte concentration of the 2.5-hour and 4-hour timepoints were comparable between the two MS sampling methods, with the pooled samples lying within the SD of the mean of the study population calculated from each participant. Hence, the pooled MS samples were a good representation of the *in vivo* analyte concentrations of the Zimbabwean study population at these timepoints, and hence could be a good way of obtaining a population PK profile using a smaller number of MS samples in resource-limited settings (**Section 1.10.2.1**).

Concerning the relationship between *in vivo* concentrations of parent PZQ and PZQ efficacy (PKPD analysis), there were no significant differences between those who cleared infection and those who did not clear infection. However, the participants who did not clear infection had lower PZQ concentrations than those who cleared infection at 4-hours. This may provide reasoning behind these participants not clearing infection, as the active PZQ was metabolised more rapidly, resulting in a 14.3% lower PZQ concentration compared to the cleared groups at 4-hours.

Therefore, the PGx analysis further investigated differences between *in vivo* concentrations with CYP genotype and determined if there were associations with PZQ efficacy (PK-PD-PGx). There were eleven CYP variants detected in this Zimbabwean study population. Yet, 72.7% of these variants had significantly different MAF from the expected MAF of European populations. The distribution of CYP variants differs substantially between ethnicities, nevertheless, African populations are the least studied in terms of PGx changes in drug treatments despite their high genetic diversity, disease burden and drug requirements (Murray *et al.*, 2012, Rajman *et al.*, 2017, Radouani *et al.*, 2020). Subsequently, these results indicate that PGx variants may be influencing drug exposure in Zimbabwean populations more than

predicted based on European drug efficacy studies. Therefore, the characterisation of underreported African populations, such as this study, enhances the genetic map of the continent and contributes relevant PGx information on drugs commonly used by those populations.

To date, there has only been one study in an African population that has investigated the PGx impact on PZQ and its metabolite concentrations *in vivo*, yet this only focused on the main 4-OH-PZQ metabolite and *S. mansoni*-infected individuals (Mnkugwe *et al.*, 2021a). Hence, upon relating the different genotypes of these eleven CYP variants to the concentrations of PZQ and the three detected metabolites to achieve aim (III), there were some key and novel PK-PD-PGx findings for this *S. haematobium*-infected Zimbabwean study population. Firstly, there was a significant association between *CYP1A2*1F* genotype and PZQ exposure, with the mean PZQ AUC of the *CYP1A2*1F* [CA & AA] genotypes significantly lower than the wild-type *CYP1A2*1F* [CC] genotype. *CYP1A2*1F* alleles have been reported in the literature to increase the activity of the *CYP1A2* enzyme (Lu *et al.*, 2020), further supporting these *in vivo* findings regarding PZQ exposure. Furthermore, the metabolic ratio, which indicates the conversion of the parent drug to a metabolite, was significantly higher in *CYP1A2*1F* [CA & AA] carriers than those with the wild-type *CYP1A2*1F* [CC] genotype for all three detected PZQ metabolites. This higher metabolite ratio in the Zimbabwean participants with a *CYP1A2*1F* [CA & AA] genotype supports increased *CYP1A2* activity. Specifically, the significantly higher 4-OH-PZQ/PZQ metabolic ratio in *CYP1A2*1F* [CA & AA] carriers indicates PZQ is being transformed into the main 4-OH-PZQ metabolite with these genotypes compared to *CYP1A2*1F* [CC]. The formation of 4-OH-PZQ via *CYP1A2* has already been shown to be a key pathway of the active (R)-PZQ enantiomer (Wang *et al.*, 2014b, Kapungu *et al.*, 2020), thus if *CYP1A2*1F* [CA & AA] decreases active (R)-PZQ it may be an influential PGx factor in decreasing the efficacy of PZQ treatment. Together, these

findings support previous studies that found that participants with at least one A variant allele had lower *in vivo* drug concentrations and low treatment efficacy (Laika *et al.*, 2010, Thorn *et al.*, 2012). Nevertheless, although these significant results indicate the PGx impact of *CYP1A2*1F* in this Zimbabwean population, there was no significant association between *CYP1A2*1F* genotype and parent PZQ concentration. There was a lower mean PZQ concentration for both the heterozygous *CYP1A2*1F* [CA] genotype and the homozygous alternate *CYP1A2*1F* [AA] genotype, but it was not statistically significant. This opens an avenue for investigation in terms of PGx impacts on PZQ efficacy as mean analyte concentration using DBS may not be completely representative of drug exposure and metabolite formation, thus further investigations are required.

Another variant of interest in the *CYP1A2* gene was *CYP1A2*1C*, a PGx variant reported to decrease the function of the *CYP1A2* enzyme (Nakajima *et al.*, 1999). Although there were no significant findings, the differences in mean concentration, AUC and metabolites ratio between *CYP1A2*1C* [GG] and the *CYP1A2*1C* [GA &AA] alternate genotypes suggest a difference in *CYP1A2* activity. The lack of significance was primarily due to the low sample size of the *CYP1A2*1C* [GG] genotype, as 97% of the study population had the *CYP1A2*1C* [GA &AA] alternate genotype. Although, this could indicate decreased *CYP1A2* metabolism and the resultant increased drug exposure may be more prevalent than expected in Zimbabwean populations. This may increase the risk of ADRs, whether during PZQ treatment or other drugs. This finding emphasises the need for further investigations into the impact of this PGx variant during a PZQ treatment in Zimbabwean populations, as the impact of *CYP1A2*1C* may be underestimated.

To date, there has been minimal data on the missense *CYP2C9*9* variant, yet it is reported to be restricted to African populations (Zhou *et al.*, 2017). The current study predicted the

His251Arg change of the *CYP2C9*9* to be a deleterious change using SIFT and GWAVA, and therefore possibly damaging to the function of the *CYP2C9* gene. Yet, regarding the literature, the SNPedia database recognised this variant as having normal *CYP2C9* function (Cariaso and Lennon, 2012), *in vitro* data suggested decreased *CYP2C9* activity (Mitchell *et al.*, 2011), and a study in an African population found the *CYP2C9*9* variant to have no significant influence on the drug metabolism (Allabi *et al.*, 2005). All of these reported findings contradict each other and contradict the findings of this study, stressing the paucity of knowledge on this PGx variant. Yet, *CYP2C9* missense variants have been shown to increase drug metabolism, and decrease drug exposure in other efficacy studies (Pratt *et al.*, 2019), so it is feasible this mechanism is occurring here. However, substrate specificity also cannot be excluded as the reason for the discrepancies in metabolic route of *CYP2C9*9*.

Based on the results of this study, there were significantly higher mean concentrations and AUC for both the (-2H)-O-PZQ and O2-PZQ metabolites in participants with *CYP2C9*9* [AG & GG] genotypes compared to the homozygous reference *CYP2C9*9* [AA] genotype. Furthermore, the increase in metabolite exposure appears to stem specifically from participants with heterozygous *CYP2C9*9* [AG] genotype compared to the homozygous reference *CYP2C9*9* [AA] genotype and homozygous alternate *CYP2C9*9* [GG] genotype. Therefore, the significantly higher concentrations of (-2H)-O-PZQ and O2-PZQ with the alternate *CYP2C9*9* [AG & GG] genotypes suggest this variant increases *CYP2C9* activity and metabolises PZQ into these metabolites more rapidly than the wild-type *CYP2C9*. These increased concentrations of metabolites can signify decreased concentrations of the schistosome-killing parent PZQ in systemic circulation, and thus the *CYP2C9*9* variant could potentially decrease PZQ efficacy. The alternate genotypes of *CYP2C9*9* have previously been reported to require higher doses of warfarin to achieve the desired effect compared to wild-type homozygotes, although the strength of this association was considered

weak due to the small sample size of the participants with *CYP2C9*9* genotypes (Asiimwe *et al.*, 2020). This correlates with the findings of this study, in that the *CYP2C9*9* variant may be increasing the production of PZQ metabolites and in response decreasing lethal PZQ concentrations. However, similarly to the warfarin study, it cannot be concluded that this finding was not due to chance due to the small sample size of this Zimbabwean study population.

Still, only minimal research has been done to conclusively characterise the role and impact of the *CYP2C9* enzyme in PZQ metabolism and the metabolic products from this enzyme in humans. The *CYP2C9* pathway has been identified as a key contributor to PZQ metabolism using human liver microsomes (HLM) and recombinant CYP (rCYPs) (Wang *et al.*, 2014b). This is contrary to a more recent study, which did not detect *CYP2C9* as a major metabolic route of PZQ in rCYPs, and determined *CYP2C9* was only a minor metabolic route in humans (Kapungu *et al.*, 2020). Yet, the reason for this discrepancy may lie in the technique of metabolite identification. The Kapunga *et al.* study (no *CYP2C9)* used PZQ depletion to indicate metabolic contributions and hence measured how much PZQ disappeared via each CYP, compared to the Wang *et al.* study (*CYP2C9* involved) which measured the quantity of metabolite formation regardless of parent PZQ depletion. As the formation of the (-2H)-O-PZQ and O2-PZQ metabolites was also utilised in this study, a greater similarity to the finding of Wang *et al.* study in this chapter was found. Further, Kapunga *et al.* focused on identifying the concentrations of the 4-OH-PZQ and the X-OH-PZQ metabolites, therefore may not have detected *CYP2C9* as a metabolic route as the formation of these metabolites is not primarily reported to be due to this pathway in humans.

Thus, from this study, the *CYP2C9*9* variant significantly impacted the *in vivo* concentrations and exposure of two PZQ metabolites, despite the unknown CYP pathway,

suggesting the *CYP2C9* enzyme is involved in the production of these metabolites. The (-2H)-O-PZQ and O2-PZQ metabolites have already been postulated to be products of the mono-oxidised PZQ metabolites based on their MS/MS spectra, which are largely metabolised by *CYP2C9* (Wang *et al.*, 2014b). Thus, the *CYP2C9* enzyme is likely involved in the production of these two PZQ metabolites. Overall, these findings suggest a potentially unexplored PGx variant in an African population that increases the *in vivo* concentrations of two underreported PZQ metabolites and potentially decreases active PZQ exposure in the process. However, a conclusive determination of the metabolic pathway of these metabolites and the function of the *CYP2C9*9* is needed.

Lastly, there were no significant associations between any CYP genotype and PZQ efficacy, something also observed in the study assessing the PGx impact on PZQ efficacy in Tanzania (Mnkugwe *et al.*, 2021a). In the current study, this was attributed to the low sample size of those who did not clear schistosome infection. Of the thirty-eight Zimbabwean participants, only two individuals did not clear infection, resulting in limited samples to correlate with the PGx information. Further, although a study population of thirty-eight participants is low, it was within the range of multiple reported PKPD studies (n<35) that evaluated PZQ concentrations *in vivo* (Westhoff and Blaschke, 1992, Masimirembwa *et al.*, 1994b, Meier and Blaschke, 2000, Pengsaa *et al.*, 2004, Lima *et al.*, 2009, Meister *et al.*, 2016b, Kovac *et al.*, 2018b). This is partly because it is challenging to recruit study participants in such time-intensive and invasive studies (Maggadani *et al.*, 2021). Hence, to improve on this study, a larger sample size would be required to confirm the causality of the associations in the current study.

While this study investigated PGx variations on the *in vivo* concentrations of PZQ and its metabolites, the confirmation of whether these CYP variants influenced the levels of the

active (R)-PZQ was impossible due to a lack of enantiomeric identification. PZQ is a racemic drug, with differing enantiomers action: the (R)-PZQ possesses antischistosomal action and the (S)-PZQ has been reported to contribute towards ADRs (see further details in **Section 1.5**). As analysed in **Chapter 3**, the exposure of (S)-PZQ was higher than the (R)-PZQ following a racemic PZQ treatment (Zdesenko and Mutapi, 2020, Minzi *et al.*, 2021), thus the mean concentrations were not representative of only the active enantiomer. Further, stereoselective metabolism of PZQ has already been reported for the multiple CYP enzymes (Wang *et al.*, 2014b), and therefore certain CYP variants in this chapter may affect only (R)-PZQ or (S)- PZQ metabolism. The formation of 4-OH-PZQ, (-2H)-O-PZQ and O2-PZQ have all been reported to be produced by the stereoselective metabolism of (R)-PZQ, therefore the increased production of these metabolites could feasibly contribute towards the decreased concentrations of the active schistosome-killing drug (Wang *et al.*, 2014b). Additionally, as *CYP3A4/5* is a CYP pathway of the (S)-PZQ (Wang *et al.*, 2014b), which contributes to ADRs such as nausea and vomiting (Meyer *et al.*, 2009), further studies should take care to identify if these variants increase or decrease (S)-PZQ as this may have a significant effect on the likelihood of ADRs following a PZQ treatment. Overall, further studies evaluating the PGx impact on PZQ efficacy in a schistosome-infected population should include the quantification of PZQ enantiomers to confirm active (R)-PZQ concentrations, as well as a record of adverse events during a PZQ treatment that may be a result of the (S)-PZQ and stereospecific PGx variation.

Concerning additional limitations to this study, three PGx variants failed the HWE test: *CYP1A2*1C* and Novel-2 of the *CYP1A2* gene, and *CYP2D6*17* of the *CYP2D6* gene. Deviation from HWE could arise from a variety of reasons such as random chance and a heterogenous population, and so a failure of the HWE test is not always indicative of a genotyping problem (Lewis, 2002). The current study population were all from the same rural

area of Zimbabwe, thus the HWE deviation may be due to population homogeneity, and so the variants were included. However, genotyping issues cannot be exluded. The study population in this chapter was from the Shamva district, geographically different to the study population in **Chapter 4** which was collected in the Mutoko and Murewa districts of Zimbabwe and detected significant PGx associations with PZQ efficacy that were not detected here. Based on African genetic diversity, these deviations may occur due to sub-population heterogeneity across Zimbabwe (Campbell and Tishkoff, 2008, Hussein *et al.*, 2022).

Additionally, the recovered concentrations of PZQ and 4-OH-PZQ from the DBS were only approximately 10% of the plasma concentrations reported by Mnkygwe *et al.* (Mnkugwe *et al.*, 2021a), despite the recovery of the d11-PZQ standard from the DBS extracts being acceptable. A study evaluating the PK profile of PZQ using DBS cards detected the (R)-PZQ at approximately 20ng/mL at 4 hours, and the (S)-PZQ at approximately 200ng/mL (Meister *et al.*, 2016b). When comparing this concentration range to the parent PZQ concentrations detected in this study to the Meister *et al.* study, both concentrations of the PZQ enantiomers lie within the range of the racemic parent PZQ concentrations detected in this study (see **Figure 5.5**). Therefore, as the DBS sample is collected directly from the capillary blood there may have been strong plasma protein binding of the analyte, causing unrepresentative DBS concentrations compared to the concentrations in venous blood (Emmons and Rowland, 2010). Thus, the differences in concentrations detected between this study and previous PK studies on PZQ were due to the lower sensitivity and sample volumes of the DBS. Despite this, the use of DBS is still a highly desirable method due to its ease of sampling, increased patient adherence and the potential for use in rural areas of Africa. However, models should be developed to extrapolate DBS capillary concentrations to the active parent PZQ plasma concentrations, allowing assessments of the PZQ efficacy outcome. This would allow a direct correlation to the plasma concentration, to determine if it was above the 312 ng/mL required

for schistosome death where capillary blood could not (Emmons and Rowland, 2010, Nleya *et al.*, 2019).

PGx is of growing importance in healthcare across the world, with PGx technology redirecting drug development to increase efficacy and prevent the occurrence of ADRs using a precision medicine approach (Olivier and Williams-Jones, 2011). Based on the results of this study, PGx variations in the PZQ-metabolising enzymes can alter the exposure of PZQ and its metabolites, providing further evidence of the PGx impact on PZQ efficacy. Thus, in patients with PGx variants that impact the PZQ-metabolising CYP enzymes, an alteration to PZQ dose or even the use of an alternative treatment that is not metabolised via these pathways may be required (metrifonate or oxamniquine (Kramer *et al.*, 2014)). For instance, these findings indicated that patients with the *CYP1A2*1F* variant (increased *CYP1A2* activity) may require higher doses of PZQ to successfully clear schistosome infection due to decreased active PZQ exposure. However, this must be performed with caution to not increase the risk of ADRs. Overall, knowledge of how PGx variations affect active PZQ concentrations and treatment outcomes is vital for maintaining and improving PZQ efficacy (Mukonzo *et al.*, 2014, Mutagonda *et al.*, 2017, Mnkugwe *et al.*, 2021a).

5.5 Key findings and conclusions

This chapter addressed **Thesis Aim 3 (Section 1.11**). Parent PZQ and three phase I metabolites (4-OH-PZQ, (-2H)-O-PZQ, and O2-PZQ) were identified using DBS which were optimised, evaluated, and shown to be a reliable approach for field-based population studies in rural areas. These analytes were related to eleven detected PGx variants. Significant associations between several *in vivo* measurements and CYP genotypes were identified. *CYP1A2*1F* [CA & AA] carriers had significantly lower PZQ exposure and a significantly higher 4-OH-PZQ/PZQ metabolic ratio, indicating increased *CYP1A2* metabolism and decreased PZQ exposure which may consequently reduce the efficacy of PZQ treatment. Furthermore, individuals with *CYP2C9*9* [AG & GG] genotypes had significantly higher mean concentration and mean AUC for both the (-2H)-O-PZQ and O2-PZQ metabolites, implying increased PZQ metabolism and decreased exposure of the schistosome-killing parent PZQ. Together, these findings provide insight into the PGx impact on PZQ exposure, and therefore the potential consequences on PZQ efficacy, in schistosome-infected patients. However, there were no significant associations with PZQ efficacy during this PK-PD-PGx analysis, largely due to the limited number of participants who did not clear schistosome infection. Overall, the significant findings concerning *CYP1A2* and *CYP2C9* indicate the role of PGx during a PZQ treatment may be underestimated in schistosome-infected patients, however, further evaluations of the findings of this study must be performed in humans with larger sample sizes to confirm this. Thus, in the future, to prevent cases of variable PZQ efficacy and improve schistosome control, PGx testing could move towards personalised PZQ treatments.

Chapter 6 - Identifying hotspots of *S. haematobium* infection and decreased praziquantel efficacy during multiple mass drug administrations in Zimbabwe

6.1 Introduction

Schistosomiasis is Africa's second most prevalent parasitic disease, infecting millions of people each year (WHO, 2022a). Humans contract schistosome infections through contact with freshwater sources contaminated with *Schistosoma* parasites and this can occur via routine day-to-day activities like washing, working, and playing (Nelwan, 2019) (**Section 1.4**). Schistosome infections are treated with praziquantel (PZQ) which reduces disease-related morbidity (WHO, 2013c) (**Section 1.5**). Schistosomiasis control programs rely heavily on the mass drug administration (MDA) of PZQ to treat schistosome- exposed individuals and control this disease in endemic areas (WHO, 2002b) (**Section 1.6.1**). Despite preventative chemotherapy (PCT) interventions, there have been areas in which infection prevalence did not decline as expected or bounced back to high levels, regardless of well-implemented MDA programs (Kittur *et al.*, 2019). Areas that do not decline in schistosome prevalence after multiple MDAs are termed persistent hotspots (PHS) of schistosome infection, however there is no standard definition of PHS (Kittur *et al.*, 2017) (**Section 1.8**). Further, the reasons why these communities become PHS is not well understood (Kittur *et al.*, 2020). PHS of *S. haematobium* infection, a dominant species in Sub-Saharan Africa, have so far been identified in multiple schistosome-endemic countries during MDA programs (Pennance *et al.*, 2016, King *et al.*, 2020). To date, PHS occurrence has been associated with decreased PZQ efficacy, treatment regimen or poor coverage, and water contact (Pennance *et al.*, 2016, Wiegand *et al.*, 2017, Kittur *et al.*, 2020).

Decreased PZQ efficacy during the treatment of schistosomiasis has been reported across Africa (Danso-Appiah and De Vlas, 2002, Tchuenté *et al.*, 2004, Alonso *et al.*, 2006, Midzi *et al.*, 2008, Barakat and Morshedy, 2010, Kabuyaya *et al.*, 2017), even after multiple rounds of MDA with PZQ (Crellen *et al.*, 2016). There is an ongoing need to monitor the efficacy of PZQ in schistosome-infected populations, and to identify the causes of variable PZQ efficacy to prevent future unsuccessful PZQ treatments (Webster *et al.*, 2014, Walker *et al.*, 2022). As there is no consensus on the definition of a 'hotspot' in schistosome research or even in infectious disease epidemiology (Lessler *et al.*, 2017), there may also be hotspots of decreased PZQ efficacy that cannot be detected using current definitions of PHS. Therefore, there is a need to further investigate if hotspots of decreased PZQ efficacy are also occurring during MDA programs and if they are a key contributing factor to persistent schistosome infections and PHS emergence.

Like several other African countries, Zimbabwe has been administering PZQ as part of a national helminth control program for nearly a decade (Mduluza *et al.*, 2020). To date, the analysis of potential hotspots in Zimbabwe has not been conducted. Hence, this chapter aimed to identify hotspots of *S. haematobium* infection in Zimbabwe using six years of annual MDA data, determining whether decreased PZQ efficacy is responsible for PHS of schistosome infection, or if other factors contribute to persistent infections. The hotspots will therefore be identified using two definitions, with the terms separated by the measurement of the hotspot: persistent hotspots of *S. haematobium* prevalence (PPHS) and hotspots of decreased PZQ efficacy (EPHS). Together, identifying and understanding the determinants of a hotspot area (PPHS or EPHS) compared to a responder area (responds as expected to MDA), and identifying if EPHS or other risk factors are the cause of PPHS, will provide valuable information to guide the PCT program managers on the concerning issues regarding the control and elimination of schistosomiasis.

6.2 Methods

6.2.1 Aims and objectives

I. Identify persistent hotspots of *S. haematobium* prevalence (PPHS) from six years of MDA data in Zimbabwe using current definitions of a persistent hotspot.

II. Identify hotspots of decreased PZQ efficacy (EPHS) from six years of MDA data in Zimbabwe using reduced therapeutic PZQ efficacy measures.

III. Determine drivers of persistent infection and decreased PZQ efficacy by comparing hotspots (PPHS and EPHS) and responders in Zimbabwe.

IV. Determine whether decreased PZQ efficacy (EPHS) or other drivers of persistent infection are responsible for the PPHS detected during the MDA program in Zimbabwe using the results of these analyses.

6.2.2 Study design and inclusion criteria

This study analysed data collected from 2012 to 2017 during MDAs conducted in Zimbabwe. All surveys were obtained from September to November, apart from the third MDA which was delayed due to logistical issues and was completed in January 2015. The inception and execution of the schistosomiasis control program in Zimbabwe are described in detail by Mduluza *et al.* (Mduluza *et al.*, 2020). Briefly, school-aged children (SAC) from schools in thirty-five villages across twenty-nine districts of Zimbabwe were recruited. Following the world health organization (WHO) guidelines detailing the pre-MDA sampling of schools, the villages were purposely selected in districts that represent three endemicities of schistosomiasis infection prevalence: high (≥50%), moderate (10–49%), and low (<10%) prevalence (WHO, 2002b).

To identify hotspots of *S. haematobium*, the SAC could be included in one or both arms of this study. For the PPHS analysis, the infection prevalence and infection intensity was calculated using six years of MDA data. Therefore, the *S. haematobium* egg count from pre-MDA and/or post-MDA data was required for SAC to be included in the PPHS analysis. For the EPHS analysis, the cure rate (CR) and egg reduction rate (ERR) were calculated using six years of MDA data. Only treated SAC (as confirmed by the school MDA registers) who were positive for schistosome infection pre-MDA and were followed up for an efficacy check post-MDA were included in the EPHS analysis. SAC who were positive for *S. mansoni* infections were excluded from both analyses as the effect of coinfections on PZQ efficacy is currently inconclusive (Kabuyaya *et al.*, 2018a). The sample size of included SAC in each hotspot analysis, and the number of districts surveyed at each MDA, is given in **Figure 6.1**.

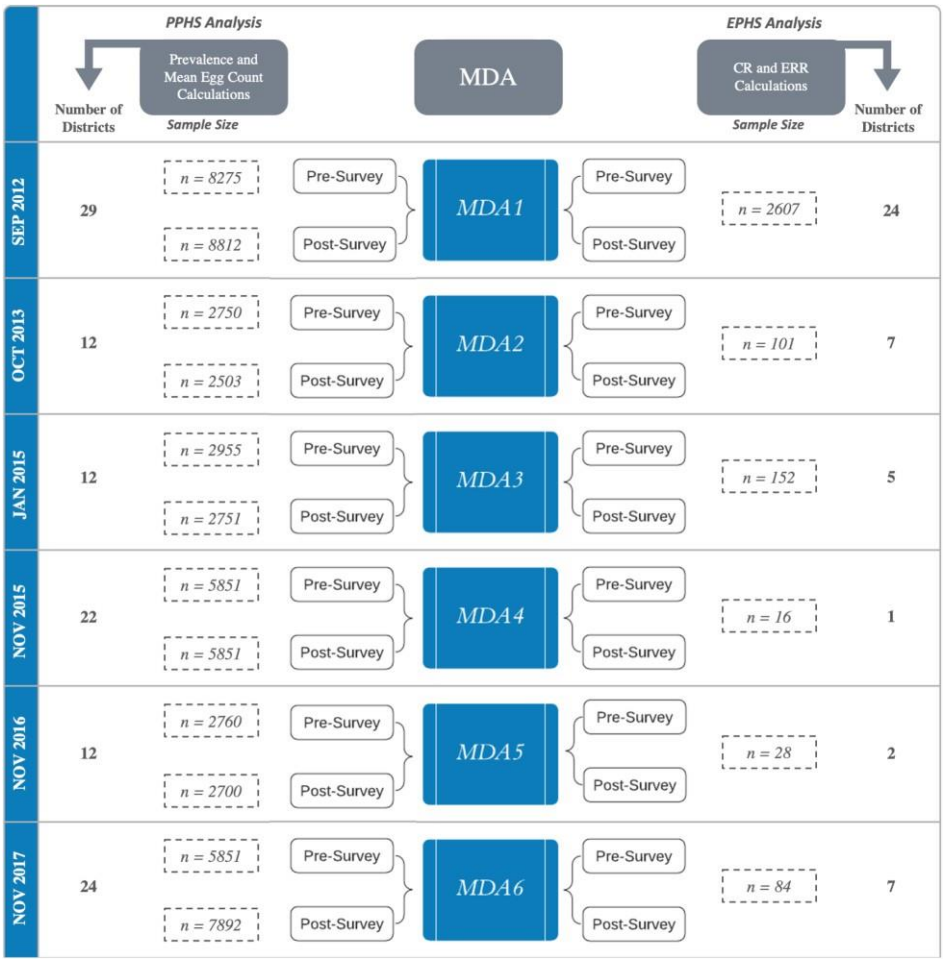


Figure 6.1: The two-pronged approach of this hotspot analysis, containing the number (*n*) of included school-aged children (SAC) from each mass drug administration (MDA) survey. PPHS: persistent hotspots of *S. haematobium* prevalence, EPHS: hotspots of decreased PZQ efficacy, CR: cure rate, ERR: egg reduction rate. The number of districts included in each survey is labelled, along with the date of each MDA.

6.2.3 Data analysis and visualisation

For the PPHS analysis, the infection prevalence (**Equation I, Section 2.3.1**) and arithmetic mean egg counts of *S. haematobium*, including the 95% confidence interval (CI), were calculated for each MDA survey for both village and district (calculated as the mean of each village). The arithmetic mean egg counts included both positive and negative children, and

was also referred to as infection intensity. For the EPHS analysis, the efficacy of PZQ treatment in clearing *S. haematobium* infections was calculated using the CR, a measure of those cured of infection upon treatment (**Equation II, Section 2.3.2.1**), and the ERR, a measure of the change in parasite egg burden upon treatment (**Equation III, Section 2.3.2.1**) (Zwang and Olliaro, 2014, Kimani *et al.*, 2018), for each MDA survey for both village and district. The calculation of the CR and ERR only included data from treated children who were positive for schistosome infection and were followed at both pre-MDA and post-MDA, as described in **Section 6.2.2**. If there was more than one village sample site per district, these datasets were combined for the district efficacy calculations. Each MDA was considered a separate data source as the PZQ efficacy was measured upon each administration. Box plots of the baseline infection prevalence and intensity of PPHS, EPHS and responders (no hotspot) were produced using GraphPad Prism version 8.2.0 (GraphPad Software, Inc.), as well as bar charts of the coverage of the MDAs. Choropleth maps of the infection prevalence, CRs and ERRs were generated using QGIS, Version [3.22.2], a shapefile of Zimbabwe and the GPS coordinates of the village sites obtained during the collection of the primary data.

6.2.4 Sample collection and parasitology

The *S. haematobium* parasitology data was diagnosed using the standard method of urine filtration and microscopy methods of Mott *et al.* (Mott, 1983, Mduluza *et al.*, 2020). The intensity of *S. haematobium* infection was categorised as light (<50 eggs/10mL) or heavy (≥50 eggs/10mL), as defined by the WHO (WHO, 2022b). Diagnosis of *S. mansoni* was conducted using the Kato-Katz thick smear procedure (Katz *et al.*, 1972). Further information regarding the procedures of parasitological diagnoses is described in **Section 2.2.3**.

6.2.5 Praziquantel (PZQ) treatment during the mass drug administration (MDA)

Regardless of infection status, SAC were co-administered a standard dose of PZQ (40mg/kg) to treat the schistosome infections, and albendazole (ABZ) to treat soil-transmitted helminths at 400mg per child (WHO, 2011a). A PZQ dose pole (further details in **Section 2.2.4**) was used to determine the number of 600mg tablets required to achieve a 40mg/kg PZQ dose. The children were given bread and juice to enhance absorption of the drug and, to ensure compliance, each child was checked by the nurses to confirm they had swallowed the tablet. The number and brand of tablets administered to each child were also recorded in a MDA register to distinguish non-compliance from treatment failure. All children followed up in this study were confirmed to have received PZQ treatment (when recorded as administered) at each MDA using these records. The treatment and health check of the SAC was performed by the nurses and school health coordinators of the national helminth control team, as is the standard Ministry of Health practice in Zimbabwe during the MDA program (Mduluza *et al.*, 2020). Throughout this study, the nomenclature of each MDA will be followed by the number representing the treatment arm, e.g., MDA1. Missing data for the current study was attributed to either completing primary school and progressing to secondary school or children transferring to other schools (Mduluza *et al.*, 2020).

6.2.6 Approaches and identification of hotspots of *S. haematobium*

6.2.6.1 Persistent hotspots of *S. haematobium* prevalence (PPHS)

Early identification of PPHS, for instance before MDA initiation or after a couple of years, could guide programmatic decision-making (Shen *et al.*, 2020). Data collected after three years of MDAs has been used to identify and predict PPHS of *S. haematobium* and *S. mansoni* in multiple studies (Ezeamama *et al.*, 2016, Kittur *et al.*, 2017, Kittur *et al.*, 2019,

Shen *et al.*, 2020). To explore PPHS emergence in Zimbabwe, the MDAs after year three of the program were each evaluated in detail as an end-point of the MDA program (King *et al.*, 2020). Thus, this analysis investigated differences in the timeframe to identify PPHS, either after three years (pre-MDA1 to pre-MDA4), four years (pre-MDA1 to pre-MDA5), or five years (pre-MDA1 to pre-MDA6). Yet, only MDA data from villages/districts that were treated more than twice within four years were assessed for the emergence of PPHS, as described by the Kittur *et al.* analyses (Kittur *et al.*, 2017, Kittur *et al.*, 2019, Kittur *et al.*, 2020). To identify PPHS, two approaches were selected.

Approach A was defined as a site with an unchanged WHO prevalence risk category (high, ≥50%; moderate, 10–49%; and low <10% prevalence) at either the pre-MDA4/5/6 survey compared to pre-MDA1. This approach was based on studies assessing *S. haematobium* and/or *S. mansoni* hotspots in Tanzania, Cote d'Ivoire, Kenya, and Mozambique, and incorporated current WHO guidelines on selecting MDA treatment frequency to control morbidity due to *Schistosoma* infection (Crompton, 2006, Kittur *et al.*, 2017, Kittur *et al.*, 2019). Villages/districts that did not meet the definition of a PPHS using ***Approach A*** were discussed in the context of increasing or decreasing in prevalence. For instance, a decrease in prevalence from 55% in pre-MDA1 to 42% in pre-MDA5 would be a meaningful decline in WHO risk category and not a PPHS. Conversely, a decrease from 42% in pre-MDA1 to 11% in pre-MDA5 would remain in the moderate prevalence WHO risk category and would be designated as a PPHS using ***Approach A***.

Approach B was defined as a site with an infection prevalence ≥ 10% pre-MDA1, which remained ≥ 5% at either the pre-MDA4/5/6 survey. This method was based on a study conducted in Zanzibar to identify hotspots of *S. haematobium* (Kittur *et al.*, 2020).

Villages/districts that did not meet the definition of a PPHS using ***Approach B*** were discussed in the context of increasing or decreasing in prevalence.

6.2.6.2 Hotspots of decreased praziquantel (PZQ) efficacy (EPHS)

Approach C classified a 'lower than expected' CR of <70% and/or an ERR of <90% after treatment with PZQ as an EPHS. The CR threshold for reduced PZQ efficacy was based on a study in Senegal investigating poor responses to PZQ during *S. mansoni* infections (Gryseels *et al.*, 2001). The ERR threshold for reduced PZQ efficacy was based on the WHO threshold (WHO, 2013a).

6.2.7 Drivers of *S. haematobium* hotspots

To determine the differences between a site that responded to an MDA (responder) and a hotspot (either PPHS or EPHS), an assessment of the drivers of a hotspot was performed. This examined whether it was possible to predict a responder site versus a hotspot site using MDA data and the relevant metadata (Kittur *et al.*, 2019). **Table 6.1** below describes the risk factors, the rationale, and the method of analysing these drivers for each hotspot.

Table 6.1: Descriptions of the factors analysed in association with the occurrence of persistent hotspots of *S. haematobium* prevalence (PPHS) and/or hotspots of decreased praziquantel (PZQ) efficacy (EPHS). This includes the rationale for assessing these risk factors, along with the specific details on the method of analysis.

Risk Factor	Rationale	Method of Analysis for Hotspot	
		PPHS	EPHS
Baseline prevalence	Predicting whether a site will become a hotspot based on baseline prevalence or infection intensity would allow for early detection and for program managers to address and remedy problems in schistosome control (Kittur *et al.*, 2020, Shen *et al.*, 2020).	Baseline prevalence at pre-MDA1 in responder districts was compared to districts identified as a hotspot.	
Baseline infection intensity		Baseline mean egg count at pre-MDA1 in responder districts was compared to districts identified as a PPHS.	Baseline mean egg count at pre-MDA1 in responder districts was compared to districts identified as a EPHS. Further, pre-MDA infection intensity of all PZQ efficacy surveys during six years of annual MDAs in Zimbabwe was compared to the resultant CR and ERR after PZQ treatment. This identified if pre-MDA infection intensity affected all PZQ efficacy surveys or was just a driver of EPHS occurrence, as reduced PZQ efficacy tends to be attributed to high pre-treatment infection intensity (Midzi *et al.*, 2008) and thus may not be specific to EPHS by definition.
Distance to the nearest freshwater source	The contribution of the distance to the nearest freshwater source as a *Schistosoma* transmission site was also evaluated as the snail vectors of schistosome infection live in the water; therefore can increase the risk of infection transmission (Angelo *et al.*, 2018).	The distances to the closest waterway were calculated using the 'Distance to the nearest hub' feature in QGIS version 3.22.2 using a shapefile containing location data on the larger bodies of water in Zimbabwe, including the rivers, streams, dams, and lakes, along with the coordinate data of each village sample site. The results were exported to Microsoft Excel to identify the shortest distance from each village to the water for analysis.	
Snail transmission score	The greater the suitability of the snail habitat, the more likely for potential schistosome transmission from a *Bulinus globosus* vector (Pedersen *et al.*, 2014a). Therefore, the spatial distribution of the freshwater snail *Bulinus globosus* was evaluated.	An extrapolation of the spatial distribution of the freshwater snail *Bulinus globosus* in Zimbabwe, the intermediate host involved in the transmission of *S. haematobium,* was performed (Pedersen *et al.*, 2014a). The extrapolated data from the Pedersen *et al.* studies (**Supplementary Table 1 in Appendix 2**) was overlaid with the villages from the current study. This overlay produced a transmission score for each village based on snail habitat suitability based on surveys conducted in 1988 and 2012 in Zimbabwe.	

Coverage of MDA	Coverage determines whether a site is a hotspot or the eligible population was just not adequately treated, resulting in persistent infections (Toor *et al.*, 2018). The WHO defines sufficient MDA coverage as the treatment of 50% of required SAC during the first round of MDA, then 75% for the following MDAs (WHO, 2022b). Poor coverage is among the first factors to evaluate when a site fails to decline in prevalence as expected (Kittur *et al.*, 2020).	The coverage of each MDA in Zimbabwe was obtained on a national and district level (when available) from the ESPEN database (http://espen.afro.who.int/). The coverage of the MDA that resulted in a PPHS at the next survey was highlighted for PPHS districts. If coverage was over 100%, this represented additional treatments during the MDAs, for instance adults and preschool-aged children (PSAC), in the district (ESPEN). For the current study, if the treatment coverage of the district in the previous MDA was low, it was postulated to contribute towards the development of a PPHS due to the sustained time-period for increased transmission and persistence of infections.	Coverage was not considered a contributing factor to the emergence of EPHS as the analysis of PZQ efficacy only included SAC who complied with treatment.
Drug holidays	Drug holidays are defined as a skipped MDA of PZQ treatment during the PCT program. Studies have found that sites with greater numbers of drug holidays have a smaller reduction in schistosome prevalence (King *et al.*, 2020). Fears of decreased drug sensitivity and drug resistance are high for PZQ due to repeated and frequent MDAs to control schistosomiasis (Summers *et al.*, 2022).	The frequency of drug holidays, either ≤3 MDAs or >3 MDAs, of PPHS compared to responder districts was assessed.	The frequency of drug holidays, either ≤3 MDAs or >3 MDAs, of EPHS compared to responder districts was assessed.
Seasonal treatment	The MDAs were administered between September and November, the season of prevalent schistosome transmission (Chandiwana *et al.*, 1987). MDA3 was delayed until January due to implementation issues (described in **Section 6.2.2**). Thus, due to this extended untreated period, the infected individuals harboured and perpetuated the transmission of schistosomiasis for longer during the rainy season (De Leo *et al.*, 2020) than the remaining five MDAs.	This analysis examined the impact of the change of season on PPHS occurrence. As infection prevalence determines the WHO risk category (**Section 1.3**), a comparison of the changes in the WHO risk category in the twelve districts followed at all six MDA surveys were compared to the baseline WHO risk category of the previous mass drug administration (MDA).	Due to a lack of consistent PZQ efficacy surveys in all six MDA (only assessed paired samples pre-MDA to post-MDA), this was not assessed as a predictor for EPHS.

6.2.8 Statistical analysis

The statistical analyses were performed using either SPSS statistical software (IBM, version 23) or Minitab Statistical Software 20. Due to the non-parametric nature of the data, differences in *S. haematobium* prevalence between pre- and post-MDA time points were analysed using a paired McNemar's test, and the differences between annual MDAs were tested for significance using a Fisher's exact test (Gillbricht, 1971). The differences in *S. haematobium* mean egg counts between pre- and post-MDA time-points were analysed using a paired two-way Student's t-test, and the difference between annual MDAs were tested for significance using an unpaired Student's t-test (Mduluza *et al.*, 2020). Additionally, $\chi 2$ tests to investigate significant associations between villages/districts with decreased PZQ efficacy (CR/ERR <100%) and their baseline infection intensity (heavy/light) was performed. The association between a hotspot and either drug holidays or the month of MDA was assessed using Fisher's Exact tests. The association between the hotspot and the baseline infection prevalence and intensity was tested using the Mann-Whitney U-test.

Linear and logistic regressions were also performed to identify potential drivers of PPHS and EPHS using a combined MDA survey dataset. Five separate stepwise linear regression models were used to evaluate the dependent variables (prevalence, mean egg counts, ERR, CR), as described in **Supplementary Tables 2 and 3 in Appendix 2**, with different combinations of predictors assessed. A binary logistic regression model (**Supplementary Table 4 in Appendix 2**) was also used, where 1=schistosome-positive (*S. haematobium* eggs≥1), and 0=schistosome-negative (*S. haematobium* eggs=0), to test the influence of two independent variables (distance to freshwater and snail transmission score) on the detection of schistosome infection. An ordinal logistic regression model (**Supplementary Table 4 in Appendix 2**) was also performed to test the influence of distance and transmission scores on the WHO risk

category. The ordinal dependent variable was coded as 0 = schistosome-negative, 1= low prevalence (<10%), 2 = moderate prevalence (10-49%), and 3 = high prevalence (≥50%). For all analyses in this study, a significant threshold of $P \leq 0.05$ was established.

6.3 Results

6.3.1 Prevalence and mean egg counts during the Zimbabwean control program

Initially, heterogeneity during the Zimbabwean national control program was assessed. Twenty-nine districts were surveyed for *S. haematobium* infection, with the prevalence, arithmetic mean egg count, and proportion of heavy to light infections calculated for each village and district (**Supplementary Table 5 in Appendix 2)**. To observe the progression of *S. haematobium* infection during the MDAs, **Supplementary Figures 1 & 2 in Appendix 2** show the prevalence of each district and the location and infection intensity of each village using a choropleth map. The results below discuss the heterogeneity in the prevalence and infection intensity of each district to observe any clear deviations from MDA success.

6.3.1.1 MDA1

Twenty-five districts (86.2%) were positive for *S. haematobium* at the pre-MDA1 survey in 2012. The baseline prevalence across Zimbabwe based on the twenty-nine districts surveyed was 33.5% (CI: 25-42.2%). The district prevalence ranged from 0% to 73.6%. Pre-MDA1, the mean egg count across the twenty-nine districts was 24.9 (CI: 16.9-32.9) eggs/10mL. The district mean egg count ranged between 0 to 88.9 egg/10mL. Only the Muzarabani district (prevalence of 73.6%) had a pre-MDA1 mean egg count of heavy intensity, having the largest initial parasite burden of the surveyed populations at 88.9 (CI: 75.33-102.53) eggs/10mL. Of the twenty-five districts positive at pre-MDA1, twenty-four significantly reduced in mean egg count ($P<0.05$) by post-MDA1, and twenty-three significantly reduced in prevalence ($P<0.0001$) after PZQ treatment. The district of Mount Darwin had a pre-MDA1 prevalence of 66% and failed to significantly decrease prevalence ($P=0.266$) post-MDA1, reducing to 24.3%. Still, Mount Darwin significantly reduced in mean egg count,

from 49.7 (CI: 39.1-60.3) eggs/10mL to 4.96 (CI: 3.2-6.7) eggs/10mL post-MDA1. However, pre-MDA1, 44% of the Mount Darwin population carried heavy infections. This was fewer heavy infections than the district of Muzarabani, where 66% of the population carried heavy infections; the highest of all surveyed in pre-MDA1. Yet, Muzarabani had cleared all infections post-MDA1. The second exception to significant prevalence reduction was Nkayi, a district of low endemicity in pre-MDA1 at 6.2%, which significantly increased in prevalence ($P<0.0001$) post-MDA1 to 7%. Furthermore, the mean egg count for Nkayi did not significantly change ($P=0.265$) post-MDA1, reducing from 1.14 (CI: 0.19-2.1) eggs/10mL to 0.77 (CI: 0.33-1.2) eggs/10mL. Overall, post-MDA1, five (17.2%) districts in Zimbabwe still carried infection: Mount Darwin, Nkayi, Rushinga, Shamva, and Mwenzi.

6.3.1.2 MDA2

Twelve districts were surveyed pre-MDA2, and seven (58.3%) were positive for *S. haematobium.* Only five districts significantly decreased in prevalence ($P<0.0001$), and four districts significantly reduced in mean egg count ($P<0.05$) post-MDA2. Muzarabani, which had the highest prevalence pre-MDA1, significantly increased in prevalence ($P<0.0001$) by 6.1% post-MDA2 compared to pre-MDA2. All were light infections, with a significant increase in mean egg count ($P<0.01$) of 0.06 (CI: 0.02-0.1) eggs/10mL. Additionally, the UMP district significantly increased in prevalence ($P<0.0001$) from 6.8% to 11.2% at post-MDA2, increasing from a low to moderate WHO risk category. Also, UMP did not significantly decrease in mean egg count (0.17 to 0.15 eggs/10mL, $P=0.725$) at post-MDA2, although there was a significant decrease at pre-MDA2 (0.17 (CI: 0.05-0.29) eggs/10mL) compared to pre-MDA1 (48.37 (CI: 36.07-60.66) eggs/10mL). The district of Nkayi increased in both prevalence (by 1.2%) and mean egg count (by 1.19 eggs/10mL) from pre-MDA1 to pre-MDA2, with heavy infections increasing by 9% despite one round of MDA

with PZQ. Both increases were insignificant (P=0.719, P=0.14) in Nkayi, and all infections were cleared post-MDA2. The Rushinga district significantly decreased in prevalence (P<0.0001) from 21.6% to 17.6%, however, it remained moderately endemic post-MDA2. Rushinga also increased in mean egg count (P=0.067) post-MDA2, from 0.75 (CI: 0.49-1) eggs/10mL to 0.98 (CI: 0.62-1.35) eggs/10mL, with all infections classified as light. Post-MDA2, three (17.2%) districts in Zimbabwe still carried schistosome infections: Muzarabani, Rushinga, and UMP.

6.3.1.3 MDA3

Twelve districts were surveyed pre-MDA3, and seven (58.3%) were positive for *S. haematobium.* Three districts were reinfected compared to pre-MDA2. The Mount Darwin and Muzarabani districts were negative for infection pre-MDA2 but significantly increased in prevalence (P<0.0001) at pre-MDA3 to 11.2% and 10.4%, respectively. The Murewha district increased from 0% prevalence in MDA2 to 2%, remaining in low endemicity. Similarly, pre-MDA3, the Rushinga and Mberengwa districts significantly increased in prevalence and mean egg count (P<0.05) compared to pre-MDA2. Post-MDA3, four of the seven infected districts significantly decreased in prevalence (P<0.0001) and mean egg count (P<0.05), except for Mutoko, Mount Darwin and Mwenzi. Mutoko significantly reduced in mean egg count (P<0.05), but only decreased from 1.8% to 0.9% in prevalence (P=0.509) Mount Darwin did not significantly change in mean egg count (2.27 to 2.33 eggs/10mL, P=0.729), and there was a 2% increase (from 17% to 19%) in heavy infections post-MDA3 compared to the pre-MDA3. The Mwenzi district significantly decreased in prevalence (P<0.0001) but did not significantly decrease mean egg count (P=0.894) post-MDA3, although the infection intensity was very low (0.002 eggs/10mL). The Chiredzi district significantly increased in prevalence (P<0.0001) from 0% to 0.46% with no significant

increase in mean egg count (P=0.318), and all were light infections. Overall, after three rounds of MDA, there were five (17.2%) districts in Zimbabwe still carrying infection, although the mean egg counts were all <2.5 eggs/10mL: Mount Darwin, Nkayi, Chiredzi, Mutoko, and Mwenzi.

6.3.1.4 MDA4

Twenty-two districts were surveyed pre-MDA4, and only the Mount Darwin district (4.6%) was positive for *S. haematobium*. Post-MDA4, both prevalence (P<0.0001) and mean egg count (P<0.01) of Mount Darwin significantly reduced, with no infection detected. However, pre-MDA4, this district's prevalence was not significantly different from pre-MDA3, at 7.8% (P=0.312). Further, despite successive MDAs, both the Mutoko and UMP districts significantly increased in prevalence (P<0.0001) by approximately 0.4% post-MDA4. However, neither district significantly increased in mean egg count and all were light infections.

6.3.1.5 MDA5

Pre-MDA5, two of the twelve districts surveyed (16.7%) were positive for *S. haematobium*, Chiredzi and UMP, and neither cleared infection post-MDA5. Chiredzi, a district with zero or low infection prevalence for the past two MDAs, returned to moderate endemicity (~12.3%), similar to the pre-MDA2 survey. Post-MDA5, Chiredzi significantly reduced prevalence (P<0.0001) to 0.9%, and mean egg count (P<0.0001) to 0.003 (CI: 0-0.01) eggs/10mL. UMP had a low prevalence of 0.4% at pre-MDA4. Post-MD4, the prevalence did not change and remained at 0.4%, with no significant differences in mean egg count either (P=0.318). The Mutoko district was negative for *S. haematobium* pre-MDA5, yet the prevalence of infection increased to 0.9% post-MDA5. However, the mean egg count did not significantly increase (P=0.158), with an average of 0.003 (CI: 0-0.01) eggs/10mL. All schistosome infections

detected both pre- and post-MDA5 were designated as light. Post-MDA5 survey, Chiredzi, UMP and Mutoko were all positive for infection.

6.3.1.6 MDA6

Nine (37.5%) of the twenty-four districts surveyed pre-MDA6 were positive for *S. haematobium*, with the prevalence ranging from 0.2% to 11.2% across the infected districts. All nine districts significantly reduced in prevalence (P<0.0001) post-MDA6, and the only districts not significantly reduced in mean egg count were those with a low initial egg count (<0.3 eggs/10mL) at pre-MDA6. Only the Chipinge district had a prevalence >10%, pre-MDA6, classifying it as moderately endemic, and post-MDA6 all districts had cleared schistosome infection. Compared to the pre-MDA1 survey, the overall prevalence and infection intensity of the country at pre-MDA6 was significantly reduced (P<0.0001) to 1.47% (CI: 0.26-2.67%) and 1.39 (CI: 0-3.9) eggs/10mL, respectively, as depicted below in **Figure 6.2.**

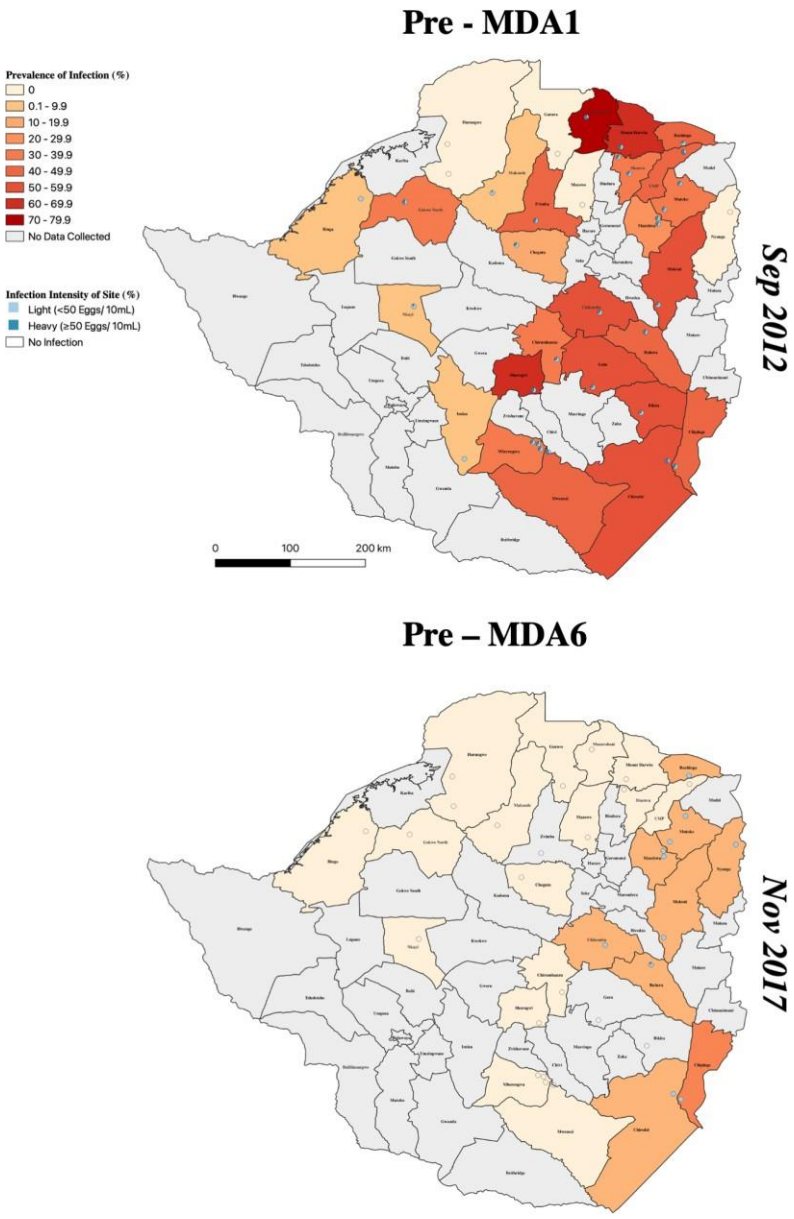


Figure 6.2: Choropleth maps depicting the geographical distribution of the prevalence of *S. haematobium* at the pre-MDA1 and pre-MDA6 surveys. The prevalence of each district is coloured to represent the prevalence (%) at that timepoint, with the village site locations highlighted by a circular data point. These data points also represent the proportion of infection intensities in the survey, with the circular pie chart indicating what percentage of infections were light (light blue) or heavy (dark blue). The date on which the MDA was carried out is depicted on the right-hand side of the choropleth maps. Both maps were generated using QGIS, Version [3.22.2]. MDA: mass drug administration.

To conclude, upon the post-MDA6 survey in 2017, the national prevalence of Zimbabwe across all districts was 0%.

6.3.2 Praziquantel (PZQ) efficacy during the Zimbabwean control program

For each MDA, the CR and the ERR were calculated to evaluate PZQ efficacy for each village and district. The PZQ efficacy per district is collated in **Supplementary Table 6 in Appendix 2** and includes forty-nine surveys of PZQ efficacy. Twenty-four districts were included from MDA1 (82.8%), seven districts from MDA2 (58.3%), five districts from MDA3 (41.6%), one district from MDA4 (4.6%), two districts from MDA5 (16.7%), and seven districts from MDA6 (29.2%). The CRs and ERRs are visualised on a map of each district in Zimbabwe in **Supplementary Figures 3 and 4 in Appendix 2**, respectively, and display the intensity of infection in every village.

The sample sizes for each survey ranged between $1 \leq n \leq 236$, and forty of the forty-nine assessments of PZQ efficacy achieved a 100% CR and ERR. Thus, of the 2988 participants included in the analysis of PZQ efficacy during the six years of the schistosomiasis control program, only 504 participants had reduced PZQ efficacy measures. Reduced PZQ efficacy measures (CR/ERR<100%) were detected across six districts in Zimbabwe, as displayed in **Table 6.2**.

Table 6.2: Measures of praziquantel efficacy (PZQ) after a mass drug administration (MDA) of praziquantel (PZQ) to treat *Schistosoma haematobium* infections.

District (Village)	Mt Darwin (Bemberi)		Rushinga (Mazowe Bridge)		Nkayi (Gonye)	Chiredzi (Mareya)
MDA	**1**	**3**	**1**	**2**	**1**	**5**
Sample Size	126	9	110	23	5	27
Cure Rate (%)	68.25	22.22	99.09	43.48	0	96.29
Egg Reduction Rate (%)	91.72	28.57	99.99	58.91	61	99.06
Pre-Mean Egg Count (95% CI)	80.13 (65-95.27)	25.93 (7.16-44.69)	43.89 (30.78-57)	3.74 (2.22-5.26)	37.27 (-10.08-84.61)	1.31 (1.03-1.59)
Post-Mean Egg Count (95% CI)	6.64 (4-9.27) [A]ii	18.52 (1.61-35.43) [A]i	0.005 (0-0.01)	1.54 (0.3-2.77)	14.53 (4.16-24.91)	0.01 (-0.01-0.04)
Light: Heavy Infections (%)	56:44	78:22	81:19	100:0	60:40	100:0

District (Village)	UMP (Kafura)		Shamva (Total)		Shamva (Gono)	Shamva (Chihuri)
MDA	**2**	**5**	**1**		**1**	**1**
Sample Size	9	1	194		149	45
Cure Rate (%)	66.67	0	97.42		96.64	100
Egg Reduction Rate (%)	82.41	4.17	99.63	**Village Analysis**	99.57	100
Pre-Mean Egg Count (95% CI)	4 (1.45-6.55)	8 (8-8) [B]	76.76 (58.21-95.31)		85.58 (62.7-108.45)	47.56 (22.49-72.63)
Post-Mean Egg Count (95% CI)	0.7 (0.11-1.3)	7.67 (7.67-7.67) [B]	0.28 (-0.11-0.68)		0.37 (-0.15-0.88)	0 (0-0)
Light: Heavy Infections (%)	100:0	100:0	66:34		64:36	76:24

The cure rate (CR) and egg reduction rate (ERR) are presented, along with the mean egg count per 10mL of urine for the baseline and follow-up surveys. Mean egg counts expressed 95% confidence interval (CI). [A] No significant change in unpaired test, from post-MDA to post-MDA, [B] No significant change in the paired test between pre-MDA and post-MDA, i) compared to MDA1 ii) compared against MDA3.

In **Table 6.2**, four of the surveys with reduced efficacy measures occurred in MDA1 (44.4%), two in MDA2 (22.2%), one in MDA3 (11.1%), and two in MDA5 (22.2%). CRs ranged from 0% to 99.1%, and ERR ranged from 4.2% to 99.9% among the districts with reduced PZQ efficacy measures. The UMP and Nkayi districts had a 0% CR, although they had a sample size of 1 and 5, respectively. Neither had a significant mean egg reduction pre- to post-MDA. For UMP, pre- to post-MDA, the ERR was minimal at 4.2%. In Nkayi, the ERR was 61%, yet the post-mean egg count was 14.53 (CI: 4.16-24.91) eggs/10mL. All other districts evaluated in this efficacy assessment significantly reduced mean egg count upon PZQ treatment, despite not clearing schistosome infection.

The Mashonaland Central province had the greatest number of surveys with reduced PZQ efficacy, with Mount Darwin, Rushinga and Shamva recording reduced CRs and ERRs. In MDA1, Mount Darwin had a CR of 68.3% and ERR of 91.7%. This district had a pre-MDA1 mean egg count of 80.13 (CI: 65-95.27) and 44% were heavy infections, the highest of any of the surveys in this analysis of reduced PZQ efficacy. Additionally, pre-MDA3, this district had 22% heavy infections, even after two rounds of MDAs, and was the only survey to remain above a mean egg count >15 egg/10mL at the follow-up efficacy survey.

Of the nine surveys with reduced PZQ efficacy, only one district had more than one village contribute to the efficacy calculations: Shamva. The district had a mean CR of 97.4% and ERR of 99.6%, with a heavy initial infection intensity of 76.76 (CI: 58.21-95.31) eggs/10mL. Shamva had two villages sampled, firstly Chihuri which had 100% CR/ERR and an initial light mean egg count of 47.56 (CI: 22.49-72.63). The other village, Gono, had a CR/ERR of 96.6% and an initial heavy mean egg count of 85.58 (CI: 62.7-108.45).

6.3.3 Hotspots of *S. haematobium* in Zimbabwe

The emergence of persistent hotspots (PHS) of *S. haematobium* infection in Zimbabwe was investigated using the prevalence, infection intensity, and measures of PZQ efficacy (CR/ERR) calculated above. To identify hotspots of *S. haematobium* infection, we separated the hotspot analysis into two (PPHS and EPHS).

6.3.3.1 Persistent hotpots of *S. haematobium* prevalence (PPHS)

Two approaches were utilised to assess PPHS. Briefly, ***Approach A*** classified PPHS as villages/districts with no change in a WHO prevalence category at either pre-MDA4/5/6 from pre-MDA1, and ***Approach B*** classified PPHS as villages/districts with a prevalence ≥ 10% pre-MDA1 that remained ≥ 5% at either pre-MDA4/5/6.

The analysis of potential PPHS based on changes in prevalence from pre-MDA1 to pre-MDA4/5/6 for each of the twenty-nine districts is in **Table 6.3.**

Table 6.3: The classification of districts based on their prevalence between multiple mass drug administration (MDA) time-periods to identify persistent hotpots of *S. haematobium* prevalence (PPHS).

		Classification							
	Time-period to Assess Hotspots	No Infection Detected	Declined One WHO Category	Declined Two WHO Categories	Cleared Infection	Increased One WHO Category	No Data Available	PPHS	Total Number of Districts
Approach A	MDA1 to MDA4	4	0	1	17	0	7	0	29
	MDA1 to MDA5	0	2	0	10	0	17	0	
	MDA1 to MDA6	3	4	3	10	1	7	**1**	
Approach B	MDA1 to MDA4	4	0	0	17	0	7	**1**	
	MDA1 to MDA5	0	1	0	10	0	17	**1**	
	MDA1 to MDA6	3	3	3	10	1	7	**2**	

To determine whether analysing the data by village would reveal a more precise mapping of these hotspots, the analysis of potential PPHS was also conducted on a village level, as seen in **Supplementary Table 7 in Appendix 2**. There were no differences in PPHS identification

between the village and district assessments. However, there were differences in PPHS identification between the two approaches, as outlined in **Table 6.4** below.

Table 6.4: Results of the two approaches to identify persistent hotspots of *S. haematobium* prevalence (PPHS).

	Approach A	**Approach B**			
MDA	***1 to 6***	***1 to 4***	***1 to 5***	***1 to 6***	
District (Village)	**Chipinge (Chitepo)**	Mount Darwin (Bemberi)	Chiredzi (Mareya)	**Chipinge (Chitepo)**	Buhera (Masocha)
Number of MDAs received by PPHS identification	**2**	3	5	**2**	2

Approach A: Based on the lack of change in WHO prevalence category, based on the Kenyan and Tanzanian studies (Shen *et al.*, 2020), Approach B: Based on prevalence ≥10% pre-MDA1 and remained ≥5% pre-MDA4/5/6, based on a study in Zanzibar (Kittur *et al.*, 2020). The **bold** text indicates the PPHS detected using both approaches.

Using ***Approach A***, one PPHS was detected in the village of Chitepo in the Chipinge district, based on the comparison of prevalence at pre-MDA1 to the pre-MDA6 survey. This PPHS had an initial *S. haematobium* prevalence of 48.8% (moderately endemicity), yet Chipinge remained moderately endemic at 11.2% at pre-MDA6. Despite this, Chipinge significantly reduced prevalence from pre-MDA1. ***Approach B*** detected four PPHS, also identifying the village of Chitepo in the Chipinge district as a PPHS based on the comparison of prevalence at pre-MDA1 to the pre-MDA6 survey. Masocha in the Buhera district was also identified in pre-MDA6 as a PPHS. Further, ***Approach B*** detected the village of Bemberi in Mount Darwin and the village of Mareya in Chiredzi as potential PPHS using the pre-MDA4 and pre-MDA5 surveys, respectively. Some districts did not fulfil either ***Approach A*** or ***Approach B's*** definition of a PPHS but were of interest (**Table 6.3**). For instance, the district of Nyanga increased by one WHO risk category from 0% prevalence in pre-MDA1 to a

prevalence of 4.5% in pre-MDA6. However, this did not fit into the definition of a PPHS utilised in this study. By combining the results from both approaches, four PPHS were detected during the six rounds of MDAs in Zimbabwe, with each PPHS presented in **Figure 6.3**.

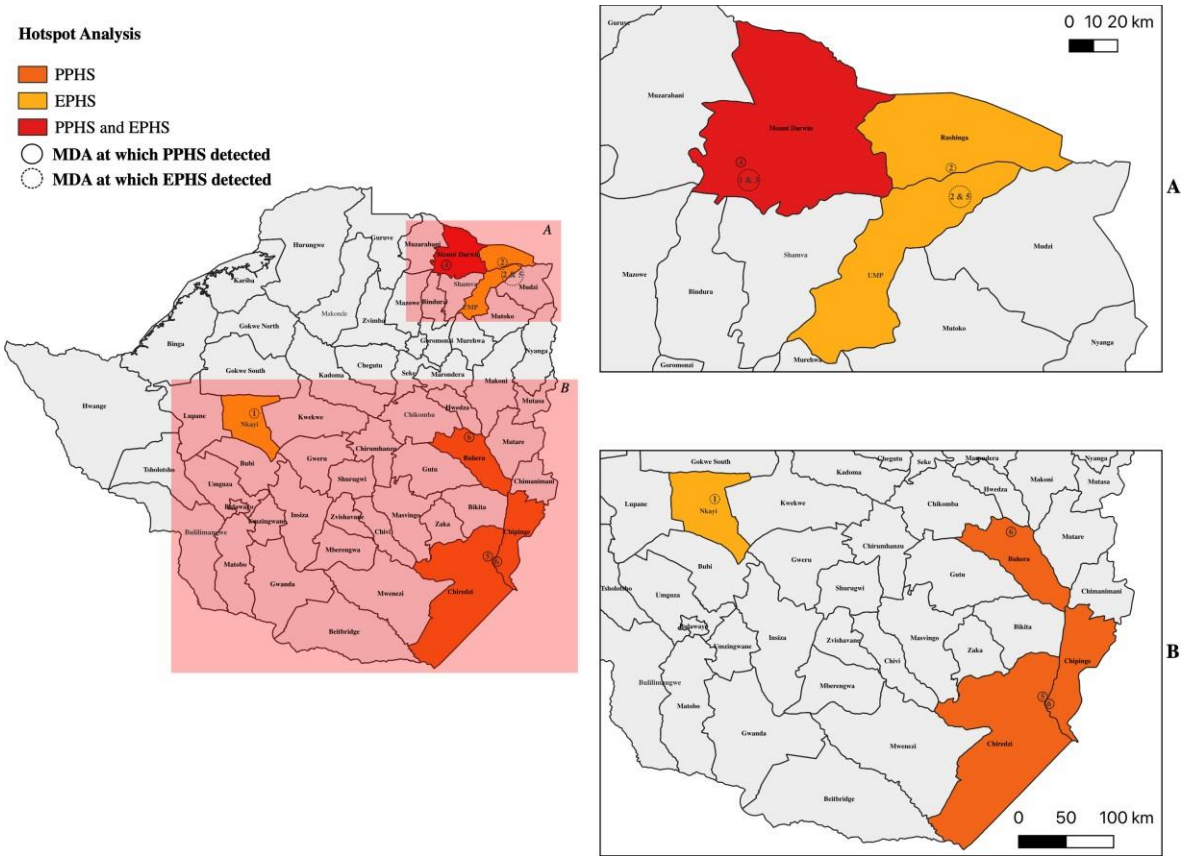


Figure 6.3: Map of Zimbabwe highlighting the districts detected as persistent hotspots of *S. haematobium* prevalence (PPHS), hotspots of decreased praziquantel (PZQ) efficacy (EPHS), and districts of both PPHS and EPHS. The villages where the surveys were conducted are labelled with a circle, containing the number of the mass drug administration (MDA) survey at which the hotspot was detected.

6.3.3.2 Hotspots of decreased praziquantel efficacy (EPHS)

To identify EPHS using this MDA data, ***Approach C*** was used to classify the villages/districts with a CR <70% and/or an ERR <90% based on the surveys in **Table 6.2**. Six EPHS were identified across four districts, as presented in **Figure 6.3**. The EPHS, along with the MDA it was detected, were Nkayi (MDA1), UMP (MDA2 & MDA5), Rushinga (MDA2), and Mount Darwin (MDA1 & MDA3). Notably, apart from Mount Darwin in MDA1, all the EPHS detected had a sample size of n<25 individuals.

6.3.3.3 Correlations between persistent hotpots of *S. haematobium* prevalence (PPHS) and hotspots of decreased praziquantel efficacy (EPHS)

As visualised in **Figure 6.3**, only one district detected both a PPHS and EPHS, however they were not identified in the same MDA. The EPHS in Mount Darwin were detected in MDA1 and MDA3, and the PPHS was detected in MDA4. Further, the remaining PPHS did not correlate with the EPHS detected, indicating decreased PZQ efficacy was not a primary contributor to PPHS emergence, thus additional drivers of persistent infections required investigation.

6.3.4 Potential causes of a hotspot

To determine the differences between a site that responded to an MDA (responder) and a hotspot (either PPHS or EPHS), an analysis of the drivers of a hotspot was conducted to determine if a responder site versus a hotspot site could be predicted based on relevant metadata. To simplify this assessment, the analysis took place on a district level.

6.3.4.1 Baseline prevalence and infection intensity

Beginning with PPHS risk factors, **Figure 6.4** shows the pre-MDA1 prevalence and mean egg counts of responder districts compared to those labelled a PPHS during this study using both PPHS approaches.

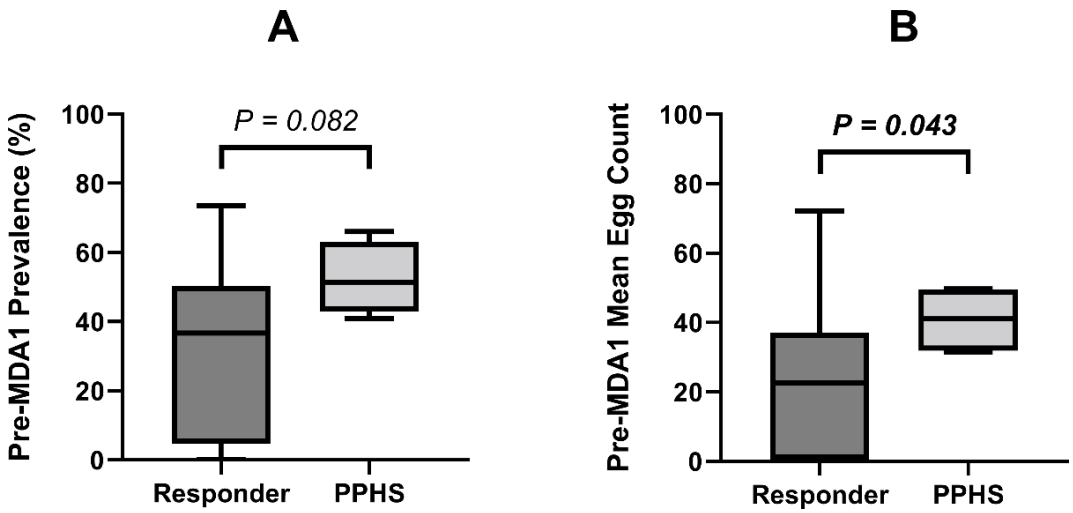


Figure 6.4: Box plots showing the baseline prevalence and mean egg count at pre-MDA1 in responder districts compared to districts identified as persistent hotspots of *S. haematobium* prevalence (PPHS). [A] Compares baseline prevalence, [B] Compares the baseline mean egg count. The PPHS were identified using the approaches of classification described in detail in this study. The Mann–Whitney U-test P-values are indicated between the two groups. MDA: mass drug administration.

The median prevalence for PPHS was higher than in responder districts, as seen in **Figure 6.4A**, however no significant relationship was detected (P=0.082). Conversely, as seen in **Figure 6.4B**, the median mean egg count of PPHS compared to responder districts were significantly higher (P=0.043).

Concerning the drivers of EPHS, **Figure 6.5** displays the pre-MDA1 prevalence and mean egg counts of responder districts compared to those categorised as a EPHS.

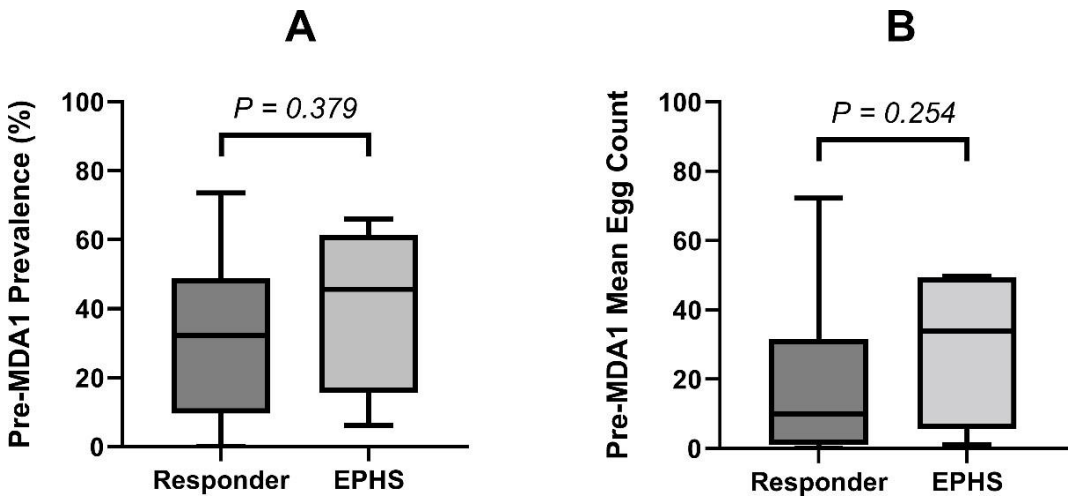


Figure 6.5: Box plots showing baseline prevalence and mean egg count at pre-MDA1 in districts that were responders compared to those that were hotspots of decreasing praziquantel efficacy (EPHS). [A] Compares baseline prevalence, [B] Compares the baseline mean egg count. The EPHS were identified using the approaches of classification described in detail in this study. The Mann–Whitney U-test P-values are indicated between the two groups. MDA: mass drug administration.

The median prevalence and mean egg count for EPHS were higher than responder districts, as seen in **Figure 6.5A and Figure 6.5B**, however no significant relationship was detected (P=0.379, P=0.254).

Additionally, as a known EPHS risk, pre-MDA infection intensity (**Section 6.2.7**) was further assessed as to whether the pre-MDA intensity of infection was the cause of any reduced CRs/ERRs, as well as the districts designated as EPHS. Firstly, the mean egg count pre-MDA was plotted against the resultant CR in **Figure 6.6A** and the ERR in **Figure 6.6B**.

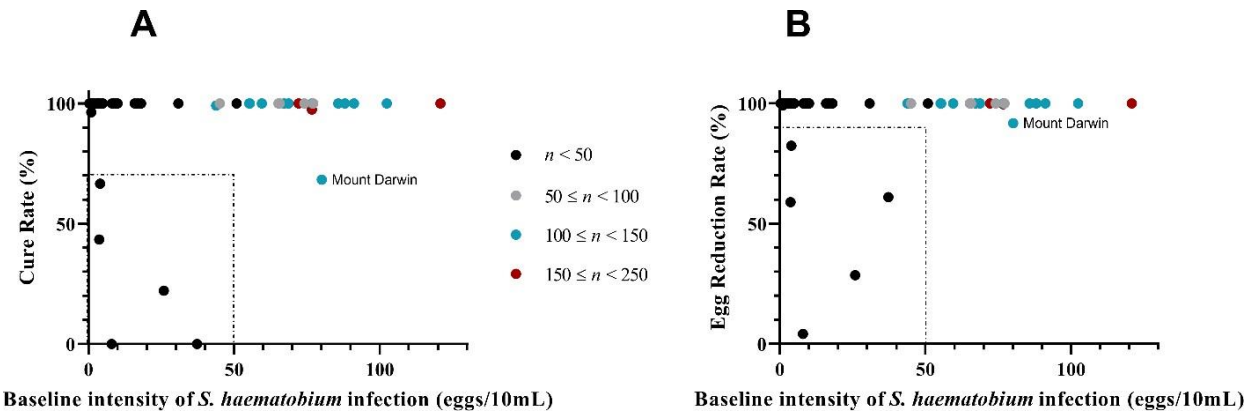


Figure 6.6: **The relationship between pre-treatment intensity of *Schistosoma haematobium* infection with the [A] cure rate (CR) and [B] egg reduction rate (ERR) after praziquantel (PZQ) treatment during six years of annual mass drug administrations (MDAs) in Zimbabwe.** The baseline intensity of eggs of individuals is given per 10mL of urine. CR denotes the percentage of *S. haematobium*-positive individuals treated with PZQ who were negative for eggs at the follow-up survey. ERR measures the effect of a PZQ treatment on all the infected individuals. The colour of the dots represents the different sample sizes (n) of the surveys: black, n <50; grey, 50≤n≤99; blue, 100≤n≤149; red, 150≤n≤249. The dotted area in which studies had either a [A] CR <70% and [B] ERR <90%, and only light pre-treatment infections in the assessed population (<50 eggs/10mL).

As shown in **Figure 6.6**, most surveys had 100% CR/ERR or very close to this value regardless of baseline infection intensity. The only EPHS with a heavy intensity of infection was Mount Darwin in MDA1. The lack of PZQ efficacy in Mount Darwin was postulated to be because of these heavy infections, however there was no significant association between infection intensity and status (P=0.172). $\chi 2$ tests found no significant association between the intensity of infection in the pre-MDA survey with infection intensity in the post-MDA survey. The remaining five EPHS carried light infections (**Figure 6.6**, dotted area), and all these surveys had a sample size n <50.

6.3.4.2 Transmission risk factors

An analysis of the relationship between known PPHS risks, the distance to the nearest waterway and either the snail transmission scores from 1988 (Model 1) or 2012 (Model 2) was performed using separate stepwise linear regression models (**Supplementary Table 3 in Appendix 2**). There were no significant predictors for prevalence (Model 1 [R^2=0.005, P=0.744], Model 2 [R^2=0.009, P=0.581]) or mean egg count (Model 1 [R^2=0.001, P=0.948], Model 2 [R^2=0.004, P=0.778]). Additionally, neither the binary nor ordinal logistic regression models had a significant relationship between the distance to the nearest waterway and either the snail transmission scores from 1988 (Model 1) or 2012 (Model 2) and schistosomiasis status (Model 1 [NR^2=0.008, P=0.684], Model 2 [NR^2=0.022, P=0.363]) or WHO risk category (Model 1 [NR^2=0.012, P=0.528], Model 2 [NR^2=0.22, P=0.289]) to distinguish a responder compared to a PPHS (**Supplementary Table 4 in Appendix 2**).

Furthermore, an analysis of the EPHS risks, the distance to the nearest waterway and either the snail transmission scores from 1988 (Model 3) or 2012 (Model 4) was performed using separate stepwise linear regression models (**Supplementary Table 3 in Appendix 2**). There were no significant predictors for CR (Model 3 [R^2=0.113, P=0.2] or ERR (Model 3 [R^2=0.151, P=0.085], Model 4 [R^2=0.152, P=0.084]). Despite each model's insignificance, the distance to the nearest waterway indicated it may be a significant predictor. Consequently, Model 5 (**Supplementary Table 3 in Appendix 2**) used distance to the nearest waterway as a singular predictor for both CR and ERR, with no significant relationship with CR (Model 5 [R^2=0.072, P=0.0504]). However, there was a significant relationship between ERR and distance to the nearest waterway of the sample sites (Model 5 [R^2=0.106, P=0.016]), as can be seen in **Figure 6.7**, however the low R^2 value indicated distance did not account for the variation in ERR.

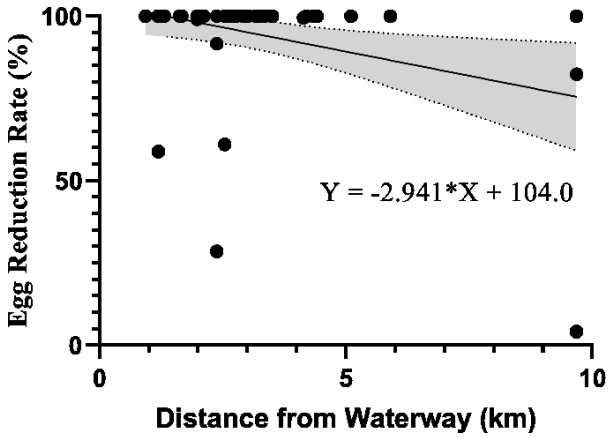


Figure 6.7: Linear regression of the relationship between the egg reduction rate and distance to the nearest waterway. This model was significant (R^2=0.106, P=0.016). The shaded area represents the 95% confidence interval (CI).

Based on Model 5's prediction, the further the sampling site from a potential transmission waterway, the lower the ERR. Nevertheless, based on **Figure 6.7,** it appears to be the occurrence of outliers which have skewed the model to significance rather than a pattern in the MDA dataset.

6.3.4.3 Coverage

The WHO defines sufficient MDA coverage as the treatment of 50% of required SAC during the first round of MDA, then 75% for the following MDAs (WHO, 2022b). In **Figure 6.8** the national and district coverage for each MDA where a PPHS was detected was assessed (**Section 6.2.7**).

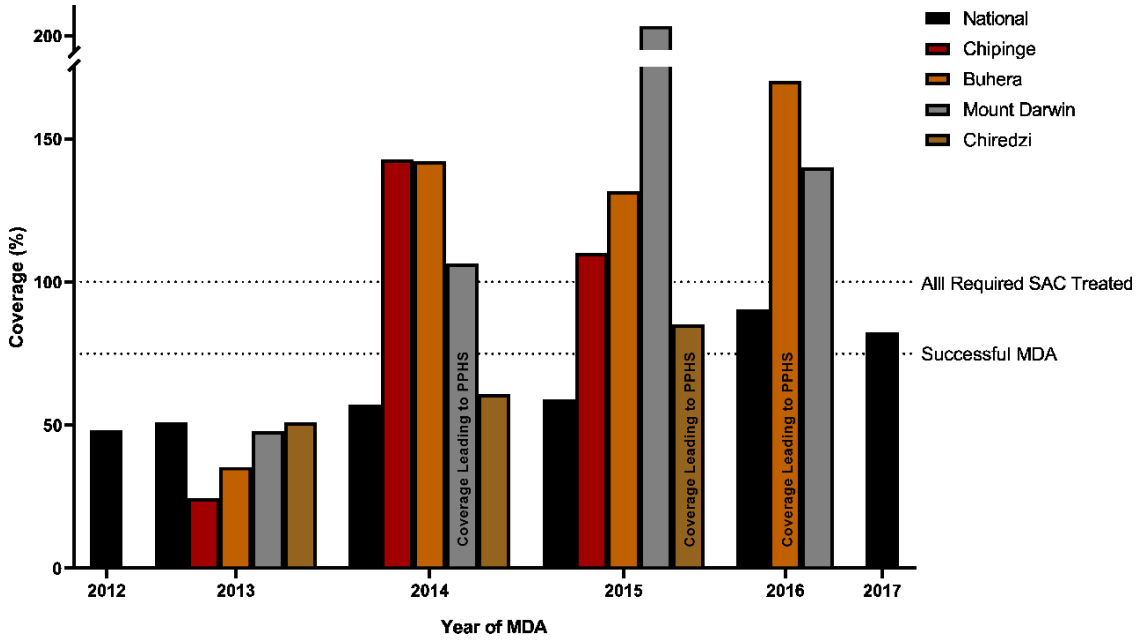


Figure 6.8: **Coverage of the mass drug administration (MDA) of praziquantel (PZQ) in Zimbabwe on a national and district level, for the detected persistent hotspots of *S. haematobium* prevalence (PPHS).** The coverage of the MDA that resulted in a PPHS at the next survey has been highlighted for these districts. This did not include when there was no infection detected or the coverage data was not available on a district level. The dotted line labelled 'Successful MDA' represents the threshold of 75% of the eligible SAC treated with praziquantel (PZQ) during the MDA. The second dotted line labelled 'All Required SAC Treated' represents the threshold of 100% of the eligible SAC treated with PZQ during the MDA, thus anything above represents additional treatments for instance adults and preschool-aged children (PSAC), in the district (ESPEN, 2022).

For the PPHS detected in the districts of Mount Darwin and Chiredzi, the national coverage in the years before their detection was below the recommended threshold for a successful MDA, at 57% and 59%, respectively. However, the coverage for both districts was above the required threshold (>75%) and hence was not considered responsible for the emergence of these PPHS. For both PPHS detected in MDA6, the national MDA coverage was 90.3% the

year before detection, and above the required threshold, thus was not considered a primary driver of PPHS emergence. Coverage was not considered a contributing factor to the emergence of EPHS as analysis of PZQ efficacy was based upon treatment compliance (**Section 6.2.7**).

6.3.4.4 Drug Holidays

The relationship between the frequency of treatment and the emergence of hotspots was assessed, focusing on the impact of drug holidays (no MDA). The proportion of PPHS that had multiple drug holidays versus those that did not is shown in **Table 6.5**.

Table 6.5: A comparison of the proportion of persistent hotspots of *S. haematobium* prevalence (PPHS) compared to the proportion of responder sites based on whether they received either more or less than three drug holidays. This was based on the number of mass drug administrations (MDAs) each district received, with the districts that received ≤3 MDAs designated as having multiple drug holidays.

Drug Holidays	No (> 3 MDAs)	Yes (≤ 3 MDAs)	P-Value
PPHS	1.8%	5.4%	0.422
Responders	41.1%	51.8%	

Significance based on Fisher's Exact Test.

This analysis found that districts that received three or fewer MDAs were more likely to be PPHS than those that received more than four MDAs, although the difference between the two groups was not significant (P=0.422). Analysis of the impact of drug holidays on EPHS is presented in **Table 6.6**.

Table 6.6: A comparison of the proportion of hotspots of decreasing PZQ efficacy (EPHS) compared to the proportion of responder sites based on whether they received either more or less than three drug holidays. EPHS are sites with cure rates (CRs) <70% and/or egg reduction rates (ERRs) <90%. Responder sites were designated as 70%≤ CR ≤100% and/or 90%≤ ERR ≤100%.

Drug Holidays	No (> 3 MDAs)	Yes (≤ 3 MDAs)	P-Value
EPHS	8.7%	4.3%	0.602
Responders	60.9%	26.1%	

Significance based on Fisher's Exact Test.

There was no significant relationship (P=0.602) between MDA drug holidays and whether the site was designated as an EPHS.

6.3.4.5 Seasonal Treatments

To determine whether the change in treatment regimen was impacted by the month of MDA (further rationale in **Section 6.2.7**), **Table 6.7** monitored the impact of each MDA on the WHO risk category of the twelve districts followed in every MDA.

Table 6.7: A comparison of the changes in the world health organization (WHO) risk category of twelve districts compared to the baseline WHO risk category of the previous mass drug administration (MDA).

	Change in WHO Risk Category compared to baseline in the previous MDA						FE to MDA2-3
Month of Treatment	**Declined 1**	**Declined 2**	**Increased 1**	**Increased 2**	**No Change**	**No Infection**	**P-Value**
October (MDA1-2)	4	1	0	0	2	5	0.097
January (MDA2-3)	**0**	**0**	**2**	**2**	**3**	**5**	-
November (MDA3-4)	1	0	0	0	0	11	0.005
November (MDA4-5)	0	0	1	1	0	10	0.186
November (MDA5-6)	1	0	3	0	0	8	0.126

Significance was based on multiple Fisher's Exact Test (FE) based on 2x2 contingency tables, assessing the change in MDAX-Y compared to MDA2-3. WHO: World health organization, MDA: Mass drug administration.

Compared to MDA2, four districts in this study increased in their WHO risk category in MDA3, with two of those increasing by two WHO risk categories. Furthermore, between MDA2 and MDA3, no districts exhibited a decline in the WHO risk category. MDA3 also had the lowest number of districts with 'no infections', tying with the initial survey in MDA1. There was a significant difference between the number of districts that changed WHO risk category in MDA2-3 compared to those who altered in MDA3-4 (P=0.005). However, this was determined to be due to the low number of infected districts in MDA4 rather than the date of the MDA. Overall, the change in treatment administration date was not determined to significantly change the number of districts that increased in WHO risk category, thus was not a key contributing factor to PPHS emergence, although an impact was observed due to the change of treatment month in MDA3.

6.4 Discussion

PCT programs and the implementation of MDAs are supported by numerous organisations and are vital in the fight for global control and the eventual elimination of schistosomiasis (Olveda *et al.*, 2016). Therefore, the identification of hotspots, such as those in this study, is of particular importance to inform on drug failures and determine risk factors that perpetuate schistosome transmission (Drew, 2016) to aid in the early detection of hotspots in the future. In the current study, the analyses and the term 'hotspot' were separated by the measure of the hotspot: i) persistent hotspot of *S. haematobium* prevalence (PPHS) and ii) hotspot of decreased PZQ efficacy (EPHS). This chapter also identified what factors, if any, contribute to the occurrence of hotspots (PPHS or EPHS) compared to sites that responded as expected to MDA treatment (responders). This particularly focused on whether decreased PZQ efficacy was responsible for PPHS, or if there are other drivers of persistent infections. Furthermore, what factors, if any, contributed to the occurrence of EPHS. To date, there have been no assessments of PPHS or EPHS in Zimbabwe using MDA data.

Four PPHS were identified in the surveyed villages of the Mount Darwin, Chiredzi, Chipinge, and Buhera districts in Zimbabwe using ***Approach A*** & ***Approach B***. Additionally, six EPHS were identified in the surveyed villages of the Nkayi, UMP, Rushing and Mount Darwin districts in Zimbabwe using ***Approach C***. There were minimal parallels between the PPHS and the EPHS detected, with no strong relationship between the two classifications detected during this current study. For the PPHS detected in the Buhera and Chipinge districts, neither reported reduced CR/ERR in any MDA (no EPHS). There was a case of decreased PZQ efficacy detected in Chiredzi in MDA5, in the same MDA as the PPHS identification, however it did not fulfil the definition of a EPHS. Further, in the district of Mount Darwin, where two EPHS were identified, both EPHS occurred before the PPHS identification was

made in MDA4. There may be associations between the number of EPHS detected in a district before a PPHS occurred, but due to the lack of correlation in other PPHS, this was not determined to be the case here. The lack of concurrence between the two types of hotspots highlights the need to characterise the drivers of PPHS and EPHS to investigate if more targeted interventions are required.

Thus, the analysis of the drivers of PPHS occurrence began with assessments of the measures used to calculate PPHS. The pre-MDA1 prevalence and mean egg count have been evaluated as predictors of PPHS in multiple other hotspot analyses (Kittur *et al.*, 2019, Kittur *et al.*, 2020). If the baseline measurements of PPHS were significantly different from responders, the identification of PPHS could be performed using the baseline survey. In the current study, the prevalence was relatively higher in PPHS districts compared to the responders, but it was not significant. This concurred with four studies on PPHS in Africa which also found no statistical differences between the baseline prevalence of PPHS and responder sites (Kittur *et al.*, 2019). Yet, the current study identified a significantly higher pre-MDA1 mean egg count in PPHS compared to responders, however to confirm this further studies on MDA datasets must be conducted to conclusively determine whether initial infection intensity is a predictor of PPHS emergence. This uncertainty rises from a study in Kenya, which also found a significant difference between infection intensity and responder sites, but determined it was not a reliable predictor (Kittur *et al.*, 2020).

Similarly, high pre-treatment parasite burden is a common factor for decreased PZQ efficacy (Midzi *et al.*, 2008), thus may be a predictor for EPHS also. Specifically, the EPHS in Mount Darwin in MDA3 was postulated to be from the high proportion of heavy infections in this population, which in turn may have stemmed from the delayed PZQ treatment in MDA3. Hence, individuals in Mount Darwin pre-MDA3 will have carried infection for longer than

during any of the other MDAs (Senghor *et al.*, 2014), contributing to the increased number of heavy infections and resultant decreased CRs and ERRs. However, there was no significant association between those individuals who did not clear schistosome infection and their pre-MDA1 infection intensity for any EPHS and was therefore not identified as a contributing factor to the EPHS detected during the MDA program.

The remaining five EPHS had a baseline 'light' infection intensity. Other studies in Zimbabwe have emphasised that the low levels of persistent infections warrant further investigation into the causes of decreased PZQ efficacy (Midzi *et al.*, 2008). In **Chapters 4 and 5** of this thesis, PGx variations have already been shown to impact active PZQ concentrations and thus a PZQ treatment outcome (Mnkugwe *et al.*, 2021a, Zdesenko *et al.*, 2022). As these EPHS were calculated from a small number of individuals, the reasoning behind these unsuccessful PZQ treatments may be due to individual PGx factors influencing their inability to clear infection. Therefore, there may not be a treatment problem in the general population, instead, a precision medicine approach may be required for these individuals with light infections who are not cured upon PZQ treatment.

The impact of drug holidays (skipped MDA) between PPHS, EPHS and responders was also evaluated. Three of the PPHS had received less than three MDAs of PZQ throughout the six years, with only one PPHS receiving more than three MDAs. Yet, despite the implied relationship between the decreased frequency of PZQ treatment and PPHS emergence, it was not significant in this study. Similarly, other studies in Cote d'Ivoire, Mozambique, and Tanzania found no significant relationship between drug holidays and PPHS, although in Kenya, drug holidays were shown to produce significantly more PPHS than those who received consecutive MDAs during a PCT program (Kittur *et al.*, 2019). Additionally, drug holidays were not associated with the occurrence of EPHS. This was important to highlight

when discussing increasing the frequency of MDAs of PZQ, as concerns of PZQ tolerance and even resistance due to natural variations of the parasite will grow as the pressures for PZQ treatment in areas of persistent and endemic infection continue to increase (Landouré *et al.*, 2012, Cupit and Cunningham, 2015, Vale *et al.*, 2017). So far, widespread resistance has not been verified with minimal cases of *S. mansoni* showing decreased sensitivity to PZQ (Ismail *et al.*, 1996, William and Botros, 2004) and no studies have indicated resistance to PZQ in *S. haematobium* (Vale *et al.*, 2017, Summers *et al.*, 2022). The lack of a significant relationship between the number of drug holidays and the emergence of PPHS or EPHS highlights the difficulties in the early identification of these hotspots, as currently drug holidays cannot be used as a predictor of hotspot occurrence. This was prominent during the PPHS analysis as 51.8% of districts in this study also received less than three MDAs and did not become PPHS.

Nevertheless, the implication of more frequent annual MDAs was also exemplified in this study. Mount Darwin was treated annually as a high-prevalence district (WHO, 2011a). Although Mount Darwin was detected to be PPHS in MDA4 after three annual rounds of PZQ and had two EPHS before MDA3, the district had cleared schistosome infection by MDA6 after five rounds of PZQ. This indicated that a higher frequency of MDAs resulted in increased control. Therefore, in the PPHS treated biennially (Buhera and Chipinge), the low frequency of treatment may have contributed to the recurrence of moderate endemicity at pre-MDA6 and the occurrence of PPHS. Both PPHS districts were at the upper end of the moderate endemicity scale at 48.8% and 40.9% at pre-MDA1, thus to prevent PPHS emergence and improve control, these districts may require an annual MDA regime like a high prevalence district. Currently, few studies have evaluated the impact of increased treatment frequency for *S. haematobium*, yet these indicate it may be more cost-effective for long-term control of this disease, particularly in high-prevalence settings (Lo *et al.*, 2015, Lo

et al., 2016). These findings, along with reports that baseline infection prevalence and intensity can reliably predict future prevalence (Kittur *et al.*, 2020), indicate that alterations to PCT programs to increase the number of MDAs in PPHS locations could improve schistosome control.

It is also important to consider the feasibility of implementing an increased number of MDAs. As PZQ has been well tolerated for decades with low risks of serious ADRs (Zwang and Olliaro, 2014), the increased use of PZQ should not pose severe risks to patients. Yet, numerous challenges could arise during a scaled-up PCT program, including shortages of implementation vehicles, increased workload for on-the-ground staff, political discrepancies, competing programs, drug availability, and inaccessibility to administration sites due to extreme terrain or weather conditions (heavy rain and floods) (Linehan *et al.*, 2011). Presently, it is already difficult to design and obtain the required coverage for a successful schistosomiasis treatment program that allows equitable access to at-risk populations (Tchuem Tchuenté *et al.*, 2017). As of 2020, the latest data from ESPEN showed that, of the fifty-eight districts in Zimbabwe that require PCT, only seven were treated in 2020, and only the Mbire district achieved effective coverage (77%) (ESPEN, 2022). Nevertheless, annual PCT programs have been ongoing in other countries for multiple NTDs (Turner *et al.*, 2021), therefore alterations to the current regimen could be a feasible and positive use of resources to control the emergence of PPHS.

Therefore, the benefits and subsequent health implications of more frequent PZQ treatment on the infected populations must be prioritised. As schistosomiasis disproportionately affects those individuals who are vulnerable and in poverty without access to adequate health services, increased PZQ treatments to improve schistosomiasis control could improve overall health-related quality of life (HRQoL). Schistosome infection has been associated with

educational, learning, and memory deficits in SAC infected with schistosomiasis compared to those who were uninfected (Ezeamama *et al.*, 2018, Mutapi *et al.*, 2021). Further, individuals with low educational attainment find it difficult to be placed in work or receive low-quality insecure roles with unstable wages (Renzaho *et al.*, 2016); all of which are associated with a better QoL.

Decreasing the number of active infections in the community will further hinder the parasite transmission cycle (Colley *et al.*, 2014). This reduces the contamination of the surrounding environment with schistosome eggs and was the premise of multiple risk factors evaluated during this study. *S. haematobium* transmission occurs seasonally, thus implementing the MDAs between September to November targets the season with the highest prevalence and intensity of infection (Senghor *et al.*, 2014). The current study indicated that MDA3, the only delayed survey, had the largest number of districts increasing in WHO prevalence risk category and the lowest number of districts carrying no infections. Although not significantly different from the other MDAs, it emphasised controlling the date of implementation of the MDA during PCT programs to prevent the prolonged and increased burden of schistosome infection on the patients and improve their HRQoL.

Here, the six-week efficacy check was purposely selected to determine the efficacy of PZQ as it is less than the latent period for *S. haematobium* (Rollinson and Southgate, 1987), removing the possibility of low CRs/ERRs due to reinfections. Some participants may have harboured different developmental stages of schistosomes simultaneously, as juvenile and immature worms can't be targeted by PZQ (Utzinger *et al.*, 2003). This manifestation could account for the increased infection prevalence of multiple districts at the post-MDA surveys, and the appearance of EPHS. Likewise, *S. haematobium* egg excretion can ensue upon schistosome death even after PZQ treatment, which could account for participants not

clearing infection at six weeks (McMahon and Kolstrup, 1979). To improve, when conducting more detailed evaluations of PPHS and EPHS, surveys should assess egg viability to fully determine whether egg excretion is representative of active infection (Webster *et al.*, 2013).

Low coverage has been reported to be a contributing factor to persistent infections (Kittur *et al.*, 2019). However, in other MDA studies in Africa, it was not useful in explaining the emergence of PPHS, with inconsistencies in the relationship between coverage and PPHS emergence (Kittur *et al.*, 2019, King *et al.*, 2020). In this analysis, low treatment coverage was not identified as a contributor to PPHS emergence. Yet, coverage may only be influential on a micro-epidemiological level, for instance if taken village by village, and this was not available for this study.

Furthermore, no significant relationship was detected between either EPHS or PPHS and increased risk of transmission from the snail host. However, the snail habitat data were obtained from surveys conducted in 1988 and 2012, therefore may not represent the current schistosome-transmission risks from the snail hosts as snail habitats can change over time (Adekiya *et al.*, 2019). Additionally, the lack of detailed snail counts from local water sources of the village sampling sites limited this analysis. Still, it does not negate the importance of snail control in interrupting the transmission and eliminating schistosomiasis (WHO, 2020a). Implementing snail control is one of the most common methods of intervention to prevent further transmission of schistosomes in the community, minimising the risk of active miracidia reaching the intermediate snail host and reducing the chances of developed cercariae contaminating freshwater sources (Chimbari, 2012). Other studies conducted in Zimbabwe have found that snail control in combination with PCT programs significantly reduces the prevalence of schistosome infection (Chimbari *et al.*, 2003).

Yet, a significant negative relationship between the ERR, but not CR, and the distance to the nearest waterway was detected, although, the R^2 value produced from this model was too low to be an accurate predictor of ERR. It was postulated that this negative relationship was skewed by the higher prevalence of the populations in village sites that were >5km from the freshwater source, as 60% of these had a moderate/high *S. haematobium* prevalence between 36-58%, with mean egg counts between 24-48 egg/10mL. Therefore, it was concluded that these populations had a reduced ERR due to their higher prevalence rather than because of their distance from a freshwater transmission site, and was not deemed a predictor of EPHS in this study. However, the distances were calculated from national data, as the micro-spatial data was not collected during the study, and consequently only indicated general exposure to potentially infected freshwater.

As transmission via the water sources is essential for infection with schistosomes, further disrupting the parasite life cycle is imperative (Chimbari *et al.*, 2003). WASH interventions are effective at decreasing the prevalence of schistosomiasis by reducing the contamination of the environment with schistosome eggs (Esrey *et al.*, 1991, Tanser *et al.*, 2018). Yet, low levels of education on schistosomiasis transmission have been shown to deter WASH practices (Omedo *et al.*, 2014). A recent study in Zimbabwe surveyed over 100 households to determine the social, cultural, and behavioural influences on schistosome infection showed that although 60% of the community had access to toilets, 36% of children did not use them and practised open defecation (Lampard-Scotford *et al.*, 2022). This stresses the importance of health education and awareness programs that target the entire community to further promote behavioural changes to decrease contamination and increase toilet use (Gyapong *et al.*, 2010). Although the current study did not gather data on the WASH practices of participants in this study, the improvement of WASH infrastructure and behaviours,

particularly in PPHS, could massively impact the control of schistosomiasis (Waite *et al.*, 2017).

Looking forward, the implementation of alternate treatment plans or drugs is essential for schistosome control, particularly if areas of EPHS and PPHS become more prevalent across Africa. In the current study, multiple districts still carried schistosome infections pre-MDA6, despite a low pre-MDA1 prevalence, something that has been observed in MDA strategies across other endemic African countries (King *et al.*, 2020). Further, within months of halting PZQ treatment programs, the prevalence and intensity of infection can return to pre-treatment levels (Olveda *et al.*, 2016). Presently, new pyrazoline derivatives with antischistosomal activities are in development to remove the reliance on PZQ, with no definitive success so far (Morais *et al.*, 2021). Yet, regardless of successful alternative drugs, MDA programs would still be required to administer new drugs regimen. In the current study, PZQ was co-administered with albendazole (ABZ), a common occurrence during PCT programs to control both schistosomiasis and soil-transmitted helminths, respectively (Hong, 2018). I have already discussed the importance of DDIs in **Chapter 3**, indicating their role in under/overdosing, adverse drug reactions (ADRs), and even fatalities (Beijnen and Schellens, 2004). Thus, as PZQ was co-administered with ABZ, DDIs must also be discussed. PZQ+ABZ acts synergistically to increase the exposure of the schistosome-killing (R)- PZQ (Lima *et al.*, 2011) and was thus not considered a contributor to either hotspot. Combination therapies to treat schistosomiasis, such as PZQ+ABZ, are gaining traction (Akrasi *et al.*, 2022). Nonetheless, increased drug exposure has been reported to increase cases of ADRs and so should be approached with caution (Kabatende *et al.*, 2022). Consequently, progress towards a long-term vaccine would be highly desirable. Vaccination programs would be the most cost-effective method of controlling schistosomiasis and introducing a population-level immunity that could interrupt the transmission of this debilitating disease (Collyer *et al.*,

2019). The introduction of a vaccine program could provide long-term schistosome control, prevent the development of PPHS and EPHS, and could hold the key to the future elimination of schistosomiasis. While awaiting this intervention, studies such as this which monitor PZQ efficacy and aim to identify PPHS and EPHS in endemic countries are vital in the continuing use of PZQ to treat schistosomiasis.

There were limitations to the current study, beginning with the approaches of identifying a hotspot. Currently, the most common approach to designate PPHS is 'a failure to achieve at least a 35% decrease in prevalence relative to the baseline and/or a 50% decrease in infection intensity relative to baseline after four years of MDAs, with the PPHS receiving at least two rounds of treatment' (Shen *et al.*, 2020). However, this was not optimal to determine a PPHS in this study, primarily as the initial prevalence of multiple districts in this program was <35%. So, any reduction less than this value designated them as a PPHS, even if they had decreased to 0% prevalence.

Moreover, throughout all the MDAs, only the Mutoko district detected *S. haematobium* infection at every treatment arm, whether pre-MDA or post-MDA survey. Yet, under the common definition of PPHS and ***Approach A& Approach B*** utilised in this study, Mutoko was not classed as a district with persistent infections in any MDA despite gaining prevalence post-treatment on many occasions. Mutoko is of particular interest regarding persistent infections as a large proportion of the study population analysed in **Chapter 4** was from this district. Therefore, the lack of PPHS identification but the persistence of schistosome infections in Mutoko may be host-related, such as the PGx factors detected in **Chapters 4 and 5**. Yet, Mutoko was not detected as an EPHS, with only two surveys available for PZQ efficacy analysis with paired pre-MDA and post-MDA data throughout the six MDAs. Thus, some individuals who were not followed up for the efficacy check (not available for EPHS

analysis), may have had an unsuccessful PZQ treatment and contributed to the infections at the next pre-MDA survey. Additionally, due to a lack of consistent annual MDA surveys, there were limits to the included data for the hotspot analyses. Although ***Approach A& Approach B*** were selected to incorporate low-prevalence districts and districts without consistent annual MDA data, these approaches still had their limitations, as shown by the persistence of the Mutoko district. Further, because of the lack of paired data for many participants, EPHS may have been missed using ***Approach C***, as also indicated in Mutoko.

Based on recent guidelines from the WHO, a PPHS would only include those areas that had less than a third relative reduction in baseline prevalence after two years of annual PCT (WHO, 2022b). Here, the Rushinga district would be a PPHS with only a 27% relative reduction in baseline prevalence after two years of MDAs. However, the PPHS approaches used in this study didn't identify Rushinga, although the EPHS analysis did. An EPHS was detected in MDA2, yet after MDA3 the Rushinga district maintained low endemicity with annual PZQ treatments. The absence of a definitive definition of a PPHS is a great hindrance to gathering conclusive results, particularly as decreased PZQ efficacy does not appear to be the main contributor. This is made more difficult by the unpredictability of PPHS. Mount Darwin and Chiredzi were only detected as PPHS in MDA4 and MDA5, respectively, implying that PPHS identified in this analysis were not consistent through a longer MDA program. So, although previous studies have reported it was possible to predict PPHS after three years of MDAs (Shen *et al.*, 2020), the current study indicates that this may not be the case and that at least six years of data may be necessary to confirm a hotspot. Overall, the fluctuations in PPHS detection timeframe create difficulties in the early identification and effective targeting of a PPHS.

Here, the lack of consistent MDAs in multiple districts, as high as 48% not treated or surveyed in MDA5, prevented accurate mapping of hotspots using national MDA data. For instance, for the PPHS in Chipinge and Buhera, the MDA2, MDA3 and MDA5 data were not administered, according to the WHO recommendations of baseline prevalence. Hence, it is difficult to ascertain whether persistent infections would be present in future MDA surveys if these districts were treated more regularly.

From the six years of MDA data, only 44% satisfied the criteria to be included in the EPHS analysis, and only around 65% of the surveys required to identify PPHS were present. This was a limited set of data compared to what could have been achieved. So, although this PCT program followed the recommended WHO guidelines regarding treatment regimens (WHO, 2011a), this eventually limited the hotspot analysis. Compared to the analysis of persistent hotspots by the Schistosomiasis Consortium for Operational Research and Evaluation (SCORE) studies (Kittur *et al.*, 2019), the lack of consistent data for the twenty-nine districts meant that some trends and predictors may have been missed or classed as insignificant due to absent data. Also, particularly for the EPHS, due to the small sample sizes of both pre/post samples, this study could not fully evaluate whether these low CRs and ERRs were on a population level or stemmed from the contributions of a small subset of individuals. Further, the hotspot analyses were also limited by the number of villages surveyed per district, as multiple districts had only one village site to represent the district population. Averages such as this have been shown to conceal considerable heterogeneity in MDA success (Kittur *et al.*, 2019). As the MDA treatment regimen in Zimbabwe is defined by the district infection prevalence (WHO, 2011a), the use of micro-spatial mapping tools to determine heterogeneity across different village sites within districts may be better to target and treat hotspots on a tailored basis. Overall, this study discussed data-driven approaches to better target PZQ

treatment to control schistosome infection, further monitor PZQ efficacy, and prevent the emergence of persistent hotspots.

6.5 Key findings and conclusions

This chapter addressed **Thesis Aim 4** (**Section 1.11**) and assessed the heterogeneity in MDA data from Zimbabwe over six years, from 2012 to 2017, identifying hotspots of prevalence and decreasing PZQ efficacy. Four PPHS were identified, and baseline infection intensity was significantly higher in PPHS than in responder districts, providing valuable information on the possibility of early hotspot detection. Additionally, six EPHS were detected, however there did not appear to be a clear predictor of decreased PZQ efficacy. As the sample sizes of the EPHS were small, individual host-related factors may be responsible for the decreased PZQ efficacy. The occurrence of EPHS was not predictive of PPHS, based on these analyses, therefore care to control the other drivers of schistosome transmission is essential in maintaining control and preventing persistent infections. Additionally, a more cohesive definition must be applied to determine a hotspot to standardise results across schistosomiasis research and improve the depth of knowledge. However, identifying a hotspot may rely on numerous MDAs, thus more frequent monitoring and analysis of PCT programs should occur to detect hotspots earlier to provide more intensive and targeted efforts in those locations. Thus, to successfully eliminate schistosomiasis, the reliance on seasonal MDAs cannot be the only control method. Approaches to achieve this include increasing the frequency of MDAs, different drug/combination therapies, utilisation of snail control, WASH interventions and sufficient schistosomiasis education to improve HRQoL. Overall, strategies for defining, identifying, and addressing hotspots in endemic countries will be critical for morbidity control and the elimination of schistosomiasis.

Chapter 7 – General Discussion

7.1 Introduction

Schistosomiasis is a global cause of morbidity and mortality, and over 93% of schistosome-infected populations reside in Sub-Saharan Africa (Colley *et al.*, 2014, WHO, 2022b). The mass drug administration (MDA) of praziquantel (PZQ) during preventative chemotherapy (PCT) is a vital intervention to reduce disease-related morbidity in endemic areas, and to eliminate schistosomiasis as a public health problem by 2030 (WHO, 2013b, WHO, 2020a). Therefore, understanding the factors that influence the efficacy of a PZQ treatment, particularly in schistosome-exposed populations where the drug is commonly used, is essential in achieving this goal. Drug efficacy can be influenced by several host, parasite or drug-related factors (Rowland and Tozer, 2011, Haga, 2017). Of these factors, a crucial determinant of drug efficacy is host drug metabolism, as it can change the systemic concentration of the active drug (Ogu and Maxa, 2000). Thus alterations to host PZQ metabolism could alter the concentration of the schistosome-killing PZQ and be a crucial determinant of PZQ efficacy (Nleya *et al.*, 2019, Mnkugwe *et al.*, 2021a).

In this thesis, I addressed key knowledge and research gaps, via a series of studies in Zimbabwean populations and analyses of published studies, to identify the factors that influence the efficacy of a PZQ treatment in a schistosome-exposed population. Here, the major findings concerning the aims outlined in **Section 1.11** are summarised and discussed in broader terms. I also consider the potential contribution of my findings in improving current schistosomiasis control and suggest how my research could be developed in the future.

7.2 Causes and consequences of variable praziquantel (PZQ) efficacy during schistosome control: what were the key gaps?

Despite the general success of PZQ treatment in controlling schistosome infections over four decades (Fukushige *et al.*, 2021), several studies in schistosome-endemic African countries reported cases of decreased PZQ efficacy (Danso-Appiah and De Vlas, 2002, Tchuenté *et al.*, 2004, Alonso *et al.*, 2006, Midzi *et al.*, 2008, Barakat and Morshedy, 2010, Kabuyaya *et al.*, 2017). As discussed in **Chapter 1**, a common determinant of variable drug efficacy is the alteration of host drug metabolism, hence many studies have postulated that reduced PZQ efficacy may be the result of alteration to the metabolism of PZQ (Nleya *et al.*, 2019, Mnkugwe *et al.*, 2021a, Summers *et al.*, 2022). In concordance with these studies, the findings of **Chapter 3**, using published studies, demonstrated that several drug and host-related factors influenced PZQ metabolism and resulted in variable levels of PZQ in circulation, potentially altering PZQ efficacy and causing adverse drug reactions (ADRs). These findings included the consequences of co-administering PZQ with other drugs, as **Chapter 3** highlighted that the investigation of drug-drug interactions (DDIs) with PZQ was minimal during treatment for schistosomiasis, particularly those relevant to schistosome-endemic populations. DDIs tend to be neglected topics in LMICs (McFeely *et al.*, 2019), yet other studies have well-reviewed the risks of DDIs during treatment for other infectious diseases, for instance, HIV (Seden *et al.*, 2013) or Tuberculosis (Bhutani *et al.*, 2005).

Here, DDIs were discussed as key determinants in variable PZQ efficacy as they can increase or decrease the exposure of PZQ (**Chapter 1**), and because there is a paucity of comprehensive knowledge regarding the impact of DDIs in Sub-Saharan Africa on drug efficacy and ADRs (Kiguba *et al.*, 2021). Multiple drugs were discussed to act antagonistically with PZQ, like the antimalarial chloroquine, which decreased the exposure of

the schistosome-killing parent PZQ upon co-administration (Masimirembwa *et al.*, 1994a). Hence, as malaria is co-endemic with schistosome infections, it must be acknowledged as an antagonistic DDI to be aware of regarding reduced PZQ efficacy, as well as in combination with other drugs utilised in Africa as reduced active drug exposure was also common (Seideman *et al.*, 1994, Munera *et al.*, 1997, Ilo *et al.*, 2006, Ilo *et al.*, 2008).

Yet, up to 80% of ADRs in Sub-Saharan Africa are due to co-administration with antiretroviral drugs (Dandara *et al.*, 2019). Although no DDIs with antiretrovirals were detected in the **Chapter 3** review of determinants of variable PZQ exposure, HIV shares the same epidemiological space as schistosomiasis (Bustinduy *et al.*, 2014). A recent study combined two antiretroviral drugs with PZQ. Efavirenz significantly reduced the AUC of PZQ by four times, and ritonavir over doubled the AUC of the inactive (S)-PZQ that causes side effects (Mutiti *et al.*, 2021). Thus, when using PZQ in HIV patients, there is a risk of both PZQ treatment failure due to decreased PZQ exposure, as well as ADRs due to increased exposure of the (S)-enantiomer that causes side effects. This DDI was not identified in this thesis but adds valuable information on the potential causes of variable PZQ efficacy and ADRs, and highlights a gap in schistosomiasis control that is being addressed by the research community.

Despite the risks highlighted in this thesis from DDIs, there is an emergence of combination therapies that could allow for smaller doses of PZQ, reducing the concern of drug resistance due to mass use, while also achieving the desired PZQ efficacy (Ferraz *et al.*, 2022). Recently, the WHO has recommended testing combinations of PZQ with ABZ in PCT programs with over five years of high coverage (WHO, 2020b), a treatment option which could control both schistosomiasis and soil-transmitted helminths (Hong, 2018). Even though the analyses in **Chapter 3** emphasised the benefits of this combination via increased PZQ

exposure, recent reports of combination therapies of PZQ and ABZ recorded higher cases of ADRs compared to separate monotherapies during an MDA of the two drugs (Akrasi *et al.*, 2022, Kabatende *et al.*, 2022). Further, the study in **Chapter 6** also co-administered these drugs during the MDA in Zimbabwe, although no data on ADRs were available for analysis. Consequently, although synergistic in these analyses and other studies, the potential risks of combination therapies to increase PZQ exposure and prevent treatment failures may outweigh the benefits of improving PZQ efficacy during schistosome control. The findings of the review in **Chapter 3** and these recent studies highlight that the acknowledgement and reporting of DDIs are increasing. So, although DDIs were not addressed further in this thesis as experimental studies focused on host genetic factors, they are a key influential factor in PZQ exposure, and thus PZQ efficacy. The increased recognition of DDI determinants of PZQ efficacy that cause variable PZQ exposure, and even ADRs, can serve as a resource to further care for patients in co-endemic areas (McFeely *et al.*, 2019).

Current drug formulation was also identified as a determinant of PZQ efficacy in the review of published studies, yet patients are still dosed with a racemic PZQ tablet (as standard during MDAs (Crompton, 2006)) resulting in lower exposure of the desired (R)-PZQ enantiomer compared to the pharmacologically inactive (S)-PZQ. This point has been highlighted in multiple recent studies (Meister *et al.*, 2014, Vendrell-Navarro *et al.*, 2020, Mnkugwe *et al.*, 2021b), however currently the only single enantiomer formulation developed is for deployment in paediatric patients (Consortium, 2022b). To reduce the risk of PZQ treatment failures due to insufficient (R)-PZQ exposure, a single enantiomer treatment should be utilised in other age groups (Bagchus *et al.*, 2019), however this is still a gap in improving schistosome control.

Notwithstanding the importance of drug-related factors, this thesis also discussed the host-related alterations including liver disease, infection status, *Schistosoma* species and age-related CYP activity that also played a role in variable PZQ efficacy. Yet, during the review of factors influencing PZQ efficacy in **Chapter 3,** there was a paucity of information on host-genetic influences, despite multiple published studies postulating variable drug exposure and PZQ efficacy was due to host-genetic or interindividual variation (Leopold *et al.*, 1978, Ofori-Adjei *et al.*, 1988, Mandour *et al.*, 1990, el Guiniady *et al.*, 1994, Masimirembwa *et al.*, 1994a, Metwally *et al.*, 1995a, Metwally *et al.*, 1995b, Jung *et al.*, 1997, Botros *et al.*, 2006, Botros *et al.*, 2010, Gotardo *et al.*, 2011, Bustinduy *et al.*, 2016, Kovac *et al.*, 2018a). No PGx studies of PZQ treatment were identified before 2020, despite PGx variants in the CYP enzymes being associated with altered drug metabolism in efficacy and toxicity studies (Evans and Johnson, 2001, McLeod and Evans, 2001). As PGx has been utilised in the individualised treatment of HIV patients in Africa (Matimba *et al.*, 2016), it has the potential to identify PGx determinants of variable PZQ exposure and efficacy. Thus, the PGx impact on PZQ efficacy during schistosomiasis treatment was investigated in **Chapters 4 and 5**.

7.3 Pharmacogenetic (PGx) impact on praziquantel (PZQ) efficacy

PGx is important in Africa to identify non-responders to drug treatment, reduce ADRs, and optimise drug dosing; all of which could revolutionise the treatment of diseases on the continent (Radouani *et al.*, 2020). Having determined there was a scarcity of PGx studies evaluating PZQ efficacy during a schistosomiasis treatment, I characterised the PGx variations in the PZQ-metabolising CYP enzymes which could alter an individual's response to PZQ. I identified four SNPs in a schistosome-exposed population that were significantly associated with PZQ efficacy across the *CYP1A2, CYP2D6* and *CYP3A5* genes, presenting novel associations between PGx variants and PZQ treatment outcome. As discussed in

Chapter 4, only the rs28371702 [CC] genotype of *CYP2D6* was significantly associated with an unsuccessful PZQ treatment, which was hypothesised to be a similar mechanism to other *CYP2D6* SNPs: increased *CYP2D6* function and decreased PZQ exposure (Wang *et al.*, 2014a). However, there were contrasting results with other drug exposure studies (Zhang *et al.*, 2021), emphasising the need for further research on this variant to identify if individuals possessing this PGx variant require higher doses of PZQ to achieve therapeutic efficacy. This is particularly relevant as *CYP2D6* is a key metabolic route of around 25% of clinically prescribed medicines, thus identifying and characterising a PGx *CYP2D6* variant such as this in a relevant population could aid the further implementation of tailored drug treatments across Africa (Twesigomwe *et al.*). Further, individuals possessing the rs951840747 [CT] genotypes of the *CYP1A2* gene were significantly associated with clearing schistosome infection, postulated to be from increased PZQ exposure. Further studies are required to ensure ADRs are not a risk for PZQ and other drugs utilised in African populations concerning this PGx variant, as ADRs have been detected with other decreased *CYP1A2* activity variants (Thorn *et al.*, 2012).

As discussed in **Chapter 5**, significant associations between the PGx variations of *CYP1A2*1F* and *CYP2C9*9* and the *in vivo* concentrations of PZQ and its metabolites further demonstrated PGx could be a key contributing factor to variable PZQ efficacy. Both PGx variants were indicative of increased CYP metabolism and decreased parasite exposure to the schistosome-killing PZQ, and concurred with other studies that evaluated the altered activity of these enzymes on drug efficacy (Lu *et al.*, 2020, Zhou *et al.*, 2021b). Nonetheless, as discussed in **Chapter 5** there was a lack of relevant *in vivo* data regarding *CYP2C9*9* (Allabi *et al.*, 2005, Mitchell *et al.*, 2011, Cariaso and Lennon, 2012), which contributes to a lack of uncertainty surrounding the function of the PGx variant. However, due to the vast genetic diversity of African populations, it is difficult to routinely characterise the functional

impact of the quantity of PGx variants in humans (Zhou *et al.*, 2018). This emphasises the importance of studies, such as this, that characterise a population that commonly uses a drug to find common PGx variants that could be crucial to any drug treatment in these populations that are mediated by CYP metabolism.

In **Chapter 5**, there were no significant associations with PZQ treatment outcome which was contrary to the study in **Chapter 4**. However, this concurred with another study which also found PGx variants (*CYP2C19*2/*3*) that significantly altered the *in vivo* concentrations of the schistosome-killing PZQ, but found no associations with PZQ efficacy (Mnkugwe *et al.*, 2021a). Therefore, although the findings from **Chapter 5** evaluated the PGx impact on PZQ exposure, it only provided insight into the suspected influence on PZQ efficacy and requires confirmation in larger sample sizes of schistosome-infected patients. In both PGx studies, multiple SNPs were found at significantly different frequencies to European and African studies, in addition to multiple novel SNPs detected. As numerous drugs are developed in European populations for use in African populations, the dosage is usually calculated without accounting for the PGx of the diverse African population it is to be used in (Tata *et al.*, 2020), hence it may lead to reduced drug efficacy or ADRs (Dandara *et al.*, 2019). PGx research is scarce in Africa (Radouani *et al.*, 2020), despite Africa carrying approximately 25% of the global disease burden, and the amplified requirements for the drugs to be used in African populations (Murray *et al.*, 2012). Hence, the results of this thesis provide valuable contributions to PGx research in Africa, especially regarding the significant differences in drug concentration and efficacy.

Although the PGx approach for 'precision public health' is novel, improving testing technologies and awareness allows approaches to precision medicine to become a feasible option in Africa (Radouani *et al.*, 2020). The improvement of training, education and genetic

testing facilities is required across Africa to address the gap in PGx clinical research, as this will have the potential to help policymakers base healthcare decisions on the genetic makeup of populations and communities (Tata *et al.*, 2020). However, there are issues in the implementation and equity of PGx testing in Africa, including the affordability, feasibility, capacity to address local burden, and technological requirements (Pang, 2003, Olivier and Williams-Jones, 2011). Despite this, many studies have argued that PGx testing is necessary to improve global public health, specifically in treating infectious diseases like schistosomiasis (Pang, 2003, Vegter *et al.*, 2008, Olivier and Williams-Jones, 2011). Further, the use of PGx data to improve treatment outcomes has been intensified in Africa over recent years (Dandara *et al.*, 2019), thus PGx could be a feasible option to improve schistosomiasis control and tailor other drug treatments across the continent.

Yet, to further characterise how PGx variations impact active PZQ concentrations and PZQ efficacy, the implementation of genetic testing, for instance during the MDA of PZQ in PCT programs, would pose a challenge (Mukonzo *et al.*, 2014, Mutagonda *et al.*, 2017). Thus evidence to support PGx implementations, such as this thesis, must continue in the field. The PGx studies in this thesis provided the first investigation in PGx of PZQ efficacy in Zimbabwe and the second in Africa (Mnkugwe *et al.*, 2021a), and highlighted significant PGx determinants of PZQ efficacy during a schistosomiasis treatment. The results of this thesis added to the paucity of knowledge on the PGx impact on a PZQ treatment in a schistosome-exposed population and provided a mechanistic basis for further investigation for treatment efficacy in larger sample sizes. The detection of variants significantly associated with PZQ efficacy **(Chapter 4)** and with the *in vivo* concentrations of PZQ and its metabolites **(Chapter 5)** exemplifies the importance of conducting PGx studies on PZQ, particularly in African populations where the drug is commonly used. Further, these findings enriched the quantity of genomic data of a relatively uncharacterised African population that

applies to multiple drug efficacy studies (da Rocha *et al.*, 2021). PGx research, identifying and reporting variants involved in drug response, like those detected in this thesis, is critical for improving health throughout Africa. This investigation provides a vital insight into the PGx reasons behind the successes and failures of a PZQ treatment and presented data that applies to schistosomiasis research and multiple drug efficacy studies.

7.4 Decreased praziquantel (PZQ) efficacy: is it to blame for hotspots of schistosome infection?

Understanding the determinants of a hotspot area (persistent infections) compared to a responder area (clears infection) during the MDA of PZQ is essential in maintaining control of schistosomiasis (Wiegand *et al.*, 2017). Persistent hotspots (PHS) of schistosome infection have been identified in multiple schistosome-endemic countries (Sang *et al.*, 2014, Pennance *et al.*, 2016, Kittur *et al.*, 2017, Kittur *et al.*, 2019, Kittur *et al.*, 2020, Shen *et al.*, 2020, Pennance *et al.*, 2022), yet the causes of PHS are not well understood (Kittur *et al.*, 2020). **Chapter 6** of this thesis identified hotspots in Zimbabwe using six years of annual MDA data and evaluated the drivers of these hotspots. Both persistent hotspots of *S. haematobium* prevalence (PPHS) and hotspots of decreased PZQ efficacy (EPHS) were identified, and EPHS were not identified as a primary cause for PPHS based on these analyses, despite being listed as a potential cause of PPHS in other hotspot analyses (Wiegand *et al.*, 2017). Still, initial infection intensity was significantly higher in PPHS than in responder districts. This concurred with a study in Kenya (Kittur *et al.*, 2019, Kittur *et al.*, 2020) and provided valuable information on the possibility of early hotspot detection and the amendment of PCT intervention strategies as necessary based on the first MDA survey. Yet, further studies must be performed to confirm this as other hotspot assessments did not find this to be a predictor of PPHS occurrence (Kittur *et al.*, 2019).

Although no associations between the detected PPHS and drug holidays were identified in these results, drug holidays have been shown to produce significantly more PPHS than those who received consecutive MDAs during a PCT program (Kittur *et al.*, 2019). Recently, the WHO has recommended that any community classified as a PPHS after two MDAs should be treated biannually if the site is highly endemic and annually if the site is moderately endemic (WHO, 2022b). Increasing the frequency of treatment in districts of both high and moderate prevalence could prove an important factor in preventing the development of PPHS, especially those detected in this thesis, as two PPHS were in moderately endemic districts and were only treated biennially.

Despite recent progress and the emphasis on cases of reduced PZQ efficacy, there remains little systematic monitoring of anthelmintic efficacy in human populations (Walker *et al.*, 2022). Six EPHS were identified in **Chapter 6**, however there did not appear to be a clear predictor of decreased PZQ efficacy, which highlights the need to further monitor PZQ efficacy and increase research to determine the factors that contribute to these occurrences during PCT. Furthermore, the implications of having a small proportion of individuals who show reduced PZQ efficacy regarding the long-term sustainability of PCT programs are still unknown and certainly warrant close longitudinal monitoring (Moser *et al.*, 2020). There is an important ongoing debate on how to address these outliers or non-responders in the context of PZQ efficacy, and further investigation of non-responsive individuals during MDAs is required to build on the studies in this thesis (Levecke *et al.*, 2020). Although PGx variations were found to have a significant impact on PZQ efficacy and the *in vivo* concentrations of PZQ and its metabolites throughout this thesis (**Chapter 4** and **Chapter 5**), due to a lack of experimental genetic or *in vivo* data it could not be concluded whether host genetic factors caused any PPHS or EPHS. Currently, the advantages of curing most of the schistosome-infected population to reduce morbidity take precedence in endemic countries,

and are prioritised over precisely targeting individuals who fail to clear schistosome infection upon a standard PZQ treatment (World Health Organization 2020b). Thus, more studies, such as those in this thesis, are required to inform policymakers of the importance of precision medicine approaches in PCT programs to increase schistosome control, as well as studies on the feasibility of implementation (Tata *et al.*, 2020).

Based on the results of **Chapter 6**, a more cohesive definition must be applied to determine a hotspot, whether PPHS or EPHS, to standardise results across schistosomiasis research and improve the depth of knowledge. It does not appear that decreased PZQ efficacy is responsible for PPHS emergence based on the results of this thesis, and no determinants of EPHS were detected during the MDA program. To further try to characterise these risk factors, MDA programs should continue to monitor PZQ efficacy, particularly in repeatedly PZQ-treated populations that are experiencing variable PZQ efficacy (WHO, 2022b). Additionally, to successfully eliminate schistosomiasis, the reliance on seasonal MDAs using PZQ cannot be the only control method, as highlighted in other hotspot studies (Kittur *et al.*, 2020). The combined utilisation of snail control, WASH interventions and sufficient schistosomiasis education to improve HRQoL will be key in implementing long-term control and elimination, particularly in hotspot settings (Chimbari *et al.*, 2003). Furthermore, there is only limited availability of PZQ worldwide for schistosome control programs, thus it is essential that the target populations and the identification of individuals who require alternate treatment regimens or are in hotspot areas are correctly identified and treated (Kura *et al.*, 2022). The evidence provided in **Chapters 4, 5 and 6** can guide NTD program managers to structure cost-effective solutions for schistosomiasis interventions, focusing on the prevention of hotspots of infection and identifying PGx variants detrimental to PZQ efficacy, to improve control and work towards the elimination of schistosomiasis.

7.5 Future directions and recommendations

The work presented in this thesis was conducted to answer a series of questions regarding the factors that influence PZQ efficacy (aims outlined in **Section 1.11**). Yet, these studies also raised issues concerning the clinical relevance of these findings in the wider field of schistosomiasis control, especially when broadening the focus away from Zimbabwe.

To confirm the associations detected in this thesis, further studies should be performed in cohorts of increased sample size, age range, and sex, with the relevant *in vivo* and PZQ efficacy measures to fully capture and inform on the PGx impact on a PZQ treatment in a schistosome-exposed population. Crucially, advances must be made to develop PGx testing in Africa. The African Pharmacogenomics Consortium (APC) highlighted in a recent report that identifying and understanding the role of PGx factors in PCT interventions is vital for improved treatment outcomes (Mukonzo *et al.*, 2014, Mutagonda *et al.*, 2017, Dandara *et al.*, 2019, Mnkugwe *et al.*, 2021a). Furthermore, this could reduce the burden of ADRs in patients with PGx variants and improve the life of the patient, and reduce the pressure on healthcare resources to treat these ADRs (Ampadu *et al.*, 2016). For this to occur, collaborations between industry, academia, non-governmental, and international organizations will be critical in overcoming the challenges (Pang, 2003). This will apply not only to schistosome control but drug use and development in Africa as a whole to increase the safety and efficacy of individual care on a precision medicine approach (Olivier and Williams-Jones, 2011). Africa possesses a vast genetic diversity (Hussein *et al.*, 2022), thus building on the studies in this thesis and obtaining further information in larger sample sizes will be important in the development of novel therapeutics for many diseases.

Also, PCT control programmes must consider continuous pharmacovigilance (ADRs, DDIs, decreased PZQ efficacy) during the MDAs administration to monitor the efficacy of PZQ in

the populations this drug is being used, a critical need that has mostly been ignored to date (Kiguba *et al.*, 2021). Including pharmacovigilance in routine MDA evaluations could improve the depth of data on variable PZQ efficacy, as well as risk factors (e.g. co-administered drugs, ecological and spatial data, or poor compliance), which could be systematically collected. As shown in this thesis, even with smaller cohorts variable PZQ efficacy was detected. Therefore, an increased number of studies and sample sizes could clarify the causality of the observed changes, collect a greater number of MDAs, and detect hotspots of concern where program-level changes could improve schistosome control and prevent morbidity in these communities.

Further surveys must also be made to understand the spatial and transmission dynamics of schistosomiasis and confirm an applicable predictor of hotspots (Cotter et al., 2019; Dlamini et al., 2019a; Sturrock et al., 2013). Collecting samples in the hotspot areas, like those detected in this thesis, could provide further insight into the drug-gene picture of PZQ treatment, and if an alteration to PCT programs must be made due to other drivers of persistent infections. Further longitudinal studies in communities suspected to be hotspots using a wider range of ages, not just the SAC targeted by MDAs, could inform on cases of persistent schistosome infection, PGx factors and variable PZQ efficacy in well-characterised populations. If PGx factors are responsible for the high number of unsuccessful PZQ treatments for schistosomiasis, then these non-responders will remain as a reservoir of infection in the community (Toor *et al.*, 2018). Yet, hotspot identification, as recommended by WHO (WHO, 2022b), is based on changes in prevalence, not PZQ efficacy measures. Thus, to improve schistosome control the determination of what causes a hotspot is essential (Shen *et al.*, 2020), a fact that I discussed during this work. Also, by assessing the impact of MDAs more frequently, valuable data for decision-making during PCT program design can

be collected, providing a rich source of information to the schistosomiasis research community (Ezeamama *et al.*, 2016).

Additionally, due to the lack of enantiomeric identification in these studies, further research to identify and provide evidence on the determinants of altered concentrations of each PZQ enantiomer, for instance, whether PGx changes influence (R)-PZQ exposure to decrease efficacy, or if PGx alters (S)-PZQ concentrations to increase ADRs (Mnkugwe *et al.*, 2021a) is imperative. As PGx factors were the target of the experimental studies in this thesis, other host factors that influence the individual response to PZQ may have been missed (Haga, 2017). Thus, a more comprehensive predictor of PZQ efficacy may involve a joint analysis of the host genetic, epigenetic and microbiome modification (GENDEP Investigators, 2013), as the gut microbiome can affect the expression of CYP enzymes (Swanson, 2015), and epigenetic modifications can impact drug response (Powell *et al.*, 2013). Furthermore, examining parasite population genetic structures across hosts and PZQ treatment regimens can ensure resistance is not the cause of variable PZQ efficacy (Lamberton *et al.*, 2010, Webster *et al.*, 2020).

Extending these findings to conduct further studies on different African populations will contribute evidence appropriate for improving the health of all people suffering from schistosome infections. By identifying determinants of variable PZQ efficacy, these factors can be controlled as much as possible to allow for a successful PZQ treatment in infected individuals. Likewise, promoting the integration of PGx into health systems and improving the ability to conduct drug efficacy studies in schistosome-infected populations could reduce long-term morbidity and ADRs; moving closer towards a personalised treatment for schistosomiasis. Additionally, by utilising MDAs to identify hotspots of schistosome infection, PCT can be better targeted to prevent unsuccessful PZQ treatments. The provision

of robust infrastructure to monitor PZQ efficacy, perform PGx testing, and identify hotspots of infection will all contribute to the control and elimination of this disease.

7.6 Conclusion

Overall, the results of this thesis provide evidence of several factors that can influence PZQ efficacy in schistosome-infected populations which must be acknowledged to improve current schistosomiasis control. This thesis provides novel information on the PGx factors that influence PZQ efficacy and indicates the role of PGx factors during a PZQ treatment for schistosomiasis may be significant and underestimated. The identification and reporting of PGx variants involved in drug response are critical for the improvement of health throughout Africa, thus to further utilise the findings of this thesis an improvement of infrastructure for PGx testing in Africa is required. Further, the investigation of hotspots of persistent schistosome infections and decreased PZQ efficacy during PCT programs provides strategies for defining, identifying, and addressing hotspots critical for morbidity control and the eventual elimination of schistosomiasis. Together, these investigations provide insight into the causes of variable PZQ efficacy and persistent infections to improve patient outcomes during schistosomiasis treatment.

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Appendix 1

Supplementary Table 1: Demographic characteristics of the 38 Zimbabwean subjects recruited to assess the pharmacokinetics of PZQ and its metabolites. This included the division of demographics by the matching performed for this study by age category, baseline infection intensity, treatment outcome and sex.

Age Category	Baseline *S. haematobium* Intensity	Treatment Outcome	Sex		Total
			F	M	
SAC (6-17 years)	*Heavy (≥50 eggs/10mL)*	Cleared	2	3	5
		Total	**2**	**3**	**5**
	Light (<50 eggs/10mL)	Cleared	12	10	22
		Not Cleared	0	1	1
		Total	**12**	**11**	**23**
	Total	Cleared	14	13	27
		Not Cleared	0	1	1
		Total	**14**	**14**	**28**
ADULTS (≥18 years)	*Heavy (≥50 eggs/10mL)*	Cleared	1	0	1
		Not Cleared	1	0	1
		Total	**2**	0	**2**
	Light (<50 eggs/10mL)	Cleared	8	0	8
		Total	**8**	0	**8**
	Total	Cleared	9	0	9
		Not Cleared	1	0	1
		Total	**10**	0	**10**
Total Participants		**Cleared**	**23**	**13**	**36**
		Not Cleared	**1**	**1**	**2**
		Total	**24**	**14**	**38**

Supplementary Table 2: Demographic characteristics of the two Zimbabwean subjects recruited to determine an extraction method of PZQ and its metabolites from a DBS card. This included the division of demographics by age category, infection intensity, and sex.

Age Category	Mean Infection intensity	Sex
		Male
SAC (6-17 years)	18.83 eggs/10mL	2

Supplementary Table 3: Column flow rate for ZIC-pHILIC column.

Time (minutes)	%A	%B
0	20	80
15	80	20
15	95	5
17	95	5
17	20	80
24	20	80

Supplementary Table 4: Mass spectrometry (MS) settings for the Thermo Orbitrap QExactive (Thermo Fisher Scientific) used for the analysis of dried blood spot (DBS) extracts.

Setting	Value
Resolution	70,000
AGC	1e6
m/z range	70–1050
Sheath gas	40
Auxiliary gas	5
Sweep gas	1
Probe temperature	150°C
Capillary temperature	320°C

Supplementary Table 5: Comparison of the known d11-PZQ internal standard (IS) concentration versus the concentration extracted from the peak area from the mass spectrometer (MS).

Samples	Average Recovered Concentration (ng/mL)	Percentage Accuracy (%)
Pooled	720.98 ± 230.15	120.16 ± 38.36
Individual	713.83 ± 95.06	118.97 ± 15.84

d11-PZQ: (deuterated-praziquantel)

Supplementary Table 6: The strength of LD based on the following test statistics: LOD, D', D' 95% CIs, and r^2. Two SNPs were determined to be in complete LD where the LOD score ≥3, the D'=1, 95% CIs of the D' value between them has a lower limit ≥ 0.7 and an upper limit ≥ 0.98, and with association measured using with $r^2 = 1$

Evidence of LD	D'	LOD	r^2	CI (95%)
COMPLETE	D' = 1	LOD ≥3	$r^2 = 1$	CI (95%) [≥0.7, ≥0.98]
STRONG	D' = 1	LOD ≥3	≥ 0.8	CI (95%) [≥0.7, ≥0.98]
INTERMEDIATE	D' = 1	LOD ≥3	< 0.8	CI (95%) [≥0.7, ≥0.98]

D' is the value of D prime between the two loci; LOD is the log of the likelihood odds ratio, a measure of confidence; r^2 is the correlation coefficient between the two loci; CI (95%) is 95% confidence interval bounds on D'.

Supplementary Table 7: Comparison of the mean concentration of praziquantel (PZQ) and the three metabolites detected for each of the timepoints collected using dried blood spot (DBS) cards. The timepoints indicated in bold represent where the means and pooled analysis are comparable and within the standard deviation (SD) of the means analysis.

Method	Timepoint (h)	Mean Concentration (ng/mL) ± SD			
		PZQ	4-OH-PZQ	Dehydro-O-PZQ	O2-PZQ
Pooled MS Sampling	0.5	21.9	85.0	9.9	17.2
Individual MS Sampling		3.6 ± 2.3	21 ± 8.1	3.6 ± 1.6	4.3 ± 1.9
Pooled MS Sampling	1.5	82.2	258.2	38.9	53.8
Individual MS Sampling		22.2 ± 13.8	130.4 ± 50.3	21.6 ± 9.8	26.4 ± 11.8
Pooled MS Sampling	**2.5**	**33.3**	**234.0**	**33.2**	**44.9**
Individual MS Sampling		**33.9 ± 21.1**	**210.1 ± 80.7**	**32.3 ± 14.8**	**39.8 ± 17.5**
Pooled MS Sampling	**4**	**33.2**	**211.3**	**31.0**	**45.0**
Individual MS Sampling		**31.9 ± 13.9**	**205.2 ± 69.8**	**31.9 ± 13.9**	**40.2 ± 14.5**

Supplementary Table 8: Noncompartmental analysis (NCA) of the parameters of the pooled MS samples versus the mean of the individual MS samples. Green highlight indicates the parameters that are within one standard deviation (SD).

Method	PZQ			4-OH-PZQ			(-2H)-O-PZQ			O2-PZQ		
	AUC (h*ng/mL) ± SD	Cmax (ng/mL)	Tmax (h)	AUC (h*ng/mL) ± SD	Cmax (ng/mL)	Tmax (h)	AUC (h*ng/mL) ± SD	Cmax (ng/mL)	Tmax (h)	AUC (h*ng/mL) ± SD	Cmax (ng/mL)	Tmax (h)
Pooled MS Sampling	165.3	82.24	1.5	772.9	258.25	1.5	108.3	38.90	1.5	156.7	53.82	1.5
Individual MS Sampling	91.2 ± 47.1	33.88	2.5	567.1 ± 195.6	210.09	2.5	88.6 ± 36.8	32.32	2.5	109.5 ± 43	40.19	4.0

Supplementary Table 9: Comparison of the mean concentration ± standard deviation (SD) of praziquantel (PZQ), 4-OH-PZQ, (-2H)-O-PZQ, and O2-PZQ concentrations (ng/mL) of those participants that had cleared or not cleared infection at each timepoint using one-way ANOVA. Green highlight indicates P<0.05 and was statistically significant.

Infection Status	Timepoint (h)	PZQ		4-OH-PZQ		(-2H)-O-PZQ		O2-PZQ	
		Mean Concentration (ng/mL) ± SD	P-value	Mean Concentration (ng/mL) ± SD	P-value	Mean Concentration (ng/mL) ± SD	P-value	Mean Concentration (ng/mL) ± SD	P-value
Cleared Infection	2.5	33.4 ± 21.2	0.718	212.1 ± 82.2	0.531	32.8 ± 15.1	0.027	40.3 ± 17.8	0.272
Not Cleared Infection		41.8 ± 24.8		174.7 ± 42.2		24.2 ± 2.6		31.1 ± 7.2	
Cleared Infection	4	34.4 ± 20.6	0.877	207 ± 64.2	0.505	32.6 ± 13.7	0.526	40.9 ± 14.1	0.552
Not Cleared Infection		29.5 ± 34.9		172.6 ± 183.3		20.1 ± 19.2		27.5 ± 22.4	

Supplementary Table 10: Comparison of the mean area-under-the-curve (AUC) ± standard deviation (SD) of praziquantel (PZQ), 4-OH-PZQ, (-2H)-O-PZQ, and O2-PZQ concentrations (ng/mL) of those participants that had cleared or not cleared infection at each timepoint using one-way ANOVA.

	PZQ				4-OH-PZQ				Dehydro-O-PZQ				O2-PZQ			
Infection Status	**Mean AUC (h*ng/mL) ± SD**	**P-value**	**Cmax (ng/mL)**	**Tmax (h)**	**Mean AUC (h*ng/mL) ± SD**	**P-value**	**Cmax (ng/mL)**	**Tmax (h)**	**Mean AUC (h*ng/mL) ± SD**	**P-value**	**Cmax (ng/mL)**	**Tmax (h)**	**Mean AUC (h*ng/mL) ± SD**	**P-value**	**Cmax (ng/mL)**	**Tmax (h)**
Cleared Infection	92.2 ± 46.6	0.71	34.36	4.0	572.4 ± 196.3	0.484	212.06	2.5	90 ± 37.2	0.326	32.77	2.5	111 ± 43.7	0.373	40.90	4.0
Not Cleared Infection	105.2 ± 75.1		41.77	2.5	471.3 ± 216.4		175.35	2.5	63.4 ± 9.1		24.15	2.5	82.7 ± 2.1		31.09	2.5

Supplementary Table 11: Linkage disequilibrium (LD) statistics for the detected variants. D' is the value of D prime between the two loci; LOD is the log of the likelihood odds ratio, a measure of confidence; r^2 is the correlation coefficient between the two loci; CI (95%) is 95% confidence interval bounds on D'. All pairwise comparisons of SNPs were < 500 kb apart, any variants which had a D' = 0 was excluded from further analysis as did not indicate LD.

Chromosome	Gene	SNP 1	SNP 2	D'	CI (95%)	LOD	r^2	Evidence of LD
15	CYP1A2	CYP1A2*1C	CYP1A2*1F	1	[0.3 ,1]	1	0.339	None
22	CYP2D6	rs1135840	rs16947	1	[0.29 ,1]	1.64	0.24	None
		rs1135840	CYP2D6*4	1	[0.05 ,0.97]	0.18	0.007	None
		rs16947	CYP2D6*4	1	[0.05 ,0.97]	0.21	0.029	None
15	CYP3A5	CYP3A5*6	CYP3A5*3	1	[0.06 ,0.98]	0.27	0.031	None

Acronyms - SNP: Single nucleotide polymorphism, CYP: cytochrome P450

Supplementary Table 12: Comparison of the mean concentration ± standard deviation (SD) of praziquantel (PZQ), 4-OH-PZQ, (-2H)-O-PZQ, and O2-PZQ concentrations (ng/mL) and the metabolic ratio (metabolite/PZQ) between the detected cytochrome P450 (CYP) genotypes using one-way ANOVA. This analysis was performed using the genotypic model. Orange highlight indicates 0.05<P<0.1. Green highlight indicates P<0.05 and was statistically significant.

					PZQ		4-OH-PZQ				Dehydro-O-PZQ				O2-PZQ			
Timpoint (h)	**Enzyme**	**Variant**	**Genotype**	**N**	**Mean Concentration (ng/mL) ± SD**	**P-value**	**Mean Concentration (ng/mL) ± SD**	**P-value**	**Ratio ± SD**	**P-value**	**Mean Concentration (ng/mL) ± SD**	**P-value**	**Ratio ± SD**	**P-value**	**Mean Concentration (ng/mL) ± SD**	**P-value**	**Ratio ± SD**	**P-value**
2.5	CYP1A2	CYP1A2*1C	0/0	1	58.1 ± -	0.25	306.3 ± -	0.232	5.4 ± -	0.506	39.6 ± -	0.627	0.7 ± -	0.473	45.4 ± -	0.747	0.8 ± -	0.434
			0/1	37	33.2 ± 21		207.5 ± 80.2		7.6 ± 3.4		32.1 ± 15		1.2 ± 0.7		39.6 ± 17.7		1.5 ± 0.9	
		CYP1A2*1F	0/0	9	44.8 ± 24.1	0.19	208.4 ± 73.6	0.917	5.5 ± 2.8	0.076	28.8 ± 11.4	0.681	0.8 ± 0.4	0.101	33.9 ± 11.3	0.497	0.9 ± 0.6	0.097
			0/1	20	29.4 ± 20		206.4 ± 77.9		8.5 ± 3.5		32.7 ± 16.1		1.3 ± 0.7		40.9 ± 19.4		1.7 ± 0.9	
			1/1	9	32.9 ± 18.6		220 ± 100.6		7.3 ± 2.7		35 ± 15.9		1.3 ± 0.8		43.1 ± 18.2		1.7 ± 1.1	
		Novel-2	0/1	38	33.9 ± 21.1	-	210.1 ± 80.7	-	7.5 ± 3.3	-	32.3 ± 14.8	-	1.2 ± 0.7	-	39.8 ± 17.5	-	1.5 ± 0.9	-
	CYP2C9	CYP2C9*9	0/0	29	33.8 ± 22.4	0.991	199.4 ± 81.4	0.098	7.1 ± 3.1	0.256	29.3 ± 12.9	0.009	1.1 ± 0.6	0.092	36 ± 14.1	0.008	1.4 ± 0.8	0.178
			0/1	7	34.6 ± 16.4		267 ± 50		9.3 ± 4.1		47.1 ± 14		1.7 ± 0.9		57.6 ± 20.6		2.1 ± 1.3	
			1/1	2	32.3 ± 28.9		165.4 ± 101.2		6.2 ± 2.4		24.6 ± 20		0.8 ± 0.1		32.6 ± 20.6		1.2 ± 0.4	
	CYP2D6	CYP2D6*4	0/0	37	33.2 ± 21	0.25	207.5 ± 80.2	0.232	7.6 ± 3.4	0.506	32.1 ± 15	0.627	0.2 ± 0.7	0.473	39.6 ± 17.7	0.747	1.5 ± 0.9	0.434
			0/1	1	58.1 ± -		306.3 ± -		5.3 ± -		39.6 ± -		0.7 ± -		45.4 ± -		0.8 ± -	
		CYP2D6*17	0/1	38	33.9 ± 21.1	-	210.1 ± 80.7	-	7.5 ± 3.3	-	32.3 ± 14.8	-	1.2 ± 0.7	-	39.8 ± 17.5	-	1.5 ± 0.9	-
		rs1135840	0/1	26	33.3 ± 22.2	0.801	213.4 ± 83.9	0.719	7.9 ± 3.6	0.309	34 ± 16.1	0.326	1.3 ± 0.8	0.2	41.6 ± 19.4	0.326	1.6 ± 1	0.272
			1/1	12	35.2 ± 19.4		203 ± 76.4		6.7 ± 2.7		28.8 ± 11.4		1 ± 0.5		28.8 ± 11.4		1.3 ± 0.7	
		rs16947	0/0	14	31.7 ± 21.9	0.627	198.6 ± 93	0.511	7.4 ± 3.6	0.876	33.1 ± 18	0.819	1.3 ± 0.8	0.533	41 ± 22.2	0.755	1.7 ± 1.2	0.496
			0/1	24	35.2 ± 21		216.8 ± 73.9		7.6 ± 3.3		31.9 ± 13.1		1.1 ± 0.6		39.1 ± 14.5		1.4 ± 0.8	
	CYP3A5	CYP3A5*3	0/0	30	33.9 ± 21.6	0.992	211.1 ± 88.5	0.879	7.3 ± 3	0.39	33.3 ± 16	0.439	1.2 ± 0.7	0.778	40.7 ± 18.7	0.524	1.5 ± 1	0.964
			0/1	8	33.9 ± 20.7		206.2 ± 43.9		8.4 ± 4.6		28.6 ± 9.5		1.1 ± 0.6		36.2 ± 11.8		1.5 ± 1	
		CYP3A5*6	0/0	22	30.6 ± 20.5	0.273	193.5 ± 77	0.139	7.6 ± 3.4	0.771	31.4 ± 15.3	0.662	1.3 ± 0.7	0.444	39.7 ± 18.7	0.965	1.7 ± 1	0.295
			0/1	16	38.3 ± 21.7		232.9 ± 82.5		7.3 ± 3.3		33.6 ± 14.7		1.1 ± 0.6		39.9 ± 16.1		1.3 ± 0.8	
		CYP3A5*7	0/0	29	34.7 ± 22.2	0.668	208.2 ± 81.3	0.795	7.3 ± 3.3	0.474	32.4 ± 15.1	0.926	1.2 ± 0.7	0.872	40 ± 18	0.898	1.5 ± 1	0.917
			0/1	9	31.2 ± 18.1		216.3 ± 83		8.2 ± 3.7		31.9 ± 14.8		1.2 ± 0.7		39.1 ± 16.5		1.6 ± 0.9	
4	CYP1A2	CYP1A2*1C	0/0	1	26.5 ± -	0.716	260.9 ± -	0.427	9.9 ± -	0.866	35.4 ± -	0.804	1.3 ± -	0.96	41.6 ± -	0.922	1.6 ± -	0.877
			0/1	37	34.3 ± 21.1		203.7 ± 70.2		8.7 ± 6.7		31.8 ± 14.1		1.4 ± 1.3		40.2 ± 14.7		1.9 ± 1.8	
		CYP1A2*1F	0/0	9	42.8 ± 17.4	0.355	198.7 ± 84.1	0.945	5.1 ± 2.3	0.14	26.5 ± 11.4	0.422	0.7 ± 0.3	0.105	33.6 ± 13.2	0.31	0.9 ± 0.3	0.121
			0/1	20	30.6 ± 20.9		208.3 ± 50.7		10.4 ± 7.6		33.2 ± 10.3		1.8 ± 1.6		42.1 ± 12.4		2.3 ± 2.1	
			1/1	9	33.2 ± 23.6		204.8 ± 96.4		8.7 ± 6		34.3 ± 21.8		1.3 ± 0.8		42.5 ± 19.4		1.8 ± 1.4	
		Novel-2	0/1	38	34.1 ± 20.9	-	205.2 ± 69.8	-	8.8 ± 6.6	-	31.9 ± 13.9	-	1.4 ± 1.3	-	40.2 ± 14.5	-	1.9 ± 1.7	-

	CYP2C9	**CYP2C9*9**	0/0	29	36.7 ± 20.7	0.13	204 ± 72.4	0.647	8 ± 6.5	0.154	30.9 ± 13.4	0.221	1.3 ± 1.3	0.096	38 ± 12.7	0.084	1.6 ± 1.7	0.119
			0/1	7	20.3 ± 9.9		220.8 ± 59		12.9 ± 6.5		39.1 ± 14.6		2.3 ± 1.3		51 ± 17.2		3 ± 1.7	
			1/1	2	45.3 ± 42.5		168.6 ± 90.7		5 ± 2.7		21.9 ± 17		0.6 ± 0.1		34.2 ± 22.6		0.9 ± 0.4	
	CYP2D6	**CYP2D6*4**	0/0	37	34.3 ± 21.1	0.716	203.7 ± 70.2	0.427	8.7 ± 6.7	0.866	31.8 ± 14.1	0.804	1.4 ± 1.3	0.96	40.2 ± 14.7	0.922	1.9 ± 1.8	0.877
			0/1	1	26.5 ± -		260.9 ± -		9.9 ± -		35.4 ± -		1.3 ± -		41.6 ± -		1.6 ± -	
		CYP2D6*17	0/1	38	34.1 ± 20.9	-	205.2 ± 69.8	-	8.8 ± 6.6	-	31.9 ± 13.9	-	1.4 ± 1.3	-	40.2 ± 14.5	-	1.9 ± 1.7	-
		rs1135840	0/1	26	34.7 ± 19.1	0.792	218.1 ± 61.4	0.093	8.8 ± 5.9	0.903	35.5 ± 13.7	0.017	1.5 ± 1	0.765	44.2 ± 13.8	0.01	1.9 ± 1.5	0.866
			1/1	12	32.8 ± 25.2		177.2 ± 81.2		8.6 ± 8.2		24.1 ± 11.4		1.3 ± 1.8		31.5 ± 12.5		1.8 ± 2.3	
		rs16947	0/0	14	36.9 ± 21.1	0.541	196.4 ± 69.5	0.56	7.4 ± 5.2	0.33	34.8 ± 17.2	0.332	1.3 ± 0.8	0.62	41.6 ± 16.1	0.659	1.6 ± 1.3	0.557
			0/1	24	32.5 ± 21		210.3 ± 71		9.6 ± 7.2		30.2 ± 11.7		1.5 ± 1.5		39.4 ± 13.9		2 ± 2	
	CYP3A5	**CYP3A5*3**	0/0	30	37 ± 21.7	0.1	208.5 ± 71.3	0.581	8.2 ± 6.4	0.366	33.6 ± 14.6	0.149	1.4 ± 1.4	0.936	41.6 ± 15.2	0.26	1.8 ± 1.8	0.698
			0/1	8	23.3 ± 13.8		192.9 ± 67		10.6 ± 7.3		25.6 ± 8.9		1.4 ± 1		35 ± 10.8		2.1 ± 1.7	
		CYP3A5*6	0/0	22	34.5 ± 24.4	0.895	189.4 ± 64	0.102	9.2 ± 7.9	0.615	31.6 ± 14.7	0.892	1.6 ± 1.5	0.412	39.5 ± 15	0.726	2.1 ± 2.1	0.388
			0/1	16	33.6 ± 15.6		226.9 ± 73.6		8.1 ± 4.4		32.3 ± 13.3		1.2 ± 0.9		41.2 ± 14.4		1.6 ± 1.2	
		CYP3A5*7	0/0	29	31.8 ± 19.8	0.234	197.3 ± 77.7	0.216	9 ± 7	0.637	31.3 ± 15.3	0.662	1.5 ± 1.4	0.619	39.5 ± 15.4	0.582	1.9 ± 1.8	0.541
			0/1	9	41.4 ± 23.8		230.6 ± 22		7.8 ± 5.2		33.7 ± 8.6		1.2 ± 1.2		42.6 ± 11.6		1.5 ± 1.5	

Acronyms - CYP: cytochrome P450

Supplementary Table 13: Noncompartmental analysis (NCA) to compare the mean area-under-the-curve (AUC) ± standard deviation (SD) of praziquantel (PZQ), 4-OH-PZQ, (-2H)-O-PZQ, and O2-PZQ using one-way ANOVA. The pharmacokinetic (PK) parameters, C_{max} and the $T_{max,}$ are also reported and this analysis was performed using the dominant model. Orange highlight indicates 0.05<P<0.1. Green highlight indicates P<0.05 and was statistically significant.

				PZQ				4-OH-PZQ				Dehydro-O-PZQ				O2-PZQ			
Enzyme	**Variant**	**Genotype**	**N**	**Mean AUC (h*ng/mL) ± SD**	**P-value**	**Cmax (ng/mL)**	**Tmax (h)**	**Mean AUC (h*ng/mL) ± SD**	**P-value**	**Cmax (ng/mL)**	**Tmax (h)**	**Mean AUC (h*ng/mL) ± SD**	**P-value**	**Cmax (ng/mL)**	**Tmax (h)**	**Mean AUC (h*ng/mL) ± SD**	**P-value**	**Cmax (ng/mL)**	**Tmax (h)**
CYP1A2	**CYP1A2*1C**	0/0	1	135 ± -	0.372	58.1	2.5	801 ± -	0.230	306.3	2.5	105.7 ± -	0.644	39.6	2.5	121.5 ± -	0.782	45.4	2.5
		0/1 & 1/1	37	91.7 ± 47.2		34.3	4	560.8 ± 194.3		207.5	2.5	88.1 ± 37.1		32.1	2.5	109.2 ± 43.6		40.2	4
	CYP1A2*1F	0/0	9	120.9 ± 51.6	0.039	44.8	2.5	555.5 ± 195.9	0.842	208.4	2.5	77.3 ± 30.4	0.299	28.8	2.5	92.5 ± 30.5	0.178	33.9	2.5
		0/1 & 1/1	29	84.2 ± 42.8		33.2	4	570.7 ± 198.8		220.0	2.5	92.1 ± 38.3		35.0	2.5	114.8 ± 45.4		43.1	2.5
	Novel-2	0/1 & 1/1	38	92.9 ± -	-	34.1	4	567.1 ± -	-	210.1	2.5	88.6 ± -	-	32.3	2.5	109.5 ± -	-	40.2	4
CYP2C9	**CYP2C9*9**	0/0	29	94.7 ± 48.1	0.68	36.7	4	544.9 ± 198.4	0.215	204.0	4	81.7 ± 31.9	0.037	30.9	4	100.1 ± 33.9	0.014	38.0	4
		0/1 & 1/1	9	87.1 ± 46		45.3	4	638.4 ± 177.7		267.0	2.5	110.7 ± 44.4		47.1	2.5	139.7 ± 56.5		57.6	2.5
CYP2D6	**CYP2D6*4**	0/0	37	91.7 ± 47.2	0.372	34.3	4	560.8 ± 194.3	0.230	207.5	2.5	88.1 ± 37.1	0.644	32.1	2.5	109.2 ± 43.6	0.782	40.2	4
		0/1 & 1/1	1	135 ± -		58.1	2.5	801 ± -		306.3	2.5	105.7 ± -		39.6	2.5	121.5 ± -		45.4	2.5
	CYP2D6*17	0/1 & 1/1	38	92.9 ± 47.1	-	34.1	4	567.1 ± 195.6	-	210.1	2.5	88.6 ± 36.8	-	32.3	2.5	109.5 ± 43	-	40.2	4
	rs1135840	0/1 & 1/1	38	92.9 ± 47.1	-	34.1	4	567.1 ± 195.6	-	210.1	2.5	88.6 ± 36.8	-	32.3	2.5	109.5 ± 43	-	40.2	4
	rs16947	0/0	14	90.6 ± 48.1	0.82	36.9	4	538.4 ± 221.1	0.498	198.6	2.5	92.2 ± 45	0.714	34.8	4	112.9 ± 53.9	0.648	41.6	4
		0/1 & 1/1	24	94.2 ± 47.4		35.2	2.5	583.8 ± 182		216.8	2.5	86.5 ± 31.9		31.9	2.5	107.5 ± 36.4		39.4	4
CYP3A5	**CYP3A5*3**	0/0	30	95 ± 47.9	0.597	37.0	4	571.5 ± 216.3	0.793	211.1	2.5	91.8 ± 39.6	0.299	33.6	4	112.4 ± 46.3	0.43	41.6	4
		0/1 & 1/1	8	84.9 ± 48.1		33.9	2.5	550.6 ± 89.5		206.2	2.5	76.4 ± 20.3		28.6	2.5	98.7 ± 26.8		36.2	2.5
	CYP3A5*6	0/0	22	86.7 ± 46.8	0.347	34.5	4	522.5 ± 178.3	0.100	193.5	2.5	86.5 ± 37.1	0.682	31.6	4	108.6 ± 44.9	0.887	39.7	2.5
		0/1 & 1/1	16	101.4 ± 47.5		38.3	2.5	628.4 ± 207.3		232.9	2.5	91.5 ± 37.3		33.6	2.5	110.9 ± 41.7		41.2	4
	CYP3A5*7	0/0	29	92.9 ± 49.3	1	34.7	2.5	557.7 ± 204.4	0.601	208.2	2.5	88.5 ± 38.6	0.974	32.4	2.5	109.4 ± 45.1	0.984	40.0	2.5
		0/1 & 1/1	9	92.9 ± 41.9		41.4	4	597.4 ± 171.3		230.6	4	88.9 ± 32.3		33.7	4	109.8 ± 37.8		42.6	4

Acronyms - CYP: cytochrome P450

Supplementary Table 14: Noncompartmental analysis (NCA) was performed to compare the mean area-under-the-curve (AUC) ± standard deviation (SD) of praziquantel (PZQ), 4-OH-PZQ, (-2H)-O-PZQ, and O2-PZQ using One-way ANOVA. The pharmacokinetic (PK) parameters, C_{max} and the $T_{max,}$, are also reported and this analysis was performed using the genotypic model. Green highlight indicates P<0.05 and was statistically significant.

				PZQ				4-OH-PZQ					Dehydro-O-PZQ				O2-PZQ			
Enzyme	**Variant**	**Genotype**	**N**	**Mean AUC (h*ng/mL) ± SD**	**P-value**	**Cmax (ng/mL)**	**Tmax (h)**	**Mean AUC (h*ng/mL) ± SD**	**SD**	**P-value**	**Cmax (ng/mL)**	**Tmax (h)**	**Mean AUC (h*ng/mL) ± SD**	**P-value**	**Cmax (ng/mL)**	**Tmax (h)**	**Mean AUC (h*ng/mL) ± SD**	**P-value**	**Cmax (ng/mL)**	**Tmax (h)**
CYP1A2	CYP1A2*1C	0/0	1	135 ± -	0.372	58.1	2.5	801 ± -	-	0.230	306.3	2.5	105.7 ± -	0.644	39.6	2.5	121.5 ± -	0.782	45.4	2.5
		0/1	37	91.7 ± 47.2		34.3	4	560.8 ± 194.3	194.31		207.5	2.5	88.1 ± 37.1		32.1	2.5	109.2 ± 43.6		40.2	4
	CYP1A2*1F	0/0	9	120.9 ± 51.6	0.106	44.8	2.5	555.5 ± 196	195.95	0.940	208.4	2.5	77.3 ± 30.4	0.554	28.8	2.5	92.5 ± 30.5	0.39	33.9	2.5
		0/1	20	81.2 ± 46		30.6	4	563.4 ± 179.2	179.22		208.3	4	90.5 ± 37.7		33.2	4	113.1 ± 46		42.1	4
		1/1	9	90.8 ± 36.4		33.2	4	586.8 ± 248.3	248.25		220.0	2.5	95.7 ± 41.8		35.0	2.5	118.5 ± 46.5		43.1	2.5
	Novel-2	0/1	38	92.9 ± -	-	34.1	4	567.1 ± -	195.59	-	210.1	2.5	88.6 ± -	-	32.3	2.5	109.5 ± -	-	40.2	4
CYP2C9	CYP2C9*9	0/0	29	94.7 ± 48.1	0.86	36.7	4	544.9 ± 198.4	198.44	0.137	204.0	4	81.7 ± 31.9	0.013	30.9	4	100.1 ± 33.9	0.007	38.0	4
		0/1	7	84 ± 38		34.6	2.5	692.4 ± 123.9	123.90		267.0	2.5	123.5 ± 36.7		47.1	2.5	153.8 ± 51.5		57.6	2.5
		1/1	2	98.1 ± 89.2		45.3	4	449.6 ± 262.6	262.57		168.6	4	66.1 ± 50.3		24.6	2.5	90.4 ± 57.8		34.2	4
CYP2D6	CYP2D6*4	0/0	37	91.7 ± 47.2	0.372	34.3	4	560.8 ± 194.3	194.31	0.230	207.5	2.5	88.1 ± 37.1	0.644	32.1	2.5	109.2 ± 43.6	0.782	40.2	4
		0/1	1	135 ± -		58.1	2.5	801 ± -	-		306.3	2.5	105.7 ± -		39.6	2.5	121.5 ± -		45.4	2.5
	CYP2D6*17	0/1	38	92.9 ± 47.1	-	34.1	4	567.1 ± 195.6	195.59	-	210.1	2.5	88.6 ± 36.8	-	32.3	2.5	109.5 ± 43	-	40.2	4
	rs1135840	0/1	26	92.1 ± 49	0.888	34.7	4	583.7 ± 200.6	200.58	0.449	218.1	4	94.5 ± 39.8	0.158	35.5	4	116.2 ± 47.5	0.146	44.2	4
		1/1	12	94.5 ± 44.6		35.2	2.5	531.1 ± 187.6	187.56		203.0	2.5	75.8 ± 26.1		28.8	2.5	94.9 ± 27.6		31.5	4
	rs16947	0/0	14	90.6 ± 48.1	0.82	36.9	4	538.4 ± 221.1	221.09	0.498	198.6	2.5	92.2 ± 45	0.714	34.8	4	112.9 ± 53.9	0.648	41.6	4
		0/1	24	94.2 ± 47.4		35.2	2.5	583.8 ± 182	181.99		216.8	2.5	86.5 ± 31.9		31.9	2.5	107.5 ± 36.4		39.4	4
CYP3A5	CYP3A5*3	0/0	30	95 ± 47.9	0.597	37.0	4	571.5 ± 216.3	216.29	0.793	211.1	2.5	91.8 ± 39.6	0.299	33.6	4	112.4 ± 46.3	0.43	41.6	4
		0/1	8	84.9 ± 48.1		33.9	2.5	550.6 ± 89.5	89.46		206.2	2.5	76.4 ± 20.3		28.6	2.5	98.7 ± 26.8		36.2	2.5
	CYP3A5*6	0/0	22	86.7 ± 46.8	0.347	34.5	4	522.5 ± 178.3	178.26	0.100	193.5	2.5	86.5 ± 37.1	0.682	31.6	4	108.6 ± 44.9	0.887	39.7	2.5
		0/1	16	101.4 ± 47.5		38.3	2.5	628.4 ± 207.3	207.25		232.9	2.5	91.5 ± 37.3		33.6	2.5	110.9 ± 41.7		41.2	4
	CYP3A5*7	0/0	29	92.9 ± 49.3	1	34.7	2.5	557.7 ± 204.4	204.40	0.601	208.2	2.5	88.5 ± 38.6	0.974	32.4	2.5	109.4 ± 45.1	0.984	40.0	2.5
		0/1	9	92.9 ± 41.9		41.4	4	597.4 ± 171.3	171.28		230.6	4	88.9 ± 32.3		33.7	4	109.8 ± 37.8		42.6	4

Acronyms - CYP: cytochrome P450

Supplementary Table 15: Chi-squared and Fisher's exact tests to assess the association between PZQ efficacy and CYP genotype using the dominant genotypic model.

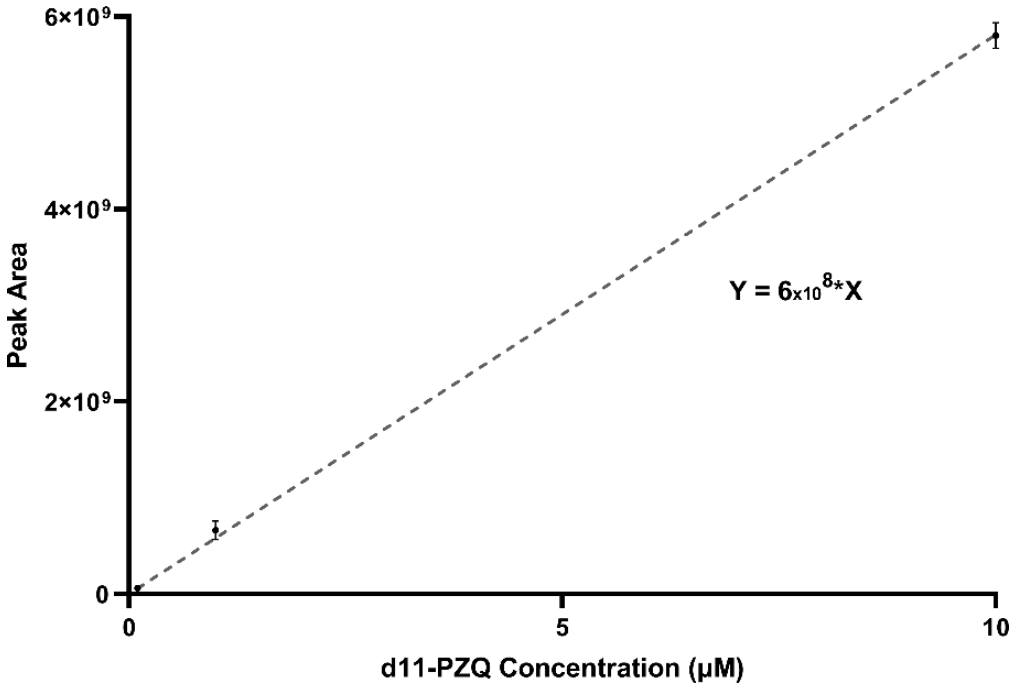
				3 Week Status	
				P-Values	
Enzyme	**Variant**	**Genotype**	**N**	**Chi-Squared**	**Fisher's Exact**
CYP1A2	**CYP1A2*1C**	0/0	1	0.811	1
		0/1 & 1/1	37		
	CYP1A2*1F	0/0	9	0.368	0.422
		0/1 & 1/1	29		
	Novel-2	0/1 & 1/1	38	-	-
CYP2C9	**CYP2C9*9**	0/0	29	0.418	1
		0/1 & 1/1	9		
CYP2D6	**CYP2D6*4**	0/0	37	0.811	1
		0/1 & 1/1	1		
	CYP2D6*17	0/1	38	-	-
	rs1135840	0/1 & 1/1	38	-	-
	rs16947	0/0	14	0.692	1
		0/1 & 1/1	24		
CYP3A5	**CYP3A5*3**	0/0	30	0.302	0.381
		0/1 & 1/1	8		
	CYP3A5*6	0/0	22	0.215	0.499
		0/1 & 1/1	16		
	CYP3A5*7	0/0	29	0.418	1
		0/1 & 1/1	9		

Acronyms - CYP: cytochrome P450

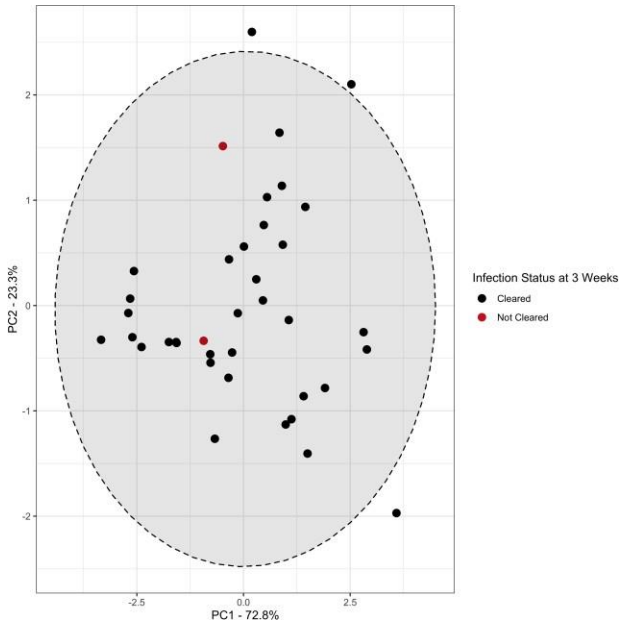
Supplementary Table 16: Chi-squared and fisher's exact tests to assess the association between PZQ efficacy and CYP genotype using the genotypic model.

				3 Week Status	
				P-Values	
Enzyme	**Variant**	**Genotype**	**N**	**Chi-Squared**	**Fisher's Exact**
CYP1A2	**CYP1A2*1C**	0/0	1	0.811	1
		0/1	37		
	CYP1A2*1F	0/0	9	0.309	-
		0/1	20		
		1/1	9		
	Novel-2	0/1	38	-	-
CYP2C9	**CYP2C9*9**	0/0	29	0.721	-
		0/1	7		
		1/1	2		
CYP2D6	**CYP2D6*4**	0/0	37	0.811	1
		0/1	1		
	CYP2D6*17	0/1	38	-	-
	rs1135840	0/0	26	0.565	0.538
		0/1	12		
	rs16947	0/0	14	0.692	1
		0/1	24		
CYP3A5	**CYP3A5*3**	0/0	30	0.302	0.381
		0/1	8		
	CYP3A5*6	0/0	22	0.215	0.499
		0/1	16		
	CYP3A5*7	0/0	29	0.418	1
		0/1	9		

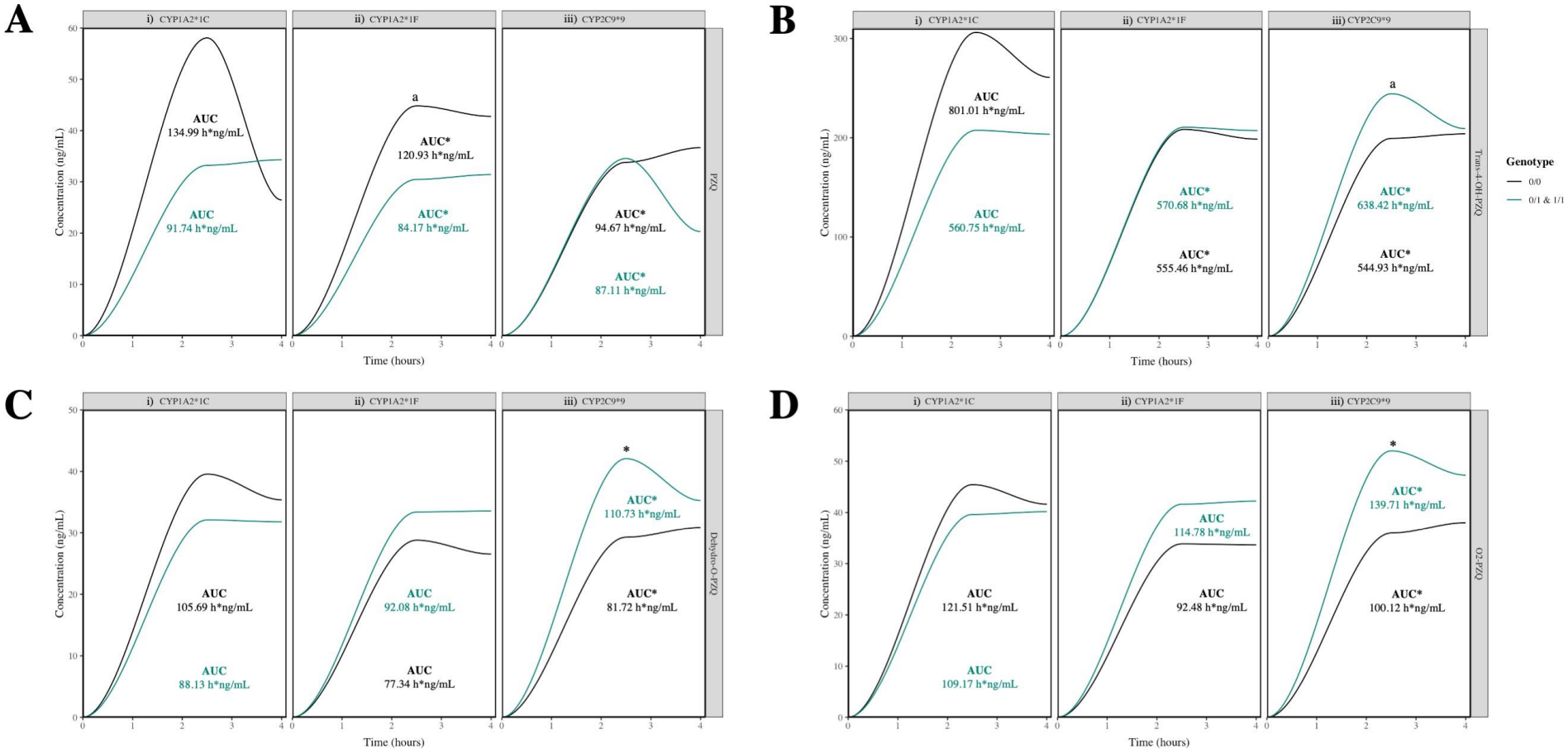
Acronyms - CYP: cytochrome P450



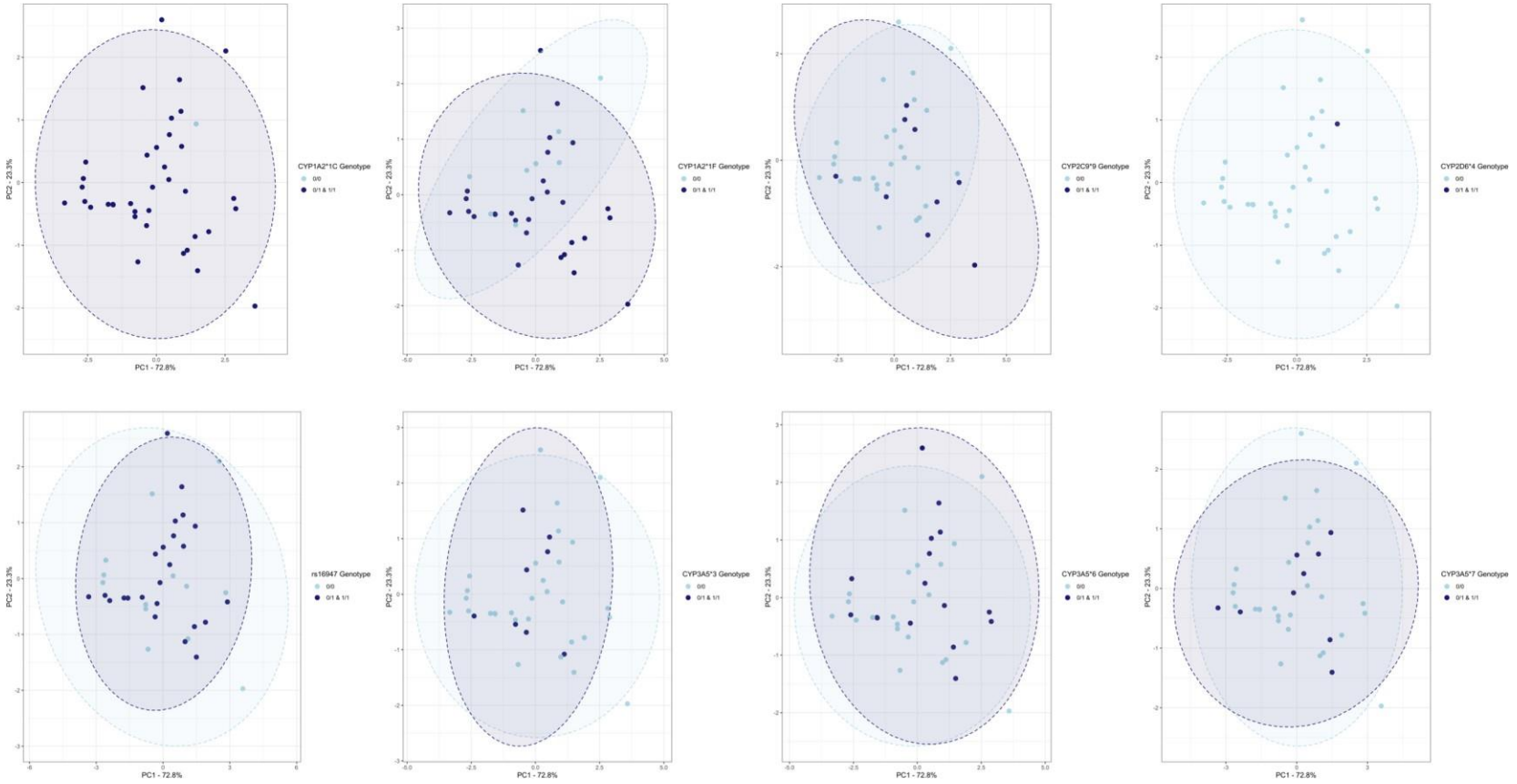
Supplementary Figure 1: Calibration curve with error bars between the replicates presented on each data point.



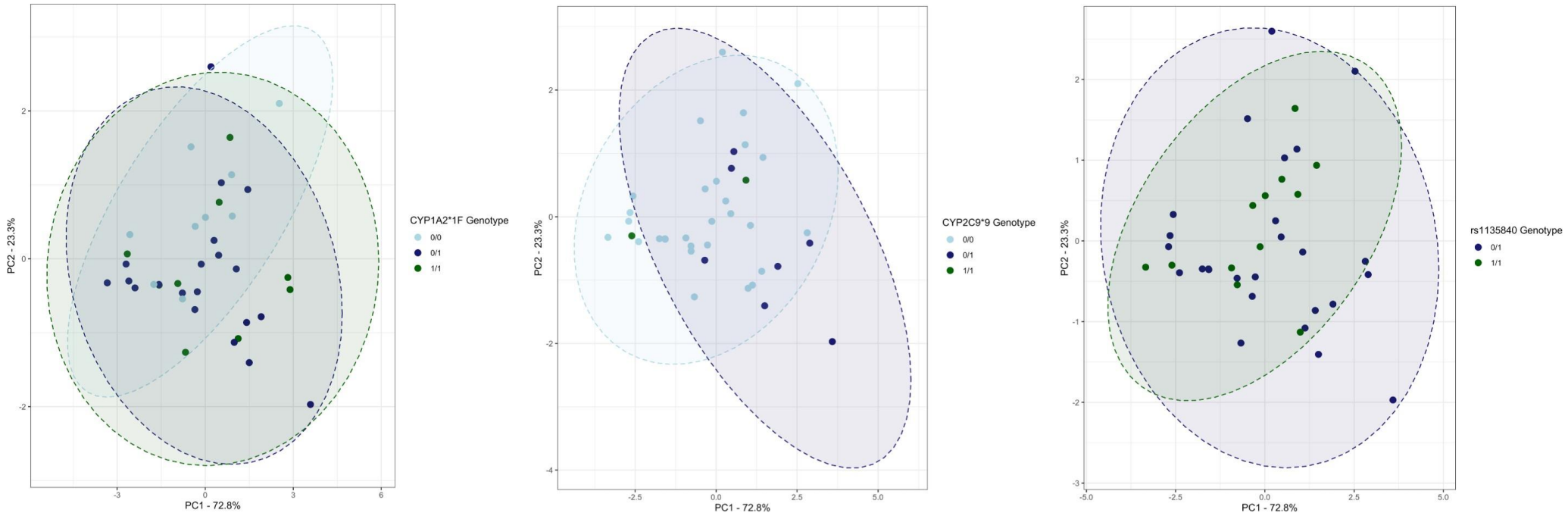
Supplementary Figure 2: Principal component analysis (PCA) plots of the concentrations (ng/mL) of praziquantel (PZQ), 4-OH-PZQ, (-2H)-O-PZQ, and O2-PZQ across samples, annotated by 3-week infection status. PC1 and PC2 explained 96.1% of the total variation in the concentration of the components. The participants who did not clear infection had ≤2 participants, and ellipses could not be drawn. Acronyms: 4-OH: 4-monohydroxy, (-2H)-O: dehydro-mono-oxidised, O2: di-oxidised.



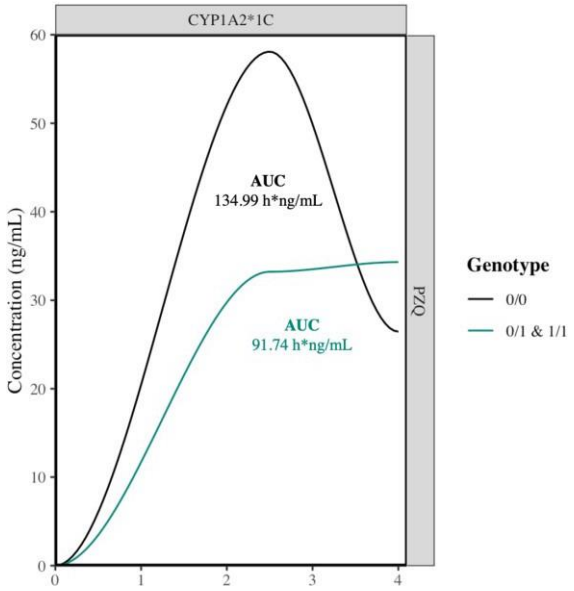
Supplementary Figure 3: Pharmacokinetic (PK) profile of praziquantel (PZQ) [A], 4-OH-PZQ [B], (-2H)-O-PZQ [C], and O2-PZQ [D] across the CYP1A2*1C, CYP1A2*1F, and CYP2C9*9 variants. The dominant genotypic model was used with the black line indicating the reference [0/0] genotype, and the green line indicating the dominant [0/1 & 1/1] genotypes. The area-under-the-curve (AUC) values are presented under the relevant curves. CYP: cytochrome P450.



Supplementary Figure 4: Principal component analysis (PCA) plots of the concentrations (ng/mL) of praziquantel (PZQ), 4-OH-PZQ, (-2H)-O-PZQ, and O2-PZQ across samples, annotated by variant and genotype. PC1 and PC2 explained 96.1% of the total variation in the concentration of the components. When a CYP genotype had ≤2 participants, ellipses could not be drawn. Acronyms: 4-OH: 4-monohydroxy, (-2H)-O: dehydro-mono-oxidised, O2: di-oxidised.



Supplementary Figure 5: Principal component analysis (PCA) plots of the concentrations (ng/mL) of praziquantel (PZQ), 4-OH-PZQ, (-2H)-O-PZQ, and O2-PZQ across samples, annotated by genotype. PC1 and PC2 explained 96.1% of the total variation in the concentration of the components. When a CYP genotype had ≤2 participants, ellipses could not be drawn. Acronyms: 4-OH: 4-monohydroxy, (-2H)-O: dehydro-mono-oxidised, O2: di-oxidised.



Supplementary Figure 6: Pharmacokinetic (PK) profile of praziquantel (PZQ) for the CYP1A2*1C, variant. The dominant genotypic model was used with the black line indicating the reference [0/0] genotype, and the green line indicating the dominant [0/1 & 1/1] genotypes. The area-under-the-curve (AUC) values are shown under the relevant curves. CYP: cytochrome P450.

Supplementary Data 1: Reference FASTA file for the alignment with the FASTQ file.

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>NC_000015.10:99768886-99769286 Homo sapiens chromosome 15, GRCh38.p14 Primary Assembly
>NC_000015.10:99772928-99773328 Homo sapiens chromosome 15, GRCh38.p14 Primary Assembly
>NC_000015.10:99783499-99783899 Homo sapiens chromosome 15, GRCh38.p14 Primary Assembly
>NC_000015.10:99783518-99783918 Homo sapiens chromosome 15, GRCh38.p14 Primary Assembly
>NC_000015.10:99784249-99784649 Homo sapiens chromosome 15, GRCh38.p14 Primary Assembly
>NC_000015.10:99784271-99784671 Homo sapiens chromosome 15, GRCh38.p14 Primary Assembly

Appendix 2

Supplementary Table 1: The transmission scores for both 1988 and 2012 of the suitability of the sample sites used in this study in Zimbabwe as a host snail habitat. Snail habitats were obtained by overlaying the spatial maps from Pedersen *et al.* (Pedersen *et al.*, 2014a, Pedersen *et al.*, 2014b) with the sample sites used in this study.

District	Village	Transmission Suitability Score 1988	Transmission Suitability Score 2012
UMP	Kafura	0.54	0.77
Bikita	Chitenderano	0.54	0.38
Gokwe North	Chireya	0.31	0
Hurungwe	Kajekache	0.31	0.15
Shurugwi	Newgato	0.62	0.69
Mazowe	Mbebi	0.69	0.38
Buhera	Masocha	0.54	0.85
Shamva	Gono	0.54	0.77
Shamva	Chihuri	0.62	0.77
Mberengwa	Zverenje	0.69	0.77
Nyanga	Chipataronga	0.77	0.77
Insiza	Insiza	0.54	0.46
Murehwa	Chingono	0.69	0.85
Mberengwa	Mukwakwe	0.54	0.69
Mutoko	Chimukopa	0.69	0.77
Zvimba	Chomutamba	0.69	0.77
Binga	Siabuwa	0.38	0
Murehwa	Chiguri	0.69	0.69
Chirumanzu	Gambiza	0.77	0.62
Makoni	Bandanyenje	0.69	0.85
Chikomba	Nhidza	0.77	0.62
Chegutu	Gadzema	0.54	0.54
Nkayi	Gonye	0.46	0.31
Mt Darwin	Bemberi	0.69	0.85
Makonde	Kanyaga	0.46	0.31
Chiredzi	Mareya	0.46	0.54
Mwenezi	Ruzongwe	0.54	0.69
Gutu	Mutendeure	0.77	0.62
Mutoko	Kushinga	0.69	0.85
Chipinge	Chitepo	0.38	0.77
Rushinga	Mazowe Bridge	0.54	0.62
Muzarabani	Muzarabani	0.31	0.38
Mberengwa	Nhenga	0.46	0.54
Guruve	Nyanhunzi	0.69	0.69
Hurungwe	Dandawa	0.31	0.15

Supplementary Table 2: Model summary and predictors of each regression model

Regression Model	*Predictors*				
	Distance to Body of Water	Transmission Score		Baseline	
		1988	2012	Prevalence	Mean Egg Count
1	x	x			
2	x		x		
3	x	x		x	x
4	x		x	x	x
5	x				

Supplementary Table 3: Model summary and coefficients of each linear regression model

Linear Regression Model	Dependent Variable	*Predictors*					Model Parameters		
		Transmission Score		Distance to Body of Water	Baseline Prevalence	Baseline Mean Egg Count			
		1988	2012				R^2	F	Sig
1	Prevalence	B=1.97, p=0.88		B=-0.676, p=0.446			0.005	0.297	0.744
	Mean Egg Count	B=-2.351, p=0.829		B=-1.68, p=0.819			0.001	0.054	0.948
2	Prevalence		B=5.763, p=0.473	B=-0.697, p=0.43			0.009	0.545	0.581
	Mean Egg Count		B=4.427, p=0.508	B=-0.205, p=0.781			0.004	0.251	0.778
3	Cure Rate	B=12.651, p=0.617		B=-3.343, p=0.044	B=-0.33, p=0.901	B=0.249, p=0.385	0.113	1.561	0.2
	Egg Reduction Rate	B=6.063, p=0.748		B=-3.035, p=0.015	B=-0.004, p=0.985	B=0.183, p=0.392	0.151	2.179	0.085
4	Cure Rate		B=1.613, p=0.920	B=-3.2743, p=0.05	B=-0.21, p=0.935	B=0.233, p=0.415	0.109	1.493	0.219
	Egg Reduction Rate		B=-4.365, p=0.714	B=-2.9233, p=0.02	B=0, p=0.99	B=0.178, p=0.403	0.152	2.188	0.084
5	Cure Rate			B=-3.1711, p=0.05			0.072	4.011	0.0504
	Egg Reduction Rate			**B=-2.941, p=0.016**			**0.106**	**6.154**	**0.016**

Supplementary Table 4: Table. Model summary and coefficients of each logistic regression model

Logistic Regression Model	Method	Dependent Variable	Predictors			Model Parameters		
			Transmission Score		Distance to Body of Water			
			1988	2012		-2 log likelihood	Nagelkerke R^2	P
1	Binary	Schistosomiasis Status	B=0.497, Wald=0.132, p=0.717		B=-0.77, Wald=0.65, p=0.42	166.76	0.008	0.684
	Ordinal	WHO Risk Category	Estimate=0.393, Wald=0.091, p=0.76		Estimate=-0.101, Wald=1.13, p=0.288	167.27	0.012	0.528
2	Binary	Schistosomiasis Status		B=1.019, Wald=1.357, p=0.244	B=-0.81, Wald=0.721, p=0.396	165.49	0.022	0.363
	Ordinal	WHO Risk Category		Estimate=0.93, Wald=1.219, p=0.27	Estimate=-0.108, Wald=1.295, p=0.255	167.27	0.22	0.289

Supplementary Table 5: Prevalence, mean egg counts and proportion of light (< 50 eggs/10mL) to heavy (≥ 50 eggs/10mL) intensity of S. haematobium infection during six mass drug administrations (MDAs) during the years 2012 to 2017 in twenty-nine districts in Zimbabwe. The data was separated into pre- and post- a PZQ treatment.

		Province	Manicaland				
		District	Bikita	Buhera	Chipinge	Makoni	Nyanga
MDA 1	Pre-	Prevalence (n)	58.46 (195) [A]	40.91 (242) [A], [D]iv, vi	48.8 (250) [A], [D]iv, vi	52.79 (233) [A], [D]ii, iii, iv, v, vi	0 (397) [D]vi
		Mean Egg Count (95% CI)	34.31 (24.56-44.06) [A]	31.57 (21.8-41.35) [A], [D]vi	34.31 (24.56-44.06) [A], [D]vi	29.27 (20.06-38.48) [A]	0 (0-0)
		Light:Heavy Infections (%)	71:29	58:42	62:38	73:27	0:0
	Post-	Prevalence (n)	0 (195)	0 (242)	0 (250)	0 (233)	0 (398)
		Mean Egg Count (95% CI)	0 (0-0) [A]	0 (0-0) [A]	0 (0-0) [A]	0 (0-0) [A]	0 (0-0)
		Light:Heavy Infections (%)	0:0	0:0	0:0	0:0	0:0
MDA 2	Pre-	Prevalence (n)	-	-	-	0 (233) [D]i, vi	-
		Mean Egg Count (95% CI)	-	-	-	0 (0-0)	-
		Light:Heavy Infections (%)	-	-	-	0:0	-
	Post-	Prevalence (n)	-	-	-	0 (191)	-
		Mean Egg Count (95% CI)	-	-	-	0 (0-0)	-
		Light:Heavy Infections (%)	-	-	-	0:0	-
MDA 3	Pre-	Prevalence (n)	-	-	-	0 (233) [D]i, vi	-
		Mean Egg Count (95% CI)	-	-	-	0 (0-0)	-
		Light:Heavy Infections (%)	-	-	-	0:0	-
	Post-	Prevalence (n)	-	-	-	0 (233)	-
		Mean Egg Count (95% CI)	-	-	-	0 (0-0)	-
		Light:Heavy Infections (%)	-	-	-	0:0	-
MDA 4	Pre-	Prevalence (n)	-	0 (242) [D]i, vi	0 (250) [D]i, vi	0 (233) [D]i, vi	0 (398)
		Mean Egg Count (95% CI)	-	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
		Light:Heavy Infections (%)	-	0:0	0:0	0:0	0:0
	Post-	Prevalence (n)	-	0 (242)	0 (250)	0 (233)	0 (398)
		Mean Egg Count (95% CI)	-	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
		Light:Heavy Infections (%)	-	0:0	0:0	0:0	0:0
MDA 5	Pre-	Prevalence (n)	-	-	-	0 (233) [D]i, vi	-
		Mean Egg Count (95% CI)	-	-	-	0 (0-0)	-
		Light:Heavy Infections (%)	-	-	-	0:0	-
	Post-	Prevalence (n)	-	-	-	0 (192)	-
		Mean Egg Count (95% CI)	-	-	-	0 (0-0)	-
		Light:Heavy Infections (%)	-	-	-	0:0	-
MDA 6	Pre-	Prevalence (n)	-	7.03 (242) [A], [D]iv	11.2 (250) [A], [D]i, iv	3.86 (233) [A], [D]i, ii, iii, vi, v	4.52 (398) [A], [D]i
		Mean Egg Count (95% CI)	-	1.19 (0.08-2.3) [A], [D]i	0.91 (0.37-1.45) [A], [D]i	0.62 (0.05-1.18) [A]	0.43 (0.14-0.72) [A]
		Light:Heavy Infections (%)	-	88:12	100:0	100:0	100:0
	Post-	Prevalence (n)	0 (195)	0 (242) [A]	0 (250) [A]	0 (233) [A]	0 (398) [A]
		Mean Egg Count (95% CI)	0 (0-0)	0 (0-0) [A]	0 (0-0) [A]	0 (0-0) [A]	0 (0-0) [A]
		Light:Heavy Infections (%)	0:0	0:0	0:0	0:0	0:0

		Province	Mash West				Mashonaland Central
		District	Chegutu	Hurungwe	Makonde	Zvimba	Guruve
	Pre-	Prevalence (n)	15.73 (267) [A], [D]iv, vi	0 (439)	9.6 (241) [A], [D]iv, vi	46.18 (248) [A]	0 (179)

		Mean Egg Count (95% CI)	8.01 (3.9-12.11) [A]	0 (0-0)	1 (0.33-1.68) [A]	39.6 (27.33-51.86) [A]	0 (0-0)
		Light:Heavy Infections (%)	71:29 x	0:0	96:4 x	59:41 x	0:0
MDA 1		**Prevalence (n)**	0 (267)	0 (439)	0 (250) [A]	0 (248) [A]	0 (179)
	Post-	**Mean Egg Count (95% CI)**	0 (0-0) [A]	0 (0-0) [A]	0 (0-0) [A]	0 (0-0) [A]	0 (0-0)
		Light:Heavy Infections (%)	0:0	0:0	0:0	0:0	0:0
		Prevalence (n)	-	-	-	-	-
	Pre-	**Mean Egg Count (95% CI)**	-	-	-	-	-
MDA 2		**Light:Heavy Infections (%)**	-	-	-	-	-
		Prevalence (n)	-	-	-	-	-
	Post-	**Mean Egg Count (95% CI)**	-	-	-	-	-
		Light:Heavy Infections (%)	-	-	-	-	-
		Prevalence (n)	-	-	-	-	-
	Pre-	**Mean Egg Count (95% CI)**	-	-	-	-	-
MDA 3		**Light:Heavy Infections (%)**	-	-	-	-	-
		Prevalence (n)	-	-	-	-	-
	Post-	**Mean Egg Count (95% CI)**	-	-	-	-	-
		Light:Heavy Infections (%)	-	-	-	-	-
		Prevalence (n)	0 (267) [D]i	0 (439)	0 (249) [D]i	-	0 (179)
	Pre-	**Mean Egg Count (95% CI)**	0 (0-0)	0 (0-0)	0 (0-0)	-	0 (0-0)
MDA 4		**Light:Heavy Infections (%)**	0:0	0:0	0:0	-	0:0
		Prevalence (n)	0 (267)	0 (439)	0 (249)	-	0 (179)
	Post-	**Mean Egg Count (95% CI)**	0 (0-0)	0 (0-0)	0 (0-0)	-	0 (0-0)
		Light:Heavy Infections (%)	0:0	0:0	0:0	-	0:0
		Prevalence (n)	-	-	-	-	-
	Pre-	**Mean Egg Count (95% CI)**	-	-	-	-	-
MDA 5		**Light:Heavy Infections (%)**	-	-	-	-	-
		Prevalence (n)	-	-	-	-	-
	Post-	**Mean Egg Count (95% CI)**	-	-	-	-	-
		Light:Heavy Infections (%)	-	-	-	-	-
		Prevalence (n)	0 (267) [D]i	0 (439)	0 (249) [D]vi	-	0 (179)
	Pre-	**Mean Egg Count (95% CI)**	0 (0-0)	0 (0-0)	0 (0-0)	-	0 (0-0)
MDA 6		**Light:Heavy Infections (%)**	0:0	0:0	0:0	-	0:0
		Prevalence (n)	0 (267)	0 (439)	0 (250)	0 (248)	0 (179)
	Post-	**Mean Egg Count (95% CI)**	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
		Light:Heavy Infections (%)	0:0	0:0	0:0	0:0	0:0

Province	**Mashonaland Central**				
District	**Mazowe**	**Mt Darwin**	**Muzarabani**	**Rushinga**	**Shamva**

MDA 1	Pre-	Prevalence (n)	0 (186)	66.02 (206) [C], [D]ii, iii, iv, v, vi	73.58 (299) [A], [D]ii, iii, iv, v, vi	44 (250) [A], [D]ii, iii, iv, v, vi	32.19 (612) [A], [D]iv, vi
		Mean Egg Count (95% CI)	0 (0-0)	49.69 (39.08-60.31) [A], [D]iii,[D]iv	88.93 (75.33-102.53) [A], [D]iii	19.31 (12.98-25.64) [A], [D]ii, [D]iii, [D]vi	24.36 (17.87-30.86) [A]
		Light:Heavy Infections (%)	0:0	56:44	34:66	81:19	67:33
	Post-	Prevalence (n)	0 (186)	24.27 (206) [illegible]	0 (320) [illegible]	1.2 (250) [illegible]	0.89 (1118) [illegible]
		Mean Egg Count (95% CI)	0 (0-0)	4.96 (3.21-6.71) [A], [E]iii	0 (0-0) [A]	0.01 (0-0.01) [A], [E]ii, [G]iv	0.35 (0.05-0.65) [A]
		Light:Heavy Infections (%)	0:0	95:5	0:0	100:0	90:10
MDA 2	Pre-	Prevalence (n)	-	0 (206) [D]I, iii, iv	0 (297) [B], [D]i, iii	21.6 (250) [A], [D]I, iii, iv, v, vi	-
		Mean Egg Count (95% CI)	-	0 (0-0)	0 (0-0) [B]	0.75 (0.49-1) [C], [D]i, [D]iii, [D]vi	-
		Light:Heavy Infections (%)	-	0:0	0:0	100:0	-
	Post-	Prevalence (n)	-	-	6.06 (298) [B], [E]iii, iv, v, vi	17.6 (250) [A], [E]i, iii, iv, v, vi	-
		Mean Egg Count (95% CI)	-	-	0.06 (0.02-0.1) [B] [F]iii	0.98 (0.62-1.35) [C], [E]i, [E]iv	-
		Light:Heavy Infections (%)	-	-	100:0	100:0	-
MDA 3	Pre-	Prevalence (n)	-	11.17 (206) [A], [D]ii, v, vi, [F]iv	10.37 (299) [A], [D]i, ii, iv, v, vi	32 (250) [A], [D]i, ii, iv, v, vi	-
		Mean Egg Count (95% CI)	-	2.27 (0.89-3.64) [C], [D]i, [F]iv	0.05 (0.03-0.07) [A], [D]I, [F]ii	2.99 (2.02-3.97) [A],[D]i, [D]ii, [D]vi	-
		Light:Heavy Infections (%)	-	83:17	100:0	99:1	-
	Post-	Prevalence (n)	-	0.49 (206) [A], [E]i, iv, v, vi	0 (298) [E]ii	0 (250) [A], [F]i, ii, iv	-
		Mean Egg Count (95% CI)	-	2.33 (0.93-3.73) [C], [E]i	0 (0-0)	0 (0-0) [A], [E]ii, [G]i	-
		Light:Heavy Infections (%)	-	81:19	0:0	0:0	-
MDA 4	Pre-	Prevalence (n)	0 (186)	7.77 (206) [A], [D]I, ii, v, vi, [F]iii	0 (298) [D]i, iii	0 (250) [B], [D]I, ii, iii, [F]vi	0 (218) [D]i
		Mean Egg Count (95% CI)	0 (0-0)	1.41 (0.47-2.35) [A], [D]i	0 (0-0)	0 (0-0) [C]	0 (0-0)
		Light:Heavy Infections (%)	0:0	88:13	0:0	0:0	0:0
	Post-	Prevalence (n)	0 (186)	0 (206) [A], [E]i, iii	0 (298) [E]ii	1.2 (250) [B], [E]ii, [F]I, iii, v, vi	0 (218) [G]i
		Mean Egg Count (95% CI)	0 (0-0)	0 (0-0) [A]	0 (0-0)	0.004 (0-0.01) [C], [E]ii, [G]i	0 (0-0)
		Light:Heavy Infections (%)	0:0	0:0	0:0	100:0	0:0
MDA 5	Pre-	Prevalence (n)	-	0 (216) [D]iii, iv	0 (298) [D]i, iii	0 (250) [D]i, ii, iii, [F]vi	-
		Mean Egg Count (95% CI)	-	0 (0-0)	0 (0-0)	0 (0-0)	-
		Light:Heavy Infections (%)	-	0:0	0:0	0:0	-
	Post-	Prevalence (n)	-	0 (196) [E]i, iii	0 (298) [E]ii	0 (250) [E]ii, [F]I, iv	-
		Mean Egg Count (95% CI)	-	0 (0-0)	0 (0-0)	0 (0-0)	-
		Light:Heavy Infections (%)	-	0:0	0:0	0:0	-
MDA 6	Pre-	Prevalence (n)	0 (186)	0 (206) [D]iii, iv	0 (298) [D]i, iii	0.4 (250) [A], [D]ii, iii, vi, [F]iv, v	0 (218) [D]i
		Mean Egg Count (95% CI)	0 (0-0)	0 (0-0)	0 (0-0)	0.001 (0-0.33) [C], [D]i, [D]ii, [D]iii	0 (0-0)
		Light:Heavy Infections (%)	0:0	0:0	0:0	100:0	0:0
	Post-	Prevalence (n)	0 (186)	0 (206) [E]i, iii	0 (298) [E]ii	0 (250) [A], [E]ii, [F]i, iv	0 (468) [E]i
		Mean Egg Count (95% CI)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0) [C]	0 (0-0)
		Light:Heavy Infections (%)	0:0	0:0	0:0	0:0	0:0

		Province	Mashonaland East				Masvingo
		District	Chikomba	Murehwa	Mutoko	UMP	Chiredzi
MDA 1	Pre-	Prevalence (n)	50.4 (250) [A], [D]iv, vi	23.46 (422) [A], [D]ii, iii, iv, v, vi	30.57 (422) [A], [D]ii, iii, iv, v, vi	47.2 (250) [A], [D]ii, iii, iv, v, vi	54.13 (218) [A], [D]ii, iii, iv, v, vi
		Mean Egg Count (95% CI)	27.82 (20.27-35.36) [A], [D]vi	17.4 (11.83-22.96) [A], [D]iii, [D]vi,	21 (15.31-26.69) [A], [D]ii, [D]iii, [D]vi	48.37 (36.07-60.66) [A], [D]ii, [D]v	49.37 (36.02-62.73) [A], [D]ii, [D]v, [D]vi
		Light:Heavy Infections (%)	69:31	60:40	64:36	43:57	48:52
	Post-	Prevalence (n)	0 (250) [A]	0 (422) [A]	0 (422) [A], [G]iii, iv, v	0 (250) [A], [E]ii, [G]iv, v	0 (218) [A], [F]iii, v
		Mean Egg Count (95% CI)	0 (0-0) [A]	0 (0-0) [A]	0 (0-0) [A]	0 (0-0) [A]	0 (0-0) [A]
		Light:Heavy Infections (%)	0:0	0:0	0:0	0:0	0:0
	Pre-	Prevalence (n)	-	0 (198) [D]i, vi, [F]iii	4.02 (224) [A], [D]i, iv, v, vi, [F]iii	6.8 (250) [B], [D]i, iii, iv, v, vi	12.39 (218) [A], [D]i, iii, iv, v, vi

		Mean Egg Count (95% CI)	-	0 (0-0)	0.04 (0.01-0.07) [A], [D]i, [F]iii, [F]vi	0.17 (0.05-0.29) [C], [D]i, [D]v	0.43 (0.2-0.66) [A], [D]i, [D]v, [D]vi
		Light:Heavy Infections (%)	-	0:0	100:0	100:0	100:0
MDA 2		**Prevalence (n)**	-	0 (198)	0 (224) [A], [G]iii, iv, v	11.2 (250) [B], [E]iii, iv, v, vi, [G]i	0 (218) [A]
	Post-	**Mean Egg Count (95% CI)**	-	0 (0-0)	0 (0-0) [A]	0.15 (0.08-0.22) [C], [E]iv, [E]v	0 (0-0) [A]
		Light:Heavy Infections (%)	-	0:0	0:0	100:0	0:0
		Prevalence (n)	-	2.02 (198) [C], [D]i, iv, [F]ii, v, vi	1.79 (448) [C], [D]i, ii, vi, [F]ii, v	0 (250) [D]i, ii, [F]v	0 (218) [B], [D]i, ii, v, [F]vi
	Pre-	**Mean Egg Count (95% CI)**	-	0.1 (-0.01-0.2) [C], [D]i, [F]vi	0.11 (0.01-0.21) [A], [D]ii, [F]i, [F]vi	0 (0-0)	0 (0-0) [C]
MDA 3		**Light:Heavy Infections (%)**	-	100:0	100:0	0:0	0:0
		Prevalence (n)	-	0 (198) [C]	0.89 (224) [C], [G]i, ii, iv, v, vi	0 (250) [E]ii, [G]iv, v	0.46 (218) [B], [F]i, iv, v, vi
	Post-	**Mean Egg Count (95% CI)**	-	0 (0-0) [C]	0.002 (0-0.01) [A], [E]iv, [E]v	0 (0-0)	0.01 (-0.01-0.02) [C], [G]v
		Light:Heavy Infections (%)	-	0:0	100:0	0:0	100:0
		Prevalence (n)	0 (250) [D]i, vi	0 (422) [D]i, iii, vi	0 (422) [B], [D]i, ii, iii, [F]vi	0 (250) [B], [D]i, ii, [F]v	0 (218) [D]i, ii, v, [F]vi
	Pre-	**Mean Egg Count (95% CI)**	0 (0-0)	0 (0-0)	0 (0-0) [C]	0 (0-0) [C]	0 (0-0)
MDA 4		**Light:Heavy Infections (%)**	0:0	0:0	0:0	0:0	0:0
		Prevalence (n)	0 (250)	0 (422)	0.47 (422) [B], [G]i, ii, iii, v, vi	0.4 (250) [B], [E]i, ii, [G]iii, v, vi	0 (218) [F]iii, v
	Post-	**Mean Egg Count (95% CI)**	0 (0-0)	0 (0-0)	0.002 (0-0) [C], [E]c, [G]v	0.02 (-0.02-0.07) [C], [E]b, [G]v	0 (0-0)
		Light:Heavy Infections (%)	0:0	0:0	100:0	100:0	0:0
		Prevalence (n)	-	0 (198) [D]i, vi, [F]iii	0 (224) [B], [D]i, ii, [F]iii, vi	0.4 (250) [C], [D]i, ii, [F]iii, iv, v	12.29 (218) [A], [D]i, ii, iii, iv, vi
	Pre-	**Mean Egg Count (95% CI)**	-	0 (0-0)	0 (0-0) [C]	0.03 (-0.03-0.1) [C], [D]i, [D]ii	0.16 (0.1-0.23) [A], [D]i, [D]ii, [D]vi
MDA 5		**Light:Heavy Infections (%)**	-	0:0	0:0	100:0	100:0
		Prevalence (n)	-	0 (198)	0.89 (224) [B], [G]i, ii, iii, iv, vi	0.4 (250) [C], [E]i, ii, [G]iii, iv, vi	0.92 (218) [A], [F]i, iii, iv, vi
	Post-	**Mean Egg Count (95% CI)**	-	0 (0-0)	0.003 (0-0.01) [C], [E]c, [G]iv	0.03 (-0.03-0.09) [C], [E]ii, [G]iv	0.003 (0-0.01) [A], [G]iii
		Light:Heavy Infections (%)	-	0:0	100:0	100:0	100:0
		Prevalence (n)	4 (250) [A], [D]i, iv	3.55 (422) [A], [D]i, ii, iv, v, [F]iii	0.24 (422) [A], [D]i, ii, iii, [F]iv, v	0 (250) [D]i, ii, [F]v	0.46 (218) [A], [D]i, ii, v [F]iii, iv
	Pre-	**Mean Egg Count (95% CI)**	0.38 (0-0.77) [C], [D]i	0.32 (0.1-0.54) [A], [D]i, [F]iii	0.03 (-0.03-0.08) [C], [D]i, [D]ii, [F]iii	0 (0-0)	0.02 (-0.01-0.05) [C], [D]i, [D]ii, [D]v
MDA 6		**Light:Heavy Infections (%)**	100:0	100:0	100:0	0:0	100:0
		Prevalence (n)	0 (250) [A]	0 (422) [A]	0 (422) [A], [G]iii, iv, v	0 (250) [E]ii, [G]iv, v	0 (218) [A], [F]iii, v
	Post-	**Mean Egg Count (95% CI)**	0 (0-0) [C]	0 (0-0) [A]	0 (0-0) [C]	0 (0-0)	0 (0-0) [C]
		Light:Heavy Infections (%)	0:0	0:0	0:0	0:0	0:0

	Province	**Masvingo**		**Mat North**		**Mat South**	
	District	**Gutu**	**Mwenezi**	**Binga**	**Nkayi**	**Insiza**	
	Prevalence (n)	51.04 (199) [A]	48.69 (267) [A], [D]ii, iii, iv, v, vi	4.72 (253) [A]	6.19 (242) [B], [D]iii, iv, v, vi, [F]ii	5.13 (248) [A]	
Pre-	**Mean Egg Count (95% CI)**	23.01 (15.04-30.98) [A]	42.87 (32.68-53.07) [A], [D]ii, [D]iii	0.47 (0.03-0.91) [A]	1.14 (0.19-2.1) [C], [F]ii	0.43 (0.03-0.83) [A]	
MDA 1	**Light:Heavy Infections (%)**	72:28	42:58	100:0	87:13	100:0	
	Prevalence (n)	0 (199) [A]	0.38 (267) [A], [G]ii, iii, iv, v, vi	0 (253) [A]	7.03 (242) [B], [E]ii, iv	0 (248) [A]	
Post-	**Mean Egg Count (95% CI)**	0 (0-0) [A]	0 (0-0) [A], [G]iii	0 (0-0) [A]	0.77 (0.33-1.2) [C]	0 (0-0) [A]	

		Light:Heavy Infections (%)	0:0	100:0	0:0	100:0	0:0
MDA 2	**Pre-**	**Prevalence (n)**	-	3.49 (267) [A], [D]i, iv , v, vi, [F]iii	-	7.44 (242) [A], [D]iii, iv, v, vi, [F]i	-
		Mean Egg Count (95% CI)	-	0.09 (0.02-0.16) [A], [D]i, [F]iii	-	2.29 (1.09-3.5) [A], [F]i	-
		Light:Heavy Infections (%)	-	100:0	-	78:22	-
	Post-	**Prevalence (n)**	-	0 (267) [A], [G]i, iii	-	0 (242) [A], [E]i	-
		Mean Egg Count (95% CI)	-	0 (0-0)	-	0 (0-0) [A]	-
		Light:Heavy Infections (%)	-	0:0	-	0:0	-
MDA 3	**Pre-**	**Prevalence (n)**	-	1.49 (267) [A], [D]i, [F]ii, iv, v	-	0 (240) [D]i, ii, [F]iv	-
		Mean Egg Count (95% CI)	-	0.03 (-0.01-0.06) [C], [D]i, [F]ii	-	0 (0-0)	-
		Light:Heavy Infections (%)	-	100:0	-	0:0	-
	Post-	**Prevalence (n)**	-	0.75 (267) [A], [G]i, ii, iv, v, vi	-	0.83 (242)	-
		Mean Egg Count (95% CI)	-	0.002 (0-0.01) [C], [G]i	-	0.45 (0-56.67)	-
		Light:Heavy Infections (%)	-	100:0	-	0:100	-
MDA 4	**Pre-**	**Prevalence (n)**	-	0 (267) [D]i, ii, [F]iii	-	0 (242) [D]i, ii, [F]iii	-
		Mean Egg Count (95% CI)	-	0 (0-0)	-	0 (0-0)	-
		Light:Heavy Infections (%)	-	0:0	-	0:0	-
	Post-	**Prevalence (n)**	-	0 (267) [G]i, iii	-	0 (242) [E]i	-
		Mean Egg Count (95% CI)	-	0 (0-0)	-	0 (0-0)	-
		Light:Heavy Infections (%)	-	0:0	-	0:0	-
MDA 5	**Pre-**	**Prevalence (n)**	-	0 (267) [D]i, ii, [F]iii	-	0 (242) [D]i, ii, [F]iii	-
		Mean Egg Count (95% CI)	-	0 (0-0)	-	0 (0-0)	-
		Light:Heavy Infections (%)	-	0:0	-	0:0	-
	Post-	**Prevalence (n)**	-	0 (267) [G]i, iii	-	0 (242) [E]i	-
		Mean Egg Count (95% CI)	-	0 (0-0)	-	0 (0-0)	-
		Light:Heavy Infections (%)	-	0:0	-	0:0	-
MDA 6	**Pre-**	**Prevalence (n)**	-	0 (267) [D]i, ii, [F]iii	-	0 (242) [D]i, vi, [F]iii	-
		Mean Egg Count (95% CI)	-	0 (0-0)	-	0 (0-0)	-
		Light:Heavy Infections (%)	-	0:0	-	0:0	-
	Post-	**Prevalence (n)**	0 (199)	0 (267) [G]i, iii	0 (253)	0 (242) [E]i	-
		Mean Egg Count (95% CI)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	-
		Light:Heavy Infections (%)	0:0	0:0	0:0	0:0	-

		Province	**Midlands**			
		District	**Chirumanzu**	**Gokwe North**	**Mberengwa**	**Shurugwi**
MDA 1	**Pre-**	**Prevalence (n)**	30.26 (195) [A]	36.68 (199) [A]	34.71 (680) [A], [D]ii, iii, iv, v, vi	62.9 (186) [A], [D]ii, iii
		Mean Egg Count (95% CI)	31.5 (18.45-44.55) [A]	24.8 (16.86-32.74) [A]	25.89 (20.01-31.78) [A], [D]ii, [D]iii	48.33 (35.28-61.38) [A]
		Light:Heavy Infections (%)	71:29	56:44	64:36	58:42

	Post-	Prevalence (n)	0 (195) [A]	0 (199) [A]	0 (680) [A]	0 (186) [A]
		Mean Egg Count (95% CI)	0 (0-0) [A]	0 (0-0) [A]	0 (0-0) [A]	0 (0-0) [A]
		Light:Heavy Infections (%)	0:0	0:0	0:0	0:0
MDA 2	Pre-	Prevalence (n)	-	-	3.35 (179) [A], [D]i, iii, iv, v, vi	0 (186) [D]i
		Mean Egg Count (95% CI)	-	-	0.12 (0.01-0.23) [A], [D]i, [D]iii	0 (0-0)
		Light:Heavy Infections (%)	-	-	100:0	0:0
	Post-	Prevalence (n)	-	-	0 (179) [A]	0 (186)
		Mean Egg Count (95% CI)	-	-	0 (0-0)	0 (0-0)
		Light:Heavy Infections (%)	-	-	0:0	0:0
MDA 3	Pre-	Prevalence (n)	-	-	11.25 (160) [A], [D]i, ii, iv, v	0 (186) [D]i
		Mean Egg Count (95% CI)	-	-	0.44 (0.21-0.66) [A], [D]ii, [D]iii	0 (0-0)
		Light:Heavy Infections (%)	-	-	100:0	0:0
	Post-	Prevalence (n)	-	-	0 (179) [A]	0 (186)
		Mean Egg Count (95% CI)	-	-	0 (0-0)	0 (0-0)
		Light:Heavy Infections (%)	-	-	0:0	0:0
MDA 4	Pre-	Prevalence (n)	-	-	0 (179) [D]i, ii, iii	0 (186)
		Mean Egg Count (95% CI)	-	-	0 (0-0)	0 (0-0)
		Light:Heavy Infections (%)	-	-	0:0	0:0
	Post-	Prevalence (n)	-	-	0 (179)	0 (186)
		Mean Egg Count (95% CI)	-	-	0 (0-0)	0 (0-0)
		Light:Heavy Infections (%)	-	-	0:0	0:0
MDA 5	Pre-	Prevalence (n)	-	-	0 (179) [D]i, ii, iii	0 (186)
		Mean Egg Count (95% CI)	-	-	0 (0-0)	0 (0-0)
		Light:Heavy Infections (%)	-	-	0:0	0:0
	Post-	Prevalence (n)	-	-	0 (179)	0 (186)
		Mean Egg Count (95% CI)	-	-	0 (0-0)	0 (0-0)
		Light:Heavy Infections (%)	-	-	0:0	0:0
MDA 6	Pre-	Prevalence (n)	-	-	0 (179) [D]i, ii, iii	0 (186)
		Mean Egg Count (95% CI)	-	-	0 (0-0)	0 (0-0)
		Light:Heavy Infections (%)	-	-	0:0	0:0
	Post-	Prevalence (n)	0 (195)	0 (199)	0 (680)	0 (186)
		Mean Egg Count (95% CI)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
		Light:Heavy Infections (%)	0:0	0:0	0:0	0:0

The mean egg count is calculated per 10mL of urine for the baseline and follow-up surveys. Mean egg counts expressed 95% confidence interval (CI). Descriptions of [X]x are as follows; [X] represents: [A] Significant decrease in paired analysis based on pre- to post-MDA, [B] Significant increase in paired test based on pre- to post-MDA, [C] No significant difference in paired analysis based on pre- to post-MDA, [D] Significant difference in pre to pre in unpaired test between MDAs, [E] Significant difference in post to post in unpaired test between MDAs, [F] No significant difference in pre to pre in unpaired test between MDAs, [G] No significant difference in post to post in unpaired test between MDAs. x) represents: i) compared to MDA1, ii) compared to MDA2, iii) compared against MDA3, iv) compared against MDA4, v) compared against MDA5, vi) compared against MDA6.

Supplementary Table 6: Sample size, cure rate (CR), egg reduction rate (ERR), pre- and post-mean egg counts, and the proportion of light (< 50 eggs/10mL) to heavy (≥ 50 eggs/10mL) intensity of *S. haematobium* infection during six mass drug administrations (MDAs) during the years 2012 to 2017 in twenty-nine districts in Zimbabwe.

		Province	Manicaland				
		District	Bikita	Buhera	Chipinge	Makoni	Nyanga
MDA1	Pre-	Sample Size	102	99	122	123	-
		Cure Rate (%)	100	100	100	100	-
		Egg Reduction Rate (%)	100	100.0	100	100	-
		Pre-Mean Egg Count (95% CI)	59.54 (44.3-74.78) [A]	77.18 (56.17-98.2) [A], [D]	67.17 (51.39-82.96) [A], [D]	55.44 (39.26-71.63) [A], [D]	-
	Post-	Post-Mean Egg Count (95% CI)	0 (0-0) [A]	0 (0-0) [A]	0 (0-0) [A]	0 (0-0) [A]	-
		Light:Heavy Infections (%)	70:30	58:42	62:38	73:27	-
MDA2	Pre-	Sample Size	-	-	-	-	-
		Cure Rate (%)	-	-	-	-	-
		Egg Reduction Rate (%)	-	-	-	-	-
		Pre-Mean Egg Count (95% CI)	-	-	-	-	-
	Post-	Post-Mean Egg Count (95% CI)	-	-	-	-	-
		Light:Heavy Infections (%)	-	-	-	-	-
MDA3	Pre-	Sample Size	-	-	-	-	-
		Cure Rate (%)	-	-	-	-	-
		Egg Reduction Rate (%)	-	-	-	-	-
		Pre-Mean Egg Count (95% CI)	-	-	-	-	-
	Post-	Post-Mean Egg Count (95% CI)	-	-	-	-	-
		Light:Heavy Infections (%)	-	-	-	-	-
MDA4	Pre-	Sample Size	-	-	-	-	-
		Cure Rate (%)	-	-	-	-	-
		Egg Reduction Rate (%)	-	-	-	-	-
		Pre-Mean Egg Count (95% CI)	-	-	-	-	-
	Post-	Post-Mean Egg Count (95% CI)	-	-	-	-	-
		Light:Heavy Infections (%)	-	-	-	-	-
MDA5	Pre-	Sample Size	-	-	-	-	-
		Cure Rate (%)	-	-	-	-	-
		Egg Reduction Rate (%)	-	-	-	-	-
		Pre-Mean Egg Count (95% CI)	-	-	-	-	-
	Post-	Post-Mean Egg Count (95% CI)	-	-	-	-	-
		Light:Heavy Infections (%)	-	-	-	-	-
MDA6	Pre-	Sample Size	-	17	28	9	18
		Cure Rate (%)	-	100	100	100	100
		Egg Reduction Rate (%)	-	100	100	100	100
		Pre-Mean Egg Count (95% CI)	-	16.9 (1.73-32.08) [A], [D]	8.12 (4.03-12.2) [A], [D]	15.93 (2.95-28.9) [A], [D]	9.52 (4.16-14.87) [A]
	Post-	Post-Mean Egg Count (95% CI)	-	0 (0-0) [A]	0 (0-0) [A]	0 (0-0) [A]	0 (0-0) [A]
		Light:Heavy Infections (%)	-	88:12	100:0	100:0	100:0

		Province	Mash West				Mashonaland Central
		District	Chegutu	Hurungwe	Makonde	Zvimba	Guruve
MDA1	Pre-	Sample Size	42	-	24	114	-
		Cure Rate (%)	100	-	100	100	-
		Egg Reduction Rate (%)	100	-	100	100	-
		Pre-Mean Egg Count (95% CI)	50.9 (28.24-73.56) [A]	-	10.08 (4.13-16.03) [A]	85.8 (61.71-109.89) [A]	-
	Post-	Post-Mean Egg Count (95% CI)	0 (0-0) [A]	-	0 (0-0) [A]	0 (0-0) [A]	-
		Light:Heavy Infections (%)	71:29	-	96:4	60:40	-

MDA2	Pre-	Sample Size	-	-	-	-	-
		Cure Rate (%)	-	-	-	-	-
		Egg Reduction Rate (%)	-	-	-	-	-
	Post-	Pre-Mean Egg Count (95% CI)	-	-	-	-	-
		Post-Mean Egg Count (95% CI)	-	-	-	-	-
		Light:Heavy Infections (%)	-	-	-	-	-
MDA3	Pre-	Sample Size	-	-	-	-	-
		Cure Rate (%)	-	-	-	-	-
		Egg Reduction Rate (%)	-	-	-	-	-
	Post-	Pre-Mean Egg Count (95% CI)	-	-	-	-	-
		Post-Mean Egg Count (95% CI)	-	-	-	-	-
		Light:Heavy Infections (%)	-	-	-	-	-
MDA4	Pre-	Sample Size	-	-	-	-	-
		Cure Rate (%)	-	-	-	-	-
		Egg Reduction Rate (%)	-	-	-	-	-
	Post-	Pre-Mean Egg Count (95% CI)	-	-	-	-	-
		Post-Mean Egg Count (95% CI)	-	-	-	-	-
		Light:Heavy Infections (%)	-	-	-	-	-
MDA5	Pre-	Sample Size	-	-	-	-	-
		Cure Rate (%)	-	-	-	-	-
		Egg Reduction Rate (%)	-	-	-	-	-
	Post-	Pre-Mean Egg Count (95% CI)	-	-	-	-	-
		Post-Mean Egg Count (95% CI)	-	-	-	-	-
		Light:Heavy Infections (%)	-	-	-	-	-
MDA6	Pre-	Sample Size	-	-	-	-	-
		Cure Rate (%)	-	-	-	-	-
		Egg Reduction Rate (%)	-	-	-	-	-
	Post-	Pre-Mean Egg Count (95% CI)	-	-	-	-	-
		Post-Mean Egg Count (95% CI)	-	-	-	-	-
		Light:Heavy Infections (%)	-	-	-	-	-

		Province	Mashonaland Central				
		District	Mazowe	Mt Darwin	Muzarabani	Rushinga	Shamva
MDA1	Pre-	Sample Size	-	126	220	110	194
		Cure Rate (%)	-	68.25	100	99.09	97.42
		Egg Reduction Rate (%)	-	91.72	100	99.99	99.63
	Post-	Pre-Mean Egg Count (95% CI)	-	80.13 (65-95.27) [A], [D]iii, [D]iv	120.86 (104.29-137.44) [A], [D]iii	43.89 (30.78-57) [A], [D]ii	76.76 (58.21-95.31) [A]

		Post-Mean Egg Count (95% CI)	-	6.64 (4-9.27) [A], [G]iii	0 (0-0) [A]	0.005 (0-0.01) [A], [E]ii	0.28 (-0.11-0.68) [A]
		Light:Heavy Infections (%)	-	56:44	34:66	81:19	66:34
MDA2	Pre-	Sample Size	-	-	-	23	-
		Cure Rate (%)	-	-	-	43.48	-
		Egg Reduction Rate (%)	-	-	-	58.91	-
		Pre-Mean Egg Count (95% CI)	-	-	-	3.74 (2.22-5.26) [A], [D]iii	-
	Post-	Post-Mean Egg Count (95% CI)	-	-	-	1.54 (0.3-2.77) [A], [E]ii	-
		Light:Heavy Infections (%)	-	-	-	100:0	-
MDA3	Pre-	Sample Size	-	9	31	80	-
		Cure Rate (%)	-	22.22	100	100	-
		Egg Reduction Rate (%)	-	28.57	100	100	-
		Pre-Mean Egg Count (95% CI)	-	25.93 (7.16-44.69) [A], [D]i, [F]iv	0.51 (0.4-0.61) [A], [D]i	9.35 (6.78-11.92) [A], [D]i	-
	Post-	Post-Mean Egg Count (95% CI)	-	18.52 (1.61-35.43) [A], [G]i	0 (0-0) [A]	0 (0-0) [A]	-
		Light:Heavy Infections (%)	-	78:22	100:0	99:1	-
MDA4	Pre-	Sample Size	-	16	-	-	-
		Cure Rate (%)	-	100	-	-	-
		Egg Reduction Rate (%)	-	100	-	-	-
		Pre-Mean Egg Count (95% CI)	-	18.13 (8.64-27.61) [A], [D]i, [F]iii	-	-	-
	Post-	Post-Mean Egg Count (95% CI)	-	0 (0-0) [A]	-	-	-
		Light:Heavy Infections (%)	-	88:13	-	-	-
MDA5	Pre-	Sample Size	-	-	-	-	-
		Cure Rate (%)	-	-	-	-	-
		Egg Reduction Rate (%)	-	-	-	-	-
		Pre-Mean Egg Count (95% CI)	-	-	-	-	-
	Post-	Post-Mean Egg Count (95% CI)	-	-	-	-	-
		Light:Heavy Infections (%)	-	-	-	-	-
MDA6	Pre-	Sample Size	-	-	-	1	-
		Cure Rate (%)	-	-	-	100	-
		Egg Reduction Rate (%)	-	-	-	100	-
		Pre-Mean Egg Count (95% CI)	-	-	-	0.33 (-)	-
	Post-	Post-Mean Egg Count (95% CI)	-	-	-	0 (0-0)	-
		Light:Heavy Infections (%)	-	-	-	100:0	-

		Province	Mashonaland East				Masvingo
		District	Chikomba	Murehwa	Mutoko	UMP	Chiredzi
MDA1	Pre-	Sample Size	126	99	129	118	118
		Cure Rate (%)	100	100	100	100	100
		Egg Reduction Rate (%)	100	100	100	100	100

		Pre-Mean Egg Count (95% CI)	55.19 (41.77-68.61) [A], [D]vi	74.16 (53.93-94.38) [A]	68.69 (52.79-84.59) [A]	102.47 (80.04-124.91) [A], [D]ii	91.22 (69.1-113.33) [A], [D]ii, [D]v
	Post-	**Post-Mean Egg Count (95% CI)**	0 (0-0) [A]	0 (0-0) [A]	0 (0-0) [A]	0 (0-0) [A]	0 (0-0) [A]
		Light:Heavy Infections (%)	69:31	60:40	64:36	43:57	48:52
MDA2		**Sample Size**	-	-	9	9	27
	Pre-	**Cure Rate (%)**	-	-	100	66.67	100
		Egg Reduction Rate (%)	-	-	100	82.41	100
		Pre-Mean Egg Count (95% CI)	-	-	1 (0.66-1.34) [A]	4 (1.45-6.55) [A], [D]i	3.46 (2-4.92) [A], [D]i, [D]v
	Post-	**Post-Mean Egg Count (95% CI)**	-	-	0 (0-0) [A]	0.7 (0.11-1.3)	0 (0-0) [A]
		Light:Heavy Infections (%)	-	-	100:0	100:0	100:0
MDA3		**Sample Size**	-	-	-	-	-
	Pre-	**Cure Rate (%)**	-	-	-	-	-
		Egg Reduction Rate (%)	-	-	-	-	-
		Pre-Mean Egg Count (95% CI)	-	-	-	-	-
	Post-	**Post-Mean Egg Count (95% CI)**	-	-	-	-	-
		Light:Heavy Infections (%)	-	-	-	-	-
MDA4		**Sample Size**	-	-	-	-	-
	Pre-	**Cure Rate (%)**	-	-	-	-	-
		Egg Reduction Rate (%)	-	-	-	-	-
		Pre-Mean Egg Count (95% CI)	-	-	-	-	-
	Post-	**Post-Mean Egg Count (95% CI)**	-	-	-	-	-
		Light:Heavy Infections (%)	-	-	-	-	-
MDA5		**Sample Size**	-	-	-	1	27
	Pre-	**Cure Rate (%)**	-	-	-	0	96.29
		Egg Reduction Rate (%)	-	-	-	4.17	99.06
		Pre-Mean Egg Count (95% CI)	-	-	-	8 (8-8) [C]	1.31 (1.03-1.59) [A], [D]i, [D]ii
	Post-	**Post-Mean Egg Count (95% CI)**	-	-	-	7.67 (7.67-7.67) [C]	0.01 (-0.01-0.04) [A]
		Light:Heavy Infections (%)	-	-	-	100:0	100:0
MDA6		**Sample Size**	10	-	-	-	1
	Pre-	**Cure Rate (%)**	100	-	-	-	100
		Egg Reduction Rate (%)	100	-	-	-	100
		Pre-Mean Egg Count (95% CI)	9.6 (0.33-18.87) [A], [D]i	-	-	-	3.33 (-)
	Post-	**Post-Mean Egg Count (95% CI)**	0 (0-0) [A]	-	-	-	0 (-)
		Light:Heavy Infections (%)	100:0	-	-	-	100:0

		Province	**Masvingo**		**Mat North**		**Mat South**
		District	**Gutu**	**Mwenezi**	**Binga**	**Nkayi**	**Insiza**
MDA1	**Pre-**	**Sample Size**	98	130	11	5	-

		Cure Rate (%)	100	100	100	0	-
		Egg Reduction Rate (%)	100	100	100	61	-
		Pre-Mean Egg Count (95% CI)	45.08 (30.67-59.48) [A]	88.05 (70.06-106.04) [A], [D]ii, [D]iii	9.98 (1.37-18.6) [A]	37.27 (-10.08-84.61) [C], [F]ii	-
	Post-	**Post-Mean Egg Count (95% CI)**	0 (0-0) [A]	0 (0-0) [A]	0 (0-0) [A]	14.53 (4.16-24.91) [C]	-
		Light:Heavy Infections (%)	72:28	42:58	100:0	60:40	-
MDA2		**Sample Size**	-	9	-	18	-
	Pre-	**Cure Rate (%)**	-	100	-	100	-
		Egg Reduction Rate (%)	-	100	-	100	-
		Pre-Mean Egg Count (95% CI)	-	2.74 (1.56-3.92) [A], [D]i, [F]iii	-	30.85 (21.53-40.17) [A], [F]i	-
	Post-	**Post-Mean Egg Count (95% CI)**	-	0 (0-0) [A]	-	0 (0-0) [A]	-
		Light:Heavy Infections (%)	-	100:0	-	78:22	-
MDA3		**Sample Size**	-	4	-	-	-
	Pre-	**Cure Rate (%)**	-	100	-	-	-
		Egg Reduction Rate (%)	-	100	-	-	-
		Pre-Mean Egg Count (95% CI)	-	1.75 (-0.93-4.43) [C], [F]ii	-	-	-
	Post-	**Post-Mean Egg Count (95% CI)**	-	0 (0-0) [C]	-	-	-
		Light:Heavy Infections (%)	-	100:0	-	-	-
MDA4		**Sample Size**	-	-	-	-	-
	Pre-	**Cure Rate (%)**	-	-	-	-	-
		Egg Reduction Rate (%)	-	-	-	-	-
		Pre-Mean Egg Count (95% CI)	-	-	-	-	-
	Post-	**Post-Mean Egg Count (95% CI)**	-	-	-	-	-
		Light:Heavy Infections (%)	-	-	-	-	-
MDA5		**Sample Size**	-	-	-	-	-
	Pre-	**Cure Rate (%)**	-	-	-	-	-
		Egg Reduction Rate (%)	-	-	-	-	-
		Pre-Mean Egg Count (95% CI)	-	-	-	-	-
	Post-	**Post-Mean Egg Count (95% CI)**	-	-	-	-	-
		Light:Heavy Infections (%)	-	-	-	-	-
MDA6		**Sample Size**	-	-	-	-	-
	Pre-	**Cure Rate (%)**	-	-	-	-	-
		Egg Reduction Rate (%)	-	-	-	-	-
		Pre-Mean Egg Count (95% CI)	-	-	-	-	-
	Post-	**Post-Mean Egg Count (95% CI)**	-	-	-	-	-
		Light:Heavy Infections (%)	-	-	-	-	-

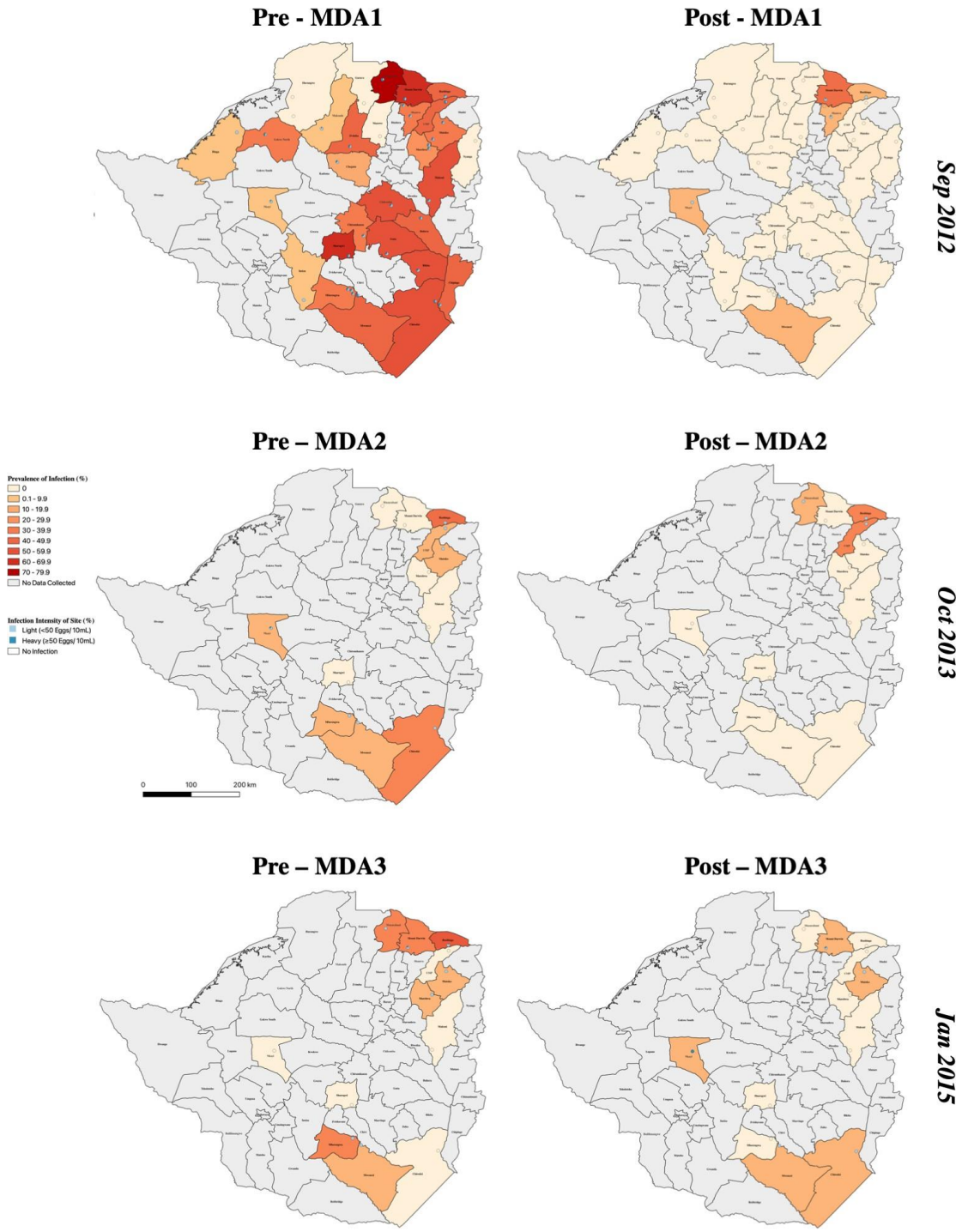
	Province	**Midlands**

		District	Chirumanzu	Gokwe North	Mberengwa	Shurugwi
MDA1	Pre-	Sample Size	59	73	236	117
		Cure Rate (%)	100	100	100	100
		Egg Reduction Rate (%)	100	100	100	100
		Pre-Mean Egg Count (95% CI)	65.68 (41.01-90.34) [A]	65.23 (47.79-82.67) [A]	72.19 (57.48-86.9) [A], [D]ii, [D]iii	76.83 (57.82-95.83) [A]
	Post-	Post-Mean Egg Count (95% CI)	0 (0-0) [A]	0 (0-0) [A]	0 (0-0) [A]	0 (0-0) [A]
		Light:Heavy Infections (%)	71:29	56:44	64:36	58:42
MDA2	Pre-	Sample Size	-	-	6	-
		Cure Rate (%)	-	-	100	-
		Egg Reduction Rate (%)	-	-	100	-
		Pre-Mean Egg Count (95% CI)	-	-	3.56 (1.2-5.91) [A], [D]i, [F]iii	-
	Post-	Post-Mean Egg Count (95% CI)	-	-	0 (0-0) [A]	-
		Light:Heavy Infections (%)	-	-	100:0	-
MDA3	Pre-	Sample Size	-	-	18	-
		Cure Rate (%)	-	-	100	-
		Egg Reduction Rate (%)	-	-	100	-
		Pre-Mean Egg Count (95% CI)	-	-	3.87 (2.65-5.1) [A], [D]i, [F]ii	-
	Post-	Post-Mean Egg Count (95% CI)	-	-	0 (0-0) [A]	-
		Light:Heavy Infections (%)	-	-	100:0	-
MDA4	Pre-	Sample Size	-	-	-	-
		Cure Rate (%)	-	-	-	-
		Egg Reduction Rate (%)	-	-	-	-
		Pre-Mean Egg Count (95% CI)	-	-	-	-
	Post-	Post-Mean Egg Count (95% CI)	-	-	-	-
		Light:Heavy Infections (%)	-	-	-	-
MDA5	Pre-	Sample Size	-	-	-	-
		Cure Rate (%)	-	-	-	-
		Egg Reduction Rate (%)	-	-	-	-
		Pre-Mean Egg Count (95% CI)	-	-	-	-
	Post-	Post-Mean Egg Count (95% CI)	-	-	-	-
		Light:Heavy Infections (%)	-	-	-	-
MDA6	Pre-	Sample Size	-	-	-	-
		Cure Rate (%)	-	-	-	-
		Egg Reduction Rate (%)	-	-	-	-
		Pre-Mean Egg Count (95% CI)	-	-	-	-
	Post-	Post-Mean Egg Count (95% CI)	-	-	-	-
		Light:Heavy Infections (%)	-	-	-	-

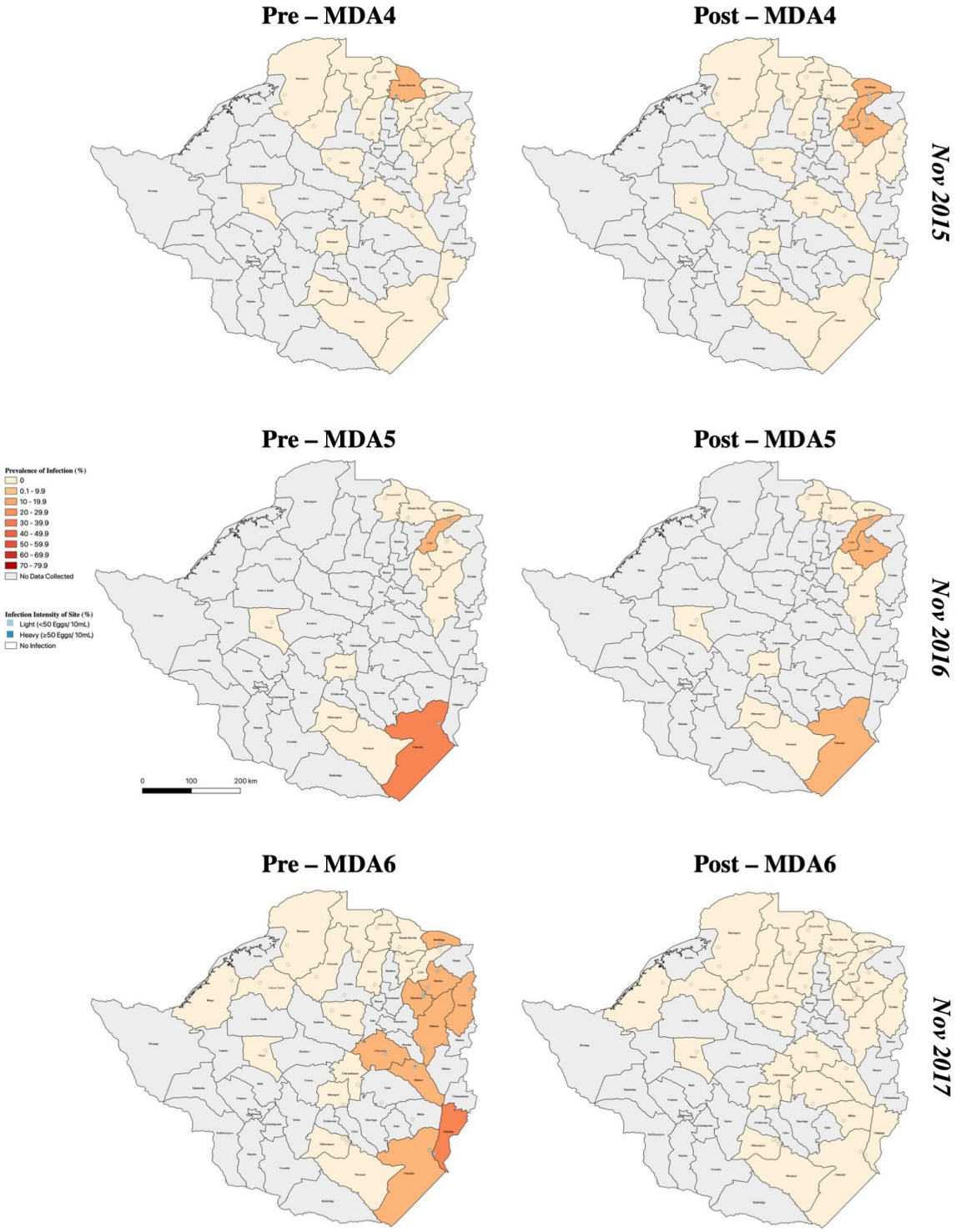
The mean egg count is calculated per 10mL of urine for the baseline and follow-up surveys. Mean egg counts expressed 95% confidence interval (CI). Descriptions of [X]x are as follows; [X] represents: [A] Significant decrease in paired analysis based on pre- to post-MDA, [B] Significant increase in paired test based on pre- to post-MDA, [C] No significant difference in paired analysis based on pre- to post-MDA, [D] Significant difference in pre to pre in unpaired test between MDAs, [E] Significant difference in post to post in unpaired test between MDAs, [F] No significant difference in pre to pre in unpaired test between MDAs, [G] No significant difference in post to post in unpaired test between MDAs. []x represents: i) compared to MDA1, ii) compared to MDA2, iii) compared against MDA3, iv) compared against MDA4, v) compared against MDA5, vi) compared against MDA6.

Supplementary Table 7: The classification of village sentinel sites based on their prevalence between multiple mass drug administration (MDA) time periods to determine whether persistent hotpots of *S. haematobium* prevalence (PPHS) could be detected in Zimbabwe using data from six years of annual treatment with PZQ.

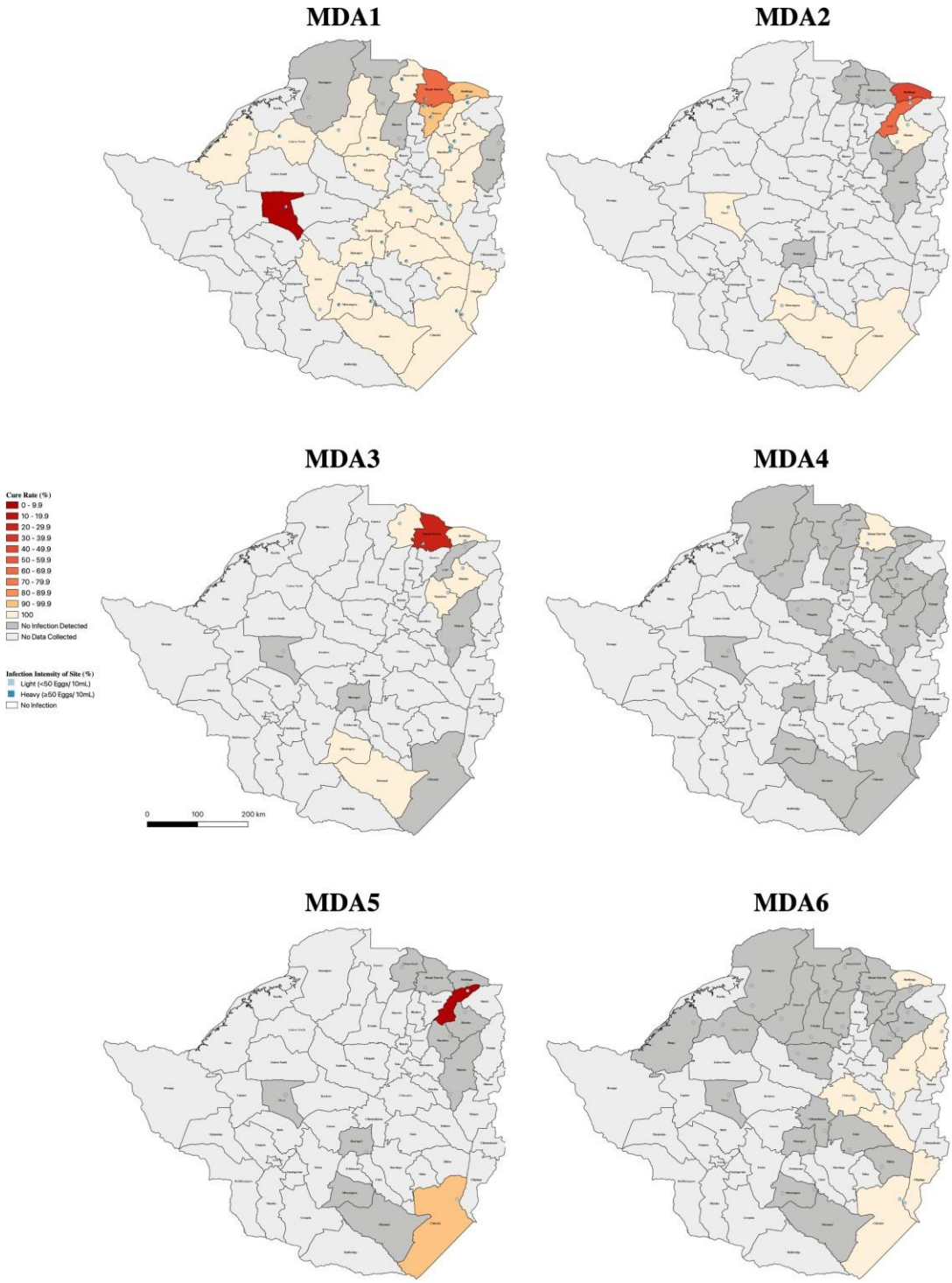
		Classification							
	Time-period to Assess Hotspots	**No Infection Detected**	**Declined One WHO Category**	**Declined Two WHO Categories**	**Cleared Infection**	**Increased One WHO Category**	**No Data Available**	**PPHS**	**Total Number of Villages**
Approach A	MDA1 to MDA4	7	0	1	17	0	10	0	35
	MDA1 to MDA5	0	2	0	10	0	23	0	
	MDA1 to MDA6	4	2	3	12	3	10	**1**	
Approach B	MDA1 to MDA4	4	0	0	17	0	7	**1**	
	MDA1 to MDA5	0	1	0	10	0	17	**1**	
	MDA1 to MDA6	3	3	3	10	1	7	**2**	



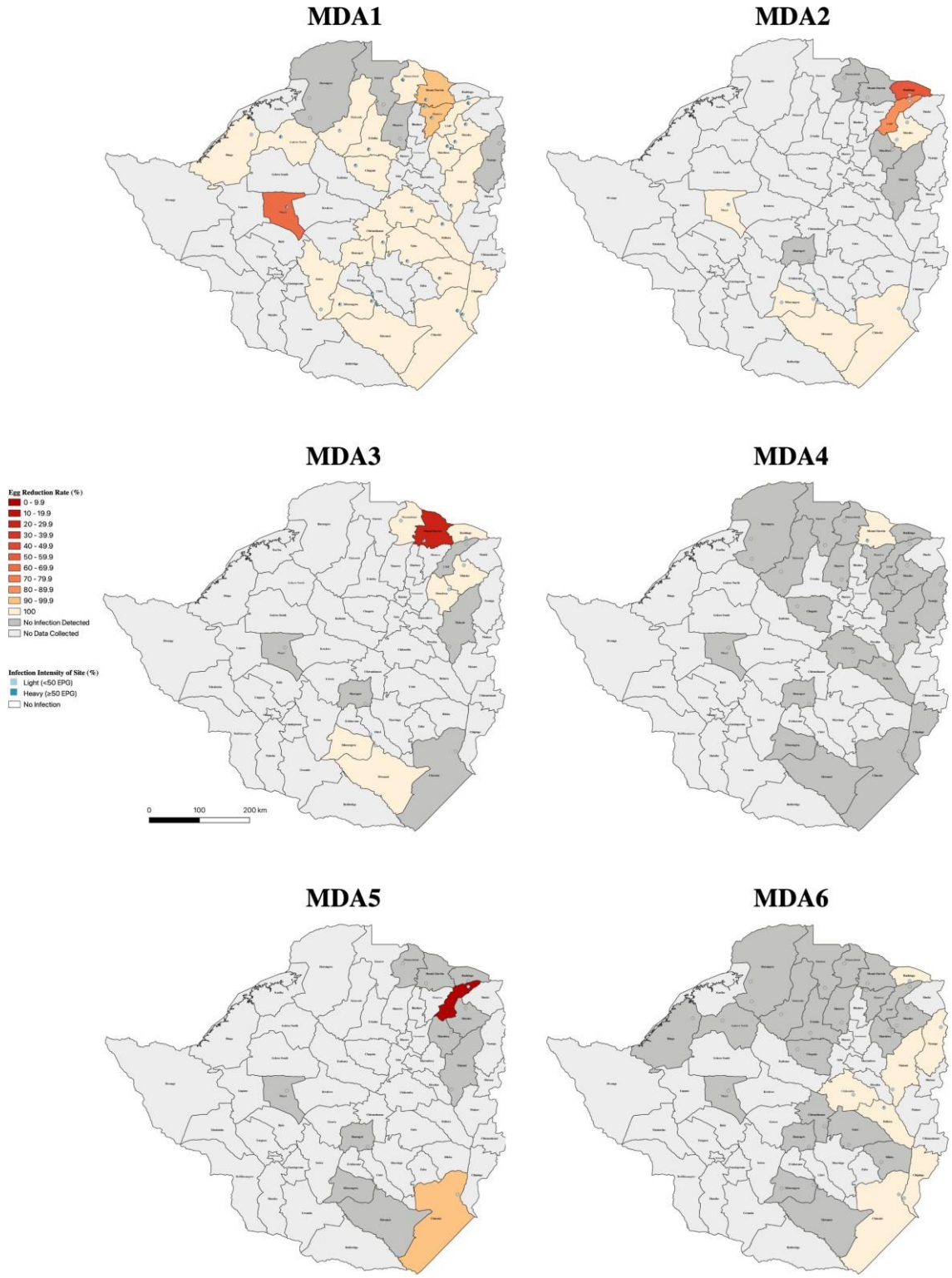
Supplementary Figure 1: The above choropleth maps depict the geographical distribution of the prevalence of *S. haematobium* at the pre- and post- surveys during rounds one, two and three of an annual mass drug administration (MDA) in Zimbabwe. The prevalence of each district is coloured to represent the prevalence (%) at that timepoint, with the sentinel site locations highlighted by a circular data point. The village data points also represent the intensity of infections at the time of survey, with the circular pie chart indicating what percentage of infections were heavy (dark blue) or light (light blue). The date at which every MDA was carried out is depicted on the right-hand side of the choropleth maps. These maps were generated using QGIS, Version [3.22.2].



Supplementary Figure 2: The above choropleth maps depict the geographical distribution of the prevalence of *S. haematobium* at the pre- and post- surveys during rounds four, five and six of an annual mass drug administration (MDA) in Zimbabwe. The prevalence of each district is coloured to represent the prevalence (%) at that timepoint, with the sentinel site locations highlighted by a circular data point. The village data points also represent the intensity of infections at the time of survey, with the circular pie chart indicating what percentage of infections were heavy (dark blue) or light (light blue). The date at which every MDA was carried out is depicted on the right-hand side of the choropleth maps. These maps were generated using QGIS, Version [3.22.2].



Supplementary Figure 3: The above choropleth maps depict the geographical distribution of the cure rate (CR) of *S. haematobium* during six rounds of annual mass drug administrations (MDAs). Each district is coloured to represent the CR (%) at that survey, with the sentinel site locations highlighted by a circular data point. The village data points also represent the intensity of infections at the time of survey, with the circular pie chart indicating what percentage of infections were heavy (dark blue) or light (light blue). The date at which every MDA was carried out is depicted on the right-hand side of the choropleth maps. These maps were generated using QGIS, Version [3.22.2].



Supplementary Figure 4: The above choropleth maps depict the geographical distribution of the egg reduction rate (ERR) of *S. haematobium* during six rounds of annual mass drug administrations (MDAs). Each district is coloured to represent the ERR (%) at that survey, with the sentinel site locations highlighted by a circular data point. The village data points also represent the intensity of infections at the time of survey, with the circular pie chart indicating what percentage of infections were heavy (dark blue) or light (light blue). The date at which every MDA was carried out is depicted on the right-hand side of the choropleth maps. These maps were generated using QGIS, Version [3.22.2].