

**INVESTIGATING SCHISTOSOMIASIS MARKERS OF INFLAMMATION AND
IMMUNE RESPONSES IN SCHOOL CHILDREN ON REPEATED MASS DRUG
ADMINISTRATION IN BANDANYENJE, ZIMBABWE**

TAWANDA JONATHAN CHISANGO

214584507



**UNIVERSITY OF TM
KWAZULU-NATAL**

**INYUVESI
YAKWAZULU-NATALI**

**A thesis submitted to the University of KwaZulu Natal, College of Health Science, in
fulfilment of the requirements for the degree of Doctor of Philosophy in Medical
Microbiology (Laboratory Medicine).**

Durban

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A thesis submitted to the University of KwaZulu Natal, College of Health Sciences in fulfilment of the requirements for the degree of Doctor of Philosophy in Medical Microbiology (Laboratory Medicine).

This is to attest that contents outlined in this thesis are the original research work done and reported by the author (Tawanda Jonathan Chisango). The research work detailed in this thesis has not been previously submitted to any institution for award of a degree or diploma. The use of other researchers/scientist's work in the text has been acknowledged accordingly.


Signature.. 

Date..31/10/2017.....

Tawanda Jonathan Chisango, Registration Number.214584507

As the candidate's supervisor, I have approved this thesis for submission.

1. Professor Takafira Mduluzi (Main Supervisor)

Signature .. 

Date....31/10/2017

Format of dissertation

This thesis was presented as a manuscript format, which included submitted journal articles and prepared journal articles under peer review that have emanated from the research project from this field.

Research Approval

Name of Research Committee	Date	Reference Number
Biomedical Research Ethics Committee	16/09/16	BE467/16
Medical Research Council of Zimbabwe	31/06/16	MRCZ/A/1958

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PREFACE AND DECLARATION

This dissertation is submitted in fulfilment of the Doctor of Philosophy degree in Medical Microbiology with the University of KwaZulu Natal, College of Health Sciences. This research has been conducted under supervision by Professor Takafira Mdluza and co-supervision by Dr Bongiwe Ndlovu during the period January 2015 to December 2017.

DECLARATION 1: PLAGIARISM

I Tawanda Jonathan Chisango declare that;

The work presented in this thesis is my original work except where otherwise stated.

This dissertation has never been submitted for any degree or examination at any other institution or university.

This dissertation does not contain any personal information, pictures, graphs or even data except if referenced or acknowledged as outside sources.

No pasted graphics, text, pictures, figures or tables from the internet are found in this dissertation unless specifically acknowledged or properly referenced.

Signed:



Date: 30 October 2017

DECLARATION 2: PUBLICATIONS AND MANUSCRIPTS

Part of this work has been presented in the format of a book chapter we worked on during review of the field of study and manuscripts; one under review in the BMC Infectious Diseases Journal and the other in manuscript, to be submitted to the Infectious Diseases of Poverty Journal.

Book Chapter

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Author contributions

TM, TJC, AFN, and AM researched and transcribed different sub-topics within the area of study. TM reviewed and compiled the sub-topics into one chapter. All the authors critically reviewed the chapter before submission.

Manuscript 1

Benefits of Annual Chemotherapeutic Control of Schistosomiasis on the Development of protective Immunity. (Submitted to the BMC Infectious Diseases Journal under review)

Authors

Tawanda Chisango, Arthur Vengesai, Agness Farai Nhidza, Bongiwe Ndlovu, Danai Zhou, Edson Sibanda, Takafira Mduluza.

Author contributions

TJC, BN& TM developed the field study design, parasitological and immunoassays interpreted & analyzed the data. AV, AFN, ES, DZ & TM conducted field and sampling work. TJC, AV, AFN & BM conducted the laboratory assays. TJC and TM conducted the initial statistical analyses. TJC compiled the data and wrote the manuscript and all authors critically reviewed the draft version of the manuscript.

Manuscript 2

***S. haematobium* infection and Chemotherapy-induced changes in IL-6 and acute phase proteins associated with inflammation in school children in a Schistosomiasis- endemic area (In manuscript, to be submitted to the *Infectious Diseases of Poverty Journal*).**

Authors

Tawanda Chisango, Arthur Vengesai, Agness Farai Nhidza, Bongiwe Ndlovu, Danai Zhou, Edson Sibanda, Takafira Mduluzza.

Author contributions

TJC, BN& TM developed the field study design, immunoassays, interpreted & analyzed the data. AV, AFN, ES, DZ & TM conducted field and sampling work. TJC, AV, AFN& BM conducted the laboratory assays. TJC and TM conducted the initial statistical analyses. TJC compiled the data and wrote the manuscript and all authors critically reviewed the draft version of the manuscript.

DEDICATION

This work is dedicated to the people I love most and owe a great deal for the sacrifices they have made for its accomplishment; my loving wife Shillah and three children Tinotenda, Trish and Darrel. A special dedication goes out to my mother and my late father who moulded me into the person I am today.

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ABBREVIATIONS

AMEC	–	Arithmetic Mean Egg Counts
APPs	–	Acute Phase Proteins
BREC	–	Biomedical Research Ethics Committee
CFPD	–	Circulating Cell-Free Parasite DNA
CR	–	Cure Rate
CRP	–	C-Reactive Protein
ELISA	–	Enzyme-Linked Immunosorbent Assay
ERR	–	Egg Reduction Rate
HRP	–	Horse Radish Peroxidase
IBD	–	Inflammatory Bowel Disease
MBP	–	Myelin Basic Protein
MDA	–	Mass Drug Administration
MRCZ	–	Medical Research Council of Zimbabwe
NTDs	–	Neglected Tropical Diseases
OPD	–	o-Phenylenediamine Dihydrochloride
PZQ	–	Praziquantel
SEA	–	Soluble Egg Antigen
Sh13	–	<i>S.haematobium</i> 13
SWA	–	Soluble Worm Antigen
TPA	–	Tissue Plasminogen Activator
WHO	–	World Health Organization
WWH	–	Whole Worm Homogenate
χ^2	–	Chi-Square Test

ABSTRACT

Background: Schistosomiasis is a devastating parasitic disease. The mainstay of schistosomiasis control is by praziquantel treatment. The study aimed to determine benefits of repeated annual chemotherapy of schistosomiasis on development of protective immunity in school children in a selected endemic rural area in Zimbabwe and evaluate acute phase proteins and IL-6 as markers of inflammation. It is fundamental to evaluate the inflammatory markers and antibody profiles in the context of *S. haematobium* infection in school children in endemic settings in order to identify markers that predispose or protect them from infections. Acute phase proteins are plasma proteins whose concentration increases or decreases as a result of infection or inflammation and their production is stimulated by IL-6. Their circulating concentrations in the body reflect the extent of inflammation. There is an increased risk of cases of direct and indirect morbidities as a result of stimulation of tissue-destructive inflammation from *S. haematobium* infection, hence the need to determine the levels of inflammatory markers in *S. haematobium* infected and uninfected children and also determine the effect of annual repeated praziquantel treatment on levels of IL-6 and acute phase proteins.

Methods: Urine specimens from 212 school children (7-13 years) were collected and examined to determine prevalence and intensity of *S. haematobium* infection at baseline, 6 weeks and 2 years following annual rounds of praziquantel treatment. The overall rate of reinfection was determined after 2 years. Blood samples from the participants were assayed for total and *S. haematobium* (*Sh13*)-specific antibodies using the multi-plex magnetic bead-based immuno-assays and sandwich ELISA, respectively before and 2 years after annual rounds of treatment. Levels of 4 acute phase proteins (ferritin, fibrinogen, procalcitonin and tissue plasminogen activator) were also measured from serum samples from the school

children using the magnetic bead–based immuno-assays at baseline and 2 years following praziquantel treatment. Sandwich ELISA was used to determine levels of IL-6 before and after treatment with praziquantel.

Results: Annual treatment with praziquantel reduced the prevalence of *S. haematobium* infection ($p < 0.05$) from 23.1% at baseline to 0.47 % after 2 years. Overall cure rate was 97.8 %. Intensity of *S. haematobium* infected children declined ($p < 0.05$) from 15.9 eggs/10ml urine at baseline to 2 eggs/10ml urine. After two years, overall rate of reinfection was 0.96 %. At baseline, total IgG4 was higher in *S. haematobium* infected children ($p = 0.042$), while all other immunoglobulins were within normal ranges. There was an increase in total IgG2 ($p = 0.044$) levels and a decrease in total IgG4 ($p = 0.031$) levels 2 years post-treatment; and no significant changes in other total immunoglobulins. *Schistosoma* infected children at baseline had an increase in anti-*S. haematobium* 13 IgG1 ($p = 0.005$) and a decrease in *S. haematobium* 13 IgG4 levels ($p = 0.012$) following treatment. *S. haematobium* infected children had marginally higher levels of procalcitonin and tissue plasminogen activator before treatment though the difference of the two was not significant $p > 0.05$ -using the Mann-Whitney non-parametric U test. Levels of ferritin and fibrinogen were lower in *S. haematobium* infected children before treatment, however the difference was also not significant $p > 0.05$ -using the Mann-Whitney non-parametric U test. There was no association between infection status or IL6 and the levels of acute phase proteins $p > 0.05$ for all acute phase proteins using the Mann-Whitney non-parametric U test at baseline and post treatment.

Discussion and Conclusion: Annual praziquantel treatment delivered to school children over 2 years significantly reduced prevalence, intensity of infection and reinfection of *S. haematobium* infection. Treatment was also observed to cause a reduction in *Schistosoma*-

specific blocking IgG4 and an increase in *Schistosoma*-specific protecting IgG1. Findings from this study suggest no bearing of *S. haematobium* infection status on level of acute phase proteins before and after treatment with praziquantel. The extent of inflammation cannot be determined using ferritin, tissue plasminogen activator and fibrinogen. Levels of IL-6 did not alter levels of the acute phase proteins. There is a need to explore other components in acute phase proteins as inflammatory markers in *S. haematobium* infections as well as explore other proinflammatory and anti-inflammatory cytokines. Findings from this study highlight the long term impact of the current Schistosomiasis control efforts which employ repeated Mass Drug Administration of praziquantel on Schistosomiasis infection, reinfection and protective immunity.

CHAPTER 1: INTRODUCTION AND RATIONALE FOR THE RESEARCH

This chapter covered the background information of the area being researched as well as relevant history on the study area. It also outlined the problem statement, study justification and purpose of the study. Study objectives and hypotheses were also covered.

1.1 BACKGROUND

Schistosomiasis commonly alluded to as bilharzia, is a devastating water-borne parasitic disease caused by infectious trematode worms of the genus *Schistosoma* [1]. It is prevalent in resource- limited settings and according to the World Health Organization (WHO), ranks second on the list of 18 Neglected Tropical Diseases (NTDs) [2]. Inadequate access to clean water coupled with poor sanitation are typical in such under privileged populations.

Six species of schistosomes cause schistosomiasis in humans, and these are *Schistosoma haematobium*, *S. mansoni*, *S. japonicum*, *S. mekongi*, *S. intercalatum* and *S. guineensis* [2].

The distribution of the six species is defined by their host snails' habitat range with each of the species having a specific range of different and suitable snail hosts. *S. mansoni* uses aquatic freshwater *Biomphalaria* whilst *S. haematobium*, *S. intercalatum* and *S. guineensis* employ the *Bulinus* snails. *S. japonicum* uses amphibious freshwater *Oncomelania* spp snails as its intermediate host and *Tricula* (*Neotricula aperta*) is used by *S. Mekongi*. *S. haematobium*, *S. japonicum* and *S. mansoni* are the major species with an estimated 93% of all cases in the world concentrated in sub-Saharan Africa [3].

Schistosomes are classified in the phylum Platyhelminthes, however unlike most other platyhelminthes, schistosomes are dioecious and exhibit marked sexual dimorphism as adults, with the slender female fitted into the gynaecophoric canal of the male, producing eggs that

are fertilised by the male. The schistosomes can live up to 40 years residing within either the perivesicular (*S. haematobium*) or mesenteric (*S. mansoni*, *S. japonicum*, and others) venules, though their average life span is 3–10 years [4].

Adult worms derive most of their energy through anaerobic glycolysis by ingesting host erythrocytes and globulins [5]. Glucose and fatty acids derived from the host are used for egg production through fatty acid oxidation [6]. Schistosomes regurgitate waste into the bloodstream as they have no anus and cannot excrete waste products. Some blood and urine-based diagnostic assays have made use of some of these excreted waste products [1].

1.1.1 Life cycle of schistosomes

In order to control and eliminate human schistosomiasis it is essential to realize the schistosome lifecycle. The human host excretes *Schistosoma* eggs into a fresh water environment through urine or faeces and on hatching, the egg releases a miracidium, a free-living and ciliated form, which remains infective for 6–12 hours. The miracidium swims towards the snail intermediate by ciliary movement and pierces its soft tissue, loses its cilia and develops into a mother sporocyst, which then produces daughter sporocysts through asexual reproduction. The sporocysts migrate to the hepatic and gonadal tissues of the snail and metamorphose into cercariae in 2-4 weeks [7]. The cercariae leave the snail intermediate host following light stimulation and swim through the water until they come into contact with human skin [8]. The cercariae penetrate the host skin using proteolytic enzymes and after shedding their tails, become young worms called schistosomula. The schistosomules leave the dermis via the venous or lymphatic vessels and migrate in the blood via the heart and lungs to the liver. These schistosomula mature in 4–6 weeks in the portal vein, mate, and migrate to the perivesicular or mesenteric venules [9].

After mating the females produce a large number of eggs which secrete proteolytic enzymes to facilitate the migration of eggs into the lumen of the bladder (*S. haematobium*) or the intestine (*S. mansoni*, *S. japonicum* and the others). The eggs are either retained in host tissues where they induce inflammation or are excreted into the environment through urine or faeces where those that reach freshwater will hatch and release the miracidia in fresh waters where the cycle commences all over again (**Figure 1.1**) [8].

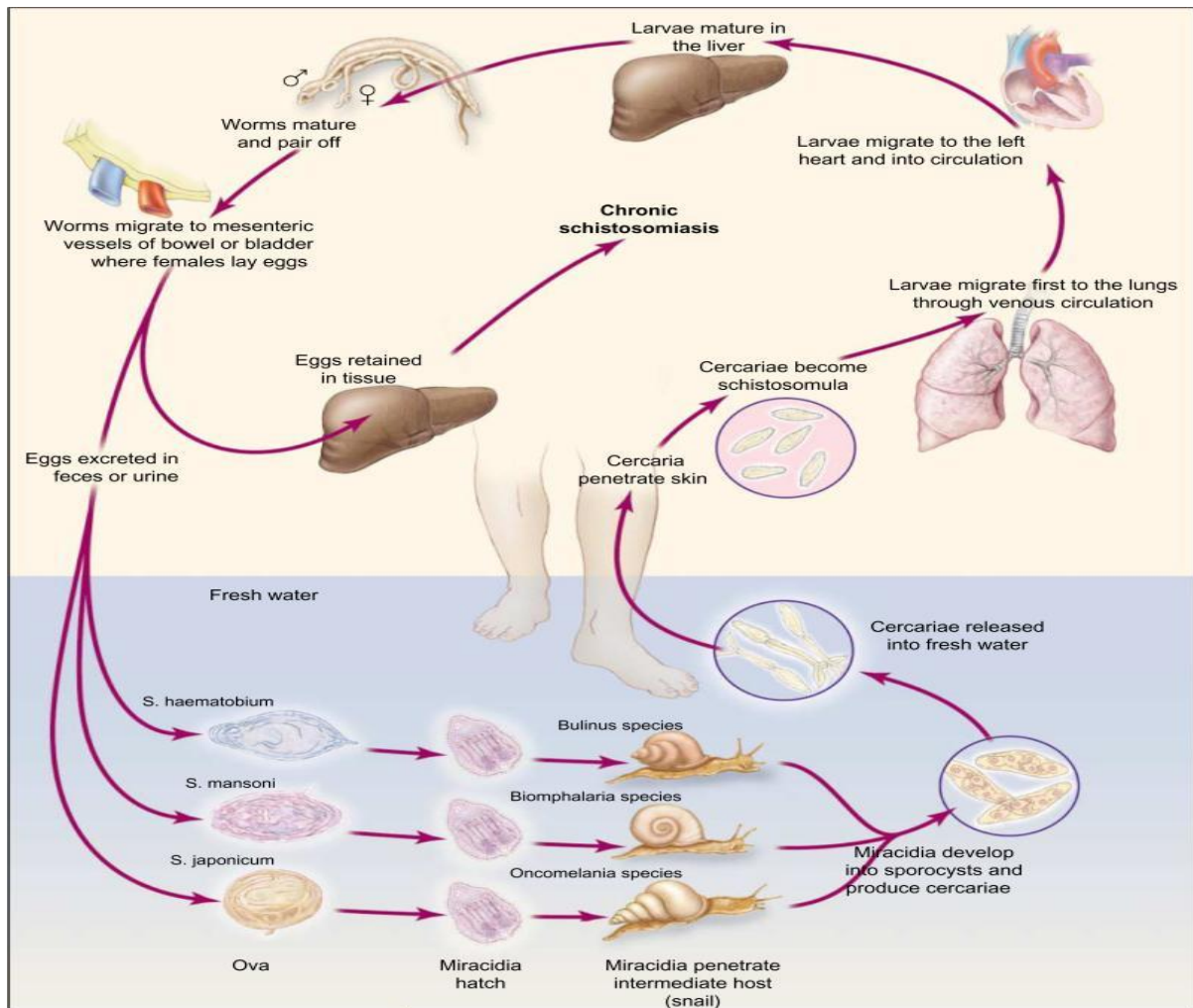


Figure 1.1: Life Cycle of Schistosomes, retrived 02/08/17, (Adopted from

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4231879/figure/F2>

1.1.2 Schistosomiasis Distribution

1.1.2.1 Global Distribution

S. haematobium is localised in Africa, India and the Middle East whilst *S. mansoni* is found in Central and West Africa, Egypt, Brazil, Venezuela, Madagascar and the West Indies. *S. japonicum* is found in China, Philippines and Indonesia whilst *S. mekongi* is found along, Mekongi river basin between Cambodia and Lao People's Democratic Republic, parts of the Congo River and in lower Guinea on the African continent. *S. guineensis* and *S. intercalatum* are localised in West and Central Africa [10, 11, 12] (**Figure1.2**).

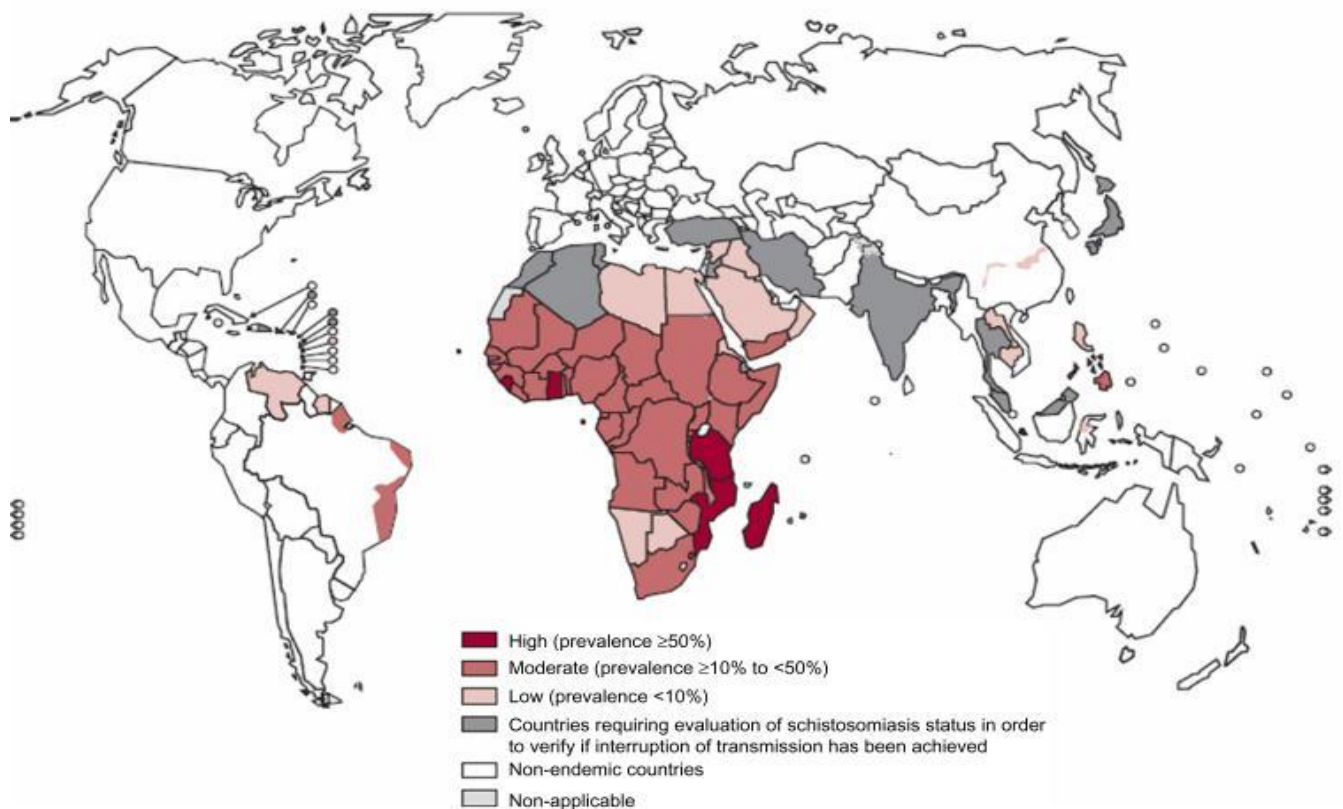


Figure 1.2: Global distribution of Schistosomiasis, retrieved 12/8/17, (Adopted from

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4231879>

1.1.2.2 Schistosomiasis distribution in Zimbabwe

In Zimbabwe, schistosomiasis is predominant in the north, north-east, south east, and eastern regions, with 11 provinces being affected with a prevalence ranging from 3.3 to 39.3% amongst 10-15 year olds according to a survey by Midzi *et al* (2014) [13]. The prevalence of schistosomiasis is low in the entire western region of the country. The distribution (**Figure 1.3**) is reflective of climatic conditions such as rain and temperature which are used in predicting infection risk and prevalence of schistosomiasis in non-surveyed areas [14].

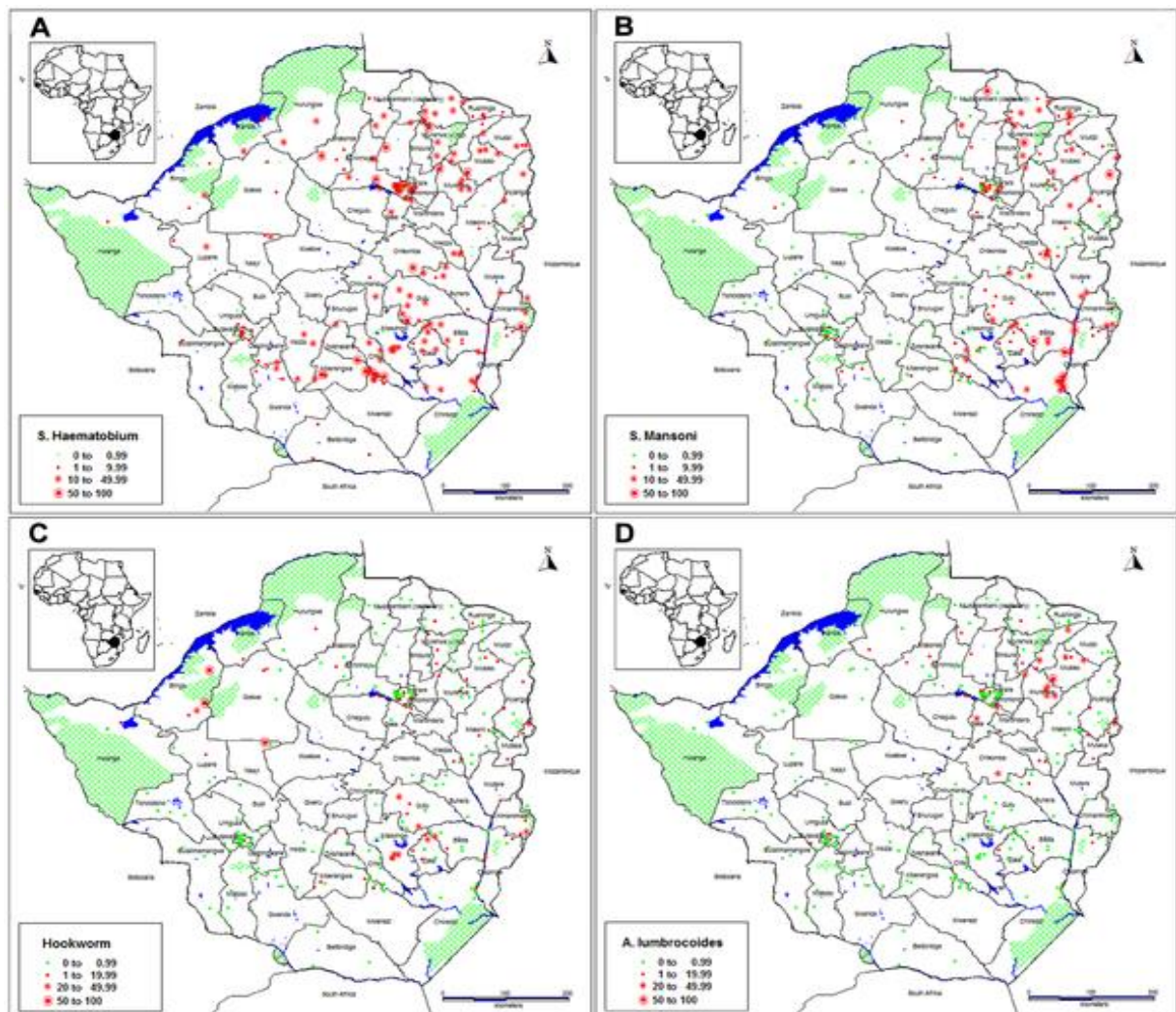


Figure 1.3: Distribution of schistosomiasis in Zimbabwe, retrieved 04/09/17, (Adopted from Midzi *et al*, 2014) [13]

1.1.3 Diagnosis of Schistosomiasis

Accurate diagnosis for schistosomiasis is necessary for swift treatment and control of the parasitic disease and should be inherent in all schistosomal control programmes. The earliest schistosomiasis diagnostic procedures involved parasitological detection of eggs in stool and urine samples and remain the gold standard for schistosomiasis diagnosis [15]. For intestinal schistosomiasis, WHO recommends the microscopy-based Kato-Katz thick stool smear technique for diagnosis of *S. mansoni*, *S. japonicum*, *S. mekongi*, and *S. intercalatum* and for the quantitative assessment of infection intensity [16].

Urine filtration and concentration of *S. haematobium* eggs, with subsequent microscopic examination is the main approach for urinary schistosomiasis diagnosis. Schistosome eggs are easy to detect and identify under a microscope owing to their oval shape with a sharp terminal spine. Immunodiagnostic techniques can be used to detect anti-schistosomal antibodies or circulating schistosomal antigens in plasma, serum, urine, or sputum. Many immunological tests are available for the diagnosis of schistosomiasis, with most techniques detecting IgG, IgM, or IgE against soluble worm antigen (SWA) or soluble egg antigen (SEA) by enzyme-linked immunosorbent assay (ELISA), indirect haemagglutination, or immunofluorescence [17]. Highly sensitive and specific PCR based assays (e.g. real-time quantitative PCR or multiplex PCR) have been developed for the detection of schistosome DNA or RNA in faeces or serum. Recent advances include the detection of egg DNA, circulating cell-free parasite DNA (CFPD), and circulating microRNAs [18].

1.1.4 Clinical Manifestations

The initial human contact with schistosomes is usually followed up by a mild prickling sensation with rash at the site of cercarial penetration [19]. The host immune system mounts an attack against the cercariae that penetrate the skin consisting of specific immunoglobulin E

antibodies, eosinophils, and macrophages that fight against the schistosomula. Destruction of the schistosomula can occur, resulting in edema and massive cellular infiltrates which cause dermatitis marked by papules, erythema, vesicles, edema, and pruritus [20]. However, the schistosomula that survive the immune attack and will migrate inside the body depositing eggs causing a hypersensitivity reaction known as Katayama fever. The acute onset of Katayama fever is accompanied by sudden, flu-like symptoms such as fever, fatigue, myalgia, headache, and non-productive cough and is common in travellers and tourists to schistosomiasis-endemic areas. Chronic schistosomiasis is the most prevalent form of the disease in endemic areas and occurs as a result of prolonged exposure to infective cercariae. In these endemic areas, children encounter their first infections by the age of 2 years and the burden of infection increases in intensity until they are young adolescents after which a general decrease occurs in adulthood. Immune reactions to *Schistosoma* eggs trapped in the tissues in chronically infected cases ultimately result in inflammation in affected tissues followed by granuloma formation [21]. The granuloma plays a protective role by preventing egg toxins and enzymes from coming into contact with tissues, however collagen deposits replace the granuloma as the immune response subsides [22]. The primary causes of morbidity in chronic *Schistosoma* infections are the large granulomas and fibrosis produced by the host's immune response against the *Schistosoma* eggs. The extent and severity of infection varies depending on the host genetics, intensity of infection and location of *Schistosoma* eggs and worms [7].

Deposition of *S.haematobium* eggs occurs in vesical and uteral walls resulting in inflammation that culminates in urinary schistosomiasis which is characterized by haematuria, dysuria, proteinuria, calcification in the bladder, obstruction of the ureter, renal colic, dark-coloured urine, and frequent and painful urination. Haematuria is so common in endemic areas that it is sometimes construed as a natural sign of puberty for boys and

confused with menses in girls [23]. Chronic infection may result in development of obstructive uropathy, renal failure, hydroureter, hydronephrosis, and bladder cancer [24, 25]. Intestinal schistosomiasis occurs as a result of inflammation caused by *S. mansoni* and *S. japonicum* eggs trapped in the walls of the large intestine and rectum. Symptoms include abdominal pain, diarrhoea and constipation. Bowel obstruction, appendicitis, and gastrointestinal perforation may develop in extreme cases [7].

Chronic inflammation caused by all *Schistosoma* species eggs trapped in tissues cause non-specific morbidities such as anaemia, malnutrition, and impaired childhood development [26,27]. These *Schistosoma*- associated morbidities are commonly associated with moderate-to-heavy egg-infection intensities and are progressive. School children and pre-school children are the most vulnerable groups to developing overt disease because of the higher rates of water-related activities, immunological status and anatomical vasculature [28, 29]. These groups harbour the largest numbers of adult worms, with copious tissue entrapped eggs causing systematic and organ-specific inflammation [1]. Fortunately, preventive chemotherapy through periodic large-scale administration of praziquantel (PZQ) to school children and other high-risk groups without prior diagnosis has resulted in suppressing the morbidities [16].

1.1.5 Control of Schistosomiasis

The World Health Assembly resolution 54.19 urged all its member nations to be actively involved in the control and ultimately the elimination of schistosomiasis [30].

Schistosomiasis can be controlled through the use of measures such as elimination of intermediate host snails, chemotherapy, basic sanitation and clean water supply, prevention of human contact with water containing infected snails and health education, either in isolation or in combination [31]. Chemotherapy using praziquantel as the drug of choice is the most

popular method of control because it significantly reduces morbidity caused by tissue-deposited eggs and transmission by reducing contamination of snail habitats by *Schistosoma* eggs. The development of vaccines is an important alternative strategy for schistosomiasis control. However, no suitable vaccine has yet been developed for widespread use. By 2020, the global health community aims to control and eliminate human helminthiases, including schistosomiasis in selected African countries, principally by preventive chemotherapy through mass drug administration of antihelminthics [30].

1.1.5.1 Chemotherapy using Praziquantel

Praziquantel, a pyrazino-isoquinoline derivative, is a WHO-endorsed drug that is highly efficacious, and has a broad spectrum against helminth infections [32]. It was discovered in 1972 and is administered at the standard oral single dose of 40 mg/kg body weight [33]. Its mechanism of action is not yet fully understood though there is evidence suggesting that it increases the permeability of schistosome membranes to calcium there by promoting tegument damage and worm paralysis [34]. However, praziquantel is only active on adult worms but not on immature worms and thus follow up treatment may be required 4-6 weeks later [1]. There have been suggestions to combine praziquantel with antimalarial drugs (artemether, artesunate) possessing anti-schistosome properties of killing immature worms [35].

Approximately 80% of the drug is rapidly absorbed from the gastrointestinal tract and metabolized by the liver then excreted through the urine and faeces [36]. The benefits of praziquantel are its high efficacy, ease of administration, relative safety, and mild to moderate side effects that include nausea, dizziness, rash, pruritus, headache, drowsiness, and abdominal pain [37]. Preventive chemotherapy is targeted toward school children in endemic areas because they are known to have a high risk of infection. In areas where the prevalence

of infection is at least 10%, preventive treatment should also be given to those who are at high risk of infection because of their occupation, such as fishermen, farmers, and irrigation workers, as well as women who may be exposed to infected waters when performing their domestic chores. Pregnant and lactating women in these areas are considered at high risk of developing morbidity due to schistosomiasis infection and should also be included in preventive chemotherapy campaigns [2].

The WHO estimated that the total number of people that required treatment for schistosomiasis was 218 million in 2015 with 92% living in the African region. The number of school children who received treatment for schistosomiasis during the same period was 46 million representing 81.2% of the total number of people treated in the African region [38]. There has been a rising interest in the control and elimination of Neglected Tropical Diseases (NTDs), and currently the control of schistosomiasis is a priority for many governments, donors, pharmaceutical companies and international agencies.

1.1.5.2 Mass Drug Administration

Mass chemotherapy is a form of treatment whereby the entire population is treated without prior individual diagnosis. The mass drug administration aims to lessen morbidity and mortality due to *Schistosoma* infection and prevent new infections by limiting transmission through reduction of the overall prevalence in the population [39]. The assumption is that mass chemotherapy results in decreased excretion of *Schistosoma* eggs, contamination of the environment, and infection of the snail population, resulting in less source of infection for humans. The advantage of mass drug administration is low diagnostic cost and the simplicity of providing treatment in schools coupled to access to infrastructure within which such programs can operate successfully however, the high cost of drugs and the delivery system

may be the major constraints. Praziquantel has been the drug of choice in recent control efforts employing mass drug administration [32].

Even though praziquantel is efficient in eradicating active infection, it does not cure everyone. As a result rapid reinfections have been reported in communities where it is unavoidable to get in contact with water infested with snails carrying the *S.haematobium* larvae [40]. Reinfection cannot be prevented by mass drug administration, and infection rates tend to return to baseline values within 24 months after chemotherapy [41]. Hence, there is need to repeat mass treatments with a frequency dependant on the endemic risk level of the community.

1.1.6 Antibody profiles and inflammation markers

The immune system of infected hosts is constantly confronted by several life cycle stages of *S.haematobium* such as penetrating cercariae, adult worms and eggs [42]. The multitude of antigenic moieties the host encounter as a result, stimulate the humoral and cellular immune responses leading to antibody production and proliferation of specific T cells [43]. The roles of particular antibody isotypes in immune defence have been revealed in mostly *in vitro* studies. IgE has been linked to resistance to reinfection as it cooperates with mononuclear phagocytes, eosinophils, and platelets to kill *Schistosoma* larvae through antibody dependant cytotoxicity. Elevated *S. haematobium* specific IgE and tissue eosinophilia are crucial in developing resistance to schistosomiasis. Studies have identified IgG2 and IgG4 as blocking antibodies that prevent IgE from binding to eosinophils preventing antibody dependant killing of schistosomes. Praziquantel kills worms exposing internal antigens which in turn alter antibody profiles with changes in humoral and cell immune responses observed following the anti-helminthic administration [44].

The immune system tries to deal with the burden of *Schistosoma* worms and eggs, however the chronic inflammation resulting from soluble egg antigens released from tissue-trapped eggs is inevitable. Inflammation is a complex process that occurs as a result of vascular tissue responding to infection, exposure to toxins or cellular injury through production and accumulation of plasma proteins, leucocytes and cytokines [45]. The degree of inflammation reflects the extent of the *S. haematobium* infection and its resolution will result in the inflamed tissue returning to its status prior to the injury. In essence inflammation is meant to control parasitic infections but if left uncontrolled culminates in tissue injury. In some cases where the *S. haematobium* persists, resolution fails to take place leading to chronic inflammation however inflammation can still persist after the removal of the parasite [45].

Chronic inflammation can be perceived as a consecutive series of separate inflammatory stimuli resulting in increased serum concentrations of acute phase proteins. The acute phase proteins are plasma proteins whose concentration increases or decreases as a result of inflammation or infection and are produced and released mainly by the liver to the systemic circulation mainly through the action of different proinflammatory cytokines (e.g., IL-6, IL-1 and TNF- α). Interleukin-6 is the chief stimulator of the production of most acute phase proteins and is important in the regulation of the immune response, inflammation, and haematopoiesis [46]. It is fundamental to evaluate the inflammatory markers and antibody profiles in the context of *S. haematobium* infection in school children in endemic settings in order to identify markers that predispose or protect them from infections. Such information has vital implications in vaccine production which has been elusive in schistosomiasis.

1.2 Problem statement

The immunology of schistosomiasis is faced with challenges where currently there are no vaccines available and insufficient knowledge of dominant antigens for future development

of vaccine targets. To this end, understanding antibody profiles and inflammatory markers such as cytokines and acute phase proteins is necessary to limit the degree of immunopathology in schistosomiasis. Children residing in the same geographical location and exposed to similar water-related activities suffer different consequences with regards to *S. haematobium* infection. Some are easily infected and reinfected whilst others never get infected. It is important to understand how this protection develops, both to aid vaccine development, and to understand the long-term implications of other control strategies which may reduce or alter natural exposure to the parasite. School children harbour the largest numbers of adult worms and tissue entrapped eggs causing systematic and organ-specific inflammation. If left untreated, the children will develop severe *Schistosoma*-related morbidities such as calcification in the bladder, obstruction of the ureter, renal colic and bladder cancer. Identifying children who are exposed to inflammatory mediators early in life allows for earlier treatment which prevents future health problems. Mass drug administration, which is the main strategy for control, does not prevent reinfection, and infection rates tend to return to baseline values within 24 months after chemotherapy. Hence, this strategy needs to be repeated with a frequency depending on the endemic risk level of the community.

1.3 Hypotheses

The following are the hypotheses of the study:

- Protection and susceptibility to *S. haematobium* infections are related to presence of total and specific *S. haematobium* protective or blocking antibodies.
- Repeated mass treatment with praziquantel alters the antibody profiles in both *S. haematobium* infected.

- Presence of inflammation markers is associated with *S. haematobium* infection status and repeated mass treatments with praziquantel modifies the level of the markers in both *S. haematobium* infected.

1.4 Research questions

Are there any variations in biochemical markers of inflammation and antibody levels in individuals on repeated annual praziquantel mass administration and is there any relationship between levels of inflammatory markers and antibodies with respect to resistance to infection/reinfection?

1.5 The General objective

The general objective of the study was to monitor antibody profiles, biochemical markers of inflammation through measurement of acute phase proteins and the proinflammatory cytokine, IL-6 in *Schistosoma*-infected, non-infected and re-infected individuals on repeated mass treatment with praziquantel.

1.5.1 Specific objectives

- i. Determine consequences of praziquantel on *S. haematobium* prevalence, intensity of infection and reinfection rates and also assess the effect on the *Schistosoma*-induced morbidity-haematuria.
- ii. To determine the effect of total and *Schistosoma*-specific antibodies on protection or resistance to *S. haematobium* infection and evaluate the effect of praziquantel treatment on the antibody profiles.
- iii. To assess the usefulness of ferritin, fibrinogen, procalcitonin and tissue plasminogen activator as inflammatory markers in schistosomiasis and determine the effect of praziquantel treatment on their levels post treatment.
- iv. To investigate the effect of the proinflammatory cytokine IL-6 on acute phase proteins in *Schistosoma* infected and uninfected individuals, before treatment and after treatment with praziquantel.

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CHAPTER 2: LITERATURE REVIEW

This chapter covered the review of the area under study, current information and previous research that had been done with emphasis on relevance of our study. The scope included epidemiology, efficacy of praziquantel and its use in mass drug campaigns. Immune responses following treatment as well as the relationship between acute phase proteins and the pro-inflammatory cytokine IL-6 in schistosomiasis were also covered.

The chapter also included some information (which was duly acknowledged) from our book chapter that was published in 2017.

2.1 Epidemiology of Schistosomiasis

Schistosomiasis is a deadly parasitic disease that remains as one of the most prevalent Neglected Tropical Diseases (NTDs). Worldwide, schistosomiasis is estimated to affect over 290 million individuals [1], with more than 779 million individuals living in high-transmission areas [2]. An estimated 120 million individuals have schistosomiasis-related symptoms and the disease accounts for over 2.8 million years lived with disabilities [1]. The disease caused by *S. haematobium* is the most prevalent form of schistosomiasis in Africa and the Middle East affecting approximately 107 million people [2]. Schistosomiasis is responsible for significant health problems and is a socioeconomic burden in most of Sub-Saharan Africa and some other tropical countries. Schistosomiasis is endemic in Zimbabwe and in 2009 the disease was included in the 2009–2013 National Health Strategy by the Ministry of Health and Child Care underscoring the significance of appreciating the distribution and burden of the disease. A nationwide cross-sectional survey was conducted by Midzi *et al* (2014) [3] in order to map schistosomiasis because of its public health importance in Zimbabwe.

In affected populations, school children harbour the highest number of eggs and intensity of schistosome infections, while a lower intensity of infection is observed in adults [4]. In children, urogenital schistosomiasis causes haematuria, dysuria, nutritional deficiencies, anaemia, growth retardation, decreased physical performance and impaired memory and cognition [5]. These factors combine to reduce school attendance and to impair educational performance, which, in turn, lead to a reduction in future productivity and wage-earning capacity [6].

2.2 Preventive Chemotherapy and Mass Drug Administration

In order to control the burden of schistosomiasis it is necessary to improve sewage disposal, access to clean water and employ preventive chemotherapy. Preventive chemotherapy with praziquantel given at 40mg/kg in school children living in schistosomiasis-endemic areas is recommended by WHO and has been given to millions of individuals [7]. Praziquantel penetrates the tegument of schistosomes and rapidly moves through the tissues causing tegument damage and worm paralysis. Work on the biochemical effects of praziquantel in mice has shown that tegumental damage in schistosomes can occur as early as 15 min after treatment and that worms take about 14–18 days to disintegrate [8]. Praziquantel is less effective on recent infections since it mainly attacks the mature schistosome worms but not the immature stages and as a result a second dose of praziquantel is recommended following the initial dose in order to kill the worms which were immature during the first treatment. Studies have suggested that a repeat dose of praziquantel, given 2 to 8 weeks after the first dose, can improve cure rates and reduce remaining intensity of infections in population-based programs [9].

Praziquantel has gained widespread usage because it is easy to administer, safe, well tolerated, cheap, can reverse schistosome-related morbidity and is highly effective against the

six schistosome species that infect humans [10]. Improving the health of school children has emerged as a policy priority in developing countries and recent control efforts have focussed their attention on mass treatment campaigns using praziquantel in school children with numerous mass drug administration programs currently being undertaken in countries such as Uganda, Sierra Leone, Burkina Faso and Mali[11, 12, 13]. Scaling up of mass drug administration was proposed in the WHO's strategic plan of 2012 and by 2020, the global health community aims to have controlled and eliminated human helminthiasis [14].

Targeting treatment in children makes a significant impact on the development of pathology since severe clinical forms of schistosomiasis infection may take many years to develop[15]. Several studies have shown that mass drug administration using praziquantel drastically reduces morbidity and transmission of schistosomiasis with a satisfactory egg reduction rate [16, 17] especially in low-to-moderate transmission areas where the risk of reinfection is generally low. The WHO recommends expression of treatment outcome in terms of egg reduction rate calculated as the difference of the arithmetic mean egg counts between pre- and post-treatment samples. In this study arithmetic mean egg counts were used to calculate the intensity of infection and egg reduction rate [16].

Cure rates for praziquantel are variable ranging from 60 to 95% [18, 19] with high cure and egg reduction rates having been reported in Côte d'Ivoire [20] and South Africa [21].

However, concerns about praziquantel efficacy have been raised with a few reports on low cure rates recorded in Senegal [22] and Ghana [23]. The discrepancies in efficacy could be linked to drug resistance, presence of pre-patent infections existing at the time of treatment in high transmission areas and the inadequacy of single dose of 40 mg/kg under conditions of extremely high intensity of *S. haematobium* infection [24].

The long-term effects of mass drug administration on infection dynamics are not yet fully understood even though the impact of *S. haematobium* infection and morbidity in affected individuals is unequivocal. As such the current longitudinal study aimed to investigate the effects of annual treatments with praziquantel over a period of two years on the infection intensity, prevalence, reinfection rates, antibody profiles and inflammation markers in school children attending Bandanyenje primary school so as to add to the body of knowledge on the epidemiology and effects of schistosomiasis treatment in Manicaland. Information on the efficacy of mass drug administration and praziquantel can assist in evaluating policies and strategies that guide schistosomiasis control activities in the district.

2.3 Reinfection

Although praziquantel is efficient in eradicating active infection, reinfection occurs rapidly after a single mass drug administration and is a challenge in communities where it is unavoidable to get in contact with water infested with snails carrying the *S. haematobium* larvae. Concerns have been raised regards mass drug administration which include failure in treating some individuals, potential for resistance to develop and spread throughout the population [22, 23]. The rate and intensity of post-praziquantel chemotherapy reinfection varies between schistosome species, socio-demography, and level of schistosomiasis prevalence [25].

Children are more rapidly reinfected and at higher rates than older children and adults [26]. These higher rates of reinfection are in part related to higher levels of water contact by children, a feature unique to most endemic settings. As such that there is a need for improved early diagnosis and treatment of children in endemic areas who seem to lack developed immune systems against the disease [27]. Control of reinfection requires repeated mass treatment where the intensity of application and the interval between treatments are

dependent on the reproductive life-expectancy of the adult worm. In the current study treatment was repeated annually for 2 years in order to control reinfection. Since the entire population does not get reinfected at the same rate, it is possible that host factors may play a dominant role in determining resistance or susceptibility to reinfection with schistosomes. In this study we analysed the general *Schistosoma*-specific antibodies and inflammation markers and tried to identify the host immune factors that either predispose or protect the host from infection or reinfection.

2.4 *S. haematobium*-induced pathology: Haematuria

The WHO recommends determination of haematuria and egg based counts for effectively identifying communities with high, moderate or low risk for schistosomiasis. Studies have shown that prevalence of *S. haematobium*-induced pathology like haematuria can be reduced after praziquantel treatment [28, 29]. Effectiveness of praziquantel treatment has been assessed through its effect on the intensity and prevalence of *S. haematobium* infection and morbidity. Although there is an association between prevalence, intensity of infection and the presence or severity of morbidity, the correlation is imperfect, and there is a need to monitor these parameters especially in individuals on repeated mass treatments. The information obtained can be useful in evaluating the effect of infection and repeated treatments on *Schistosoma*-induced morbidities.

Studies of morbidity reduction related to drug treatment have had some conflicting results which may be a reflection of differences in follow-up after treatment, methods used to measure morbidities, the *Schistosoma* species, the presence of co-infections (especially malaria), the type of population and the region, the initial prevalence of infection, the incidence of reinfection, and other factors [29,30].

2.5 Immune responses and Chemotherapy

Studies on the effects of chemotherapy on helminths indicate that the drug treatment can facilitate the development of resistance to re-infection. The action of the praziquantel results in a transient increase in antigens from damaged worms, resulting in an increase in the specific immune responses (both humoral and cellular) which assist in killing the damaged and paralysed worms. In the absence of reinfection, the levels of both worm and egg antigen would eventually decline. Chemotherapy-induced changes in responses to infection have been reported on a number of occasions [31, 32], with frequent treatments thought to enhance the development of protective immune responses. Lympho-proliferative responses have been shown to increase following chemotherapy in *S.mansoni* and *S. haematobium* infected communities [32]. Grogan *et al.* (1996) [33] reported that levels of anti-soluble egg antigen (SEA) IgE, antiwhole worm homogenate (WWH) IgE and IgG4 increased following treatment whilst a study in Sudan reported a significant increase in anti-WWH IgG1 and IgG4 following treatment of schistosomiasis [34].

Studies of the effects of chemotherapy on other helminths indicate that the drug treatment can facilitate the development of resistance to reinfection through enhanced production of protective antibodies and decline in blocking antibodies. Key information on the humoral immune status of individuals can be determined routinely by measuring total immunoglobulin levels. Studies have realized the association between total immunoglobulins in chronic diseases associated with inflammation such as diabetes [35, 36]. Very little has been done on the association between the levels of total immunoglobulins and schistosomiasis, which is also a chronic disease that is also associated with inflammation. Indeed treatment of schistosomiasis has shown to be associated with subsequent changes in humoral and cell immune responses though focus has been mainly on *Schistosoma*- specific antibodies. Analysing such changes in the context of both total and specific antibodies will

assist in interpreting the differences that occur in children living in endemic areas as well as improving current understanding of the development of acquired immunity to schistosome infections and vaccine development. In order to establish the relationships between total serum immunoglobulins and *S. haematobium*-specific antibodies and to better understand their turnover after praziquantel treatment, we quantitatively measured the total immunoglobulins and specific antibody profiles in school children before and after treatment.

2.6 Acute Phase Proteins and IL-6

Chronic inflammation subsequent to infection with *S. haematobium* appears to be the major source of burden in individuals with schistosomiasis. There is an increased risk of cases of direct and indirect morbidities such as anaemia, growth deficiencies, physical fatigue and diminished cognitive development as a result of stimulation of tissue- destructive inflammatory and granulomatous reactions from *S. haematobium* infection [37, 38]. In the control of parasitic diseases, immune responses and consequently the inflammatory processes are meant to eliminate the harmful agent and restrict tissue damage but in some situations it may end up triggering pathological repercussions that can also effect injury and illness itself. In some instances inflammation can persevere even after the harmful agent has been eliminated, giving rise to chronic inflammation [39]. Chronic inflammation can be seen as a continuous series of distinct and consistent inflammatory stimuli which results in increased serum concentrations of acute phase proteins and proinflammatory cytokines [40] which seek to inhibit the infection [41].

Induction of inflammatory reactions has been found to occur as a result of *Schistosoma* infection. The extent of inflammatory tissue damage as well as diagnostic and prognostic information in some human diseases can be resolved by measurement of acute phase proteins [42]. However there is inadequate information regarding the use of acute phase proteins in

parasitic infections like schistosomiasis in humans. This is unusual considering the fact that parasitic infections elicit considerable inflammation. Although perpetual and continuous inflammation is presumed to be a hallmark of schistosomiasis, very few studies have actually examined the acute phase proteins simultaneously with the proinflammatory cytokine IL-6 in *S. haematobium* infections. Acute phase proteins are released as a result of the action of cytokines such as IL-1, IL-6, and TNF- α produced by T-lymphocytes, macrophages, monocytes, endothelial cells, and fibroblasts [43] at the site of inflammatory lesions. Although it is evident that a number of proinflammatory and anti-inflammatory cytokines are involved in the inflammatory response, available data indicates that IL-6 is the supreme stimulator [44]. In endemic areas there is constant exposure of worms and egg burden which constantly triggers inflammation which can result in acute phase proteins production being chronic.

There is unequivocal evidence that praziquantel treatment minimizes worm burden thereby reducing the amount of deposited eggs culminating in an overall decline in inflammation related to the immunopathological reactions to the eggs. Therefore, praziquantel should be able to reduce chronic inflammation especially if administered regularly as in mass treatment campaigns. Knowledge of inflammation markers is necessary in assessing and identifying children who are likely to develop chronic pathologies later on in life. Identifying such individuals at an early stage who are vulnerable to inflammation allows better prognosis and prevents the use of invasive, costly and time consuming procedures to determine those suffering from pathological complications of chronic inflammation conditions. The ultimate goal of disease monitoring is to identify individuals at risk in order to treat earlier.

We initially determined *S. haematobium* infection status then measured four different types of acute phase proteins that have not been commonly measured in other studies and IL-6 at

baseline to assess inflammation in both *S. haematobium* infected and *S. haematobium* uninfected school children. Most studies have investigated one acute phase protein at a time and not much information regards the overall effect of *S. haematobium* infection on inflammation has been realised as the acute phase proteins have different half-lives hence the need to use four different markers in our study. Secondly we established whether repeated rounds of praziquantel treatment for 2years results in changes in the acute phase proteins and IL-6. Knowledge on inflammation markers and how they respond to treatment can assist in development of therapeutic interventions that may help alleviate or relive development of chronic inflammation and the associated morbidities.

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CHAPTER 3: GENERAL METHODOLOGY

This chapter gave a detailed account of the steps to be taken in investigating the research objectives in this study. It included information on the study design, study area and population. The criteria used in the inclusion and exclusion of participants in the study were covered. In addition it covered specimen collection, specimen processing, data analysis and ethical considerations.

3.1 Study area and population

The study was carried out in Bandanyenje Primary School located in a schistosomiasis-endemic setting in the Manicaland Province of Zimbabwe. It is located approximately 217 Km south of Harare with latitude and longitude of 7°1'N 38°35'E. In Bandanyenje, safe water and sanitation coverage are poor. Villagers depend on perennial rivers as their water source thus exposing the majority of their population to infection. The rivers that provide habitats for *Schistosoma* intermediate host snails lead to moderate to high transmission rates of *Schistosoma* parasites. Bathing, swimming, fetching water, and laundry are the main water contact activities for school children in these areas. The area is endemic to *S. haematobium* with a provincial overall schistosomiasis prevalence of 23.8% [1]. The study population included a random sample of 212 (105 boys and 107 girls) aged between 7–13 years who were attending Bandanyenje primary school.

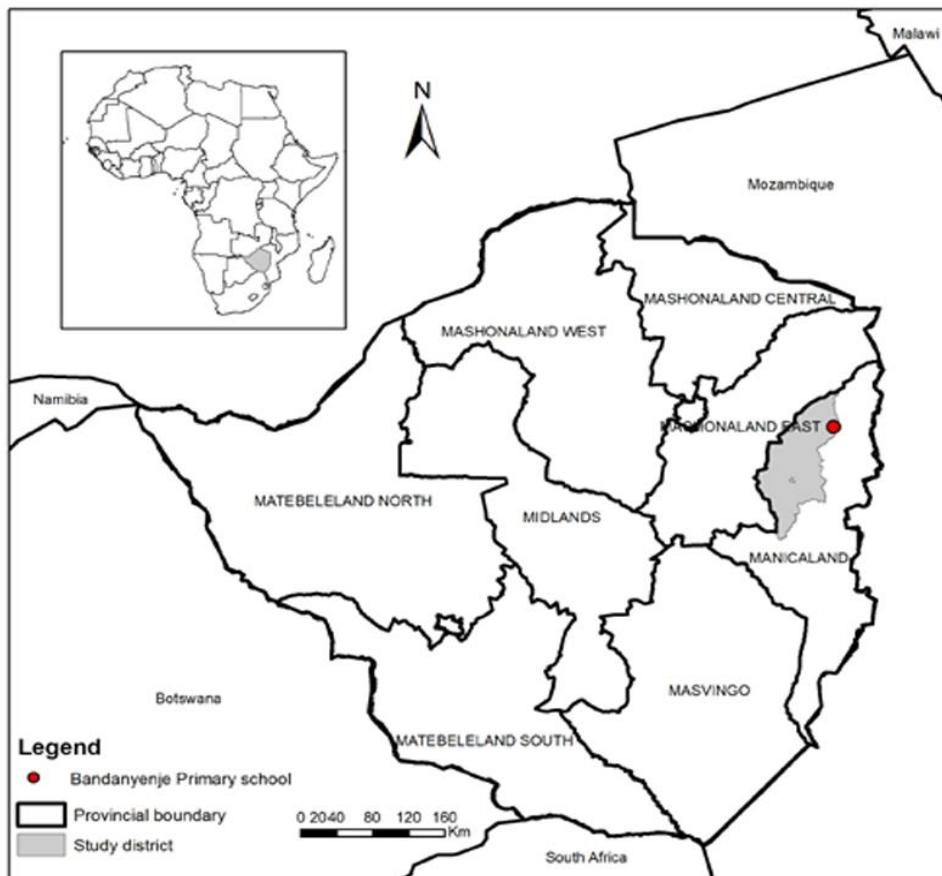


Figure 3.1: Map showing location of study site in Zimbabwe

3.2 Study design

This study design is a “before and after experiment “. It is a longitudinal survey conducted before and after treatment with praziquantel. All the children in the study were treated regardless of infection status as the study was part of the National Schistosomiasis Control programme, were all children were required to take part unless their guardians declined. The following parameters were assessed.

Prevalence and intensity: A urine and stool examination was conducted in all children selected for the study at baseline, at 6 weeks and 2years after. A child was considered infected by *S. haematobium* if at least one egg was found in a urine sample during

microscopic examination [2]. Egg reduction rate (ERR) calculated as the difference of the arithmetic mean egg counts were measured in pre- and post-treatment samples [3].

Reinfection: This involved microscopic evaluation of urine at baseline, 6 weeks and 2 years following treatment. Re-infection cases were defined as those that were positive for schistosomiasis at baseline and became negative on the second examination at 6 weeks reverting to positive 2 years later [4].

Praziquantel treatment: At baseline, praziquantel (40 mg/kg) [5] was administered to all children selected, regardless of infection status. At 6 weeks and 2 years after treatment, urine samples were collected from each child to assess the efficacy of the treatment. Any individual was considered cured if no egg was found in the urine sample examined.

Antibody profiles: Total and *Schistosoma*-specific antibody levels were measured at baseline and 2 years after repeated rounds of annual treatment with praziquantel.

Inflammation markers: Acute phase proteins: procalcitonin, tissue plasminogen activator, ferritin and fibrinogen were measured at baseline and 2 years after repeated rounds of annual treatment with praziquantel.

3.3 Inclusion and exclusion criteria

3.3.1 Inclusion criteria

In order to be included in the study, participants had to meet the following criteria: 1) be aged 7–13 years at recruitment, 2) had provided at least two urine and one stool sample on three consecutive days at each follow-up time point for the duration of the two year study period, 3) be negative for intestinal soil transmitted helminths and *S. mansoni*, 4) have successfully

taken the praziquantel tablets prescribed to them and, 6) parents/guardians had given consent for their children to be included in the study.

3.3.2 Exclusion criteria

Children were excluded from the study if they were outside the 7-13 age range, did not provide the required stool and urine samples, positive for intestinal soil transmitted helminths and *S. mansoni* and if their parents/guardians did not consent. Those who failed to successfully take the praziquantel tablets were also excluded from the study.

3.4 Sample collection and examination

3.4.1 Urine examination and collection

S. haematobium was determined using urine specimens collected from each child on three consecutive days. The urine samples were processed for urinary schistosomiasis using the filtration technique following Mott *et al.*, 1982 [6]. Generally, specimens were collected around noon (between approximately 1000hrs and 1300hrs), time for best results. The samples were transported to the laboratory for microscopic examination of *S. haematobium* eggs on the same day using the urine filtration method and microscopy. Briefly, 10 ml of well-mixed urine was aspirated and slowly forced through a filter membrane. The filter was removed and placed on a slide, covered with a cover slip and examined under a light microscope. The number of eggs on the entire filter was counted and recorded as the number of eggs/10 ml urine. The same procedure of urine examination was carried out at baseline, 6 weeks and 2 years post treatment. A sample with the number of eggs greater than zero in 10 ml of urine was classified as being positive *S. haematobium* infection. The prevalence was defined as the number of infected children with *S. haematobium* over the total number screened [4].

Intensity of *S. haematobium* infection was expressed as number of eggs per 10 ml (EP10ml) of urine. Infection intensities were classified into three categories: (1) light infections (<10 EP10ml), (2) moderate infections (10EP10ml<x<50EP10ml and (3) heavy infections (≥ 50 EP10ml).

As a measure for *Schistosoma*-induced pathology, haematuria was determined in urine samples collected at baseline and 2 years following treatment with 40mg/kg praziquantel. A Combur-Test (Roche Diagnostics GmbH, Mannheim, Germany) reagent strip was used to detect the presence of blood in the urine.

3.4.2 Stool collection and examination

A single stool sample was collected from each child for microscopic examination on the same day to determine *S. mansoni* and soil transmitted helminthiasis (STH) infections following the Kato Katz technique as modified by Peters *et al.*, (1980) [7]. Stool examination was carried out at baseline and all children who were positive *S. mansoni* and STH were excluded from the study.

3.4.3 Blood collection and examination

About 5mls of venous blood was collected from each participant before praziquantel treatment and at each post treatment survey. Serum was collected and used in the determination of total, *Schistosoma*- specific antibodies, acute phase proteins and IL-6.

3.4.3.1 Determination of total antibody levels

50ul 1x beads were added to a 96 well micro titer plate and washed 2x with the Bio-rad wash buffer. The standards, samples and controls were diluted (Bio-rad Antibody diluents) and 50 ul added to the wells and incubated in the dark at room temperature with shaking at 850 rpm for 1 hour. After incubating the beads, samples, standards, blank, and controls the plate was

washed three times with 100ul wash buffer. 1x detection antibodies were added to the assay plate and incubated in the dark for 30min with shaking at 850rpm. The plate was washed 3x and Streptavidin –PE was added. The assay plate was incubated for 10 min at room temperature with shaking at 850rpm. The plate was washed 3x and the beads were re-suspended for plate reading. The Bio-plex manager software was used for running the assay, data acquisition and analysis.

3.4.3.2 Specific antibody determination

A standard indirect ELISA was optimized and used to quantify the amount of antibodies IgG1, IgG4 and IgE produced directly against *S.haematobium* 13 antigen. The pre-treatment serum samples were the first to be analyzed followed by post treatment serum samples. The *S. haematobium*13 antigen was diluted with a 7.4 pH coating buffer after which 100ul of the diluted antigen was added to each well in a 96 well microtiter plate. The plates were covered with a parafilm and placed in the fridge at 4 degrees Celsius for 18 hours. The following day the plates were washed three times with Tris Buffer Saline at three minutes interval. Egg albumin solution was used to block the non-specific binding sites and the plates were allowed to shake for an hour. After an hour plates were washed three times again at three minutes interval.

The serum samples were diluted with a dilution buffer. For IgG1, 25ul of the sample were added to 225ul of the dilution buffer and for IgG4, 10ul of the sample was added to 490ul of the dilution buffer. 100ul of the diluted samples was added to the microwells in duplicate. The other six microwells were reserved for positive, negative controls and blank in duplicate. The incubation period was 2 hours. After 2 hours the plates were removed from the shaker and washed for three times again. About 10ul of the antibody solution was added to 10ml of the diluents buffer. Eventually, 100ul of the mixture was added to the ELISA plate and

incubated for an hour at room temperature. The plates were washed for six times at this stage. About 100ul of the o- Phenylendiamine Dihydrochloride (OPD) substrate was added to each well containing horse reddish peroxidase enzyme and incubated for 30 minutes in the dark. The enzyme reaction was stopped with sulphuric acid and the readings were measured at 450nm wavelength.

3.4.3.3 IL-6 determination

The analysis of IL-6 in the serum samples was done by sandwich ELISA. 100 ul of coating antibody was added into the wells of an ELISA plate. The plate was then covered with adhesive to prevent evaporation and incubated overnight at 4°C. After incubation the plate was turned over to remove the coating antibody and was washed thrice with the wash buffer. The plate was dried by blotting on a paper towel to remove residual wash solution. 200ul of 5% egg albumin was added to all the wells to block nonspecific binding sites. The plate was covered with adhesive tape and incubated for 1 hour at room temperature on a plate shaker. After incubation the plate was turned over to remove the coating antibody and was washed thrice with the wash buffer. The plate was dried by blotting on a paper towel to remove residual wash solution. 100 ul of the sample was added into wells in duplicate. The plate was covered with adhesive tape and incubated for 2 hours at room temperature on a plate shaker. After incubation the plate was turned over to remove the sample and was washed thrice with the wash buffer. The plate was dried by blotting on a paper towel to remove residual wash solution. 100 ul of biotin conjugated antibody was added into the wells. The plate was covered with adhesive tape and incubated for 1 hour at room temperature on a plate shaker. After incubation the plate was turned over to remove the detection antibody and was washed thrice with the wash buffer. The plate was dried by blotting on a paper towel to remove residual wash solution. 100 ul of Horse Radish Peroxidase (HRP) antibody was added into the wells. The plate was covered with adhesive tape and incubated for 1 hour at room temperature on a

plate shaker. After incubation the plate was turned over to remove the HRP conjugate antibody and was washed six times with the wash buffer. The washing was done thoroughly to remove all unbound HRP conjugate antibodies. The plate was dried by blotting on a paper towel to remove residual wash solution. 100 ul of OPD substrate was added to the plate and incubated for 20 minutes in the dark. The plate was covered with a foil paper to prevent light from interrupting with the reaction. After incubation 20ul of the stop solution was then added to each well. The plates OD was read at dual wavelength for cytokine analysis (405nm and 630nm) in an ELISA plate reader.

3.4.3.4 Acute Phase Proteins determination

There has been an interest in using more than one inflammatory biomarker in various parasitic diseases due to the varying half lives of the biomarkers. Parasitic infections have been found to be associated with increased serum concentrations of procalcitonin and ferritin. To the best of our knowledge, procalcitonin, ferritin, tissue plasminogen activator and fibrinogen have not been investigated in children residing in schistosomiasis endemic areas. Serum levels of ferritin, fibrinogen, tissue plasminogen activator and procalcitonin, were determined in the school children by the magnetic bead-based immuno-assays using the Bio-Plex Pro™ human acute phase 4-plex immunoassay complete commercial kits. 25ul 1x beads were added to a 96 well micro titer plate and vacuum filtration was carried out. The standards, samples and controls were diluted and 50 ul added to the wells and incubated in the dark at room temperature with shaking at 850 rpm for 1 hour. After incubating the beads, samples, standards, blank, and controls vacuum filtration was done and the plate was washed three times with 100ul wash buffer. 25ul of 1x detection antibodies were added to the assay plate and incubated in the dark for 30min with shaking at 850rpm. The plate was washed 3x and Streptavidin –PE was added. The assay plate was incubated for 10 min at room

temperature with shaking at 850rpm. Vacuum filtration was done and the plate was washed 3x and the beads were re-suspended in assay buffer prior to reading the plate. The Bio-plex manager software was used for running the assay, data acquisition and analysis.

3.5 Statistical analysis

Data was entered into the computer using Microsoft Excel spread sheet and exported to SPSS for windows version 16.0 (SPSS Inc., Chicago, Illinois, USA). The Pearson chi-square test was used to determine the association between the prevalence of *S. haematobium* infections, arithmetic egg counts and age orgender. Student t-test was used to compare differences in the prevalence and intensity of infection (eggs/10ml urine) before and after treatment. A value of $p < 0.05$ was considered as statistically significant.

To determine if there was a significant difference between pre-and post-treatment of the total immunoglobulin as well as the specific antibody profiles a comparison of means using a paired t-test was conducted. The hypothesis that there were no differences between pre-treatment and 2 years post treatment antibody profiles was tested. P value was set at $p < 0.05$.

Levels of serum levels of ferritin, fibrinogen, tissue plasminogen activator, procalcitonin and IL-6 at baseline and 2 years post-treatment were compared using student t test to determine the effect of praziquantel treatment on acute phase proteins and IL-6. The Mann–Whitney non parametric test was used to determine the effect of *S. haematobium* infection on the levels of ferritin, fibrinogen, tissue plasminogen activator, procalcitonin and IL-6. A value of $p < 0.05$ was considered to indicate a significant difference in statistical analyses.

3.6 Ethical Approval

Ethical approval and clearance of the study was obtained from the Biomedical Research Ethics Committee (BREC – UKZN), approval code, BE 467/16 (appendice 7.2) as well as the

Medical Research Council of Zimbabwe (MRCZ), approval code, MRCZ/A/1958 (appendice 7.1). The aims, objectives and procedures of the study were explained to the parents/guardians of the recruited children in the local language (Shona), when they were invited to participate. Written informed consent was obtained from all the guardians of the school children in Shona (appendice 7.4) and English (appendice 7.5). Participation was voluntary and the parents/guardians had the right to withdraw their child/children at any time point from the study. Treatment was administered by trained medical personnel and the children were closely monitored. Written consent to transport participants' specimens to UKZN for antibody profiles and acute phase protein determination was also obtained from parents/guardians (appendice 7.3).

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CHAPTER 4: Benefits of Annual Chemotherapeutic Control of Schistosomiasis on the Development of protective Immunity

The first three chapters presented the background information in the area of study as well as the purpose carrying out this research (**Chapter 1**) and the current and previous findings from other researchers in the field (**Chapter 2**). In the third chapter, a comprehensive account of the investigations to be carried out in order to meet all the objectives was outlined.

Several studies have investigated the efficacy of praziquantel in treatment of schistosomiasis, however scaling of repeated mass treatment campaigns has recently been adopted in Zimbabwe especially in the area under study. Reinfection with *S. haematobium* is common in individuals given a single dose, more so in moderate to high transmission areas. Children residing in the same area and exposed to the same *Schistosoma*-infested waters are infected and reinfected with *S. haematobium* differently which points towards, though not limited to, host immunity and genetic factors. We established the relationship between the humoral responses and the infection status and determined whether protective or blocking antibodies are responsible for the observed differences. The impact of repeated treatment on schistosomiasis prevalence, reinfection rates and more importantly on protective and blocking antibodies was determined.

This chapter focused on the first two objectives and combined the effect of repeated mass treatment with praziquantel on prevalence, intensity of infection and reinfection rates. It then analysed the antibody profiles in *S. haematobium* infected and uninfected individuals in order to determine if any antibody isotypes protect or predispose an individual to infection as well as to ascertain the effect of repeated treatment on the antibody profiles.

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Benefits of Annual Chemotherapeutic Control of Schistosomiasis on the Development of protective Immunity

Tawanda Chisango¹, Arthur Vengesai², Agness Farai Nhidza¹, Bongiwe Ndlovu¹, Danai Zhou³, Edson Sibanda⁴, Takafira Mduluza^{1,2}.

¹School of Laboratory Medicine and Medical Sciences, College of Health Sciences, University of KwaZulu-Natal; KwaZulu-Natal South Africa

²Biochemistry Department, University of Zimbabwe, Harare, Zimbabwe

³Medical Laboratory Sciences, College of Health Sciences, University of Zimbabwe, Harare, Zimbabwe

⁴Scientific and Industrial Research and Development Centre, 1574 Alpes Road, Box 6640, Harare, Zimbabwe

Corresponding author: Tawanda J Chisango. Email address: chisangotawanda@yahoo.com.

4.1: Abstract

Background: Schistosomiasis is a devastating parasitic disease. The mainstay of schistosomiasis control is by praziquantel treatment. The study aimed to determine benefits of annual chemotherapy of schistosomiasis on development of protective immunity in school children in a selected endemic rural area in Zimbabwe.

Methods: Urine specimens from 212 school children (7-13 years) were collected and examined to determine prevalence, intensity and reinfection of *S. haematobium* at baseline, 6 weeks and 2 years following praziquantel treatment. Blood samples from the participants were assayed for total and *S. haematobium* (*Sh13*)-specific antibodies before and 2 years after annual treatment.

Results: Annual treatment reduced the prevalence of *S. haematobium* infection ($p < 0.05$) from 23.1% at baseline to 0.47 % after 2 years. Overall cure rate was 97.8 %. Intensity of infection declined ($p < 0.05$) from 15.9 eggs/10ml urine at baseline to 2 eggs/10ml urine. After two years, overall rate of reinfection was 0.96 %. At baseline, total IgG4 was higher in *S. haematobium*-infected children ($p = 0.042$), while all other immunoglobulins were within normal ranges. There was an increase in total IgG2 ($p = 0.044$) levels and a decrease in total IgG4 ($p = 0.031$) levels 2 years post-treatment; and no significant changes in other total immunoglobulins. *Schistosoma*-positive children at baseline showed an increase in anti-*S. haematobium* 13 IgG1 ($p = 0.005$) and a decrease in *S. haematobium* 13 IgG4 levels ($p = 0.012$) following treatment.

Conclusion: Annual praziquantel treatment delivered to school children over 2 years significantly reduces prevalence, intensity of infection and reinfection of *S. haematobium* infection. Treatment was also observed to cause a reduction in *schistosoma*-specific blocking IgG4 and an increase in *Schistosoma*-specific protecting IgG1.

4.2 Introduction

Schistosomiasis is a devastating neglected tropical disease (NTD) that begins when infective cercariae from freshwater snails pierce the skin as a result of exposure to infested water [1]. Such a scenario is typical in resource limited settings with poor sewage disposal and inadequate supply of clean water. It is estimated that over 250 million people are infected with schistosomiasis, worldwide. Approximately 93% of the infected people reside in sub-Saharan Africa [2], where school children carry the heaviest burden and account for the highest prevalence and intensity of schistosome infections [3]. Children are mainly affected because of their higher rates of water related activities, immunological status and anatomical vasculature [4, 5]. Such young children suffer from haematuria, dysuria, nutritional deficiencies, anaemia, growth retardation, decreased physical performance and impaired memory and cognition as a result of extreme schistosome infections [6, 7].

The Ministry of Health and Child Care in Zimbabwe included schistosomiasis in the 2009-2013 National Health Strategy in 2009 underlining the importance and the urgent need to control the disease. A nationwide schistosomiasis survey was conducted in Zimbabwe and the overall prevalence of *S. haematobium* was reported as 18.0% while that of *S. mansoni* was 7.2%. Manicaland region, where this study was conducted has a moderate burden of the parasitic disease at 23.8% prevalence of schistosomiasis [8].

Chemotherapeutic control using praziquantel is aimed particularly at school-age children living in schistosomiasis-endemic areas. The treatment and control measure for schistosomiasis has been recommended by the World Health Organization (WHO) [9] as an interim available control strategy, since no vaccines are as yet available. Praziquantel is a pyrazino-quinoline derivative and has gained widespread usage because it is easy to administer, safe, well tolerated, cheap, can reverse schistosome-related morbidity and is

highly effective against the six schistosome species that infect humans [10, 11]. Mass drug administration using the anti-helminthic drug praziquantel has been the major focus of recent control efforts [12], with the principal aim of reducing morbidity and clearing sources of recontaminating the environment. Scaling up of mass drug administration has been proposed in the WHO's strategic plan of 2012 as a way of managing schistosomiasis morbidity by 2020 [13]. Schools have been targeted for mass treatments because of the increased benefits of reducing infection burdens in children compared to adults and the simplicity of providing treatment [14]. Many studies have shown that praziquantel drastically reduces morbidity and transmission of schistosomiasis especially in low-to-moderate transmission areas where the risk of reinfection is generally low [15 - 17] despite a few reports of treatment failures [18, 19].

Although praziquantel is efficient in mitigating or eradicating active infection, not everyone is cured. As a result, rapid reinfections have been reported and remain a challenge in communities where it is unavoidable to get in contact with water infested with snails carrying the *S. haematobium* cercariae [20]. Treatment using praziquantel must be repeatedly administered for an indefinite time period in order to maintain low reinfection levels [21], however the long-term effects on infection dynamic and immune status are not yet fully understood. In our current study, repeated annual rounds of praziquantel were administered to children attending Bandanyenje primary school. Although the overall prevalence and distribution of schistosomiasis have been reported in the province, less emphasis has been given to the effect of repeated rounds of praziquantel and the subsequent prevalence, intensity of infection and re-infection rates in Bandanyenje. Information on the efficacy of praziquantel and the infection rates may help in evaluating policies and strategies that guide schistosomiasis control activities in the district. Thus, we assessed the effect of annual treatment with praziquantel over 2 years on the prevalence and reinfection rates in school

children attending Bandanyenje primary school in Manicaland province of Zimbabwe. The intensity of *Schistosoma* infection, using the major symptom, haematuria, as an indicator was also evaluated.

Several studies have reported the chemotherapy-induced changes in responses to *Schistosomal* infection [13, 22, 23]. Frequent treatments are thought to enhance the development of protective immune responses. Studies of the effects of chemotherapy on other helminths indicate that the drug treatment can facilitate the development of resistance to reinfection through enhanced production of protective antibodies and decline in blocking antibodies. Key information on the humoral immune status of individuals can be determined routinely by measuring total immunoglobulin levels. Several studies have realized the association between total immunoglobulins in chronic diseases associated with inflammation such as diabetes [24, 25]. Very little has been done on the association between the levels of total immunoglobulins and schistosomiasis, which is also a chronic disease that is associated with inflammation. Indeed treatment of schistosomiasis has shown to be associated with subsequent changes in humoral and cellular immune responses although focus has been mainly on *Schistosoma*- specific antibodies [22]. Analysis of such changes in the context of both total and *Schistosoma*-specific antibodies will assist in interpreting the differences that occur in children living in endemic areas as well as improving current understanding of the development of acquired immunity to schistosome infections and vaccine development. In order to establish the relationships between total serum immunoglobulins and *S.haematobium*-specific antibodies and to better understand their turnover after praziquantel treatment we quantitatively measured the total immunoglobulins and specific antibody profiles in school children before and after two annual rounds of treatment using praziquantel in order to determine benefits of annual chemotherapy of schistosomiasis on development of protective immunity in the school children.

4.3 Methodology

Study area and population

The study was carried out in Bandanyenje Primary School located in a schistosomiasis-endemic area in the Manicaland Province of Zimbabwe. The school is approximately 217 km South-East of Harare with latitude and longitude of 7°1'N 38°35'E. Villagers living in Bandanyenje community depend on perennial rivers as their water source thus indicating an increased likely exposure of the majority of their population to infection. The study population included a random sample of 212 (105 boys and 107 girls) aged between 7–13 years who had provided at least two urine and one stool sample on three consecutive days at each follow-up time point for the duration of the two year study period. All school children who successfully took praziquantel tablets prescribed to them were included in the study and those school children outside the 7-13 age range, did not provide the required stool and urine samples and failed to successfully take the praziquantel tablets were excluded from the study.

Parasitology and blood sampling

A school-based longitudinal intervention study was conducted and involved examination and treatment of the study population at baseline, 6 weeks and at 2 year follow up surveys. Stool and urine samples were collected at baseline and at follow up, for each examination time-point over three consecutive days. The urine samples were processed for urinary schistosomiasis using the filtration technique following Mott *et al.*, 1982 [26] methods. The stool samples were processed following the Kato Katz technique by Peters *et al.*, (1980) [27]. A sample with the number of eggs greater than zero in 10 ml of urine was classified as being *S. haematobium*-positive. Those found to be infected with *S. mansoni* were excluded from the study. The prevalence was defined as the number of infected children with *S. haematobium* over the total number screened. Intensity of *S. haematobium* infection was expressed as

number of eggs per 10 ml (ep10ml) of urine. Infection intensities were classified into three categories: (1) light infections (<10 ep10ml), (2) moderate infections (10ep10ml<x<50ep10ml and (3) heavy infections (≥ 50 ep10ml). Blood samples were obtained from the children, the serum separated and used to determine total immunoglobulins and antibody profiles against *S. haematobium*13.

Determination of total antibody profiles

About 50 μ l 1x beads were added to a 96 well micro titer plate and washed 2x with the Bio-Rad wash buffer. The standards, samples and controls were diluted (Bio-Rad Antibody diluent and 50 μ l added to the wells and incubated in the dark at room temperature with shaking at 850 rpm for 1 hour. After incubating the beads, samples, standards, blank, and controls the plate was washed three times with 100 μ l wash buffer. 1x detection antibodies were added to the assay plate and incubated in the dark for 30min with shaking at 850rpm. The plate was washed 3x and Streptavidin –PE was added. The assay plate was incubated for 10 min at room temperature with shaking at 850rpm. The plate was washed 3x and the beads were re-suspended for plate reading. The Bio-plex manager software was used running the assay, data acquisition and analysis.

Determination of *S. haematobium* (Sh13) specific antibodies

A standard indirect ELISA was optimized and used to quantify the amount of antibodies IgG1, IgG4 and IgE produced directly against *S. haematobium* 13 antigen. Both pre- and post – treatment samples were analyzed followed on the same plate. Briefly, the plates were coated with *S. haematobium*13 antigen at 5ng/ml over night at 4°C. Diluted serum was added at 1:10 for IgG4, 1:50 for IgG1 and IgE, dilutions. Monoclonal anti-human IgG conjugated to peroxidase was used to detect the presence of anti-*S. haematobium* 13 IgG1, IgG4 and IgE

antibodies. Six microwells were reserved for positive, negative controls and background blank containing myelin basic protein (MBP) in duplicate. This was due to the *S. haematobium* 13 suspension contain MBP. About 100 µl of the o-Phenylenediamine Dihydrochloride (OPD) substrate was added to each well containing horse reddish peroxidase (HRP) enzyme and incubated for 30 minutes in the dark. The enzyme reaction was stopped with sulphuric acid and the absorbance was read at 450nm with 630nm as reference wavelength.

Haematuria

As a measure for *Schistosoma*-induced pathology, haematuria was determined in urine samples collected at baseline and 2 years following treatment with 40mg/kg praziquantel. A Combur-Test (Roche Diagnostics GmbH, Mannheim, Germany) reagent strip was used to detect the presence of blood in the urine.

Treatment

Treatment was carried out by qualified health teams of trained nurses and medical officers as part of the treatment campaign. All school children were treated with a single dose of 40mg/kg praziquantel at baseline, and thereafter without considering the infection status after 1 and 2 years.

Statistical analysis

Data was entered into the computer using Microsoft Excel spread sheet and exported to SPSS for windows version 16.0 (SPSS Inc., Chicago, Illinois, USA). The Pearson chi-square test was used to determine the association between the prevalence of infections, arithmetic egg counts with age or gender. Student t-test was used to compare differences in the prevalence and intensity of infection (eggs/10ml urine) before and after praziquantel treatment. A value of $p < 0.05$ was considered as statistically significant. To determine if there was a significant difference between pre- and post-treatment of the total immunoglobulin as well as the specific

antibody profiles a comparison of means using a paired t-test was conducted. The hypothesis that there were no differences between pre-treatment and 2 years post treatment antibody profiles was tested. P value set at $p < 0.05$.

Ethical Approval

Ethical approval and clearance of the study was obtained from the Medical Research Council of Zimbabwe (approval code, MRCZ/A 1958) and the Biomedical Research Ethics Committee (approval code, BE467/16) of UKZN. The aims, objectives and procedures of the study were explained to the parents/guardians of the recruited children in the local language (Shona), when they were invited to participate. Written informed consent was obtained from all the guardians of the school children. Participation was voluntary and the parents/guardians had the right to withdraw their child/children at any time point from the study. Treatment was administered by trained medical personnel and the children were closely monitored.

4.4 Results

Baseline prevalence, intensity and reinfection

Of the 233 school children from Bandanyenje primary school who volunteered and provided urine and stool samples, *S. mansoni* was diagnosed in (9%) 21/233 and Soil Transmitted Helminths in 0.4% (1/233) and these children were not included in the study. The remaining 212 (105 boys and 107 girls) aged between 7-13 were recruited in the study and were successfully traced and re-examined at both follow-ups with complete sets of longitudinal parasitological data on *S. haematobium* infection. The overall pre-treatment prevalence of *S. haematobium* infection was 49(23.1 %) with 20(19%) boys and 29(27.2%) girls as determined using the urine filtration technique. The age group of 10-13 years as well as the girls had the highest prevalence 25.6% and 27.1 %, respectively. However the differences in infection status between age groups ($\chi^2=0.158$, $p=0.691$) and boys and girls ($\chi^2=1.891$,

p=0.169) were not significant (**Table 4.1**). The majority of infected children in the both age groups had light infections with more heavy infections being observed in the 7-9 age (10.5%) group than the 6.7% in the 10-13 age group. However the intensity of infection had no age group ($\chi^2 = 2.594$, p=0.273) or sex-related pattern ($\chi^2 = 0.297$, p=0.862). The proportion of boys (10%) that had a heavy infection was greater than girls (6.9%) though not statistically significant (**Table 4.1**).

There was an overall decline in *S. haematobium* prevalence from 23.1% at baseline to 0.47% at 6 weeks post-treatment and, 0.47% 2 years post-treatment (p< 0.05). A total of 211(99.5%) children were egg negative after treatment. The *S. haematobium* prevalence significantly decreased to 0.47% (p<0.05%) following 2 years of 3 annual rounds of praziquantel treatment. The overall Cure Rate (CR) was 97.8 % and Average Egg Mean Count (AEMC) was 15.9 egg/10ml urine at baseline and reduced to 2 egg/10ml urine 2 years post-treatment (**Table 4.2**). The study of reinfection involved the 211 children who were cured 6 weeks after the first praziquantel treatment. Of these *S. haematobium* negative, only 1 boy (0.96%) contracted the infection 2 years after the 6 week treatment (**Table 4.3**).

Haematuria

Haematuria was detected in 32(15.1%) of the study participants of which 23(71.9%) were *S. haematobium* positive. The presence of *S. haematobium* eggs showed statistically significant association ($\chi^2 = 20.38$, p<0.05) with haematuria whilst there was no association between sex and haematuria ($\chi^2 = 0.08$, p=0.929). At 2 years post treatment, the extent of haematuria decreased from 100% to 0% (**Table 4.4**).

Total and *S. haematobium* specific antibodies

Baseline levels of total IgG4 were significantly higher in *S. haematobium* positive compared to *S. haematobium* negative children (**Figure 4.1**). At 2 years after treatment, Serum IgG2 increased and serum IgG4 decreased after treatment with praziquantel ($p= 0.044$ and 0.031 , respectively). There were no significant changes in total serum IgG1, IgG3, IgM and IgA. Levels of *S. haematobium* IgG4 were significantly higher in *S. haematobium* positive when compared to *S. haematobium* negative ($p<0.05$). Following chemotherapy, significant decreases of *S. haematobium* IgG4 were observed. Specific *S. haematobium*-IgE levels before treatment were higher than the *S. haematobium* IgE levels though no significant changes were noted before and after treatment in both *S. haematobium* uninfected and infected children (**Figure 4.2**). The protective *S. haematobium* IgG1 increased significantly in *S. haematobium* infected children following treatment.

4.5 Discussion

At baseline survey, the prevalence of *S. haematobium* infection in the study area was observed to be low at 23.1% (**Table 1**) compared to other areas in Zimbabwe [8]. However, the prevalence was within the range of the average prevalence within the province (Manicaland, 23.8%) as determined by a nationwide survey that was conducted by Midzi *et al* (2014) [8]. The low prevalence of *S. haematobium* reported in this study could reflect the on-going control efforts in the area e.g. elimination of intermediate host snails and health education. Lately, constant awareness campaigns advocated during the annual mass drug administration exercise compounded by improved use of technology in rural areas have been part of efforts by the Ministry of Health to control schistosomiasis which had been neglected for a long time. This could have resulted in decreased contact with infested waters by children in the area. There is a need to maintain the low reinfection levels to prevent the

development of morbidities associated with high *S. haematobium* prevalence. The WHO guidelines recommend treatment with praziquantel once every 2 years in school children and high risk groups in communities with a schistosomiasis prevalence of 10% to 50%. The same groups should be given praziquantel once annually in communities where prevalence is 50% and above [28].

No association was found in the prevalence between boys (19%) and girls (27.1%), as well as between the different ages in this study (**Table 4.1**). This pattern of infection may be an indication of equal exposure of both genders and the ages examined in the study due to similar water contact activities. The findings are consistent with those found in a similar study in Ethiopia [29]. Other studies have shown either boys or girls to be significantly more affected. Differences in prevalence between boys and girls arise due to cultural, behavioural and social factors within an area with the most affected sex being the one having more water contact activities [30].

Infection intensity is a better indicator of morbidity associated with schistosomiasis than prevalence as it reflects the number of worms infecting the individual and it is also a more reliable marker of treatment success defined as the removal of egg-laying worms [31, 32]. The baseline intensity of infection in this study was moderate (**Table 4.2**). The baseline infection intensity in our study indicated low infection levels comparable with findings from similar studies in Niger [16, 33].

Praziquantel remains the drug of choice for the treatment of schistosomiasis in spite of cases of low cure rates that have been reported in some areas [34]. We observed a significant decline in prevalence after the first dose at 6 weeks exhibiting a satisfactory efficacy with praziquantel after the first dose (**Table 4.2**). In this study, our results demonstrated a significant impact of praziquantel in treatment of *S. haematobium* with cure rates of 97.8%

($p < 0.05\%$) following 2 years of repeated annual rounds of praziquantel treatment. This is comparable to findings of similar studies that have reported a higher efficacy of praziquantel when administered as two or three treatments spaced at certain time intervals [14, 35]. The pronounced cure rates where 100% cure rate is achieved after 2 years could be due to the study period and the infection prevalence of the area as stipulated by the WHO, baseline infection intensity, brand of praziquantel used, geographical location/variation, repeated rounds with enhanced killing of schistosomula and the method of laboratory diagnosis employed, ie microscopy with egg determination does not rule out presence of the worm.

Total prevalence of re-infection 2 years post-treatment was 0.96% (**Table 4.3**). It has been hypothesized that, after repeated rounds of infections and praziquantel chemotherapy, humans slowly acquire protective immunity to *S. haematobium* leading to partial resistance to re-infection [36]. Treatment with praziquantel boosts adult worm immunoglobulin E (IgE) antibodies which are associated with resistance to re-infection [17]. In contrast to our findings, other studies observed a rapid and high re-infection rate a few weeks following treatment especially in high transmission areas [37]. Presence of haematuria before treatment was correlated with the presence of *S. haematobium* eggs in urine. In response to treatment with single dose of praziquantel, haematuria fell from 100% to 0% at 2 years post-treatment (**Table 4.4**). Most studies measure haematuria within weeks or months whereas in our study haematuria was measured after two year following 3 annual rounds of treatment. Studies in Ghana and Kenya have also reported significant decreases in haematuria after praziquantel treatment [38, 39].

Our findings suggest that children at Bandanyenje primary school are at moderate risk of the morbidity caused by the moderate *S. Haematobium* prevalence according to WHO threshold and we recommend a biennial mass drug administration with praziquantel. There is mounting

evidence that anti-helminthic treatment using praziquantel not only transiently reduces infection, but also has longer term benefits in terms of morbidity control and the development of parasite-specific immune responses associated with resistance to reinfection [18, 40]. It is widely accepted that the changes in *Schistosoma*-specific immune response occur following treatment of schistosomiasis with praziquantel [7, 41]. Praziquantel penetrates the tegument of worm tissues and rapidly moves through damaging the tegument and causing paralysis of the worm [42]. The increase in antigens released from dying worms as a result of praziquantel induced-tegument damage is believed to trigger this change in both the cellular and humoral immune responses [43].

In this study following chemotherapy there were changes in both the total and *S. haematobium*-specific antibodies. There was a significant increase in total IgG2, total and *S. haematobium*-specific IgG1 and a significant decline in total and *S. haematobium*-specific IgG4 while there were no significant changes in all the other antibodies (**Figure 4.1 and Figure 4.2**). Studies done in individuals with chronic schistosomiasis have revealed elevated levels of total immunoglobulins [44]. We observed an increase in both the total and specific IgG1 which was maintained over the 2 years, which suggest that IgG1 could have been stimulated by antigens produced from the worms after chemotherapy. Khalife *et al.*, (1989) [45] demonstrated the eosinophil-dependent killing of schistosomula as a result of IgG1. This could explain the significant increase of IgG1 following treatment with praziquantel and the significant clearance of infection at 6 weeks and thereafter 2 years later. The release of sub-surface antigens and a decline in egg counts following treatment result in stimulation of IgG1 production. A similar increase in specific IgG1 following chemotherapy was noted by Mutapi *et al* (1998) [46]. They observed an increase in IgG1 following treatment with praziquantel, which they attributed to a switch from IgA specific antibodies to IgG1 response

that occurs in children. The potential mechanism for this switch is not fully understood, but it is believed that changes in cytokine levels in response to antigen release from damaged parasites may cause this isotype switch from IgA to IgG1 [23]. The switch occurs naturally as worms die but takes a long time as a result of life span of schistosomes. The switch is accelerated by the praziquantel treatment which actually causes partial protection to reinfection as seen by the 0.96 % reinfection rate 2 years post treatment noted in this study.

Studies have demonstrated the protective role played by IgE, which surprisingly in this study showed no significant change in both the infected and non-infected group at baseline.

Following treatment there were no significant changes ($p < 0.05$) in IgE in both *S.*

haematobium infected and uninfected (**Figure 4.1**), though the levels remained higher than blocking IgG4 levels that decreased significantly following chemotherapy. Similar trends though in *S. mansoni* were observed by Walter *et al.*, (2006) [47]. They demonstrated that following treatment, adult worm-specific IgG4 levels decreased, while worm-specific IgE are maintained at pre-treatment levels or increases even in children, who more readily become reinfected, treatment is less likely to increase the IgE/IgG4 ratio. There is need therefore to carry out further studies especially taking into account cytokine levels which are key to facilitating the production of both the total and *S. haematobium*-specific antibodies.

In our study *S. haematobium* uninfected children had significantly lower levels ($p < 0.05$) of the blocking antibody IgG4 at baseline than the infected children, this was observed for both total and *S. haematobium*-specific IgG4 (**Figure 4.1 and Figure 4.2**). Blocking antibodies like IgG4 which have been observed to develop early in life have been shown to predispose children to infection. Studies on *Schistosoma*-infected populations have reported that anti-*Schistosoma* IgG4 levels in infected children are associated with higher parasite burdens and parasite susceptibility [48, 49]. IgG4 is known to interfere with IgE-induced mast cell and eosinophil degranulation through preventing the binding of IgE to the effector cells thereby

preventing killing of schistosomula [45], IgG4 will also block IgG1 and IgG3 mediated killing of schistosomula by human eosinophils *in vitro* [36]. Elevated IgG4 levels observed in *S. haematobium* infection compared to uninfected children increase predisposition to infection. Following treatment there was a significant decrease ($p < 0.05$) in IgG4 highlighting the importance of praziquantel in lowering IgG4 which then results in a significant decline in infection at 6 weeks and 2 year post treatment. There is need therefore to carry out further studies especially taking into account cytokine levels which are key to facilitating the production of both the total and *S. haematobium*-specific antibodies.

4.6 Conclusion

This study confirms findings from previous work by other research groups that treatment reduces *S. haematobium* egg burden and alters immune responses. There was a significant reduction in prevalence, intensity to infection and reinfection. Since the study area is a moderate zone of transmission without any specific current control program, treatment with praziquantel once every 2 years may keep the infection at low level of transmission. Two years after praziquantel treatment significant changes occur in total and specific IgG1 as well as total and specific IgG4 changes. These findings suggest consequences of praziquantel long term effects in changing the overall protective immunity of these school children and benefits of the repeated mass drug administration.

Competing Interests:

The authors declare that they have no competing interests.

Authors' contributions

TJC, BN& TM developed the field study design, immunoassays & analyzed the data. FM generated the *S. haematobium* 13. AV, AFN, EPS, DZ & TM conducted field and sampling

work. TJC, AV, AFN& BM conducted the laboratory assays. TJC and TM conducted the initial statistical analyses.

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Tables Legend

Table 4.1: Baseline prevalence and intensity of *S. haematobium* infection.

Table 4.2: Prevalence, cure rate and Average Egg Mean Count at baseline, 6 weeks and 2 years following treatment with praziquantel.

Table 4.3: Re-infection cases of *S. haematobium* post-praziquantel treatment.

Table 4. 4: Occurrence of haematuria at baseline and 2 years after praziquantel treatment.

Figures Legend

Figure 4.1: Concentration of total antibodies at baseline and 2 years post-praziquantel treatment.

Figure 4.2: Mean OD values of *S. haematobium* specific antibodies at baseline and 2 years post praziquantel treatment.

Table 4.1: Baseline *S. haematobium* infection characteristics, prevalence and intensity of infection.

Parameter	<i>S. haematobium</i> Infected	<i>S. haematobium</i> Uninfected	χ^2	P value	Light Infection	Moderate Infection	Heavy Infection	χ^2	P value
Sex			1.891	0.169				0.297	0.862
Boys	20(19%)	85(81%)			11(55%)	7(35%)	2(10%)		
Girls	29(27.1%)	78(72.9%)			20(69%)	7(24.1%)	2(6.9%)		
Age Group			0.158	0.691				2.594	0.273
7-9	19(20%)	76(80%)			13(68.4)	4(21.1%)	2(10.5%)		
10-13	30(25.6%)	87(74.4%)			18(60%)	10(33.3%)	2(6.7%)		

Table 4.2: *S. haematobium* infection prevalence, cure rate and Average Mean Egg Count at baseline, 6 weeks and 2 years following treatment with praziquantel.

Variable	Baseline	6 weeks post treatment	2 years post treatment
Prevalence	49(23.1%)	1(0.47%)	1(0.47%)
Cure rate		98%	97.8%
Boys	20(19%)	1(0.47%)	1(0.47%)
Cure rate		95%	94.7%
Girls	29(27.1%)	0	0
Cure rate		100%	100%
7-9	19(20%)	1(0.47%)	1(0.47%)
Cure rate		94.7%	94.4%
10-13	30(25.6%)	0	0
Cure rate		100%	100%
Average Mean Egg Counteggs/ 10ml urine	15.9	86.7	2

Table 4.3: Re-infection cases of *S. haematobium* post-praziquantel treatment.

	<i>S. haematobium</i> infected	<i>S. haematobium</i> uninfected	Reinfection Cases
6 weeks			
Boys	1(0.95%)	104(99.05%)	-
Girls	0(0%)	107(100%)	-
2 years			
Boys	1(0.95%)	103(99.03%)	1(0.96%)
Girls	0(0%)	107(100%)	0(0%)

Table 4.4: Occurrence of haematuria at baseline and 2 years after praziquantel treatment.

	Before Treatment		2 years Post treatment	
	<i>S. haematobium</i> Infected	<i>S. haematobium</i> Uninfected	<i>S. haematobium</i> Infected	<i>S. haematobium</i> Uninfected
Boys	9(45%)	5(5.9%)	0	0
Girls	14(48.3%)	4(5.1%)	0	0
7-9	8(42.1%)	3(3.9%)	0	0
10-13	15(50%)	6(6.9%)	0	0

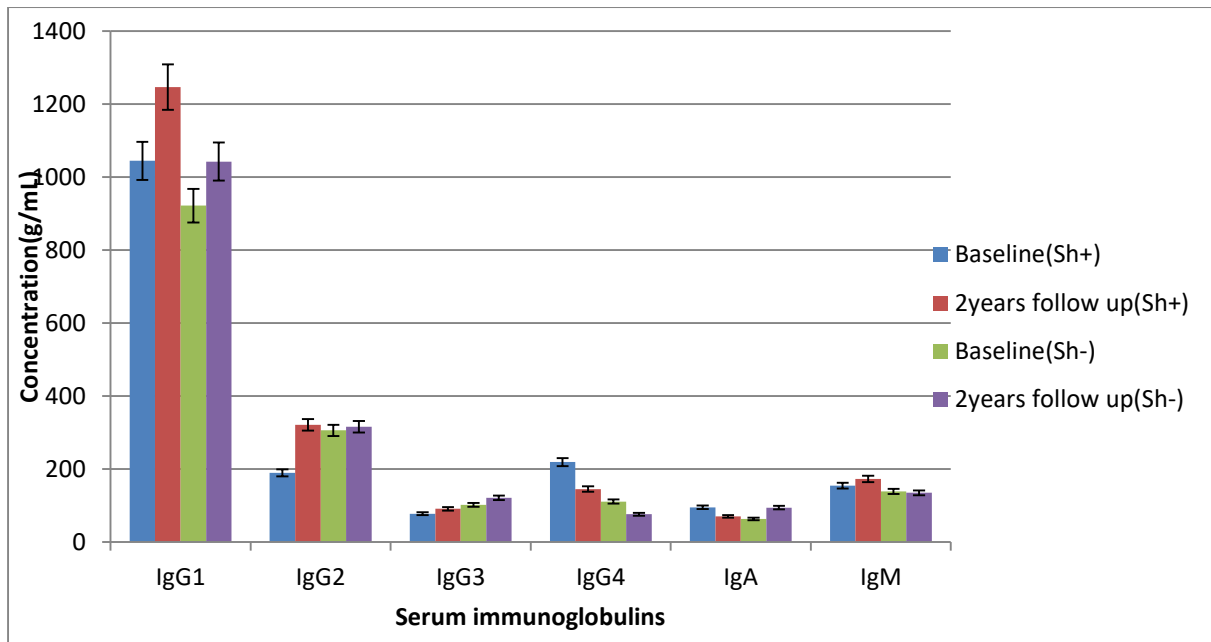


Figure 4.1: Profiles of total antibodies at baseline and 2 years post-praziquantel treatment

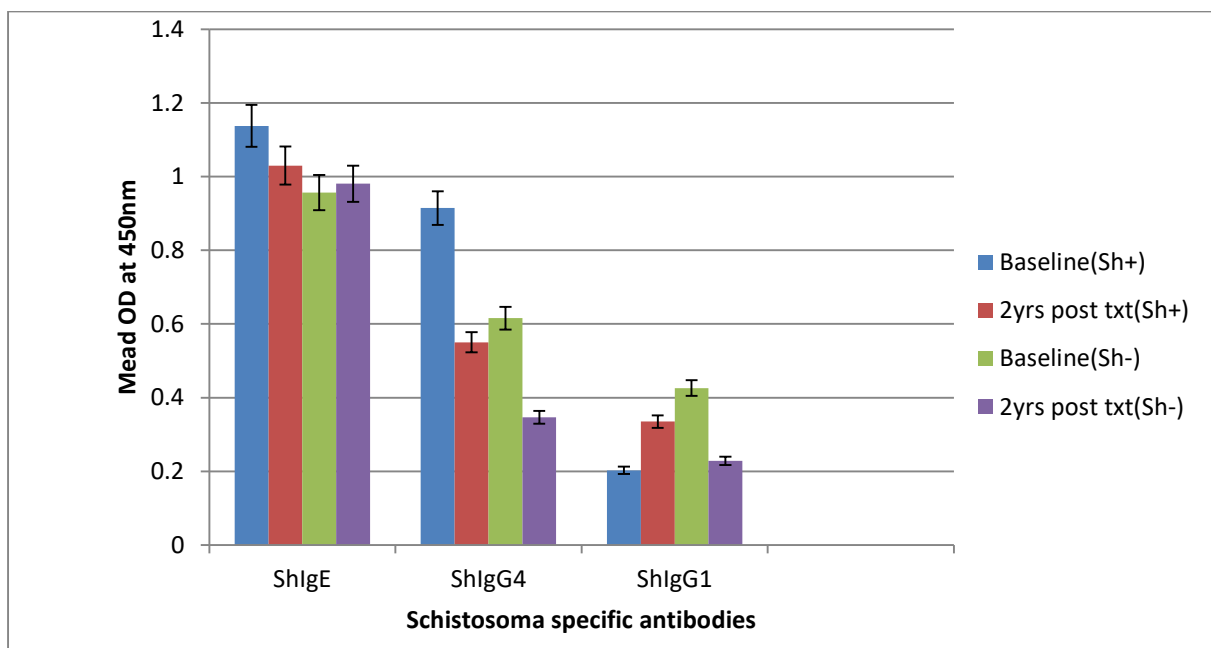


Figure4.2: Profiles of *S. haematobium* specific antibodies at baseline and 2 years post praziquantel treatment

CHAPTER 5: *S. haematobium* infection and Chemotherapy-induced changes in IL-6 and acute phase proteins associated with inflammation in school children in a Schistosomiasis- endemic area

After having identified key research gaps with respect to lack of reliable inflammation markers associated with schistosomiasis, this Chapter explored acute phase markers that had not yet been investigated to the best of our knowledge. Chronic stimulation of inflammation is common in children continuously exposed to *Schistosoma*-infested waters as a result of the increase in tissue entrapped eggs that are released. The increase in stimuli that elevates inflammation is known to increase production of acute phase proteins. The level of IL-6, the chief stimulator of production of acute phase proteins was measured before and after treatment with praziquantel to establish the effect of repeated treatment on inflammation.

We also established if there is a link between the information gathered from the preceding chapter on antibody profiles and inflammation markers. We investigated whether individuals who are protected against *S. haematobium* by virtue of elevated protective antibodies are also protected against inflammation and also established if those individuals prone to reinfection have elevated inflammatory markers which would be expected as they are continuously under attack by *S. haematobium*.

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Title: *S. haematobium* infection and Chemotherapy-induced changes in IL-6 and acute phase proteins associated with inflammation in school children in a Schistosomiasis-endemic area

Tawanda Chisango¹, Bongiwe Ndlovu¹, Arthur Vengesai², Agness Farai Nhidza¹, Danai Zhou³, Edson Sibanda⁴, Takafira Mduluza^{1,2}.

¹School of Laboratory Medicine and Medical Sciences, College of Health Sciences, University of KwaZulu-Natal; KwaZulu-Natal South Africa

²Biochemistry Department, University of Zimbabwe, Harare, Zimbabwe

³Medical Laboratory Sciences, College of Health Sciences, University of Zimbabwe, Harare, Zimbabwe

⁴Scientific and Industrial Research and Development Centre, 1574 Alpes Road, Box 6640, Harare, Zimbabwe

Corresponding author: Tawanda J Chisango. Email address: chisangotawanda@yahoo.com.

5.1 Abstract

Background: Acute phase proteins are plasma proteins whose concentration increases or decreases as a result of infection or inflammation and their production is stimulated by IL-6. Their circulating concentrations in the body reflect the extent of inflammation. There is an increased risk of cases of direct and indirect morbidities as a result of stimulation of tissue-destructive inflammatory caused by *S. haematobium* infection, hence the need to determine the levels of inflammatory markers in *S. haematobium* infected children and also determine the effect of repeated annual mass treatment on levels of IL-6 and acute phase proteins.

Methodology: Urine specimens from 212 school children were collected and examined to determine prevalence of *S. haematobium* at baseline and 2 years following annual rounds of praziquantel treatment. Levels of 4 acute phase proteins were measured from serum samples from the participants using the magnetic bead-based immuno-assays at baseline and 2 years following praziquantel treatment. Sandwich ELISA was used to determine levels of IL-6.

Results: The overall pre-treatment prevalence of *S. haematobium* infection was 23.1 % at baseline and 0.47 % after 2 years of annual treatments. *S. haematobium* infected children had marginally higher levels of procalcitonin and tissue plasminogen activator before treatment though the difference of all three was not significant $p > 0.05$ using Mann-Whitney non-parametric U test. Levels of ferritin and fibrinogen were lower in *S. haematobium* infected children before treatment, however the difference was also not significant $p > 0.05$ using Mann-Whitney U test. There was no association between infection status or IL-6 and the levels acute phase proteins $p > 0.05$ for all acute phase proteins using the Mann-Whitney test.

Discussion/Conclusion: Findings from this study suggest no bearing of *S. haematobium* infection status on level of acute phase proteins before and after annual treatment with praziquantel. The extent of inflammation cannot be determined using ferritin, tissue

plasminogen activator and fibrinogen. Levels of IL- 6 did not have any bearing on the levels of the acute phase proteins. There is a need to explore other acute phase proteins as inflammatory markers in *S. haematobium* infection.

5.2 Introduction

Schistosomiasis is a water-borne parasitic disease with a global disease burden calculated at 24–56 million disability-adjusted life-years lost [1]. Chronic inflammation subsequent to infection with *S. mansoni* and *S. japonicum*, and *S. haematobium* appears to be the major source of burden in individuals with schistosomiasis. Subtle morbidities such as anaemia, growth deficiencies, physical fatigue and diminished cognitive development occur as a result of the inflammation [2, 3, 4, 5, 6]. There is an increased risk of direct and indirect cases of morbidities as a result of stimulation of tissue- destructive inflammatory and granulomatous reactions from *S. haematobium* infection. In children of school going age infected with *S. haematobium* anaemia associated with chronic inflammation is worsened by blood loss seen as gross and micro haematuria. In addition to this example of direct morbidity, the *Schistosoma*-infected host can be indirectly predisposed to greater susceptibility to other pathogens. For example, there is an increased risk to HIV acquisition in individuals with friable sandy patches that are common in female genital schistosomiasis caused by *S. haematobium* infections [5, 7].

In the control of parasitic diseases, immune responses and consequently the inflammatory processes are meant to eliminate the harmful agent and restrict tissue damage but in some situations it may end up triggering pathological repercussions that can also effect injury and illness itself. In some instances inflammation can persevere even after the harmful agent has been eliminated, giving rise to chronic inflammation [8]. It is evident that immune responses and cytokine responses generated during the various stages of the life cycle of the parasite account for most of the morbidities culminating in chronic inflammation against schistosome antigens that are released from eggs trapped in tissues [9,10]. The location and number of eggs lodged in the tissues initially determine the magnitude of inflammation and with time

the pathology associated with fibrosis and organ damage [11]. In *S. haematobium* endemic areas, children are exposed to the parasite through water related activities such as swimming and those infected continuously have eggs being deposited in their tissues and consequently the immunopathological reactions against these eggs trapped leads to an increase in acute phase proteins and inflammation. When the agent triggering inflammation occurs repeatedly the acute phase response and may be continuously activated and become chronic as the case in *S. haematobium*-endemic areas where there is constant exposure of worms and egg burden. Immune reactions to *Schistosoma* eggs trapped in the tissues in chronically infected cases ultimately result in inflammation in affected tissues followed by granuloma formation [11].

Praziquantel has become the drug of choice in the treatment of schistosomiasis and it is dispensed through mass drug administration programmes around Africa. The drug is effective against all schistosome species with minimal detrimental effects to the host and it is also effective against other trematode and cestode infections [12]. The mechanism of action is not yet fully understood though there is evidence suggesting that it increases the permeability of schistosome membranes to calcium thereby promoting tegument damage and worm paralysis [13]. The dying parasites are then removed from the host and destroyed by circulating immune cells. There is unequivocal evidence that praziquantel treatment minimizes worm burden thereby reducing the amount of deposited eggs culminating in an overall decline in inflammation related to the immunopathological reactions to the eggs and therefore praziquantel should be able to reduce chronic inflammation especially if administered regularly.

Knowledge of inflammation markers is necessary in assessing and identifying children who are likely to develop chronic pathologies later on in life. Chronic inflammation can be seen as a continuous series of distinct and consistent inflammatory stimuli. In such conditions,

increased serum concentrations of acute phase proteins and proinflammatory cytokines are generally observed [14].

Acute phase proteins are a group of plasma proteins derived primarily from the liver and they are involved in the inhibition of infection, mediating systemic effects like fever, leucocytosis, increased cortisol, decreased serum iron, and many others [15]. The extent of inflammatory tissue damage as well as diagnostic and prognostic information in some human diseases can be resolved by measurement of acute phase proteins [16,17]. Bacterial infections [18], neoplasia, [19] and inflammatory bowel diseases (IBD) have employed measurements of acute phase proteins in their prognosis [20]. However there is inadequate information regarding the use of acute phase proteins in parasitic infections like in human *Schistosoma* infection [21, 22]. This is unusual considering the fact that parasitic infections elicit considerable inflammation.

Circulating microbial products are well known inducers of acute phase proteins [23], thus, in *S. haematobium* infected children the microbial products from the natural death of the schistosomes and those released following praziquantel treatment might induce overproduction of acute phase proteins. In this study, fibrinogen, ferritin and tissue plasminogen activator are going to be evaluated before and after treatment with praziquantel. Acute phase proteins have varying half-lives, rising and falling at different times, which limit the use of only one biomarker in determining inflammation. Thus the simultaneous measurements could be most effective in identifying individuals prone to chronic inflammation.

Traditional biomarkers such as C-reactive protein, serum amyloid A and haptoglobin have produced conflicting results in terms of correlation between inflammation and schistosomiasis and insufficiently sensitive or specific enough to guide treatment decisions in

infectious diseases. Some studies have reported a direct relationship between schistosomiasis and presence of the acute phase proteins [24, 25, 26] whilst other researchers have found no link between the two. To our knowledge the combination of tissue plasminogen activator, fibrinogen, ferritin and procalcitonin has not been evaluated in individuals living in schistosomiasis endemic areas. Recently, there has been interest in the potential use of procalcitonin and ferritin as inflammatory markers in various infectious diseases as a result of the increase of both retrospective and prospective studies that consistently have documented elevated serum concentrations of procalcitonin and ferritin in various parasitic infection.

Acute phase proteins are released as a result of the action of cytokines such as IL-1, IL-6, and TNF- α produced by T-lymphocytes, macrophages, monocytes, endothelial cells, and fibroblasts at the site of inflammatory lesions. Although it is evident that a number of proinflammatory and anti-inflammatory cytokines are involved in the inflammatory response [27], available data indicates that IL-6 is the supreme stimulator [21, 28]. Acute inflammation turns into chronic inflammation if the activity of IL-6 perseveres. However, in knockout mice incapable of expressing IL-6, the role of IL-6 in triggering the production of acute-phase proteins depends on the nature or site of the inflammatory stimulus; the response is largely inhibited in IL-6 knockout mice injected with turpentine but is normal when bacterial lipopolysaccharide is the inflammatory stimulus [22]. Unrestrained and sustained action of cytokines is potentially harmful. Anti-cytokine therapies are thus useful in light of the role of proinflammatory cytokines in inflammation-related pathologies and this may have far reaching consequences in schistosomiasis vaccine development which has been elusive [29].

Identifying individuals at an early stage who are vulnerable to inflammation allows better prognosis and prevents the use of invasive, costly and time consuming procedures to determine those suffering from pathological complications of chronic inflammation conditions. It has been demonstrated that biomarkers; C-reactive protein and faecal

calprotectin can be used to evaluate disease status in patients with inflammatory bowel disease (IBD) though endoscopic evaluation which is expensive and invasive is the gold standard [30]. The goal of disease monitoring is to identify individuals at risk in order to treat earlier.

Although perpetual and continuous inflammation is presumed to be a hallmark of schistosomiasis, very few studies have actually examined the acute phase proteins simultaneously with the proinflammatory cytokine IL-6 in *S. haematobium* infections. In this study *S. haematobium* infections were first determined then ferritin, fibrinogen, tissue plasminogen activator, procalcitonin and IL-6 were determined at baseline to assess inflammation in both *S. haematobium*-infected and *S. haematobium* uninfected school children. Secondly we established if repeated rounds of praziquantel treatment for 2 years results in changes in the acute phase proteins and IL-6.

5.3 Methodology

Study population and design

The study was carried out at Bandanyenje primary school, a rural school situated in Manicaland Province in Zimbabwe. The study population comprised of 212 (105 boys and 107 girls) aged between 7–13 years, who were permanent residents of the area. The study design is a “before and after treatment”. Serum samples from the participants were taken and measurement of acute phase proteins and IL-6 before and after praziquantel treatment.

Parasitology and blood sampling

A school-based longitudinal intervention study was conducted and involved examination and treatment of the study population at baseline, 6 weeks and at 2 year follow up surveys. Stool and urine samples were collected from 212 school children at baseline and follow up on three

consecutive days and were processed for schistosomiasis using the Kato Katz by Peters *et al.* (1980) [31] and the filtration technique Mott *et al.* (1982) [32] respectively. Blood samples were collected from the 212 children at baseline and 2 years post-treatment and their serum was used to determine acute phase proteins and IL-6.

Praziquantel Treatment

All 212 school children were given praziquantel (40mg/kg body weight) at baseline regardless of the infection status. Praziquantel was administered at the same dose in all the children annually for 2 years.

IL-6 determination

The analysis of IL-6 in the serum samples was done by sandwich ELISA. 100 ul of coating antibody was added into the wells of a 96 well ELISA plate. The plate was then covered with adhesive to prevent evaporation and incubated overnight at 4°C. After incubation the plate was turned over to remove the coating antibody and was washed thrice with the wash buffer. The plate was dried by blotting on a paper towel to remove residual wash solution. 200ul of 5% egg albumin was added to all the wells to block nonspecific binding sites. The plate was covered with adhesive tape and incubated for 1 hour at room temperature on a plate shaker. After incubation the plate was turned over to remove the coating antibody and was washed thrice with the wash buffer. The plate was dried by blotting on a paper towel to remove residual wash solution. 100 ul of the sample was added into wells in duplicate. The plate was covered with adhesive tape and incubated for 2 hours at room temperature on a plate shaker. After incubation the plate was turned over to remove the sample and was washed thrice with the wash buffer. The plate was dried by blotting on a paper towel to remove residual wash solution. 100 ul of biotin conjugated antibody was added into the wells. The plate was covered

with adhesive tape and incubated for 1 hour at room temperature on a plate shaker. After incubation the plate was turned over to remove the detection antibody and was washed thrice with the wash buffer. The plate was dried by blotting on a paper towel to remove residual wash solution. 100 ul of Horse Radish peroxidase (HRP) antibody was added into the wells. The plate was covered with adhesive tape and incubated for 1 hour at room temperature on a plate shaker. After incubation the plate was turned over to remove the HRP conjugate antibody and was washed six times with the wash buffer. The washing was done thoroughly to remove all unbound HRP conjugate antibodies. The plate was dried by blotting on a paper towel to remove residual wash solution. 100 ul of o- Phenylenediamine Dihydrochloride (OPD) substrate was added to the plate and incubated for 20 minutes in the dark. The plate was covered with a foil paper to prevent light from interrupting with the reaction. After incubation 20ul of the stop solution was then added to each well. The plates OD was read at dual wavelength for cytokine analysis (405nm and 630nm) in an ELISA plate reader.

Acute Phase Proteins determination

Serum levels of ferritin, fibrinogen, tissue plasminogen activator and procalcitonin, were determined by the magnetic bead-based immuno-assays using the Bio-Plex Pro™ human acute phase 4-plex immunoassay complete commercial kits. 25ul 1x beads were added to a 96 well micro titer plate and vacuum filtration was carried out. The standards, samples and controls were diluted and 50 ul added to the wells and incubated in the dark at room temperature with shaking at 850 rpm for 1 hour. After incubating the beads, samples, standards, blank, and controls vacuum filtration was done and the plate was washed three times with 100ul wash buffer. 25ul of 1x detection antibodies were added to the assay plate and incubated in the dark for 30min with shaking at 850rpm. The plate was washed 3x and Streptavidin –PE was added. The assay plate was incubated for 10 min at room temperature

with shaking at 850 rpm. Vacuum filtration was done and the plate was washed 3x and the beads were re-suspended in assay buffer prior to reading the plate. The Bio-plex manager software was used for running the assay, data acquisition and analysis.

Statistical analysis

Data was analysed using SPSS statistical software v16. Levels of serum levels of ferritin, fibrinogen, tissue plasminogen activator, procalcitonin and IL-6 at baseline and 2 years post-treatment in the school children were compared using student t test to determine the effect of praziquantel treatment on acute phase proteins and IL-6.

The Mann–Whitney non parametric *U*-test was used to determine the effect of *S. haematobium* infection on the levels of ferritin, fibrinogen, tissue plasminogen activator, procalcitonin and IL-6. A value of $p < 0.05$ was considered to indicate a significant difference in statistical analyses.

5.4 Results

212(105 boys and 107 girls) aged between 7-13 were recruited in the study and were successfully traced and re-examined at both follow-ups with complete sets of longitudinal parasitological data on *S. haematobium* infection. The overall pre-treatment prevalence of *S. haematobium* infection was 49(23.1 %) with 20(19%) boys and 29(27.1%) girls as determined using the urine filtration technique (**Table 5.1**). There was an overall decline in *S. haematobium* prevalence from 23.1% at baseline to 0.47% at 6 weeks post-treatment and, 0.47% 2 years post-treatment, an overall 87.1% reduction over 2 years ($p < 0.05$). A total of 211(99.5%) children were egg negative after treatment.

No significant changes were observed in ferritin, tissue plasminogen activator and procalcitonin among the *S. haematobium* positive and negative children ($p > 0.05$, Mann-

Whitney non parametric test at baseline (**Figure 5.1**). Following repeated rounds of annual treatment changes occurred in the levels of the acute phase proteins though they were all insignificant with respect to infection status $p>0.05$ - Mann-Whitney non parametric test (**Figure 5.3**). Tissue plasminogen activator and procalcitonin levels increased slightly following treatment. In *S. haematobium*-uninfected children, levels of procalcitonin decreased following treatment though it was insignificant ($p>0.05$) whilst the ferritin and tissue plasminogen activator marginally increased following treatment.

The Mann-Whitney test was used to determine the effect of *S. haematobium* infection on the levels of acute phase proteins. *S. haematobium*-infected children had slightly higher levels of procalcitonin and tissue plasminogen activator before treatment though the difference of all three was not significant $p>0.05$ - Mann-Whitney non parametric test (**Figure 5.1**). Levels of ferritin and fibrinogen were lower in *S. haematobium* positive children before treatment, however the difference was also not significant $p>0.05$ (**Figure 5.1 and Figure 5.2**).

Infection status had no bearing on the levels acute phase proteins, using the Mann-Whitney non parametric test, ferritin levels ($p=0.364$), tissue plasminogen activator ($p=0.192$), procalcitonin ($p=0.232$) were shown to be unrelated to *S. haematobium* infection. Fibrinogen levels were within normal ranges for both *S. haematobium* positive and negative children and there the Mann-Whitney test could not be performed because there was only one group (**Figure 5.2**).

In order to determine the effect of treatment on the acute phase proteins the student t test was performed. In *S. haematobium* positive children there was a decrease in ferritin levels following treatment though it was insignificant $p=0.357$, however in *S. haematobium* negative children there was a slight increase which was also not significant ($p>0.05$). There was an insignificant increase in procalcitonin and tissue plasminogen ($p=0.226$ and $p=0.186$

respectively) following praziquantel treatment in both *S. haematobium* and positive and negative children (**Figure 5.3**).

At baseline level of IL-6 was higher in *S. haematobium* infected children than in *S. haematobium* uninfected children though the difference was insignificant ($p < 0.05$) (**Figure 5.4**). Following treatment there was an increase in IL-6 in *S. haematobium* uninfected children and a decrease in IL-6 in *S. haematobium* uninfected children, however the changes were insignificant $p < 0.05$ in both cases. The Mann Whitney test was used to determine the effect of IL-6 on the levels of the acute phase proteins. IL-6 levels did not have an effect on the levels of ferritin, procalcitonin and tissue plasminogen activator ($p = 0.334$, $p = 0.134$ and $p = 0.847$, respectively using Mann-Whitney test). Effect of IL-6 on fibrinogen levels could not be determined by the Mann-Whitney test because fibrinogen levels were within normal ranges for all the children.

5.5 Discussion

The *S. haematobium* prevalence was 23.1% which is slightly lower than 23.8% recorded in the province by Midzi *et al*, 2014 [33]. The prevalence of *S. haematobium* infection declined from 23.1% to 0.47% following repeated rounds of annual praziquantel treatments (**Table 5.1**). This decline in prevalence resulted in a decline in the *S. haematobium* worms laying the eggs because of worm damage caused by praziquantel. The eggs are responsible for invoking and determining the magnitude of inflammation within tissues [34]. The low levels of acute phase proteins post treatment reflect the low prevalence of infection 2 years following repeated rounds of annual praziquantel treatment. If less children are infected (less exposure of *S. haematobium* eggs in tissues), the extent of inflammation will be reduced since the tissue-trapped eggs are responsible for inflammation.

The current study was the first in reporting the relationship between fibrinogen, ferritin, procalcitonin and tissue plasminogen activator and *S. haematobium* infection in school-going children. The effect of IL-6 on the circulating four acute phase proteins was also reported for the first time in this study. This study was motivated in part by the limited information regarding relationships between biomarkers of inflammation and *S. haematobium* infection. Contrary to what was expected, we observed no association between *S. haematobium* infection status and the inflammatory markers (**Figure 5.1 and Figure 5.2**); fibrinogen, ferritin, tissue plasminogen activator and procalcitonin before treatment with praziquantel. Using the Mann-Whitney non parametric test all four acute phase proteins did not show any association with *S. haematobium* infection status. Repeated exposure to inflammatory agents, common in schistosomiasis endemic settings where there is frequent exposure to cercariae infested water is expected to result in constant elevations in acute-phase proteins and other inflammatory markers. Previous studies had reported relationships between *S. haematobium* and other acute phase proteins albeit with conflicting results. It is against this background that we evaluated the levels of the four acute phase proteins. Positive associations between levels of acute phase proteins such as C-reactive protein and hepcidin and *Schistosoma* infections have been reported [24, 25]. Ferritin has also been reported to be raised in inflammatory diseases [26] but such an association was not evident in our study though in the reported case the prevalence of *S. haematobium* was high. Our observation can be attributed to the moderate prevalence in our study group or simply just that other markers like C-reactive protein are better markers than the markers we evaluated. The expectation that a combination of more than one measure would perform substantially better than any single one was not supported by our observations. Following repeated treatment with praziquantel, no significant changes were observed in the acute phase proteins regardless of infection status (**Figure 5.3**).

Serum concentration of acute phase proteins typically peak within 24 to 48 h after the initiation with a decline coinciding with the recovery from the infection. It has been noted that acute phase proteins decline within 4–7 days after the initial stimulus if no further stimulus occurs and that repeated exposure to the agent stimulating inflammation results in chronic inflammation with levels of acute phase proteins being continuously elevated [35]. Circulating acute phase levels are felt to be a reflection of the response to pro-inflammatory cytokines such as IL-6 [15, 28]. There were no significant differences in circulating IL-6 between *S. haematobium* infected and uninfected school children and the levels in both groups were not high (**Figure 5.4**). Inflammation with T-cell activation is a distinct characteristic of *S. haematobium* infection and as such, markers of inflammation are expected to be in circulation [36, 37]. A Th2 immune response is induced by schistosomes through Th1 down-regulation via increased IL-6 production. Host macrophages recognize the larvae of schistosomes which induces secretion of IL-6. In children who are *S. haematobium* positive induction of the Th2 response would be expected resulting in increased production of IL 6 [38].

Following treatment there were no significant changes in the level of IL-6 (**Figure 5.4**). This was unexpected especially considering the fact that studies have shown that treatment of schistosomiasis with praziquantel induces noticeable changes in cytokine levels [39, 40]. Marked changes in cytokines such as IL-6, IL-4, IL-5 and IL-10 occur following treatment of schistosomiasis induces marked changes in cytokine levels resulting in a shift to Th2 responses which have been associated with resistance to reinfection [41]. Exploring other cytokines not included in this study (i.e., IL-4, IL-5, and IL-13) is important in understanding the immune response to multiple parasitic infections and should be integrated in future research efforts.

Our study revealed novel insights in the use of the four acute phase proteins in evaluating inflammation in *S. haematobium*, albeit with some minor limitations such as sample size and availability of resources. General limitations of assessing serum levels of inflammatory markers such as acute phase proteins and cytokines are the relatively non-specificity, short half-life, nonspecific induction, and serum levels not reflecting biologic activity [42]. Notwithstanding these limitations, serum levels of some of these biomarkers have yielded important insights in the level of inflammation in parasitic diseases.

5.6 Conclusion

The central question in this study was whether IL-6, ferritin, fibrinogen, tissue plasminogen activator and procalcitonin are suitable as identifiers for inflammation in *S. haematobium* infection and whether their levels would change following repeated rounds of annual praziquantel treatments. Our results showed no significant association between *S. haematobium* infection status and level of the four acute phase proteins ferritin, fibrinogen, tissue plasminogen activator and procalcitonin. However, marginal increases were observed in levels of procalcitonin and tissue plasminogen activator in *S. haematobium* infected children. Treatment using praziquantel did not significantly affect the levels of the four acute phase proteins. There is a need to identify and select of the most appropriate biomarkers of inflammation in children since identifying individuals who are vulnerable early stages of inflammation allows better prognosis and prevents the use of invasive, costly and time consuming procedures to determine those suffering from pathological complications of chronic inflammation conditions in future.

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List of Abbreviations

CRP	_	C- Reactive Protein
TPA	_	Tissue Plasminogen Activator
WHO	–	World Health Organization
MDA	–	Mass Drug Administration
PZQ	–	Praziquantel
MDA	–	Mass Drug Administration
APPs	--	Acute Phase Proteins

Declarations

Ethics approval and consent to participate

This manuscript draws from our work which received ethical approval from The Medical Research Council of Zimbabwe (MRCZ/A/1958) and BREC (BE467/16) (appendice 7.1 and 7.2). All information pertaining to the discussion was obtained prior to written consent by the parents/guardian of the children participating in the study.

Consent for Publication

Consent to publish was contained in the individual parental consent that the data would be collectively published as patterns not containing individual information. Also the approval from the MRCZ regulatory gives permission to publish the research findings so as to share outcome with the international research community.

Availability of Data and Material

Data sets generated or analysed during the current study are accessible for sharing.

Competing Interests

The authors declare that they have no competing interests.

Authors' contributions

TJC, BN& TM developed the field study design, acute phase proteins and IL 6 immunoassays & analyzed the data. While AV, AFN, EPS, DZ &TM conducted field and sampling work. TJC, AV, AFN&BM conducted the laboratory assays. TJC and TM conducted the initial statistical analyses. All authors contributed to the manuscript.

Authors' Information

Takafira Mduluza is a Professor at the University of Zimbabwe & has 25% Fractional Professor position at The School of Laboratory Medicine and Medical Sciences, University of KwaZulu Natal, Durban, South Africa. **Dr Bongiwe Ndlovu** is a lecturer in the School of Laboratory Medicine and Medical Sciences, College of Health Sciences, University of KwaZulu-Natal; South Africa

Tawanda Chisango, Agness Farai Nhidzaand Edson PhD students in the School of Laboratory Medicine and Medical Sciences, College of Health Sciences, University of KwaZulu-Natal; Durban, South Africa. **Arthur Vengesai** is the Graduate Research Assistant on the project in the Biochemistry Department, University of Zimbabwe, Harare, Zimbabwe. **Dr Danai Zhou** is a lecturer in the Medical Laboratory Sciences, College of Health Sciences, University of Zimbabwe, Harare, Zimbabwe.

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Tables Legend

Table5.1: Overall prevalence of *S. haematobium* infection in the study population according to gender at baseline and 2 years post praziquantel treatment.

Figures Legend

Figure 5.1: Mean concentration of acute phase proteins in *S. haematobium*-infected and uninfected children at baseline.

Figure5.2: Mean concentration of fibrinogen in *S. haematobium* infected and uninfected children at baseline.

Figure5.3: Mean concentration of acute phase proteins in *S. haematobium* infected and uninfected children at baseline and 2 years post praziquantel treatment.

Figure 5.4: Mean IL-6 OD values in *S. haematobium* infected and uninfected children at baseline and 2 years post praziquantel treatment.

Table 5.1: Overall prevalence of *S. haematobium* infection in the study population according to gender at baseline and 2 years post praziquantel treatment.

Variable	Baseline	6 weeks post treatment	2 years post treatment
Prevalence	49(23.1%)	1(0.47%)	1(0.47%)
Cure rate		98%	97.8%
Boys	20(19%)	1(0.47%)	1(0.47%)
Cure rate		95%	94.7%
Girls	29(27.1%)	0	0
Cure rate		100%	100%

Figure 5.1: Mean concentration of acute phase proteins in *S. haematobium*-infected and uninfected children at baseline

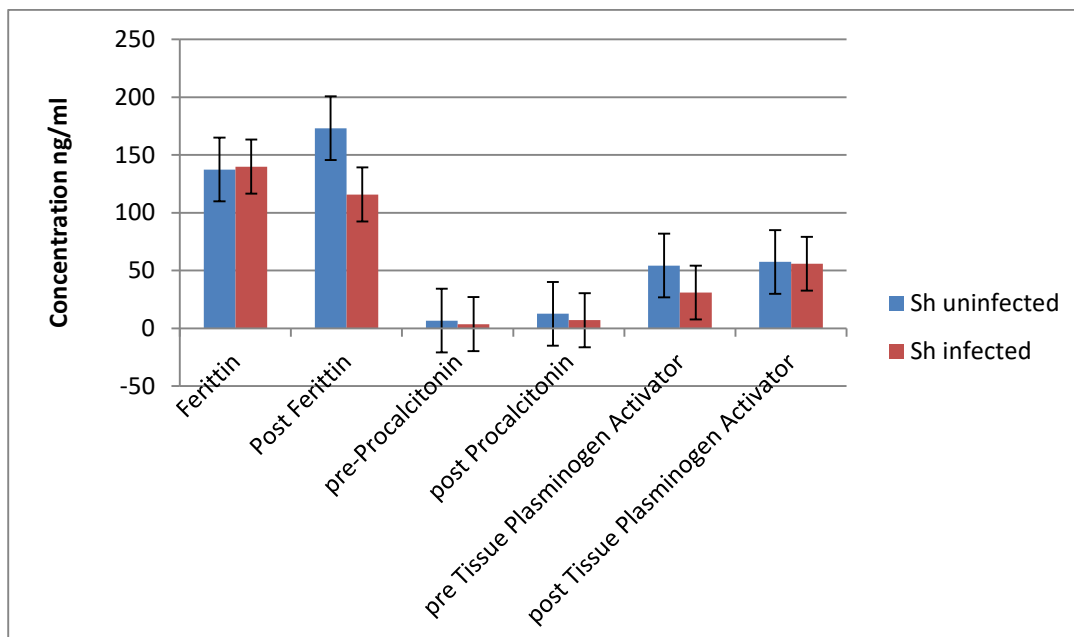


Figure 5.2: Mean concentration of fibrinogen in *S. haematobium* infected and uninfected children at baseline

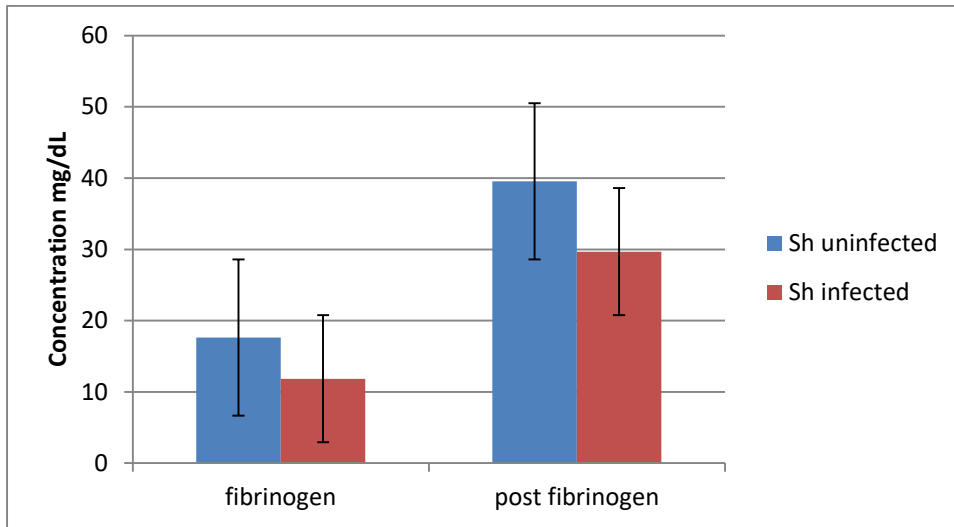


Figure 5.3: Mean concentration of acute phase proteins in *S. haematobium* infected and uninfected children at baseline and 2 years post praziquantel treatment.

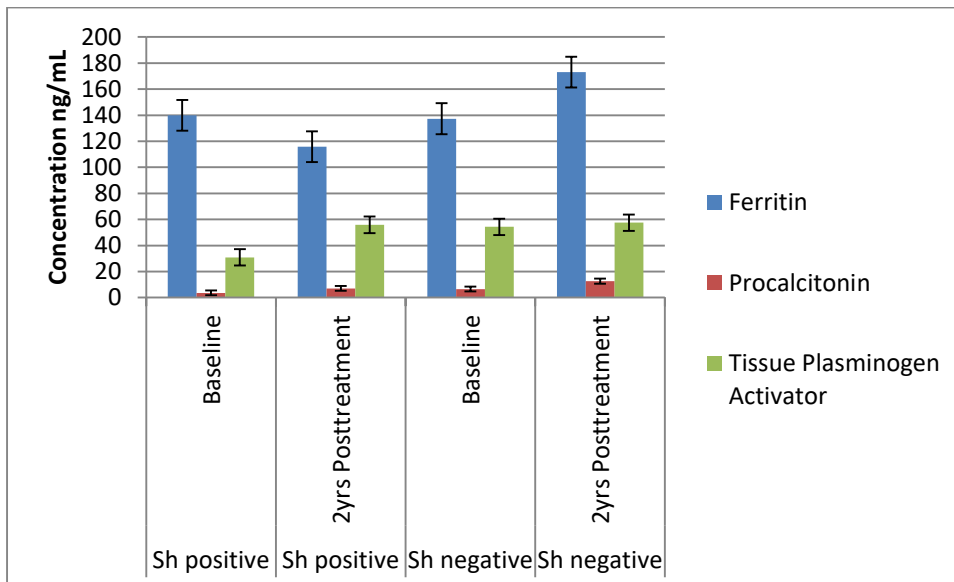
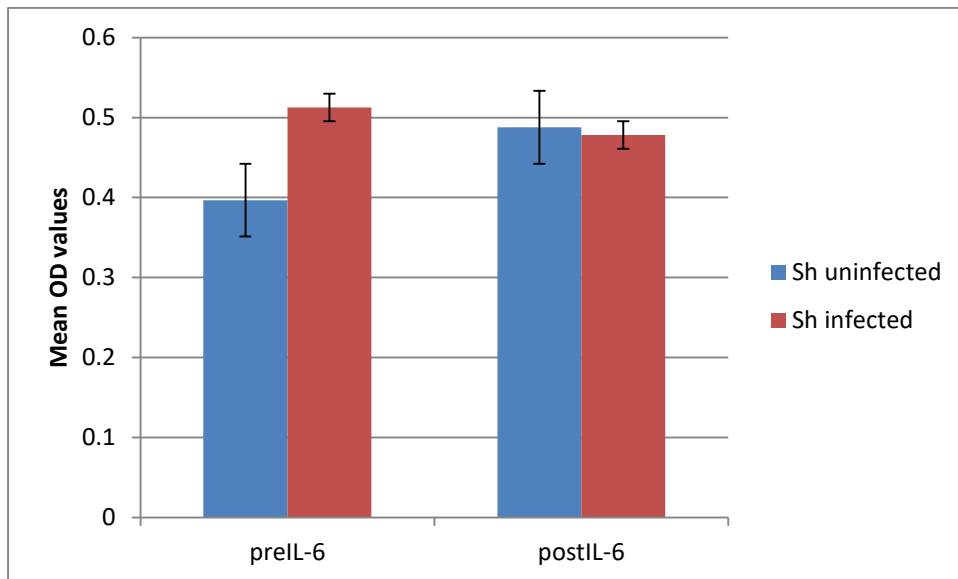


Figure 5.4: Mean OD values for IL 6 in *S. haematobium* infected and uninfected children before and after treatment with praziquantel.



CHAPTER 6: OVERALL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

6.1 General overview

This study investigated the schistosomiasis markers of inflammation through the use of four acute phase proteins and antibody profiles in school children on repeated annual mass treatment using the anti-helminthic drug praziquantel in Bandanyenje. Schistosomiasis is a devastating parasitic disease which if untreated results in severe morbidities such as obstructive uropathy, renal failure, hydronephrosis, and bladder cancer later on in life [1, 2]. Prevalence of *S. haematobium* in our area of study, at 23.1% was marginally lower than that of the overall prevalence in Manicaland province (23.8%) [3]. The prevalence of *S. haematobium* reported in this study could reflect on-going control efforts in the area with the Ministry of Health taking part in schistosomiasis awareness campaigns. Zimbabwe was experiencing dry spells during the study period with erratic rainfall patterns because of the drought. This could have attributed to the moderate prevalence because of the reduced transmission of *S. haematobium* when water levels are low. There is a need to maintain a low prevalence as this prevents *Schistosoma*-related morbidities as the children grow up. Children are mainly affected because of their higher rates of water related activities, immunological status and anatomical vasculature [4, 5] and infected children suffer from haematuria, dysuria, nutritional deficiencies, anaemia, growth retardation, decreased physical performance and impaired memory and cognition as a result of extreme schistosome infections [6]. Presence of haematuria, measure of schistosome-induced pathology, before treatment was correlated ($\chi^2=20.38$, $p<0.05$) with the presence of *S. haematobium* egg count in urine. In our study haematuria, was reduced from 30% (*S. haematobium*-infected) and 12.1% (*S. haematobium*-uninfected) to 0%, 2 years after treatment highlighting the effectiveness of praziquantel.

In our study, the pronounced cure rates after 2 years of annual treatment could be attributed to the study period, baseline infection intensity, geographical location, repeated rounds with enhanced killing of schistosomula and the method of laboratory diagnosis employed, ie microscopy with egg determination does not rule out presence of the worm. After annual praziquantel treatment for 2 years the prevalence was 0.47%. This reflects the efficacy of praziquantel as well as the benefits of repeated annual treatment. Infective cercariae are destroyed at a faster rate in children who regularly take inpraziquantel. Schools have been targeted for mass treatments because of the increased benefits of reducing infection burdens in children compared to adults and the simplicity of providing treatment.

Our group also wanted to determine whether there are any antibody profiles that protect or predispose children to *S. haematobium* hence general and *S. haematobium* specific antibodies were measured in both *S. haematobium* positive and negative children. Adult schistosome worms live for many years in the human blood stream, but treatment disrupts the integrity of the worm's outer tegument exposing a range of formerly cryptic antigens to the host's immune system. The post-treatment IgE levels induced by cryptic antigens can be more strongly associated with re-infection immunity and multiple rounds of treatment have been found to increase resistance [7, 8]. At baseline there was an increase in total IgG4 whilst all the other immunoglobulins were within normal ranges. Other studies have revealed hypergammaglobunaemia with noteworthy increases in IgG4 and IgE though in *Schistosomiasis* infected individuals [9, 10]. In our study group, upon treatment there was an expected significant decline in IgG4 though there was an unexpected absence of changes in *Schistosoma*-specific IgE. IgG4 is known to block the crosslinking of IgE and prevent the antibody dependant cytotoxicity of parasites however; the increase in total immunoglobulins is non-specific and can be affected by other invading pathogens, which is common in children living in poverty-stricken areas. We also observed an increase in both the total and

specific IgG1 which was maintained over the 2 years, which suggests that IgG1 could have been stimulated by antigens produced from the worms after chemotherapy. Khalife *et al.*, 1989 [11] demonstrated the eosinophil-dependent killing of schistosomula releases sub-surface antigens which stimulates IgG1 production. This could explain the significant increase of IgG1 following treatment with praziquantel. IgG1 is stimulated by praziquantel induced release of sub-surface antigens as well as a decline in eggs produced. An increase in IgG1 following treatment with praziquantel was observed by Mutapi *et al.*, 1998 [12] which they attributed to a switch from IgA specific antibodies to IgG1 response that occurs in children. The switch is accelerated by the praziquantel treatment which actually causes partial protection to reinfection as seen by the low 0.96 % reinfection rate 2 years post treatment in this study. Praziquantel has been shown to boost responses in IgG1, IgE and cytokines IL-4 and IL-5 which have been associated with protection against reinfection [13]. Re-infection in our study was very low (0.96%) and this can be attributed to the repeated treatment with praziquantel, lifestyle changes in children due to increased awareness of dangers of schistosomiasis and also to the moderate prevalence (23.1%) in the region.

Most studies have reported IgE association with resistance to reinfection with significant increases in *Schistosoma*-specific IgE in individuals who are resistant to reinfection with *S. haematobium*, however this was not evident in our study. Production of large amounts of specific IgE is a characteristic feature of schistosomiasis [14] and IgE mediated killing has been observed in animal experiments [15]. Several studies have shown that low levels of reinfection are correlated to higher serum levels of *Schistosoma*-specific IgE [16, 17].

We were interested in evaluating the four acute phase proteins and their association with IL-6, a pro inflammatory marker. However from our results we could not establish a direct relationship between the pro inflammatory cytokine IL-6 and acute phase biomarkers. This could have been linked to the short half live of acute phase proteins coupled with the fact that

the prevalence and intensity of infection and reinfection was low. Following annual praziquantel treatment, there were no significant changes in acute phase proteins and IL-6. This is in contrast to what other studies have reported. Marked changes in cytokines such as IL-6 have been shown to occur following treatment of schistosomiasis with a shift to Th2 responses which have been associated with resistance to reinfection [18]. Some studies have reported elevated levels of acute phase proteins such as C-reactive protein and hepcidin in schistosomiasis infected individuals with a decrease in the levels of the acute phase proteins occurring following treatment with praziquantel [19, 20]. Our observation, which is in contrast to such observations, can be attributed to the moderate prevalence in our study group or simply that other markers like C-reactive protein are better markers than the markers we evaluated.

There were some interesting cases from the study group with notable results. Only 1 child was still infected after 2 years and interestingly this child had a heavy infection at 6 weeks after the initial treatment. Not only was the individual still infected after repeated treatments, he had an antibody response significant of susceptibility to infection and reinfection and had a post treatment increase in all the four acute phase proteins. He had the highest levels of the blocking *Schistosoma*-specific IgG4 antibodies that were still very high after repeated rounds of treatment. He also had very low protective *Schistosoma*-specific IgE antibodies. IgE levels are known to be associated with protection against infection by schistosomes resulting in resistance to infection and reinfection. Interestingly at baseline he had undetectable levels of all for acute phase proteins, however after 2 years all the acute had risen with high levels recorded for fibrinogen, tissue plasminogen activator and procalcitonin. Such results can indicate unique host factors that make the individual susceptible to infection or social activities the child engages in which predispose him to infection. The level of IL-6 in this individual was also high at baseline and following treatment, however the IL-6 values of the

other *S. haematobium* positive and negative children showed insignificant associations with acute phase proteins.

6.2 Strengths and Limitations

We managed to follow up 212 children over 2 years and the school children were providing the required samples needed for us to conduct our studies. Other studies have recorded high attrition rates as children drop out of such longitudinal studies making it difficult for researchers to draw conclusion from such studies. This could be attributed to the sample population which involves school children who rarely migrate to different locations once they start school.

In antibody profiling and acute phase proteins determination we used the multi-plex magnetic bead assay which is highly sensitive and can measure the concentrations of several analytes in a small sample with great precision. The only challenge was that the specimens had to be stored after sampling and then transported from Zimbabwe to University of KwaZulu Natal, South Africa for analysis.

A case-control approach to this study would have strengthened the study objective of realising the importance of repeated mass treatments using praziquantel on antibody profiles and inflammation markers. Comparison of untreated schistosomiasis-exposed children with those on mass treatments could have reinforced the impact of mass treatment campaigns. However, our study was part of the National Schistosomiasis Control programme which required the treatment of all children regardless of exposure status.

6.3 Conclusion

In conclusion, our study showed that repeated annual treatments reduce *S. haematobium* infection significantly with corresponding significant changes in total and specific *S.*

haematobium antibodies. These findings suggest consequences of long term effects of praziquantel in changing the overall protective immunity of these school children and benefits of the repeated mass drug administration. Significant levels of the blocking and protective antibodies were detected post treatment which also indicated immunizing effect of the praziquantel treatment. While no clear trends could be deduced from the profiles of other total and specific antibodies. Our results show no significant association between *S. haematobium* infection status and level of the four acute phase proteins ferritin, fibrinogen, tissue plasminogen activator and procalcitonin. Treatment using praziquantel did not significantly affect the levels of the four acute phase proteins

6.4 Recommendations

The WHO guidelines recommend treatment with praziquantel once every 2 years in school children in communities with a schistosomiasis prevalence of 10% to 50% as such school children in Bandanyenje should be given praziquantel biennially [21]. Our findings suggest that administration of praziquantel should be biennially according to the WHO recommendation.

Exploring other cytokines not included in this study (i.e., IL-4, IL-5, and IL-13) and important in understanding the immune response to multiple parasitic infections should be integrated in future research efforts. Anti-cytokine therapies are useful in light of the role of proinflammatory cytokines in inflammation-related pathologies and this may have far reaching consequences in schistosomiasis vaccine development which has been elusive [22].

Our results clearly stress the limitations of acute phase biomarkers for the purpose of identifying individuals experiencing inflammatory states who are likely to be affected by *Schistosoma*- associated morbidities later on in life. We also suggest that further research efforts should be pursued to identify other appropriate biomarkers that would perform more

efficiently in endemic populations. Identifying individuals who are vulnerable in the early stages of inflammation allows for better prognosis and prevents the use of invasive, costly and time consuming procedures to determine those suffering from pathological complications of chronic inflammation conditions in future.

6.5 References

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Chapter 7: APPENDICES

7.1: Ethical approval by MRCZ

Telephone: 791792/791193
Telefax: (263) - 4 - 790715
E-mail: mrcz@mrcz.org.zw
Website: <http://www.mrcz.org.zw>



Medical Research Council of Zimbabwe
Joshua Tongogara / Mazoe Street
P. O. Box CY 573
Causeway
Harare

APPROVAL

REF: MRCZ/A/1958

25 February 2016

Mr Tawanda Chisango
Chinhoyi University of Technology
P. Bag 7724
Chinhoyi

**RE: Investigating Schistosomiasis markers Of Inflammation And Immune Responses In Individuals On repeated
Mass Drug Administration**

Thank you for the application for review of Research Activity that you submitted to the Medical Research Council of Zimbabwe (MRCZ). Please be advised that the Medical Research Council of Zimbabwe has **reviewed** and **approved** your application to conduct the above titled study.

This approval is based on the review and approval of the following documents that were submitted to MRCZ for review:-

- a) Full proposal

• **APPROVAL NUMBER** : MRCZ/A/1958
This number should be used on all correspondence, consent forms and documents as appropriate.

- **TYPE OF MEETING** : Full Board
- **EFFECTIVE APPROVAL DATE** : 15 March 2016
- **EXPIRATION DATE** : 14 March 2017

After this date, this project may only continue upon renewal. For purposes of renewal, a progress report on a standard form obtainable from the MRCZ Offices should be submitted three months before the expiration date for continuing review.

• **SERIOUS ADVERSE EVENT REPORTING:** All serious problems having to do with subject safety, must be reported to the Institutional Ethical Review Committee (IERC) as well as the MRCZ within 3 working days using standard forms obtainable from the MRCZ Offices or website.

• **MODIFICATIONS:** Prior MRCZ and IERC approval using standard forms obtainable from the MRCZ Offices is required before implementing any changes in the Protocol (including changes in the consent documents).

• **TERMINATION OF STUDY:** On termination of a study, a report has to be submitted to the MRCZ using standard forms obtainable from the MRCZ Offices or website.

• **QUESTIONS:** Please contact the MRCZ on Telephone No. (04) 791792, 791193 or by e-mail on mrcz@mrcz.org.zw

Other

- Please be reminded to send in copies of your research results for our records as well as for Health Research Database.
- You're also encouraged to submit electronic copies of your publications in peer-reviewed journals that may emanate from this study.

Yours Faithfully

MRCZ SECRETARIAT
FOR CHAIRPERSON
MEDICAL RESEARCH COUNCIL OF ZIMBABWE



PROMOTING THE ETHICAL CONDUCT OF HEALTH RESEARCH

7.2: Ethical approval by UKZN-BREC



16 September 2016

Mr T J Chisango (214584507)
Discipline of Medical Microbiology
College of Laboratory Medicine and Medical Sciences
chisangotawanda@yahoo.com

Title: Investigating schistosomiasis markers of inflammation and immune responses in individuals on repeated mass drug administration.

Degree: PhD

BREC Ref No: BE467/16

EXPEDITED APPLICATION

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application received on 17 August 2016.

The conditions have been met and the study is given full ethics approval and may begin as from 16 September 2016.

This approval is valid for one year from 16 September 2016. To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2015), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at <http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx>.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be RATIFIED by a full Committee at its next meeting taking place on 11 October 2016.

We wish you well with this study. We would appreciate receiving copies of all publications arising out of this study.

Yours sincerely


Professor V Rambiritch
Deputy Chair: Biomedical Research Ethics Committee

cc supervisor: mduluzat@ukzn.ac.za

cc postgraduate administrator: jdudhra@ukzn.ac.za

Biomedical Research Ethics Committee

Professor J Tsoka-Gwegweni (Chair)

Westville Campus, Govan Mbeki Building






Postal Address: Private Bag X54001, Durban 4000

Telephone: +27 (0) 31 260 2486 Facsimile: +27 (0) 31 260 4609 Email: brec@ukzn.ac.za

Website: <http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx>



100 YEARS OF ACADEMIC EXCELLENCE

Founding Campuses:  Edenwood  Howard College  Medical School  Pietermaritzburg  Westville

7.3: Specimen storage informed consent form

University of Zimbabwe



SPECIMEN STORAGE INFORMED CONSENT FORM

STUDY TITLE:

**Investigating Schistosomiasis Markers Of Inflammation And Immune Responses In
Individuals On Repeated Mass Drug Administration**

Protocol Version: Ver1. 01.11.2012

SPECIMEN STORAGE

Consent Version

(English)

PRINCIPAL INVESTIGATOR: Mr T. Chisango

PHONE: 0772731322

INTRODUCTION:

You have decided to take part in the investigational research study named above, sponsored by the University of Zimbabwe and Ministry of Health & Child Welfare. While your child is in this study, blood 2.5 – 5 mls will be collected from your child. You are kindly being asked to agree to the storage of these samples for use during the study and after the study has ended. We may also ship these samples to another laboratory outside Zimbabwe if the need comes. This consent form gives you information about the collection, storage, and use of these samples. These samples will be useful in the first next 5 years the Mass Drug Administration of praziquantel research programme is running.

The study staff will talk to you about this information. Please ask if you have any questions. You will be asked to sign or make your mark on this form to indicate whether you agree to have your child's samples stored and tested. You will be offered a copy of this form to keep.

YOUR PARTICIPATION IS VOLUNTARY:

Allowing your samples to be stored is completely voluntary. You may decide not to have any samples stored other than what is needed to complete this study and still be in this research study or any future study.

Even if you decide now that your samples can be stored for the duration of the research, you may change your mind at any time. If this happens, you must tell the study staff that you have changed your mind. If you decide not to have your samples stored or used for future research, they will be destroyed at the end of the study.

PURPOSE:

The specific research to be done on your blood samples will be to analyse for markers of allergic reactions after treatment. The samples will only be used to look for antibodies as markers of reaction and effect of treatment on the infections, damage caused by infection, or how your body reacts to the infection. For example, the tests may look at cells, proteins, and other chemicals in your body. Tests may also examine your genes (DNA), since they might affect your response to parasitic infection in important ways. For example, your genes may make you more or less susceptible to becoming infected, your responses to infection or to treatment stronger or weaker, or make development of allergic reactions progress faster or slower. No other kinds of genetic test will be done by anyone on your stored specimens without first explaining the test to you and obtaining your permission.

The study researchers do not plan to contact you or your regular doctor with any results from tests done on your stored samples. This is because research tests are often done using experimental procedures, so the results may not help for making decisions on managing your health. In the very rare case that a specific test result gives important information about your health, the researchers will contact you. If you wish to be contacted with this type of test result, you must give the study staff any change to your contact information. If you have a regular doctor and you want the study staff to tell this doctor your child's test results, you must give the study staff your doctor's contact information.

Your samples will not be sold or used directly to produce commercial products.

Research studies using your samples will be reviewed by the Medical Research Council of Zimbabwe.

PROCEDURES:

Each time your child's blood is drawn, up to 5 mL (which is about 1.5 teaspoons) of the sample may be stored. For each sample of blood, part of the sample will be tested immediately and the rest will be stored.

Your blood will be stored safely and securely in a storage facility at the University of Zimbabwe-Biochemistry Department. Only the people who work at the facility and approved researchers will have access to your samples. The people who work at the facility will not have any information that identifies you. The approved researchers may be given information about you such as your age and sex, but they will not be given your name or any other information that identifies you. Your samples may be shipped to approved researchers who work outside of Zimbabwe, if the need arises for some specialized assays not available in Zimbabwe. There is no time limit on how long your samples will be stored but we anticipate during duration of the MDA.

RISKS and/or DISCOMFORTS:

There are few risks related to storing your samples. When tests are done on the stored samples there is a rare but possible risk to your privacy. It is possible that if others found out information about you that is learned from tests (such as information about your genes) it could cause you problems with your family (having a family member learn about a disease that may be passed on in families).

POTENTIAL BENEFITS:

There are no direct benefits to you from having your child's samples stored. You and others could benefit in the future from research done on these samples.

CONFIDENTIALITY:

To keep your information private, your samples will be labelled with a code that can only be traced back to your study clinic. Your name, where you live, and other personal information will be protected by the study clinic. When researchers are given your stored samples, they will not be given your personal information. Every effort will be made to keep your personal information confidential.

Efforts will be made to keep your study records and test results confidential. You will be identified by a code, and personal information from your records will not be released without your written permission. Any publication of this study will not use your name or identify you personally.

PROBLEMS OR QUESTIONS:

For questions about the storage of your samples, contact:

- The PI: Tawanda J Chisango
Biochemistry Department,

University of Zimbabwe
Churchill/University Ave
P.O. Box MP 167
Mount Pleasant, Harare
Ph: 0773633682

For questions about your rights as a research subject, contact:

- ***Medical Research Council of Zimbabwe
National Institute of Health Research***

Cnr Mazoe Street/ Josiah Tongogara Avenue

Harare

Ph :+263 4 791792, 791193

Cell : +263 912 433 166

SIGNATURE PAGE

CONSENT FOR SPECIMEN STORAGE

Please carefully read the statements below (or have them read to you) and think about your choice. No matter what you decide it will not affect whether you can be in the research study, or your routine health care.

_____ I agree to have samples of my child's blood sample, stored and used for future testing related to allergy & autoimmunity.

_____ I agree to have samples of my child's blood sample, stored and used for future testing related to allergy and autoimmunity. However, I do not agree to have genetic testing performed on my samples.

_____ I do not agree to have samples of my child's blood, stored and used for future testing related to allergy and autoimmunity.

Name of Child (please print)

Parent's/Guardian's Name (print)

Signature or Mark and Date

Study Staff Conducting
Consent Discussion (print)

Study Staff Signature and Date

Witness Name (print)
(As appropriate)

Witness Signature and Date

7.4: Informed consent form for for parental consent (Shona version)



MRCZ/A/1958

BVUMIDZO YEVABEREKI

MUSORO WECHIRONGWA

**KUONGORORWA KWEUVHUTIRO MUVANA VARIKURAPWA CHIRWERE
CHECHIPFUNGA**

Mutungamiri wechirongwa: **Mr T Chisango** (MSc)-0772731322

Vatevedzi : **Professor Takafira Mduluza**, [PhD]- 0773633682

Dr B Ndlovu. [PhD]- 27-31-788424574

Zvamunofanira kuziva nezvechirongwa ichi:

- Tinokupai chibvumirano ichi kuti muverenge pamusoro pechinangwa, zvingangonetsa, uye nezvingangobatsira muongororo iyi.
- Zvirongwa zvinoitwa zvinozobva pane ruzivo rutsva rwakanakisa rwatinenge tavanarwo
- Chinangwa chikuru cheongororo ndechekuwana ruzivo rutsva runozobatsira pane ramangwana.

- Hatingavimbisi kuti ongororo iyi ichakubatsirai.
- Munekodzero yekuramba kupinda/ kuve , kana yokubvuma kupinda izvozvi, uye yekushandura pfungwa dzenyu pamberi apo.
- Chero sarudzo yamaita, hazvikanganisi mabatirwo enyu enguva dzese
- Ndapota nyatsodzverai chibvumirano chino nemazvo. Bvunzai mibvunzo musati maita sarudzo- hamumanikidzwi kupinda.

Chinangwa cheongororo

Muri kukumbirwa mvumo yekuti mwana wenyu apinde muongororo iyi nokuti muri mubereki/ munhu mukuru anogara mudunhu rino. Muongororo/ mutsvakiridzo iyi tiri kuedza kuona zvinoitika kana munhu anwa mapiritsi anorapa bharaiziya . Vana vari muzvikoro ndivo vachange vachipihwa mapiritsi aya, zvichireva izvo vanogona kubuda munyawiri muviri wese. Isu vechirongwa chino tinoda kuongorora zvinokonzeresa munyawiri uyu.

Mafambiro echirongwa

Kana mabvumidza mwana kupinda muongororo/tsvakirodzo iyi muchange murimumwe wechikwata chevanhu vari munzvimbo dzakasiyasiyana vachange vachimirira vamwe vavo nezveongororo iyi yekuti vamwe vanobuda munyawiri asika vamwe hapanachinoitika. Tichabvunzazve vana vechikoro ava kuti tigoziva nzvimbo dzavanopinda mumvura. Izvi zvinotibatsira kuti tigoongorora kunobva chirwere ichi chebharazuya.

Zvinganetsa

Hapana zvinganetsa pakupinda muongororo iyi. Hurukuro ingangotora maminitisi mashanu kanasvika gumi enguva yavena. Tichatorazve ropa ringanhoita sipunu rimwe kana maviri ekuti tizoshandisa kuongorora zviratidzwa zvwanyawiri urwu.

Zvamunotarisirwa kuwana

Hapana chatingati muchawana semuripo nekuva kwenyu muongororo/tsvakiridzo iyi. Muchawana ruzivo rutsva runogona kukubatsirai kuchengetengetedza mhuri yenyu iri mutano. Zvekare, pfungwa dzenyu nezvamakasanganirana nazvo zvingangozobatsira kuti kuve nezvirongwa zvirinani zveutsana/kushambidzika munharaunda yenyu kana kune dzimwe nzvimbo dzenyika.

Muripo

Hamubadharwe kuva muongororo iyi/ hapana muripo nokuva mutsvangiridzo iyi.

Zvichabuda zvitsva muongororo

Tichagoverana nemi zvichawanikwa muongororo ino uye zimwe zvitsva zvatinenge tawana pamusoro pe tsvakiridzo iyi [yebharaziya nekurapwa kwayo nekubuda munyawiri].

Kuchengetedzwa kwezvinyorwa

Tichanyora pasi zita nekero yenyu, nozvimwewo zviri maererano nemi pamapepa. Hapana mumwe munhu anogona kuona zvinyorwa izvi kunze kwevari muongororo. Tichashandisa nhamba kwete zita, kuti vamwe vakuzivei. Magwaro ose ane zita nekero nezvimwe zviri maererano nemi zvamuno tiudza zvinochengetedzwa zvakakiyirwa, pane dzimwe nguva vaongorori vemitano yeongorori vangade kuona kuti ongororo iri kuitwa nemazvo here, ava ndivo vangazotarisa mapepa aya. Vanhu ava vanosanganisira veMedical Research Council of Zimbabwe kana kuti VeUniversity of Zimbabwe. Vese ava vanosungirwa kuchengetedza mazita enyu akavandika . kuchengetedzwa kwakasimbiswa hatingakukomakedze nekuti mapepa emaresearch /ongororo haangadzivisi kana achidiwa navehurumende kana vematare emutemo. Tichaedza nepatinogona kuchengetedza zviri maererano nemi zvakawandika. Zvinobuda semhinduro yeongororo iyi kana zvinoshambadzirwa, hazvibudi zvine mazita enyu kana remwana kana zvimwe zvinoita kuti muzivikanwe kuti makanga muri mune chino chirongwa.

Kuda kwenyu

Mwana haamanikidzwi kupinda muongororo iyi. Kana masarudza kuti apinde muongororo iko zvino makasununguka kumuti abude muongororo iyi chero nguva.

Kana muine mibvunzo monogona kundibvunza ndakasununguka kuipindura.

Munogonawo kubvunza vakuru vakuru [Professor Takafira Mduluzi] veongororo vane mazita akanyorwa pazasi apa kana paine zvamusanganzwisisi maererano neongororo iyi ikozvino kana mave muongororo

Ndivanaani vandinofonera kana ndiine mubvunzo kana dambudziko

Kana muine mibvunzo maererano neongororo taurai navakuru veongororo vanonzi ma[principal investigator] [Tawanda Chisango] panhamba dzinoti [0772731322]

Kana muine mibvunzo nezvekodzero dzenyu muchiongororo, ridzai rinhare kune
veMedical Research Council of Zimbabwe

Josiah Tongogara/Mazoe Street

Causeway, Harare

Telephone [04 791 193/ 7907 15/791791]

Zvinorevei kunyora [kusaina] zita rako

Ini , nyakusaina pazasi, ndaudzwa chinangwa, mafambiro, zvingangonetsa nezvingangobatsira muongororo iyi. Ndapihwa rimwe reiri gwaro rekugara naro. Ndapihwa mukana wekubvunza mibvunzo chero nguva ipi zvayo. Ndazvisarudzira uye ndabvuma kuti mwana ave muongororo iyi. Isarudzo yangu kuti mwana abude muongororo, ndinoziva kuti hazvikanganise basa rangu kana chinzvimbo changu munharaunda. Ndinovimbisa kuti ndichatevedza zvandinenge ndakumbirwa kuita navashandi, navakuru vakuru veongororo iyi. Ndinovimbisa kuudza vashandi veongororo pakare pakarepo kana ini ndiine zvinetswa pandinenge ndiri muongororo.

Zuva ramurikusainira mwana kupinda muongororo, zvichireva zuva ranhasi, RINOFANIRWA kunge riri mukati memazuva akaratidzwa pachidhindo chiri papepa rimwe nerimwe. Mazuva akaratidzwa aya anotaridza kuti pepa rino riri kufambiswa munguva yakatenderwa, kureva kuti muchange muri muongororo iyi.

Zita remwana (Nyorai nemavara makuru)

Zita remubereki (Nyorai nemavara makuru)

Zuva

Zita kana chiratidzo chearikumirira mwana

Zuva nenguva

Hukama nemwana arikupinda muchirongwa

Hwitinesi – pane avo vasingagoni

kuverenga kana kunyora

Zita remumiririri arikutora bvumo ino

Rimwe remagwaro echibvumirano ichi rakasainiwa rinofanirwa kuti 1] richengetedzwe mufaira nemukuru mukuru weongororo ,2] riphwe kune uyo asarudza kupinda muongororo ne3] riiswe mufaira rekurapwa kweuyo asarudza kupinda muongororo.

7.5: Informed consent form for parental consent



MRCZ/A/1958

INFORMED CONSENT FORM
FOR PARENTAL CONSENT

PROJECT TITLE

**Investigating Schistosomiasis Markers Of Inflammation And Immune Responses In
Individuals On Repeated Mass Drug Administration**

Principal Investigator: **Mr T Chisango** (MSc)-0772731322

Co-Investigator(s): **Professor Takafira Mduluza**, [PhD]- 0773633682

Dr B. Ndlovu. [PhD]- 27-31-788424574

ADD THE FOLLOWING PARAGRAPH TO ALL CONSENT FORMS

MORE THAN TWO (2) PAGES LONG (BEFORE ADDITION OF THIS PARAGRAPH)

What you should know about this research study:

- We give you this consent so that you may read about the purpose, risks, and benefits of this research study.
- Routine care is based upon the best known treatment and is provided with the main goal of helping the individual patient. The main goal of research studies is to gain knowledge that may help future patients.
- We cannot promise that this research will benefit your child. Just like regular care, this research can have side effects that can be serious or minor.
- You have the right to refuse to allow your child to take part, or agree for your child to take part now and change your mind later.
- Whatever you decide, it will not affect your child's regular care.
- Please review this consent form carefully. Ask any questions before you make a decision.
- Your choice to allow your child to participate is voluntary.

PURPOSE

You are being asked to allow your child to participate in a research study of **bilharzia treatment and allergy**. The purpose of the study is to observe your child before and after **treating bilharzia and observing if there is any development of allergy**. Your child was selected as a possible participant in this study because **this is the age that is commonly found infected with bilharzia and also their health and learning ability is greatly affected**. Also the children at this school will get the treatment from the currently bilharzia treatment underway throughout the country. We need to monitor their health status before and after the treatment is given. The information is important in our country for any likely development of allergy as caused by the worms when they die after treatment. We would like

to get information for preparedness in the future activities of the mass treatment exercise by The Ministry of Health and Child Welfare. **This study will have a total of about 600 children recruited to represent others in the schools and we will have school children recruited from selected schools in other provinces.**

MRCZ/A/1958

PROCEDURES AND DURATION

If you decide to allow your child to participate, your child will be asked a few questions taking less than 5 minutes so that we know where they get in contact with water in rivers or streams and if there are things that cause allergy in their daily lives. Thereafter the child will be asked to provide urine and stool in small bottles so that we will be able to diagnose if they are infected with bilharzia and some worms. This process will be done over 3 consecutive days so that we don't miss any infection. A small blood sample 2,5 -5 mls will be taken from them and will be used if they are infected with malaria and if there are markers that show any reaction to agents that cause allergy. The same procedure will be conducted before they receive treatment and 6 weeks after treatment to observe any changes to treatment and the development of allergic markers. We will monitor the children for any development of allergy throughout the exercise of bilharzia and worms treatment every year for the next 5 years.

RISKS AND DISCOMFORTS

The study does not involve any risks besides a temporary discomfort that may be experienced by child while taking venous blood due to the needle prick. However, this procedure is a common and normal as practised at most health centres and will be performed by qualified medical personnel from the Ministry of Health.

BENEFITS AND/OR COMPENSATION

Your child will benefit from this program by being one of the few children that will receive close monitoring and examination from the mass treatment of bilharzia and worm throughout the country. The study team cannot manage to examine closely everyone, the reason we have to select a few to represent others.

ALTERNATIVE PROCEDURES OR TREATMENTS

Praziquantel is the only drug available for treating bilharzia. Every child will receive this treatment for free. Treatment for the other worms would also be free.

CONFIDENTIALITY

If you indicate your willingness for your child to participate in this study by signing this document, any information that is obtained in connection with this study that can be identified with your child will remain confidential and will be disclosed only with your consent, and when appropriate, your child's permission. Under some circumstances, the MRCZ and the local Institutional Review Board may need to review patient records for compliance audits.

ADDITIONAL COSTS

There are no costs involved with this study

IN THE EVENT OF INJURY

In the event of injury resulting from your child's participation in this study, treatment will be offered by the study.

VOLUNTARY PARTICIPATION

Participation in this study is voluntary. If you decide not to allow your child to participate in this study, your decision will not affect your or your child's future relations with research group and this institution, its personnel, and associated hospitals. If you decide to allow your child to participate, you and your child are free to withdraw your consent and assent and discontinue participation at any time without penalty.

ADDITIONAL ELEMENTS

None

OFFER TO ANSWER QUESTIONS

Before you sign this form, please ask any questions on any aspect of this study that is unclear to you. You may take as much time as necessary to think it over.

AUTHORIZATION

YOU ARE MAKING A DECISION WHETHER OR NOT TO ALLOW YOUR CHILD TO PARTICIPATE IN THIS STUDY. YOUR SIGNATURE INDICATES THAT YOU HAVE READ AND UNDERSTOOD THE INFORMATION PROVIDED ABOVE, HAVE HAD ALL YOUR QUESTIONS ANSWERED, AND HAVE DECIDED TO ALLOW YOUR CHILD TO PARTICIPATE.

The date you sign this document to enroll your child in this study, that is, today's date, **MUST** fall between the dates indicated on the approval stamp affixed to each page. These dates indicate that this form is valid when you enroll your child in the study but do not reflect how long your child may participate in the study. Each page of this Informed Consent Form is stamped to indicate the form's validity as approved by the MRCZ.

Name of Parent (please print) _____
Date

Signature of Parent or legally authorized representative _____
Time

Relationship to the Participant

Signature of Witness

Signature of Research Staff

(Optional)

YOU WILL BE GIVEN A COPY OF THIS CONSENT FORM TO KEEP.

If you have any questions concerning this study or consent form beyond those answered by the investigator, including questions about the research, your rights as a research Participant or research-related injuries; or if you feel that you have been treated unfairly and would like to talk to someone other than a member of the research team, please feel free to contact the Medical Research Council of Zimbabwe on telephone 791792 or 791193.

MRCZ/A/1958