Diverse Antigen-specific
Antibody and Atopic Responses
In Schistosomiasis and Malaria
Co-infected Individuals.

A thesis submitted in fulfilment of the requirements for a Master of Philosophy Science degree. University of Zimbabwe.

By: Noah H Paul (R048084L)

Biochemistry Department, 2013

Supervisor: Prof. Takafira Mduluza
Co-Supervisor: Dr. Farisai Chidzwondo
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Abstract
The study was part of a huge schistosomiasis survey investigating schistosomiasis in the human model and was conducted in the Mashonaland East Province of Zimbabwe (31°30'E; 17°45'S) at Magaya and Chitate schools where S. haematobium is endemic and sporadic P. falciparum transmission. The study aimed at determining schistosomiasis and malaria infection prevalences, antibody response modulation during co-infections and the effect of helminths and helminth treatment on co-infections and allergic disorders. A community based longitudinal intervention study was conducted that involved examination and treatment of the study population at baseline, 6 weeks, 6 months, and 12 months follow up surveys. Urine and faecal samples were collected at baseline on three consecutive days and were processed for schistosomiasis using the Kato Katz, Formol-ether concentration method. Blood samples were obtained and the serum was separated and used to determine immunological profiles against diverse anti-parasite antigen (S. haematobium and P. falciparum). Malaria infection was detected by thick film slide microscopy. An allergen reaction test, skin prick, was done on mother and child only to determine the reaction to six different allergens on the child/parent’s forearm. A single dose of praziquantel was given to infected participants at recommended dose of 40mg/kg body weight. Data was captured using SPSS 8.0. The study established that the sampling areas differed in levels of intensity and prevalence of schistosome infection. Schistosome infection levels were significantly higher in Magaya (prevalence = 68%), than in Chitate (prevalence = 14%, p<0.001) with mean infection intensity of 58 eggs/10ml urine and 15 eggs/10ml urine respectively (p < 0.001). Overall, S. haematobium infection prevalence in the study population was 56%. All the participants investigated produced IgM antibodies directed against all the three life stages of the S. haematobium (cercariae, adult worms and eggs). Malaria infection was not detected by microscopy, and by rapid test kit, although anti-P. falciparum schizonts antibodies were detected. The prevalence of allergies in the population was 19 % and 12 % of those were allergic to the house dust mite allergy. The prevalence of autoimmune reactivity was significantly higher in Chitate the (WHO classification) moderate infection area 48% than in Magaya the high infection area 22% (p < 0.001). The prevalence of autoimmune reactivity was significantly lower in schistosome positive people compared to schistosome negative people (Magaya, p < 0.05 and Chitate p < 0.05). There was huge evidence that lack of knowledge was hampering eradication efforts from questionnaire assessment of the knowledge, attitudes and practices of the communities. Praziquantel was remained highly efficacious in schistosomiasis treatment, with 98% efficacy. The inclusion of infants into the praziquantel mass treatment scheme proved to be a safer risk to take as only 4/84 of those assessed complained of the side effects of praziquantel treatment. The detection of malaria in these low transmission set ups has to be augmented with sub-microscopic PCR detection, since the current treatment regimen and the low transmission pattern may make it difficult for correct diagnosis of malaria. The study also demonstrated that a balance between levels of atopic IgE and IgG4 plays an important part in regulation of atopic disorders. Anti-schistosome treatment with praziquantel did not result in an increase in atopic responses 6 months after treatment in children aged 6 months to 5 years of age. Praziquantel treatment resulted in a significant increase in parasite-specific IgE, which has been associated with resistance to re-infection. The success of control programmes in this area will be greatly increased by educating parents/guardians of children on bilharzia, malaria and other related parasitic infections.
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My sincere gratitude also goes to my mom, Elizabeth and dad, Augustine! Pastor Joey! To my little sister Lucy as well as my wife Kundayi.

This work is dedicated to my brother, Michael Ephraim.
Declaration

I Noah Herbert Paul do certify, that I did all the work published in this thesis. I also state I did the work on the collection of samples with the help of field orderly’s from the National Institute of Health Research.

Signed

[Signature]

Date _____/November_/2013

Supervisor

[Signature]

Date: ____/November/ 2013

Co-supervisor

[Signature]

Date: ___/November/ 2013
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Chapter One

Introduction
Chapter One: Introduction

1.0 Introduction.

Schistosomiasis or bilharzia is a tropical disease that can cause serious, long-term illness. Parasitic flatworms, called schistosomes, cause schistosomiasis. There are three major species namely; Schistosoma haematobium, (S. haematobium), Schistosoma mansoni (S. mansoni) and Schistosoma japonicum, (S. japonicum) and two less common species that produce infection in humans. Of the major species, S. mansoni and S. japonicum, can provoke intestinal and hepatic complications. Urinary schistosomiasis predominantly leads to renal and bladder complications, although occasionally, it results in liver disease. The minor species include S. mekongi and S. intercalatum, both of which can induce intestinal and liver disease. The intermediate host is the snail which lives in fresh water in the tropics, in which they must first be infected by the miracidium and mature in the freshwater snails, which are their “intermediate hosts”. Humans are infected by schistosomiasis through skin contact with contaminated fresh water in which certain types of snails that carry schistosomes are living for example the B. globusus that carries S. haematobium which causes urinary schistosomiasis. The parasites can penetrate the skin of persons who are using the water for washing or bathing, swimming, or work activities such as fishing, rice cultivation, or irrigation. After six weeks of infection, worms grow inside the blood vessels of the body and produce eggs. Some of these eggs travel to the bladder or intestines and are passed into the urine or stool (Demeure et al., 1983). The symptoms of infection are a rash or itchy skin. Fever, chills, cough, and muscle aches can begin within 1-2 months of infection. Most people have no symptoms at this early phase of infection mainly due to allergic modifications in innate immunity (Zaccone et al., 2003). The lack of more visible early symptoms of infection makes the worms so evasive that the immune response that is mounted will be complex. Later symptoms are related to the number and location of parasite eggs in the body.
Eggs travel to the liver or pass into the intestine or bladder, causing symptoms related to these organs. In rare cases, eggs can travel to the brain or spinal cord and cause seizures, paralysis, or spinal cord inflammation (Amiri et al., 1992; Strickland, 1994). The only best method to prevent the disease is vigorous human education and improvement on standards of living. An estimated 200 million people are infected worldwide (WHO, 2000).

The presence of widespread schistosomiasis in a country is usually a sign of problems in sanitary waste disposal and treatment. The long-term illnesses that result from the infection can have serious consequences for a country's socio-economic development. Schistosomiasis is an increasing problem around the world as countries build and develop new agricultural and water resources and as more people are exposed to infection (WHO, 1993).

The eggs of the schistosomes in the excreta of an infected person open on contact with water and release a parasite, the miracidium. To survive, this motile form must find a fresh water snail. In the snails, the parasites grow, reproduce, and are released into the water, where they can live for about 48 hours (Kloetzel, 1967).

In the snail host, the miracidium infects the host and produce cercariae. The snail then secretes the cercariae into the surrounding water. Cercariae penetrate an individual's skin within a few seconds, continuing their biological cycle once they have made their way to the victim's blood vessels. Within 30 to 45 days, the parasite is transformed into a long worm which is either male or female; the two live in copula for the rest of their lives. The female lays from 200 to 2000 eggs per day over an average of 5 years, according to the species (Butterworth et al., 1985).

Only about a half of the eggs are excreted in the faeces in intestinal schistosomiasis, or in the urine for the urinary schistosomiasis. The rest stay in the body, damaging other vital organs like the liver. It is the eggs and not the worm itself that cause damage to the intestines, the
bladder and other organs. The eggs cause pathology, the severity of which is related to the worm burden and intensity of the host response (Butterworth et al., 1985). Figure 1 below summarizes the lifecycle of schistosomiasis.

1.0.1 Schistosomiasis life cycle

![Figure 1. Life cycle of schistosomiasis showing the stages in the definitive and intermediate hosts. The shape of the ova according to the Schistosoma species is also shown distinctively and the shapes of the corresponding intermediate snail hosts. Adapted from CDC](https://www.cdc.gov/schistosomiasis/images/life_cycle_of_schistosomiasis.png)

Humans are infected by schistosomiasis through skin contact with contaminated fresh water in which certain types of snails that carry schistosomes are living for example the *B. globusus* that causes urinary schistosomiasis (Fig. 1.1). The parasites can penetrate the skin of persons
who are using the water for washing or bathing, swimming, or work activities such as fishing, rice cultivation, or irrigation. The most recent estimates suggest mortality rate to be directly attributable to schistosomiasis in sub-Saharan Africa at 280 000 per year (WHO, 2012). Millions of people have clinical symptoms which most of them will not be aware of as compared to other diseases like malaria. The World Health Organization estimates that up to 200 million people are infected world-wide, and 85% of these are in sub-Saharan Africa, 600 million more are at risk of infection, and thus, schistosomiasis remains an important public health problem (WHO, 2012). Schistosomiasis, *Plasmodium falciparum* malaria and the soil transmitted helminthiasis (STH) are found to be co-existent in the developing countries. These diseases have been associated with malnutrition, poverty and squalor, thus, sometimes referred to as diseases of the poor. They fall in the category of the neglected diseases. According to WHO, approximately 85% of the 200 million people infected with schistosomiasis inhabit in the sub-Saharan Africa (Chitsulo *et al.*, 2007).

It has been hypothesized that schistosomiasis co-infection with *Plasmodium falciparum* malaria and soil transmitted helminthiasis may modulate the immune response to schistosomes leading to increased susceptibility to clinical malaria severity of other cases (Buck *et al.*, 1978).

This hypothesis has resulted in a multitude of research of which the data is still largely conflicting, (Nacher 2013).

1.1 **Schistosomiasis epidemiology**

The disease schistosomiasis is found in these parts of the world; Africa, South America, the Middle East, in Southern China, and in Southeast Asia.
The Zimbabwean picture of schistosomiasis distribution is illustrated below, in Fig. 1.2 below.
The distribution of schistosomiasis in Zimbabwe: 1985 mapping

Figure 1.2: Distribution of schistosomiasis in Zimbabwe. The percentage distribution was reported by Taylor and Makura (1982). The distribution was divided into three regions according to prevalence with Area A: above 60%, Area B: 30 – 59% and Area C: below 30%
The distribution is mainly north eastern and southern Zimbabwe. We cannot rule out the presence of schistosomiasis infection in the western zone of Zimbabwe, as other publications by Ndhlovu and co-workers, have reported the presence of bilharzia in the west of Zimbabwe. The study area of Murewa is also under schistosomiasis according to the mapping of 1982 (Fig. 1.2).

School-aged children, including adolescents, and pre-school children tend to harbour the greatest numbers of intestinal worms and schistosomes and as a result experience growth stunting and diminished physical fitness as well as impaired memory and cognition (Woolhouse, 1998). The peak age group has been reported to be the age range 11-13 years (Anderson and May, 1993). Urinary schistosomiasis infection is also aggregated according to age and there is a convex age-infection intensity relationship both within and between helminth-infected populations. In general, worm burdens increase from birth with repeated infection events. Infection intensity peaks in adolescence, after which worm burdens decline; although most adults harbour some parasites due to non-sterile immunity and repeated exposure to infection throughout life (Smithers and Terry, 1996). Thus, children tend to be considered as susceptible, whilst adults are more resistant to new infection.

A comparison of the age-infection intensity relationship in areas with high infection intensity to areas with lower infection intensity provides the most convincing evidence for the role of protective immunity in determining aggregation patterns of urinary schistosomiasis. Researchers have shown that the age at which peak infection intensity occurs is younger in populations with higher intensity infection (Woolhouse et al., 1991; Mutapi et al., 1997). This pattern, known as the ‘peak shift’, indicates that exposure to a higher ‘dose’ of parasite proteins from a young age, as would be the case in areas of high infection intensity, accelerates the development of protective immunity meaning that resistance develops and
worm burdens decline at an earlier age within the population (Butterworth et al., 1994; Hagan, 1996).

These adverse health consequences combine to impair childhood educational performance, reduce school attendance, and account for the observation that hookworm and other helminthic diseases reduces future wage-earning capacity (Bleakley, 2007). Hookworm and schistosomiasis are also major determinants of reduced worker productivity (Christian et al., 2004). Such chronic, disabling, and often disfiguring effects of helminths translate into enormous poverty-promoting effects and represent a major reason why we still have poverty rooted in a downward cycle of destitution and neglect (Hotez and Ferris, 2006).

The high medical, educational, and economic burden of helminthic infections, together with their co-endemicity with malaria and AIDS, provides an important rationale for launching a global assault on parasitic worms (Hotez, 2007). However, the tools we currently have for controlling worm infections are limited. Only four drugs - albendazole, oxamniquine, praziquantel, and ivermectin - were developed to treat helminthiases (Chirac and Torreele, 2006), and of these, praziquantel is the most widely uses and highly efficacious against all helminthic diseases. Together with diethylcarbamazine and mebendazole, these drugs represent almost our entire pharmacopeia for combating the most common infections in the world.

The dearth of available anti-helminthic agents partly reflects the very modest commercial markets for drugs targeting human helminth infections. It also partly reflects how remarkably little we know about the unique biochemical metabolism of parasitic worms and the mechanisms by which worms evade human host defences, establish chronic infections, and cause adverse maternal and child health (Dunne et al., 1992; Crompton and Nesheim, 2002). Indeed, the diseases caused by infection with helminths are considered neglected tropical
diseases (NTDs), and the study of these diseases receives less than 1% of global research funds (Hotez, 2007).

1.2 Epidemiological resistance

It has been noted that there is usually a fall in the prevalence and intensity of infection in older age groups, which suggests an acquired immunity which is age related. Some evidence suggests that this may be due to reduced water contact but this in itself is not enough explanation (Taylor and Makura, 1987; Butterworth et al., 1992; Dessien et al., 1992). Human immune responses to schistosome infection have been characterized in detail, but there has been controversy over the relative importance of ecological factors (variation in exposure to infection) and immunological factors (acquired immunity) in determining the relationships between levels of infection and age typically found in areas where infection is endemic.

Independent effects of exposure and age on the rates of re-infection with *Schistosoma haematobium* after chemotherapy have been demonstrated in Zimbabwe (Woolhouse et al., 1991; Ndhlovu et al., 1996; 1998). This age effect could be the result of acquired immunity to infection. Indeed, allowing for variation in exposure and age, low rates of re-infection. Acquisition of this immunity seems to be related to the cumulative effects of repeated infection and provides only partial protection. These characteristics are consistent with immuno-epidemiological data for both *S. mansoni* and *S. haematobium* infections of humans (Dessein et al., 1992; Woolhouse et al., 1999).
1.3 Effector mechanisms of resistance

Immunological effector mechanisms are directed towards the schistosomula (Butterworth et al., 1987; Hagan et al., 1991). Antibody-dependent cellular cytotoxicity (ADCC) plays a central effector role in dealing with the adult worm and the eggs. IgE is involved and has been associated with resistance to human schistosome infections (Dunne et al., 1992). Other antibodies have been found to have key roles as well for example the IgG1 and 2 has been linked to schistosomula killing (Butterworth et al., 1985; Karanja et al, 1997).

1.4 Schistosomiasis control initiative

A World Health Assembly 2000 (WHA, 2001) resolution called for the frequent and periodic de-worming of children in developing countries. The call stressed on the inclusion of school-aged children, treating with albendazole or mebendazole and praziquantel, together with environmental control measures. Among the proven benefits of de-worming are improvements in child growth and physical fitness, cognition and school attendance, as well as improvements in haemoglobin and iron status. Since the adoption of the resolution, there has been an increasing effort worldwide to also target preschool children, given evidence that de-worming also results in significant improvements in their nutritional status, as well as in developmental milestones (WHA, 2001; WHO, 2001). In addition, antenatal de-worming increases maternal haemoglobin, resulting in greater rates of infant survival (WHA, 2001). In response to the WHA resolution, two important public–private partnerships, the WHO Partnership for Parasite Control and the Schistosomiasis Control Initiative (SCI), were established to implement national-scale de-worming programs (WHO, 1998). Additional plans are in progress to integrate de-worming with other neglected tropical disease-control measures (WHO, 1998). More recently, the WHO has been calling for schistosomiasis eradication (WHO, 2012).
Owing to the control of snail vector populations, the safe disposal of human excrement, and the availability of efficacious drugs, helminth parasites have been largely eradicated as a public health concern in developed countries. Zimbabwe has had vigorous campaigns against schistosomiasis. More anti-schistosomiasis projects have been established for example in the Chiredzi lowveld (Chimbari, 2013). Unfortunately, however, in developing countries, where these types of control measures are often not yet practical, helminths remain a significant biomedical problem.

1.5 Parasite survival in hostile environment

Many helminth parasites are long-lived and cause chronic infections. The immune response that develops during this time often proceeds to cause pathologic changes that in many helminth infections are the primary cause of disease. The parasites survive in an environment that we would assume to be hostile to them. They do so by tricking the host into developing an ineffective immune response, then finding a suitable niche for maturation and propagation and to do so without killing or unduly harming the host (Smithers and Terry, 1969). Conversely, the host has to ideally generate an effective immune response to expel the parasite, and minimize its harmful effects, while not sacrificing its ability to effectively respond to other pathogens.

The host immune system evolved in the context of a parasite-replete environment and the balance of immune effector and regulatory cell populations are at least partly a consequence of on-going responses to infectious organisms that can often simultaneously invade host tissues. Such dynamic host–pathogen interactions exist throughout much of the world, but in industrialized countries infectious diseases are better controlled because of increased hygiene, the administration of vaccines at an early age and the widespread use of antibiotics.
Although this has resulted in marked reductions in chronic and severe disease, recent studies indicate a possible adverse effect of this enhanced control of infectious diseases that leads to an increase in inflammatory disorders (Bentwich et al., 1996).

One mechanism by which this unfavourable result occurs has been suggested by an extension of the “hygiene hypothesis”, which proposes that a dysregulated immune response might develop in individuals who were not exposed to helminth infections, increasing the likelihood of the development of allergic and autoimmune diseases (Fallon and Mangan, 2007; Wilson and Maizles, 2007). Studies have supported this model as the administration of helminths can down modulate autoimmune and allergic inflammation, whereas clearance of the helminth infection can result in a resurgence of these diseases (Holt, 2000; Yazdanbakhsh and Matricardi, 2004). Understanding the helminth-induced immune regulatory mechanisms that control certain inflammatory diseases might point the way towards future treatments for these increasingly common immune disorders.

1.6 Antigens

The potential to use schistosome antigens in vaccination and immunodiagnostics has led to isolation and cloning of antigens that can be used in stimulating protective immune response (Capron et al., 2005), mostly the adult worm antigen vaccine candidates; for example, the glutathione S-transferase (GST) and the soluble worm antigen preparation (SWAP) have been emphasized. Egg antigens - the soluble egg antigen (SEA) play a huge role in pathogenesis (Bergquist and Colley, 1998).
1.7.0 Immuno-pathology of the helminth infections

Many helminth parasites are long-lived and cause chronic infections. The immune response that develops during this time often proceeds to cause pathologic changes that in schistosomiasis infections are the primary cause of disease (Terry and Smithers, 1975). In the portal vasculature, the female worms lay eggs that are intended for transmission across the intestinal wall into the gut lumen and from there to the outside of the host. However, because blood flow in the portal system is towards the liver, many of the eggs are carried to that organ where they become lodged in the sinusoids.

The egg antigens released induce a marked Th2 response that leads to the development of granulomatous lesions in the liver (Cheever et al., 2000). We have demonstrated the development of the granuloma in a hamster model (Paul et al., 2010, unpublished data) and the studies on the host-protective nature of these lesions has been demonstrated in a mouse model of infection with the human parasite. Infected mice that lack CD4+ cells are incapable of making granulomas and die due to the toxic effects on hepatocytes of certain egg proteins (Dunne and Doenhoff, 1983; Ammiri et al., 1992).

By surrounding the egg, the granuloma essentially segregates it from the hepatic tissue and allows continuing liver function. In the long term, as the eggs die and the granulomas resolve, fibrosis can develop (Cheever et al., 2000). This can lead to increased portal blood pressure and the development of portal varices. Bleeding from varices is the most common cause of death due to schistosomiasis. Analysis of the role of Th2 cytokines during infection using the mouse model of human schistosomiasis has revealed that IL-13 plays a role of central importance in the development of fibrosis (Chiaramonte, 1999; Cheever et al., 2000). Treatment with soluble interleukin-13 receptor (IL-13R), which inhibits the effect of IL-13 (Chiaramonte, 1999), or immunization prior to infection with egg antigen plus IL-12 to
induce a Th1 response and thereby minimize production of IL-13 (Wynn, 2003) significantly ameliorates fibrosis and morbidity.

The situation in humans is less clear, but segregation analysis applied to the severe fibrosis associated with portal hypertension phenotype revealed the effect of a major gene that mapped to 6q22-q23, close to the gene that encodes the alpha chain of the gamma interferon (IFN-γ) receptor (Dessein et al., 1999). This result is consistent with findings in murine schistosomiasis where IFN-γ and the Th1 response can protect against severe fibrosis by preventing alternative macrophage activation and thereby limiting the fibrosis-enhancing effects of the Th2 response (Hoffman et al., 1998; Hesse et al., 2000).

Examination of the immunology of helminth infections reveals a number of characteristics that are generally conserved across all species. The current understanding of the immune response to helminth infections has largely come from the study of well-defined laboratory models of infection in rodents. Protective immunity to helminths depends on T lymphocytes. It is now well established that the CD4+ subset of T cells plays a major role in the generation of the host protective response that expels the worms, and that CD4+ T cells regulate many of the inflammatory and immune parameters that accompany expulsion of the parasites from the gut.

Th1 cells produce cytokines that are involved in controlling intracellular pathogens. They also contribute to inflammatory responses (Harnett and Harnett, 2006; Voehringer, 2006). Th2 cells contribute to B cell activation and antibody production, eosinophil differentiation and recruitment. Therefore, parasitic worm infection is often associated with high levels of IgE, IgG1 and IgG4, and robust eosinophil and mast cell responses. Th2 immune responses to helminth infections can prevent the survival of invading parasites during a homologous secondary infection (Finkelman et al., 2004), expel adult parasites from the gut (Dillon et al.,
2004) and allow host to survive even if the immune response has failed to eradicate the parasites. The Th2 immune response can also cause serious pathology and liver damage (Finkelman et al., 2004; Mduluza et al., 2008) a strong Th2 response can cross regulate by the suppression of Th1 and modulate diseases that are characterised by a Th1 response (Ndhllovu et al., 2006).

1.8 Plasmodium falciparum malaria

Malaria cases are about 300 to 500 million per year, causing an estimated 1-2.7 million deaths (Malaney, 2002). Ninety per cent of these deaths occur in sub-Saharan Africa, mostly among children younger than five years (WHO, 1999). Malaria is endemic to over 100 nations; Africa and territories in Asia, Latin America, the Middle East, and the South Pacific. It is caused by a protozoan parasite that is transferred by the bite of an infected Anopheles mosquito (Fig. 1.3). Plasmodium falciparum (P. falciparum) is by far the deadliest of the five vectors causing human Plasmodia malarial species. Other species include malariae (P. malariae), ovale (P. ovale), vivax (P. vivax) and recently knowlesi (P. knowlesi). Of the plasmodia, P. vivax is the most widespread. P. malariae and P. ovale, although also significant, cause fewer cases and less severe forms of the disease. Symptoms of malaria include fever and shivering, pain in the joints, headache, weakness, and repeated vomiting. In severe cases, convulsions and kidney failure can result (Hoffman, 1996). Complications of P. falciparum malaria include acute anaemia and cerebral malaria. In some patients who seemingly recover, another attack of malaria may occur if the treatment does not completely clear the parasite from the blood and liver (Gallup and Sachs, 2001).

P. falciparum destroys red blood cells, which can cause acute anaemia that makes it worse than other types of malaria. Adherence to cells in certain tissues may cause problems within
those organs, such as the lungs, kidneys and brain. A major complication of *P. falciparum*, cerebral malaria, can lead to coma, transient or permanent neurological effects, and death. Compared to *P. vivax*, *P. falciparum* is less widespread, but more likely to result in severe complications and be fatal. Below is a summary of the vicious malaria life cycle.

**Malaria life cycle**

*Figure 1. 3:* Malaria life cycle showing detailed developmental stages of the parasites in the human host and the insect vector. *Adapted from CDC.*

### 1.8.1 Malaria epidemiology in Zimbabwe

Malaria remains an important public health problem in Zimbabwe, with transmission being generally unstable and seasonal. *Plasmodium falciparum* is the major primary species of malaria parasite, accounting for 97% of confirmed cases, with *P. ovale* and *P. malariae*
occurring in 3% of cases, sometimes in mixed infections (Roll Back Malaria, 2004). The main vector mosquito is *Anopheles arabiensis*. The highest transmission occurs along international border areas, especially in the north to Zambia and the East to Mozambique. The borders to the west to Botswana and to the south, South Africa support little transmission but are epidemic prone.

The central highlands are largely malaria free (GoZ/RoC, 2006). Stratification of malaria transmission patterns by district was undertaken in 2002. The classification of malaria transmission in Zimbabwe is relative to other areas of the country. Even the areas of highest malaria incidence in Zimbabwe fall below the international criteria for "low transmission 3". Nonetheless, as transmission drops with the scaling up of key interventions, a larger proportion of the population will be at risk of unstable and epidemic malaria (GoZ/WHO, 2008). Thus, the move to universal access for core interventions means that the entire population in affected areas is now targeted (Roll Back Malaria, 2004).

In Zimbabwe, 72% of the population lives in malaria unstable areas, whereas 28% is under the malaria free region. National malaria incidence was 126 cases per 1000 persons per year in 2007 (GoZ/UNICEF, 2007). The number of cases of clinical malaria/fever reported annually ranges from approximately 1.5 million to 1.8 million cases per year (GoZ/UNICEF, 2005).

1.8.2 Malaria control initiative and immunity

Research has been currently centred on trying to understand the interactions between the malaria parasites with the human host and understanding how immunity to malaria is acquired. This aspect remains fundamental to malaria vaccine development research program. Immune responses of children and adults to malaria have shown the distribution of IgG
antibody subclasses that has led to suspicions of the role of helminthic infections on acquisition of immunity to malaria (Roussilhon et al., 2000; Druilhe and Sokhna, 2005, Mutapi et al., 2008). A cascade of observations led progressively over time to corroborate this suspicion. The pattern of acquisition of resistance to malaria and the role of antibodies should be reminded first. Children in hyper endemic areas are at high risk of dying from malaria until the age of five. Though this risk then decreases, they remain susceptible to malaria attacks until they reach the ages of 15–20 years (McGregor and Wilson, 1988). By the time they are young adults, those who have survived achieve a remarkable state of premunition where they are able to control parasite growth below the threshold at which clinical symptoms occur. They have acquired immunity, but at remarkably low speed. It has been conceptually difficult for a long time to understand the reason for this long delay: the question being how malaria antigens could be so poorly immunogenic that daily exposure to outstandingly high parasite loads for many years would be required before individuals are protected against the disease. This immunity is due to antibodies (Theisen et al., 2008).

Protection can be induced by passive transfer of IgG from malaria-immune adults to malaria patients. In order to understand the underlying mechanism antibody responses between those protected and a study to compare those not protected was carried out (Sabchareon et al., 1991). It was found that this protection was associated with a novel immune mechanism called ‘‘Antibody Dependant Cellular Inhibition (ADCI)’’ in which effective antibodies act in a monocyte (MN)-mediated manner (Bouharoun-Tayoun et al., 1990).

Among non-protected individuals aged 1 to 15 years, non-cytophilic classes of antibodies IgG2, IgG4, and IgM are the most abundant. This is in stark contrast to responses in protected adults who have been found to have twice as much cytophilic IgG1 and IgG3 subclasses compared to the non-cytophilic classes (Bouharoun-Tayoun and Druilhe, 1992). Thus,, the balance of antibodies is more critical for protection than their abundance. This
indicated that those protected individuals had acquired the ability for an IgG class-switch from a predominantly non-cytophilic to a cytophilic predominance. Further studies pinpointed IgG3 against MSP3 as the antibody response most strongly associated with protection and how helminth infections may alter responses to malarial antigens (Roussilhon et al., 2000).

The helminths have been postulated to play a vital role in modulating immune response during vaccination the impact of worm load and the extent of infection still remains to be investigated in malaria surveys or Phase IIb vaccine trials, (Christian Roussilhon, 2010).

1.8.3 Malaria and helminth co-infections

Approximately two billion people living in malaria-endemic areas also have various helminth infections. Concurrent infection with malaria and helminths is common. Both non-synergistic and protective benefits of co-infections have been reported in human populations (Lwin et al., 1982; Helmy et al., 1998; Yoshida et al., 2000 Nacher, et al., 2002; Spiegel et al., 2003; Chitsulo et al., 2004; Le Hesran et al., 2004; Sokhna et al., 2004; Noland et al., 2005). In animal models, similarly mixed profiles have been described. Although the mechanisms contributing to protective outcomes are not clearly defined, significant modulation of Th1 cytokines and anti-malaria antibodies has been reported during co-infection (Mutapi et al., 1997; Woolhouse et al., 1991; Gandhi, 2001). We therefore hypothesized that such alterations to host response during concomitant helminth infection may also modulate the intensity of malaria parasite transmission, also because helminth infection has been found to increase the duration of malaria parasitaemia (Anderson and May, 1993; Strickland, 1994).
There has been a general frame shift approach in the epidemiological investigation of polyparasitism, with a particular focus on multiple helminth species and more recently, on Plasmodium-helminth co-infection (Booker et al., 2000; Midzi et al., 2008, Midzi et al., 2009). Studies across multiple epidemiological settings have shown recently that polyparasitism is the norm rather than the exception and occurs at different frequencies than would be expected under assumptions of independence (Booth and Bundy, 1995; Booth et al., 1998b). Interactions between parasites in humans can be synergistic or antagonistic. For example, studies have demonstrated a positive association between intensity and concurrent infection of helminth species, suggesting that individuals harbouring multiple helminth species also harbour the most intense infections (Brooker et al., 2000). It is conceivable therefore, that polyparasitism may have a greater impact on morbidity than single species infections since morbidity is typically related to infection intensity for most parasite species.

Multiple species infections may also increase susceptibility to other infections. However, the health impacts of polyparasitism have not been studied sufficiently despite their potential significance for public health. The geographical and socio-economic distribution of malaria overlaps with areas in which a number of helminth parasites are also endemic. It is the norm in these areas for co-infection to occur and a growing body of literature reflects this (Cox, 2001; Spiegel et al., 2003; Mwangi et al., 2006). The influence of co-infection on the immune response may result in either exacerbation or amelioration of disease (Spiegel et al., 2003; Sokhna et al., 2004; Lyke et al., 2005). It is therefore crucial to understand the host-parasite relationship in the context of multiple infections, if vaccine design and drug administration programmes are to be managed effectively (Hotez et al., 2006). Animal models accurately reflect many pathological aspects of malaria-helminth co-infection with
regard to impact on disease outcome and also provide the opportunity to further examine immunological mechanisms in detail (Su et al., 2005).

A strong rationale for developing a vaccine that simultaneously targets both hookworms and schistosomes has been raised, due to the similarities in the patho-biology of both parasites, their ability to cause anaemia and their co-endemicity in sub-Saharan Africa, Brazil and East Asia. A multivalent anti-helminthic vaccine for hookworm infection and schistosomiasis would represent an important new tool for combating the poverty related disease. Hookworm and schistosomiasis are also important diseases during pregnancy, causing neonatal prematurity, reduced neonatal birth weight, and increased maternal morbidity and mortality. In sub-Saharan Africa, the helminthiases are frequently co-endemic with malaria (Kaiser et al., 2002). Indeed, it is a frequent case for an individual to be co-infected with the malaria-causing parasite and one or more parasitic worm (Needham et al., 1998). Such co-infections have additive effects, such as severe anaemia (Maizles et al., 1998) and synergistic effects, such as increased transmission of the malaria-causing parasite, HIV and/or increased susceptibility to infection with these pathogens as well as cause an exacerbated progression of these two killer diseases (Steinmann et al., 2006).

1.8.4 Immuno-pathology of malaria co-infection with schistosomiasis

Helminth infections are prevalent throughout the tropical regions where malaria parasites are also transmitted. Co-infections with helminths and malaria parasites are frequently observed, and it is therefore important to consider that hypo-responsiveness caused by helminth infection might affect the immune response against malaria parasites and the course of infection. In murine models of malaria infection, concomitant infection with S. mansoni resulted in increased malarial parasite load. Although schistosome parasites are
phylogenetically distinct from nematodes, helminth co-infection studies that investigate *S. mansoni* provide evidence that cross-reactivity is relevant in other co-infection systems and can have a strong impact on disease severity. Naus and co-workers (2003), reported the induction of cross-reactive IgG3 antibodies that recognize both *Plasmodium falciparum* and *S. mansoni* antigens. Later the *S. mansoni* antigen (SmLRR) was identified that is recognized by both malaria and *S. mansoni* singly infected hosts. Interestingly, it has been observed that the two infections induce different antibody isotypes to antigen: cross-reactive malaria driven IgG3 and helminth driven IgG4.

In areas co-endemic for these two parasites, exposure to malaria and subsequent induction of the cross-reactive IgG3 response seems to increase the risk of developing hepatosplenomegaly in schistosome infected individuals (Naus *et al.*., 2003). Although schistosome parasites are phylogenetically distinct from nematodes, helminth co-infection studies that investigate *S. mansoni* provide evidence that cross-reactivity is relevant in other co-infection systems and can have a strong impact on disease severity.

There have been very few studies of immunological parameters during co-infection with helminths and *Plasmodium falciparum* malaria (Nacher, 2002). Since so many people are infected with helminth parasites, there is little doubt that many individuals become exposed to non-helminth pathogens while harbouring chronic helminth infections. Individuals living in areas where helminths are endemic often carry more than one species of worm infection.

Egg output for an individual helminth species is often higher in individuals carrying mixed infections than in individuals carrying single-species infections (Booth *et al.*, 1998; Needham
This may reflect higher intensities of infection and so a higher risk of morbidity. It is clear from existing reports that helminth infection can make mice far more susceptible to certain pathogens against which Th1 responses are protective and more resistant to pathogens against which Th2 responses are protective (Curry et al., 1995; Marshall et al., 1999, Karanja et al., 1998).

Significant associations have also been demonstrated between helminth infections that appear not share obvious transmission pathways and some of these associations are not explained solely by household or environmental effects (Ellis et al., 2007). Furthermore, the intensity of helminth infections alters the risk of multiple-species infection (Howard et al., 2002, Tchuem et al., 2003; Ellis et al., 2007) indicating that immunological and/ or genetic factors may be involved in regulating resistance and susceptibility to helminth-helminth co-infections.

There is speculated possibility that co-infections with Schistosoma and Plasmodium species may have synergistic effects on organ pathology. In a study in Kenya, children with the highest levels of anti-\textit{P. falciparum} immunoglobulin IgG3 and highest \textit{S. mansoni} egg counts were significantly more likely to present with splenomegaly (Booth et al., 2004b).

1.9 Co-infections and allergic disorders

An issue of interest is that of how the presence of on-going helminth infection affects the likelihood of the development of immunological disorders, such as autoimmunity and allergy. It has been hypothesised that in the cleaner environments, allergic disorders prevalence is high as compared to the dirtier environment. Data from studies addressing the issue of autoimmunity are still descriptive but giving some clues. It has been argued that a failure to acquire helminthic parasites with the decreased prevalence of helminthiasis results in
increased prevalence of autoimmune intestinal diseases and other forms of autoimmunity (Aderem and Ulevitch, 2000). The relationship between immune responsiveness to allergens and/or allergic symptoms and helminth infections is interesting and yet puzzling to immunologists (Sibanda, 2003). There are several possible explanations for this paradox, including the production of IgG antibodies that block access of allergenic antigen to specific IgE (Aderem and Ulevitch, 2000; Else et al., 2000), but in an exciting development, studies have correlated increased IL-10 levels resulting from chronic urinary schistosomiasis with reduced expression of house mite allergy in African children (Dunne et al. 1992, Else et al. 2000).

Helminth infections may induce strong IgE responses, which in combination with high antigen levels would be expected to lead to allergic symptoms and possibly anaphylaxis (Araujo et al., 2000; Knopf, 2000). However, helminth-infected individuals rarely have allergic reactions to these parasites and appear to suffer less from allergic disorders in general than do helminth-free individuals (Lynch et al., 1999). One possible explanation for this paradox is production of IgG antibodies that block access of allergenic antigens to specific IgE. In an exciting recent development, studies have correlated increased IL-10 levels resulting from chronic urinary schistosomiasis with reduced expression of house mite allergy in African children (van den Biggelaar et al., 2000; King, 2001; Yazdanbakhsh et al., 2001).

1.9.1 Vaccination and co-infections

Work on vaccine development against certain helminth parasites are already in progress, with schistosomiasis perhaps receiving the most attention. This program has led to the development of several defined vaccines against schistosomes that have been tested in animals and at least one of which is now in human trials (Capron and Capron, 1992;
Bergquist and Colley, 1998). While this represents a significant step forward, it is important to note that there is no certainty that any of these vaccines will reach the market and that during development these vaccines have been shown to be only partially effective. The challenge remaining is to renew momentum for continued work on vaccine development.

The hope is that, in conjunction with a greater appreciation of immune response induction mechanisms, our understanding of basic helminth biology, the nature of polyparasitism and co-infections in an individual will improve sufficiently in the coming years and allow the rational development of more efficacious vaccines (Maizles et al., 1999). Intriguingly, the application of anti-helminth vaccines is more advanced in veterinary medicine than in human medicine, with a history of the use of attenuated vaccines against certain nematode parasites and more recently the development of recombinant protein and DNA vaccines for example against the sheep tapeworm *Taenia ovis* (Drew et al., 2001). Concurrent helminth infection alters optimal vaccine-induced responses in humans and livestock; however, the consequences of this condition have not been adequately studied especially in the context of a challenge infection following vaccination.

Demands for new and effective vaccines to control chronic and emerging diseases, and the need for rapid deployment of vaccines for bio-security concerns requires a systematic evaluation of confounding factors that limit vaccine efficacy (Kullberg et al., 1992). One common albeit overlooked confounder is the presence of gastrointestinal nematode parasites in populations of humans and livestock targeted for vaccination. This is particularly important in areas of the world where helminth infections are prevalent, but the interplay between parasites and emerging diseases that can be transmitted worldwide make this a global issue.
1.10 Framework for the current research study

The quantitative and epidemiological information has given so much information on age- and exposure-time dependent acquired resistance to the schistosomes, *Plasmodium falciparum* malaria infections and the soil transmitted helminthiasis in man. A little information has been given on their co-infections and co-habitation in a single host. The information available is still falling short to paint a full visible picture of the role played by the immune response to regulate those interactions between the host and the parasite that give rise to pathological cases. Those tools will be so important in vaccine development and improved diagnosis as well as an effective drug administration. The present study was oriented to cover the entire age range in the population infected with the schistosomes, malaria and the soil transmitted helminths. With the overall goal being to understand the immunology of co-infections and contribute new information on immunological mechanisms and elucidate synergies and antagonisms associated with co-harbouring these diseases in one host. The findings would add an understanding on the markers of immunity to native habitants of the area who have proved to be resistant to chronic schistosomiasis and malaria together with hookworms which are prevalent in their area.

1.10.1 Study Rationale

Successful studies have been carried out in the animals but with a few studies done on the human host about polyparasitism. Schistosomiasis, malaria and soil transmitted helminths are prevalent within our communities and eradicating them will improve the health and social well-being of the communities in the endemic areas. Schistosomiasis has been in existence for quite a long time now, but no vaccine has yet been developed for this disease, therefore, there is need to investigate correlates of immunity in a way that would enhance vaccine development targeted at any of these helminthic diseases. Although sanitation and clean
water are traditionally considered important factors in the control of parasitic helminth infections, their sole impact on reducing the intensity of schistosomiasis is often minimal, especially in the absence of other public health interventions. The vaccines that have been designed and put to trial have mostly targeted reduction in morbidity, rather than sterile immunity, a stronger argument to produce only a partially protective vaccine has been put forward (Hagan et al., 1991). Vaccines have proved an excellent means of cost-effective control of many infectious diseases. Rapid re-infection, about 6 weeks in endemic areas, demands continuing treatment and drug delivery requires an infrastructure which must be both elaborate and reliable in the long term. Expanded chemotherapy programmes increase the risk of drug-resistance; and a control approach based on chemotherapy followed by vaccination would integrate short-term effect with long-term protection (Mduluza et al., 2001, Mutapi et al., 1998). Thus, understanding the immunology of individuals in health and disease will help complete the puzzle for vaccine development strategy.

1.10.2 Why study protective immunity

The anti-helminthic drugs currently being used though curative, have presided over high rates of re-infection and numerous rounds of mass treatment will be necessary to reduce the levels of infection below those necessary to sustain transmission. Indeed, it can be anticipated that such alterations in the levels of infection in communities might have a dramatic impact on the degree of their immunity to helminths. This could result in either a higher degree of protection against re-infection with schistosome worms, or conversely, resulting in less protection through drug resistance and becoming a potential impediment to elimination.

The better understanding of the protective immune mechanisms active in helminth-endemic populations is thus, important not only to make more precise predictions about the eventual
success of elimination efforts, but also to alert the national control programmes to the potential problems that might arise from altered immunity in treated communities. In addition, the re-infection despite treatment also argues for sustaining the efforts to generate a vaccine that might still be a cost-effective way both to boost the effectiveness of drugs in eliminating helminths and then to help to prevent its recrudescence, particularly as the force of infection.

Therefore, the main study hypothesis was that:

During co-infection, of schistosomiasis and malaria, Praziquantel treatment alters schistosomes/malaria-specific antibody responses and from the hygiene hypothesis- that praziquantel treatment increases atopic responses.

Other hypotheses in the study were:-

1. Schistosome infection is associated with morbidity in the age group 5-15 years (evidenced in haematuria, proteinuria and stunted growth).

2. Praziquantel treatment alters effector immune responses.

1.10.3 Questions to be addressed in this research

We set out to investigate, how is it that individuals living in the same area with similar water contact patterns and activities show different susceptibility to helminthic infections? Given that all other parameters are constant, what may be important is to understanding the human immunity. How is it that some individual residents of transmission area develop immunity to infection early and in contrast to corresponding individual within the same area? Do these infections exist singly or as co-infection? We know that each helminth has its own pathological outcome. Is there synergism against certain parasitic antigens leading to
immunomodulation of helminth/protozoan malaria illness during co-infections? What are the immunological responses during single parasitic infection and during co-infection and against the different parasite life cycle stages? What are the profiles of selected antibodies to different parasite life cycle stages and the immune response contribution to protective immunity; and how do the profiles change post treatment?

1.10.4 The overall aim of the study:
To determine the parasite infection intensity, prevalence levels, and co-infection profiles of the recruited study population, and to investigate the impact of anti-parasite treatment on polyparasitism on the immune and allergic responses.

1.10.5 The specific objectives:
We were determined to observe the distribution of polyparasitism in the study area through determination on presence of ova in urine and stool samples and parasites in blood samples. We also wanted to examine presence of co-infection in term of schistosomiasis and Plasmodium falciparum infections. To determine humoral immune responses profiles to different parasite life cycle antigens before and after treatment with anti-parasitic drugs. Since treatment would be an integral aspect, we set out to understand the effect of treatment on the immune responses and during re-infection. To observe the allergic reactions in a selected population in the participants in the area that is also exposed to parasitic infections and to understand the knowledge of parasitic infections in the community and also the contribution to the general health of a selected children cohort.
Chapter Two

Materials and Methods
Chapter two: Materials and Methods

2.0 Study area and population

2.1.1 Study design
The study design employed was a community based longitudinal intervention study that involved examination and treatment of the study population at baseline, 6 weeks, 6 months, and 12 months follow up surveys, respectively.

2.1.2 Sample size
The sample sizes for the study was calculated using the previous prevalence of S. haematobium, 58.7% and malaria, 23.5% observed in Murewa district from previous studies (GoZ, 2000). The following formula was used: \( n = \left(\frac{z}{\delta}\right)^2 P (1-P) \), where \( n \) = the sample size required, \( Z \)-statistic = 1.96, \( \delta \) (margin of error) = 0.05 and \( P \) = proportion or prevalence of the disease (58.7%, 23.5% respectively). The optimum sample size (\( n = 373 \)) was estimated. This was adjusted by 30% to \( n = 485 \) considering possible loss due to follow up.

2.1.3 Inclusion and exclusion criteria
All children from grades 1 through to 7 and secondary school children were eligible for the study. Children severely sick and those who could not provide stool, urine or blood samples were excluded from the study.

2.1.4 Ethical consideration
The Medical Research Council of Zimbabwe gave ethical approval and the University of Zimbabwe gave institutional approval for the study. In addition, provincial, district medical
and education directors, chiefs, councillors and village headmen granted permission. General information regarding the nature of study and objectives was explained to the community and study participants.

Feedback and consent was sought at schools and village meetings. Inclusion of children into the study took place after free individual, parental and school authority informed consent. Children joined the study voluntarily and were allowed to drop out at any time they wished without any prejudice.
2.1.5 The study area

The study was conducted in the Mashonaland East Province of Zimbabwe (31°30’E; 17°45’S) where *S. haematobium* is endemic. It was part of a huge survey by our group to try and understand the schistosomiasis from the mouse model to the human model. The study was mainly aimed at evaluating the hypotheses arising from schistosomiasis studies in mouse models. Mashonaland East Province was conveniently chosen for the study due to its geographical location characterised by high annual rainfalls, wet soils and malaria endemicity (*Zimbabwe National Health Profile, 2002*). These conditions are conducive for survival of schistosome species, STHs and malaria vector mosquitoes (*Midzi et al., 2009*).

Mashonaland East was also conveniently chosen due to the known high schistosomiasis endemicity in the province (*Taylor and Makura, 1985*). Multistage sampling technique was used to select the districts, wards, and schools in which the study was conducted. Using rotary method, Murewa District was randomly selected from seven districts in Mashonaland Province. Schools in Ward 7 were listed and three primary schools selected using the same technique. Every child at each school was eligible except those who were not willing to participate in the study.

The study area is characterized by high temperature ranges 20 °C to 32 °C. The participants have been involved in an on-going study of the immune-epidemiology of human schistosomiasis (*Mduluza MRCZ/ A/1408; 2008*). Permission to conduct the work in the province was obtained from the Provincial Medical Director (PMD) and informed consent obtained from all participants or their guardians/parents in case of children. The villages were selected because health surveys regularly conducted in the region by the PMD showed presence of infection with very low helminths and *S. mansoni* prevalence (<5%). Prior to our
study the selected villages had not been included in the National Schistosome Control Programme which is run by the Ministry of Health and Child Welfare in Zimbabwe and therefore had not received treatment for schistosomiasis or other helminth infections; meaning that we could study natural immune responses in the absence of drug-altered schistosome responses.

The main activity in these villages is subsistence farming and human water contact is frequent with at least four contacts per person per week, due to insufficient safe water sources and sanitation facilities (Mutapi et al., 2007). Drinking water is fetched from open wells while bathing and washing is conducted in the main rivers running through the villages. Most families maintain a garden located near the river where water is fetched for watering the crops, further increasing water contact. The schools surveyed were secondary school and its feeder primary school; Magaya and Chitate Schools in Murewa were both in close proximity to rivers.

2.1.6 The study population

2.1.6.1 Children under 5 years’ recruitment

Mass helminth control programmes as well as immunology studies in children in Africa are largely school based so as to take advantage of existing infrastructure serving an accessible population. These children tend to be aged 6 years and above which means non-enrolled children below the age of six were being excluded from such programmes. To date no studies have detailed the implementation of, or epidemiological, pathological, immunological consequences of praziquantel treatment of children aged 5 years and below.

Therefore, in this study we set out to investigate this niche. In the view of other studies that have demonstrated that; i.) Praziquantel works synergistically with the host’s immune system and treatment with praziquantel releases a large dose of parasite antigens to the
immune system at a single time point, ii.) Praziquantel treatment alters schistosome specific responses and; iii.) Treatment of helminthic infections can increase atopy;

The investigation proposed to assess the efficacy, safety, and effect of praziquantel treatment on immune responses directed against schistosome and unrelated (allergens) antigens in this neglected age group.

2.1.6.2 Children under 5 years’ hypothesis and questions

The work was intended to answer the following questions: we wanted to establish if pre-school children also infected with helminths? And if they do, do pre-school-age children need treatment? Finally we wanted to ascertain what will be the immunological consequences of treating children under the age of 5?

The hypotheses were:

Schistosome infection is associated with morbidity in this age group (haematuria, proteinuria and stunted growth). Praziquantel causes adverse reactions immediately after treatment in schistosome-infected children. Praziquantel treatment increases atopic responses and alters schistosome specific responses.

2.2 Parasite material

Lyophilised soluble S. haematobium cercariae (CAP), egg (SEA) and adult worm antigen (SWAP/WWH) were obtained from the Theodor Bilharz Institute (Egypt) and reconstituted as previously described elsewhere (Mutapi et al., 1997; Mduluza et al., 2001). Sh13 and P.
*falciparum* schizont were obtained from Edinburgh courtesy of Dr F. Mutapi. The parasite strain is one used for previous *S. haematobium* immune-epidemiology studies in this and other populations in Zimbabwe (Mutapi *et al.*, 1997, 2007; Mduluza *et al.*, 2001).

### 2.3 Parasitology samples from all participants

To aid our sampling, class registers were obtained from the school teachers and names extracted for recruiting. Every child in the school register was eligible to participate if willing. The students were assigned study identities that remained the same up to the end of the study. The method of obtaining stool and urine sample was clearly demonstrated to the participants, with children under six years and below getting help from the senior students as well as monitoring by their class teachers. The participants were each given urine and stool specimen bottles labelled with their respective number in the study. Stool and urine specimens were collected from each participant on 3 consecutive days and assayed for *S. haematobium*, *S. mansoni* and geo-helminths using standard procedures as detailed below.

Intensities of *S. haematobium* infection were calculated from at least 2 urine samples for a maximum of 3, collected over three consecutive days, and those of *S. mansoni* calculated from mean intensities of at least four Kato-Katz slides for a maximum of 6, two from each of the 2–3 stool samples collected over consecutive days. The participants were diagnosed as schistosomiasis positive if a single egg of urinary schistosomiasis was observed under the microscope.

### 2.4 Blood samples

About 10 ml of venous blood was collected into EDTA coated tubes for plasma and non-coated tubes for serum. Blood processed for serum was allowed to clot first. Both sets of
samples were processed by centrifugation at 1800 rpm for ten minutes and plasma/ serum was collected into labelled tubes and stores at -20°C for antibody assays later. Caution was extremely exercised to minimise blood to blood contact and contamination. Personal protective equipment was used. These samples were stored at -20°C and freighted frozen on dry packs to University of Zimbabwe where they were thawed for the first time for the assays described here. Serum was used in all the assays. A thick smear slide was prepared upon blood collection for microscopic detection of *Plasmodium falciparum* parasites.

Parasitaemia was estimated by counting the number of asexual parasites in random microscope fields containing 200 white blood cells. In order to be included in the study for blood sampling, participants had to meet all the following criteria: i.) Have provided at least 2 urine and 2 stool samples on 3 consecutive days; ii.) Given at least up to 5 ml of blood for serological assays and preparation of a thick smear slide for microscopic detection of *Plasmodia* parasite.

### 2.5 Treatment intervention

Children infected with any of the schistosome species were treated with praziquantel at 40 mg/kg body weight. For STHs were treated with a single albendazole tablet 400 mg as a single dose. Bread and orange juice (500 ml/child or participant) were given as supplementary food following swallowing of the tablets in order to reduce the nauseating effect of praziquantel. Children were also taught about malaria, soil transmitted helminthiasis and schistosomiasis risks. The participants were asked to continuously seek prompt malaria treatment based on recognition of signs and symptoms of malaria that include fever, headache, nausea, general malaise, and joint pains.
Teachers were asked to continue school health education on schistosomiasis, soil transmitted helminths and malaria; and to encourage children to seek medical care promptly when they felt signs and symptom of malaria irrespective of the presence of the research team. As is standard in all such study designs, after collection of the required samples, all participants were offered treatment with the recommended dose of praziquantel at 40 mg/kg of body weight. No participant presented with malaria during the examinations while it was expected that as an infection that can present in an acute form most of the participants may have been accessing treatment from the local health centres as prescribed by the Ministry of Health in Zimbabwe.

2.6. Parasitology samples

2.6.1 Parasitological techniques

Urine and faecal samples were collected between 1000 hr. and 1400 hr. in separate wide mouth plastic specimen bottles correspondingly labelled with the laboratory identification numbers assigned to each individual.

The samples were processed within two hours of collection. Diagnosis of intestinal helminths (S. mansoni, hookworms, T. trichiura and A. lumbricoides) was based on the detection of worm eggs in faeces, respectively using the Kato Katz (Katz et al., 1972) method and Formol-ether faecal concentration (FEC) (Cheesbrough, 1998) method for stool. Infection from S. haematobium was diagnosed using the urine filtration technique as described by Mott et al., (1982) as well as the haematuria check using the dip stick method.
2.6.2 Kato Katz method for intestinal schistosomiasis

A small amount of faecal matter (40 mg) was taken from the specimen bottle and squeezed past a screen/sieve using an orange stick to let off a smooth faecal material at the back side and debris remained on the inside. The smooth material was taken through to fill a template hole avoiding air bubbles to a slide that had the specimen number on it. The template was carefully lifted leaving the cylindrical drop on the slide. Cellophane material in malachite green which had been soaked overnight was layered on top of the stool sample. Another clean slide was used to sandwich the sample was gently squeezed in a circular motion to give a very thin but smooth circular smear. The slide was placed on the bench with the cellophane upwards to allow for the evaporation of water while the glycerol cleared the faeces. Hookworms were examined within 60 minutes of the slide preparation. *Schistosoma mansoni* was examined later during the course of the surveys.

The egg counts were adjusted by multiplying by 24 to give eggs per gram (epg). It is assumed that the standard hole holds 41.7 g of faecal matter. The samples were processed in the same day with the help of the NIHR staff who were recruited in sample collection, see Figure 2.1 below.

**Parasitology samples processing, Kato Katz method.**
Figure 2.1: Members of the research team from NIHR assisting by performing the Kato Katz technique for stool parasitology determination.

The same procedures were repeated on three consecutive days in order to prevent misdiagnosis due to day-to-day variation of egg excretion.

2.6.3 Formol-ether faecal concentration method (FEC) for STHs.

Infection status with intestinal helminths for each individual was decided based on the combination of results from parasitological techniques Kato Katz techniques and the FEC.
method. The stool specimens were processed as follows: firstly about one gram portion of each specimen collected on a single day was preserved in a tube containing 10% formalin. The preserved specimens were processed using the formal ether concentration technique (Mott, 1983; Cheesbrough, 1991). The contents of the specimen bottle were mixed by vortexing for 30 seconds. The cap was removed and a filter funnel unit was attached. The filter funnel and specimen bottle were tilted at approximately 30 degree angle to allow the specimen to flow into the centrifuge tube and a sufficient volume of specimen was filtered so that 1 ml of sediment remained after centrifugation. The filtration unit was plugged with the cap and the remaining faecal specimen was disposed. Normal saline, 0.85% NaCl was added to the centrifuge tube. Centrifugation was done at 2000 rpm for 10 minutes. The supernatant fluid was decanted, retaining the faecal sediment at the bottom. Some saline was added to bring the tube to contents 14 ml. The tube was spun at 2000 rpm for 10 min. The supernatant was decanted and ethyl acetate was added to the centrifuge tube till the 8 ml mark added topping up with ethyl acetate to the 15 ml mark and shaking was done for 30 seconds. Centrifugation was done at 2000 rpm for 10 min. Four top layers of: ethyl acetate; debris plug; saline; and faecal sediment emerged. The tube was inverted to pour off the supernatant fluid and debris layer.

The pellet was re-suspended with normal saline. A drop of Lugol’s iodine was placed on a slide to which was added one drop of the re-suspended faecal material. The slide was covered and examine immediately under a microscope using the 10X objective, then a 40X objective for a clearer observation.

2.6.4 Screening of Urine

The urine specimen was collected between 1000 hours and 1400 hours of the same day for the three consecutive days. It has been found that urinary egg excretion follows a daily rhythm with the peak at noon. The participants brought in labelled urine specimens. The
urine sample was mixed and 10 ml of the sample was extracted and forced through a Nytrell filter membrane (pore size 12-20 µm). Schistosome eggs size approximately 150 by 60 µm, are unable to pass through and they are trapped within. The membrane was loosened from its holder and placed on a slide then smeared with 10 % Lugol’s iodine solution. The filter membrane was examined under a microscope using the 10X objective. Egg counts were expressed as eggs /10 ml urine (ep10ml).

2.6.5 Dip stick method

Urine that is stained red with blood is easy to assess by sight. Normally coloured urine must be assessed using more sophisticated techniques – and the dipstick technique is used. The dipstick technique indicates whether microscopic traces of blood that are invisible to the naked eye are present in a urine sample. In terms of sensitivity, the dipstick technique is comparable to that of the urine filtration kit. A subject was considered positive for helminth infection if eggs were detected for at least one helminth.

2.6.6 Malaria slide preparation

Thick and thin smears of malaria slides were prepared from fresh blood and the remaining blood was used for plasma separation. The slide was labelled in pencil on the frosted part with participant’s study identity number, place and survey number. The slides were left to dry in air after which they were fixed in methanol and stored in slide boxes which were labelled with survey number and date.
The prepared slides were entered in a log book. *Plasmodium falciparum* was diagnosed by microscopic examination of thick blood films after staining the smears with Giemsa stain (Cheesbrough, 1998). The presence of either ring forms or gametocytes was conclusive diagnosis of *P. falciparum* infection.

2.7 Anthropometric data (Questionnaire administration)

The height and weight of the school children participating in the study was measured on the first survey (S0) and during the follow-up surveys. History of additional previous treatment to helminths was investigated during the questionnaire. Socio-demographic data that includes age, gender was recorded onto the questionnaire. Ages of participants were obtained from children and the class registers provided by teachers.

2.8 The skin prick test

The allergen reaction test was conducted on mother and child only and not on any other child bearer. This was carried out to determine the reaction to the six solutions namely the negative control – normal saline, positive control- histamine, grass mix, the house dust mite allergen- *Derp. Pteronyssinus, Altenaria tenius* and *Aspergillus flavas*. These were administered to the child/parent on their forearm.

The participant’s arm was fixed in a motionless position by holding the arm and cleaned using a sterilizing swab. A scheme was drawn on the inner forearm as illustrated in the Fig. 2.2, a small drop of the allergen was dropped in the labelled zones 1-6 and the skin was pricked using a sterile sharp object. The forearm was blotted to dry and the time was indicated as on the Figure 2.2 below. The results of the radius of the disk that developed
were measured 15 minutes after by the same person and were recorded on a form (Fig. 2.2 insert). In the event of itchiness an antihistamine cream was rubbed on the test area after the reading of the result had been done. The plan used to carry out the skin prick test was drawn on the forearm of the participant as illustrated below.

![Figure 2.2: The Skin Prick Test Scheme](image)

**Figure 2.2**: The Skin Prick Test Scheme (insert showing inflamed skin after 15 minutes, the wheal radius was taken and noted down.)

### 2.9 Data management and analysis

Data was captured using SPSS 8.0. Frequency tables together with 95% confidence interval (CI) were obtained. The percentage reduction of the prevalence of parasites from baseline to
12 months follow up survey and arithmetic mean egg counts were calculated. The methods for the analysis of variance (ANOVA) which include the Chi Square ($\chi^2$), Kruskall-Wallis, the Kendall and Student’s t-test tests were used where appropriate. We analysed the differences between children successfully followed up and those lost to follow up using the independent T-test for mean egg counts and age, the $\chi^2$ test for difference in proportions. Paired t-test was used to analyse longitudinal data.

Schistosomiasis infection intensity was classified according to the World Health Organization guidelines (WHO, 2002). *S. haematobium* infection intensity was classified into light infection intensity for 1-49 eggs per 10 ml urine (eggs/10 ml urine). Heavy infection intensity greater than or equal to 50 eggs/10 ml urine, *S. mansoni*, light infection intensity for 1-99 eggs per gram of stool (epg stool), moderate infection intensity for 100-399 epg of stool. Heavy infection intensity for greater than or equal to 400 epg stool. Single infection referred to infection with schistosomes (*S. haematobium* and/or *S. mansoni*), or *P. falciparum*. Infection with at least one parasite (co-infection) referred to infection with at least any of the single infections *P. falciparum* + schistosomes.

2.10 Laboratory processing of blood samples

2.10.1 Plasma and serum collection

One blood sample for plasma/ serum was collected by centrifuging the blood at 1800 rpm (Kubota centrifuge) for 10 minutes and the collected plasma was put into labelled tubes and frozen. The blood from the schools was refrigerated overnight at 4 °C for clot formation and was then centrifuged after 12 hours at 1000 rpm for ten minutes to obtain serum. The serum
was carefully collected and put in respectively labelled cryotubes and frozen. All the samples collected were recorded in a plasma/serum record book with sample ID, date and place.

2.10.2 Determination of anti-schistosomes (egg, worm, cercariae and Sh13) antibodies

Circulating levels of IgA, IgE, IgG, IgM, IgG1, IgG2, IgG3, and IgG4 directed against the following *S. haematobium* antigens Sh13, SEA, SWAP, GST and CAP were detected by indirect enzyme linked immunosorbent assays (ELISA). Preliminary titrations with varying antigen concentrations, serum and secondary antibody dilutions were conducted to determine optimal conditions to carry out the ELISA. Sera from endemic normal people that are heavily exposed to infection but negative for schistosome eggs were used. Negative controls from British people who had never travelled to schistosome endemic areas and a pool of sera from the whole population. These titration assays allowed determination of the serum dilution and antigen concentration giving the best discrimination between negative and positive controls. The following ELISA protocol was thus, developed. Nunc maxisorp microtitre plates were coated with 100 µl antigen (5 µg/ml CAP and SWAP, and SEA) diluted in phosphate buffered saline (PBS) (Gibco Invitrogen) and incubated at 4 °C overnight. These were then blocked with 100 µl/well of skimmed milk (Marvel) (5% milk in PBS) /0.03% Tween 20) for 1 hr. at room temperature and washed three times in PBS/Tween 20, which was used for all washes. About 100 µl of serum was added to each well at varying dilutions depending on the antibody/antigen combination. We used 1:40 dilution for CAP IgE, IgG3, 1:50 dilution for SEA IgG1, IgG3, IgG4, 1:100 dilution for CAP IgA, IgG1, IgG4, SEA IgE, all SWAP antibodies, and 1:200 for CAP IgM, SEA IgM and SEA IgA) and the plates were incubated for 2 hours at 37 °C and then washed three times.

About 100 µl of isotype-specific monoclonal antibody was added at varying dilution depending on the antigen. We used 1:1000 dilutions for IgA for all antigens, 1:1000 dilution for IgE against CAP and SEA and 1:250 against SWAP, 1:1000 dilutions for IgM for CAP
and SEA and 1:2000 for SWAP, 1: 500 dilution for IgG1 against CAP and 1:1000 dilutions against SEA and SWAP. 1:1000 dilutions against SEA, 1:500 dilutions for IgG3 against all antigens and 1:500 dilutions of IgG4 against CAP and SWAP and 1:1000 against SEA. These isotype-specific monoclonal antibodies were conjugated to horse-radish peroxidise and obtained from different companies IgA (Dako, Denmark, P0216), IgE (Sigma, A9667), IgG1, IgG3, IgG4 (The Binding Site, UK, AP006, AP007, AP008 and AP009, respectively) and IgM (Dako, Denmark, P0215). IgG1, IgG3, and IgG4 were biotin conjugated and these had a further step**. Plates were incubated for 2 hours at 37 °C, washed six times and 100 µl of ABTS substrate solution (KPL, Canada) was added. The reaction was allowed to take place at 37 °C for 15 min for all isotypes except those directed against SWAP which were incubated for 30 min, before the absorbance was read at 450nm (Bio-Rad®, Mames-la-Coquette, France). Three negative controls used in the titration assays were included on each ELISA plate and all samples were assayed in duplicate. Appendix 2 for detailed monoclonal and antigen dilution.

**With exception, the biotin conjugated antibodies had the following steps: after the washing of plates 6X with washing buffer, 100 µl/ well of Streptoavidin Horse Radish Peroxidase diluted 1:2000 in the dilution buffer but without Tween was added. The plates were then incubated at 37 °C for 1 hour. The plates were washed 6X followed by the addition of 100 µl/well of the TMB substrate. The reaction was stopped by the addition of 25 µl/ well of 0.2M H₂SO₄ and the optical density was measured at 450nm.

2.12.3 Determination for anti-Plasmodium falciparum (schizont and merozoites) antibodies

ELISA plates (Nunc-Immulon, Denmark) were coated with 50 µl/well of 50 ng/ml antigen for recombinant antigens and 1 µg/ml for both crude antigens in 60 mM carbonate-bicarbonate buffer (pH 9.6) and incubated overnight at 4°C. Plates were blocked with 100
μl/well of skimmed milk (5% milk in phosphate buffered saline (PBS)/0.03% Tween 20) for 1 hr. at room temperature and washed three times in PBS/Tween 20, which was used for all washes. About 100 μl of serum was added to each well at 1:100 dilutions for all assays except for MSP119 which was 1:200; plates were incubated overnight at 4°C and then washed three times. 100 μl of subclass-specific monoclonal antibody was added at 1:1000 dilutions for the detection of IgG1 and IgG3 (The Binding Site, UK).

Plates were incubated overnight at 4 °C, washed six times and 100 μl of ABTS substrate solution (KPL, Canada) was added, before the absorbance was read at 405 nm. Two negative controls from European volunteers who had never travelled to malaria or schistosome endemic areas were used on each plate.

2.12.4 Allergic antibody assays in under five children and their family members

Autoimmune reactivity was assessed by measuring serum antibodies directed against nuclear antigens including RNP, Sm, SSA, SSB, Scl-70, Jo-1, CENP-B, Ribosomal P, DNA and histones using a routine diagnostic ELISA kit (REAADS ANA Test Kit, Cat 10876, corgenix.co.uk). The level of autoimmune reactivity in Units/ml (U/ml) was calculated using negative, positive and calibrator control samples supplied by the manufacturer. As instructed, autoimmune status was designated positive if the calculated value was above the cut-off (21 U/ml) and negative if the value was 21 U/ml or below. HIV status was determined using the USAID-approved HIV1+2 Double Check Gold rapid serological tests. Positive HIV status was confirmed by a second assay using a different rapid test, Determine HIV 1/2 Ag/Ab Combo. The anonymous testing for HIV was used to exclude the infected form the allergenic and antibody tests.
Chapter Three

Results and Discussion
Chapter Three: Results and Discussion

3.1 The study area and population

The rivers in the two communities differed in their temporal distributions - those around the Magaya community are mostly perennial while those around Chitate are seasonal - leading to different schistosome transmission dynamics. We mapped the area for rivers and nearest water contact sites Appendix (Fig. 3.33).

Study area- The figure below shows the location of the study area on the Zimbabwean map and the positioning of the study sites from each other. Of note is the presence of perennial rivers in the area, which is conducive to maintain the lifecycle of schistosomiasis, and transmission of malaria.
Figure 3. 1: Map of Zimbabwe showing the location of the study villages and the GPS mapping of the study site
3.1.1 Water contact sites
The main water contact sources were determined from the administered questionnaire and in consultation with the local environmental health technician (Fig. 3.2). This revealed contribution of water contact sources as the reason for differences in the level of infection in the two communities. In the high infection area (Magaya) there are perennial rivers providing habitats for the vectors all year round while the streams in the low infection area (Chitate) are mostly seasonal.

Figure 3. 2: Distribution of the perennial rivers, streams and water contact sites around Magaya and Chitate. Chitate was situated as shown near the sources of some seasonal tributaries supplying the main perennial river which is about 5 km away.

3.2 Anthropometric data and awareness of schistosome infection
The aims and procedures of the project were fully explained to participants and their parents/guardians at the beginning of the study and written consent was obtained from the
participants and/or parents/guardians before samples were obtained. A questionnaire to obtain the knowledge about the parasite infections was successfully administered to guardians (parents/grandparents) accompanying children aged from 6 months to 5 years, bringing a total of 137 children to participate in the study.

Preliminary analyses indicate that the guardians were more informed about malaria and childhood infections (at least 84% of children had received vaccinations for BCG, mumps, measles, rubella and diphtheria); than they were of schistosomiasis (Fig 3.3). Guardians did not know what bilharzia was and whether or not one could catch bilharzia after praziquantel treatment but knew most of malaria symptoms and that they could contract malaria even after treatment. Fewer parents knew how bilharzia was contracted (26%) compared to more than 65% ($\chi^2=5.574$, df=1, p=0.018) who knew how malaria was contracted as shown in Figure 3.4.
Figure 3.3: Knowledge of bilharzia and malaria infection and treatment among the guardians of the under 5 years old children. Catch: % knows how disease is contracted: know: % know what it is: tmt: % have been treated before.

The questionnaire also indicated that all parents responded that they took their children to the local clinic for medical attention when unwell as opposed to self-treating at home with herbal or other medicine, traditional healer or other sources.

All parents also indicated that they sought medical attention for themselves at the local clinic. In order to ascertain the source of knowledge, we asked the parents where they had learnt about bilharzia and the majority had learnt about it in school and other community based activities proved an important source of information as shown in the Table 3.1 below.
Table 3.1: Community sources of information about schistosomiasis

<table>
<thead>
<tr>
<th>Source of Knowledge</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family</td>
<td>0</td>
</tr>
<tr>
<td>School</td>
<td>35</td>
</tr>
<tr>
<td>Books</td>
<td>0</td>
</tr>
<tr>
<td>Health worker</td>
<td>25</td>
</tr>
<tr>
<td>Nowhere</td>
<td>14</td>
</tr>
<tr>
<td>Community meetings</td>
<td>22</td>
</tr>
<tr>
<td>Other</td>
<td>4</td>
</tr>
</tbody>
</table>

The parents were also asked if their children had suffered from a list of symptoms including those related to schistosomiasis and a few parents indicated that their children were passing blood with urine as shown in Table 3.2 below.
Table 3.2: Distribution of the symptoms normally felt by the children

<table>
<thead>
<tr>
<th>Symptoms normally felt by the children</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood in urine</td>
<td>3</td>
</tr>
<tr>
<td>Difficulty in urinating</td>
<td>10</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>54</td>
</tr>
<tr>
<td>Pain in joints</td>
<td>9</td>
</tr>
<tr>
<td>Frequent urge to urinate</td>
<td>24</td>
</tr>
</tbody>
</table>

Most parents reported that their children complained more of diarrhoea (54%). The schistosomiasis-related symptoms were also reported difficulty in urinating 10% and blood in urine (3%). Of note was that the books of the educational curriculum contributed none of the knowledge on schistosomiasis.

3.3 Parasitology and treatment.

In case of infants, sample bottles were given to the guardians on three consecutive days to collect samples from the infants; at least 2 samples were acceptable for schistosomiasis diagnosis. All samples thus, collected were brought back to the collection points the next day for processing before distributing more containers for the next samples. In the case of infants, levels of parasite specific- antibodies directed against cercariae, egg and adult worms were used as an indicator of exposure to infection and therefore were also used for diagnosis.
of schistosome infection giving a larger study sample sizes as it included children who had failed to provide urine/stool samples.

This is the limitation of the Kato Katz and the urine filtration methods in infants, since it is not always reliable that one will get a urine specimen or a stool specimen when required. This approach was recently validated for *S. mansoni* infections (da Frota et al., 2010) and has proved successful in other previous *S. haematobium* studies (Woolhouse et al., 2000). After the parasitology examination of the whole population in the 2 areas, it was established that the study locations differed in intensity and prevalence of schistosome infection.

Schistosome infection levels were significantly higher in Magaya (prevalence = 69%, 95% CI 63% - 75%) than in Chitate (prevalence = 14%, 95% CI 11% - 18%) ($\chi^2 = 187$, df=1, p<0.001) with mean infection intensity of 58 eggs/10ml urine (Standard error of the mean, SEM = 8.02) and 15 eggs/10ml urine (SEM = 4.17) respectively ($F_{1,613} = 138.2$, p < 0.001).

According to the WHO’s classification of severity of infection, Magaya has high infection (prevalence greater that 50%) and Chitate has moderate infection (50%> prevalence>10%) (WHO, 2002). Infection levels followed the typical age-infection pattern originally described for schistosome infections in 1934 (Fisher, 1934) in which infection rises with age to peak in childhood/early adulthood before declining as shown in Figure 3.4. There were no stools positive for *S. mansoni* or other soil transmitted helminths by microscopy for the children aged 5 years and below. The infection prevalence was evaluated across the age groups of the recruited population and was reported as below.
School-aged children displayed the highest burden. This could be due to the long-term cumulative effect of schistosome infection and to the slow development of immunity only evidenced after many years of exposure when puberty occurs (Capron, 1992). The two study areas’ infection intensity was compared and the figure below illustrates the respective distribution.

**Figure 3.4:** *S. haematobium* infection intensity and prevalence of the whole population examined from the study.
Figure 3.5: *S. haematobium* infection intensity in the whole population according to age groups. The data includes the adults from the same area. Sample sizes were Chitate = 681 of which 115 were aged 5 years and below. Magaya n= 482, of which 25 were aged 5 years and below.

The age groups 0-10 and 11-20 in the high infection area maintained high infection intensity in all age groups. The decline in the infection intensity after 21 (Fig 3.5) years may be attributed to the development of protective immunity due to chronic infection which leads to the antibody class switching between IgE and IgG4 which are associated with protection to schistosomiasis infection.

3.4 Infection epidemiology

*Schistosoma haematobium* infection prevalence in the study population was 56% with a mean infection intensity of 34 eggs/10 ml of urine with a standard error (SE) of the mean of 5.5
eggs/10 ml urine (range 0-676 eggs/10 ml urine). Although these infection levels are moderate relative to reports from other areas in Zimbabwe, (Dunne et al., 1992) the World Health Organisation denotes this prevalence level as high and infection intensity also as high as defined by having more than 10% of the population with more than 50 eggs/10 ml of urine since 45 of the 227 participants had more than 50 eggs per 10 ml urine (WHO, 2002). Infection rose to peak in childhood (11-12 years) followed by a sharp decline in infection intensity while prevalence fell more gradually as shown in Figure 3.4. Infection levels in this population peaked at lower levels and in older children compared to high infection areas in Zimbabwe (Dunne et al., 1992). In our study area, that is Chitate and Magaya, there were no other helminths that were detected by microscopy.

There was no malaria infection as observed using positive thick smears and confirmed by the rapid test kit. All participants investigated produced IgM antibodies directed against all the three life stages of the parasites (cercariae, adult worm and eggs) while the least prevalent antibody response was the IgE antibody response to all three life stages (Figure 3.6).

Numbers of people producing responses against the cercariae and egg stages were high for all antibodies and were greater than the number of people producing antibodies directed against adult worm
Figure 3.6: Prevalence of antibody responses in the whole population as determined by ELISA against diverse parasite specific antigens: cercariae antigen protein – CAP; Whole worm homogenate – SWAP (WWH) and Soluble Egg antigen – SEA.

The diagram above shows the population’s response to schistosome antigens. The results show that the participants of the study areas are highly exposed to the parasites. The antibody profiles and responses indicate that individual have high parasite exposure and possibly infection in their lifetime as residents of the area (Fig. 3.6). However, the IgE responses are adaptive and mostly protective and can only be significantly elevated post treatment after infection.
More than 60% of the population responded to the schistosome antigens from the different life stages of schistosomiasis. The first stage of schistosomiasis presents the cercariae antigen; the second stage presents the egg antigen and finally the worm antigen. Water contact per day is very high and since the family chores entails that one has to do the gardening, it is inevitable that schistosomiasis infection could have been avoided. Probably it could have been better avoided, if the source of irrigation water was a clean, sunk borehole. In our investigations in the study we even scooped the wells and identified the snails that we scooped. We could identify the *B. pfeiferri* species. The shallow wells are also not exempted from transmission of bilharzia. The populations immunity towards the worm antigens portrayed clearly the immune evasion by the schistosomes.

The diagram below shows the change in infection intensity in Chitate, 6 weeks after a single dose of praziquantel to the infected individuals.
Figure 3. 7: Showing reduction in infection intensity in individuals examined living in the low transmission area –Chitate. The data shown indicate the levels in the whole population (6 months to 80 years) examined for the areas

The area of Chitate was a low infection area and treatment significantly reduced the infection intensity. In Chitate, the ages included parents/ guardians who accompanied infants below 6 years to participate in the skin prick test for allergies. Their samples were also included in the evaluation for immunological variation. The figure 3.7 above shows that treatment was highly effective.

The diagram below shows the change in infection intensity 6 weeks post treatment in a high infection area of Magaya.
In Magaya, a high infection area, praziquantel treatment reduced the infection intensity so significantly, 6 weeks post treatment Fig 3.8. Praziquantel proved to be highly effective in a single dose against schistosomiasis. This is also in line with previous work by Midzi et al., 2008 in Zimbabwe showing treatment efficacy and the reduction in infection intensity.

3.5 Praziquantel treatment

Children were all treated with praziquantel tablets at the dose of 40 mg/kg body weight. For infants the tablets were crushed to form a powder which was administered by the
parents/guardians. Bread and orange juice were given and taken just before and after taking the praziquantel tablets. All parents/guardians reported back 24 hours later with the children for the 24 hour check-up on side effects developing from praziquantel administration in children 5 years and below. The diagram below shows treatment coverage in the study community.

![Bar chart showing treatment coverage per age-group in the study villages](image)

**Figure 3.9:** Treatment coverage per age-group in the study villages

Treatment coverage was assessed among the treated school-going children as shown above. The treatment coverage was not that high in Chitate due to unforeseen circumstances-teachers were on job action, and students did not show up at school. To mitigate, praziquantel tablets were given to the Chitate clinic, a primary health care centre.
3.5.1 Short–term side effects of praziquantel treatment

An adverse effects questionnaire was administered to all guardians of children receiving praziquantel treatment 24 hr. after treatment to determine if the children suffered any treatment-related adverse effects. A preliminary check in the field indicated that most children did not suffer adverse reactions to praziquantel treatment.

The results from the questionnaire data post treatment reported very minor side effects, but a high willingness for treatment. Only 4 out of 84 children reporting back 24 hours after treatment (4.8%) reported side effects of headache, loss of appetite, stomach ache and general weakness occurring within 24 hours after taking praziquantel tablets.

This was less than what was reported in older primary school children (Midzi et al., 2008) and that reported from *S. mansoni* studies in endemic areas (Raso et al., 2004, van den Biggelaar et al., 2000). The low percentage of children suffering side effects is likely to be related to the low levels of infection they were carrying since previous studies show that the frequency and severity of side effects is proportional to the intensity of schistosome infection (Raso et al., 2004; van den Biggelaar et al., 2000).

3.5.2 Treatment efficacy in the whole population 6 weeks post–treatment

Treatment uptake was lower in Chitate, the low infection area, (51%) compared to Magaya (81%). This was due to operational reasons, in Chitate treatment was disrupted by reduced attendance to school due to schools being closed during a teacher’s strike which was an unforeseen occurrence. To ensure that infected people did receive treatment; praziquantel tablets were provided to the Nursing Sister at the local clinic for dispensing to the
community. Of the treated populations, there was a reduction in infection prevalence and intensity in both areas although this was significant only for Magaya. This is unsurprising since the changes in infection levels in the low infection areas would be too small to detect any statistically significant differences.

3.5.3 Treatment efficacy in children aged 6 months - 10 years (6 weeks post –treatment)

To assess the efficacy of praziquantel treatment in children under 5 years old, the changes in infection intensity and prevalence observed in children under 5 years after treatment were compared to those observed in older children (6 to 10 years). A reduction in *S. haematobium* infection intensity was observed in children aged 6 months - 5 years as well as in children aged 6-10 years. Sample sizes of children providing sufficient number of parasitology samples was less than for the whole study population.

Interestingly, an increase in infection intensity was observed in untreated children aged 5 years and below in the high infection area Fig. 3.10 below;
Figure 3.10: Reduction in infection intensity in children aged 6 months to 10 years. S0 is *S. haematobium* infection intensity (egg count) at baseline. S1 is *S. haematobium* infection intensity 6 weeks post-treatment Chitate; low infection area.

Infection intensity was significantly reduced in both treated and untreated age groups as shown in Fig. 3.10. The efficacy of praziquantel in all age groups was still high. The result also demonstrated the need to treat for schistosomiasis as a way of breaking the schistosomiasis life cycle.
Figure 3.11: Reduction in infection intensity in children aged 6 months to 10 years. S0 is *S. haematobium* infection intensity (egg count) at baseline. S1 is *S. haematobium* infection intensity 6 weeks post-treatment in Magaya - high infection area.

The high infection area of Magaya had good treatment coverage. Among those treated, we could see a decline in the infection intensity, as was seen in Chitate also. Treatment reduced infection intensity.
The prevalence of infection remained the same at 6 weeks post treatment in children under 5 years untreated at baseline whereas in treated children the prevalence was nil in both areas (Figure 3.12 and 3.13) below.

**Figure 3.12:** Changes in prevalence in children aged 6 months-10 years 6 weeks after treatment in Chitate.

Praziquantel treatment greatly reduced the prevalence as shown in the graphs Fig. 3.12, 3.13.
Figure 3. 13: Changes in prevalence in children aged 6 months-10 years 6 weeks after treatment in Magaya.

We anticipated the prevalence to drop off to zero six weeks post treatment, even in this age group, 0-6 years. The discord in the result (Fig 3.13) could have been due to the low uptake of treatment in the age group due to fear by the parents after the side effects of treatment were clearly explained to the guardians.

The children 0-5 yr. missed treatment, therefore there was no change in prevalence 6 weeks post treatment. The other age groups experienced a reduction in infection prevalence as was anticipated 6 weeks post treatment.
3.6 Schistosome-related morbidity before and after praziquantel treatment

Haematuria was measured in the children before treatment and at 6 weeks after treatment using urinary dipsticks (Néphur®, Boehringer, Mannheim, Germany). Very few children showed intense haematuria, while some of the most intensely infected children especially in the high infection area, Magaya, showed some haematuria.

No haematuria was observed in the treated children 6 weeks post-treatment, including the 3% whose parents reported macro haematuria in the questionnaire. The weight and height of participating children was measured and compared with the post-treatment changes in these two measures across the age range. The change observed in the children aged 6 months-5 years was compared to the whole population to determine if the effects of praziquantel on growth measures were age dependent.

In all groups weight and height increased 6 weeks following treatment (Figures 3.14 and 3.15 below). While the increase in weight was lower in the children aged 5 years and below, the increase in height was equivalent in children aged 16 to 20 years. We could not attribute these changes entirely to praziquantel treatment alone since an unplanned and therefore unforeseen supplementary feeding programme providing the children with a single meal of bulgur wheat and pulses was started in the schools by an independent organization a week following praziquantel treatment.

3.6.1 Effect of anti-worm treatment on height and weight of children 6 weeks post treatment

From the demographic data that was collected pre- and post-treatment and also during follow-ups, the following diagram was produced to illustrate the impact of de-worming in the school-going children.
In just 6 weeks after de-worming, significant changes were noted in the weight for the age group that is highly infected that is 11-15 years. This demonstrated an improvement in the well-being of the treated children.
There was no significant change in height to all the age groups, 6 weeks post treatment. It has been established that infection with schistosomiasis leads to anaemia (Midzi et al., 2007) and the removal of the worms which will be constantly laying about 300 eggs a day, is important in elevating haemoglobin in the individual. It is the egg that causes much of the morbidity as seen in the haematuria as the eggs lodge in the walls of the bladder. These growth patterns that were improved may not solely be attributed to de-worming alone.

**Figure 3.15:** Changes in height 6 weeks post treatment with praziquantel
3.7 Atopic responses before and after praziquantel treatment

Allergic responses to allergens common in Zimbabwe were tested. The overall prevalence of skin prick reactivity was 19% and the most prevalent allergic response was directed against the house dust mite with 12% of the population reacting against it. Interestingly, participants positive for schistosome infections were not reactive against any of the allergens, supporting earlier studies in the Gabon as well as the hygiene hypothesis stating that helminth infections stimulate immune responses that protect against atopy (David et al., 2004). Shown in Figure 3.16 is data from the Chitate community which is a low schistosomiasis infection area. Note-the data included parents/guardians who accompanied infants to participate in the study.

Figure 3.16: Levels of reactivity against common allergens in Chitate - the low transmission area
The responses to the common allergens followed a similar pattern to the age-infection curve described previously, with a peak in the age range 11-15 and declining in the age range 20+.

Below is Figure 3.17, showing the assessment of atopic reactivity in the same population of Chitate. Chitate was a low infection area and a few individuals had huge schistosome infection intensity, therefore a lot of individuals were above the cut-off point, making them positive for atopy. Particularly those who had zero infection intensity showed that they were ANA positive. The tendency to produce IgE antibodies in individuals who have a slight exposure to allergens is referred to as atopy. Since these participants come from a low transmission area, it is possible that they may be exposed to low doses of worm infection, making them hypersensitive and therefore, atopic (Rujeni et al., 2011).
Figure 3.17: Relationship between individuals’ anti-nuclear antibody (ANA) reactivity with schistosome infection intensity in Chitate, the low schistosome infection area. The cut-off point for auto-reactivity is represented at 21 U/ml. Participants whose ANA values are above cut-off are denoted as positive for auto-reactivity.
Figure 3.18: Relationship between individuals’ anti-nuclear antibody (ANA) reactivity with schistosome infection intensity in Magaya, the high schistosome infection area. The cut-off point for auto-reactivity is represented at 21 U/ml. Participants whose ANA values are above this cut-off point are denoted as positive for auto-reactivity.

The overall prevalence of autoimmune reactivity / the proportion of people with titres above the cut-off point were 38% (95% CI 34% to 42%). We also determined if there were significant differences in the prevalence of autoimmune reactivity in the two areas. The prevalence of autoimmune reactivity was significantly higher in Chitate the moderate infection area 48% (95% CI 43% to 53%) than in Magaya the high infection area 22% (95% CI 17% to 28%) ($\chi^2 = 41.2$, df = 1, p < 0.001).

It was also noted that, the prevalence of autoimmune reactivity was significantly lower in schistosome positive people compared to schistosome negative people (Magaya, $\chi^2 = 3.8$, df = 1, p < 0.05 and Chitate $\chi^2 = 5.1$, df = 1, p < 0.05). The levels of autoimmune reactivity decreased with increasing schistosome infection intensity in both villages (Figure 3.19 &
Schistosome infection intensity was significantly associated with autoimmune reactivity ($F_{1,612} = 4.1, p < 0.05$) and that this association was negative ($\beta = -1.7$, 95% CI -3.7 to 0.3). Levels of autoimmune reactivity were higher in the moderate infection area than in the high infection area ($F_{1,612} = 47.3 p <0.001$) but this did not affect the relationship between autoimmune reactivity and schistosome infection intensity (interaction term between village and infection intensity was not significant $F_{1,612} = 0.2, p = 0.63$). Furthermore, neither age nor HIV status had a significant association with autoimmune reactivity ($F_{1,612} = 2.3, p = 0.127$ and $F_{2,612} = 2.3, p = 0.127$ respectively). Atopic reactivity were also determined serologically by measuring levels of IgE directed against the house dust mite and this confirmed the skin prick test (SPT) results of a negative association between schistosome infection intensity and atopic responses (Fig 3.19 & Fig 3.20). The prevalence and intensity of atopic responses remained unchanged 6 weeks post-treatment with praziquantel.
Figure 3.19: The prevalence of auto-immune reactivity with age groups in Chitate community

With age, the prevalence of auto-immune reactivity decreases, particularly after 21 years, where also according to the age-infection intensity curve, there is a decline in the schistosomiasis infection. Slow acquisition of immunity against helminths could lead to the decrease in auto-immunune reactivity.
Figure 3. 20: The prevalence of auto-immunity in the two set ups of varying infection intensities. (Black bars = no infection, white bars = mild infection, grey bars = heavy infection) in the two villages. Bars represent 95% confidence intervals

In both villages the prevalence of autoimmune reactivity differed significantly with the severity of schistosome infection (Chitate $\chi^2 = 14.4$, df = 2, p = 0.002, Magaya $\chi^2 = 4.5$, df = 2, p = 0.037). Further statistical analyses showed that in both villages, the proportion of auto-reactive people declined with increasing severity of schistosome infection (Figure 3.20),
although the differences in autoimmune reactivity between people carrying no schistosome infections compared to mildly infected people was not significant in either village (Magaya $\chi^2 = 2.1$, df = 1, p = 0.097 and Chitate, $\chi^2 = 0.01$, df = 1, p = 0.534). In both villages heavily infected people had a lower prevalence of auto-reactivity compared to mildly infected (Magaya, $\chi^2 = 0.785$, df = 1, p = 0.253 and Chitate, $\chi^2 = 10.4$, df = 1, p = 0.001) and uninfected people (Magaya, $\chi^2 = 3.9$, df = 1, p = 0.036 and Chitate, $\chi^2 = 14.2$ df = 1, p < 0.001). A cohort of 102 school children was examined 6 months post treatment with the anti-helminthic drug. Before treatment, schistosome infection prevalence was 75% (76 individuals) amongst these children. The prevalence fell to 0%, 6 weeks post treatment. After the treatment, 11% was re-infected with schistosomiasis, and of these 2/11 children with low infection were ANA positive.

The remainder of the children who were ANA positive 6 months post treatment were negative for schistosome infection, also described by Mutapi et al., 1999. Post treatment the prevalence of ANA reactivity in children had increased significantly from 12.7 % (13 individuals) to 27.5 % (28 Individuals) ($\chi^2=25.9$, p<0.001, df=1) as shown below. The mean level of auto-immune reactivity increased significantly $t= -5.165$, df=101, p<0.001). The association between ANA reactivity/ status with schistosome infection intensity/ status was not formally evaluates due to the low number of cases of re-infection. The diagram below (Fig 3.21) shows the prevalence or autoimmune reactivity before and after schistosomiasis treatment.
Figure 3. 21: Comparison of the prevalence of auto-reactivity before and six months after anti-helminth treatment with praziquantel in a subgroup of participants from Magaya showing a significant increase in autoimmune reactivity after anti-helminthic treatment.

We examined a cohort of 102 school children within the study 6 months following treatment with the anti-helminthic praziquantel to remove adult schistosome parasites. Before treatment schistosome infection prevalence was 75% amongst these children. This fell to 0%, 6 weeks after treatment. Six weeks after treatment 11% of the children had become re-infected with schistosomes, of these, 2 out of 11 children with low infections were ANA positive.
The remaining children who were ANA positive 6 months after praziquantel treatment (Mutapi et al., 1999) were all negative for schistosome infection. We found 6 weeks following anti-helminth treatment the prevalence of ANA reactivity in treated children increased significantly from 12.7% to 27.5% ($\chi^2 = 25.9, p < 0.001, df = 1$) as shown in Figures 3.17 & 3.18.

The mean level of autoimmune reactivity in the participants also increased significantly ($t = -5.165, df= 101, p < 0.001$). The association between ANA reactivity/status with schistosome infection intensity/status was not formally tested due to the small sample sizes of people who became re-infected. In 613 people naturally exposed to the blood fluke helminth parasite causing schistosomiasis, the results showed that autoimmune reactivity was inversely associated with current infection intensity but is independent of host age and sex status. Autoimmune reactivity increased 6 months after anti-helminthic treatment. The implications of these findings are relevant to understanding both the aetiology of autoimmune diseases and in predicting the long-term consequences of large-scale schistosomiasis control programs.

We further investigated the role of the schistosome and atopic-specific antibodies before and after treatment, and the results were as below, Fig. 3.22.
Figure 3. 22: The profiles of the main antibodies in schistosome immunity against whole worm antigen in the low transmission area (Chitate) and high transmission area (Magaya).

At baseline, the IgM level was high in both areas, though in the high transmission area it was not significantly elevated 6 weeks post treatment as was observed in Chitate. This sheds light into the effect of the different levels of antigen exposure in the areas and the impact of treatment on the levels of the antibodies, as well as the theory of immune evasion by schistosomes. The levels of IgE and IgG4 were not much elevated before and after treatment in Chitate circumstances. Although in Magaya there was a significant difference in the IgE levels at baseline and post treatment. This could explain the concept of low doses of antigen leading to IgE hypersensitivity and allergic response unlike in Magaya, in which chronic Th2 mediated anti-schistosome response, is expected.
The elevated level of IgE is as a result of the antigen presentation from the action of praziquantel on the helminth.

**Figure 3. 23:** Change in antibody responses directed against the cercariae antigens in Magaya (high transmission) and Chitate (low transmission) for baseline S0 and post treatment S1

The responses in Chitate clearly showed that the parasite burden is low within the population. Although IgM levels were high, the population exposed was low, and after treatment, IgM levels were low, probable due to initiation of memory and immune evasion. Only IgE was significantly different in Chitate for all the antibodies, pre and post treatment in both set ups. This could be due to the small dose of antigen which stimulates production of IgE which leads to hypersensitivity.
**Figure 3.24**: The profiles of the main antibodies in schistosome immunity against the egg antigen in the low transmission area (Chitate) and high transmission area (Magaya).

The egg is the antigen that causes serious pathology in schistosomiasis infection, stronger IgG4 response to egg was noticed in the Magaya population, IgE was also strong pre-treatment in Magaya than in Chitate. This could explain the body’s acute response to the schistosome egg antigen to try and neutralise it through IgE and IgG4. It also supports the theory of class switching in chronic schistosomiasis infection where there are cytokines produce a skew from IgE to IgG4.

Most individuals in both the high and low transmission set ups had shown evidence of exposure to schistosomiasis. The IgM levels against the cercariae antigen were high both
before and post treatment in Magaya and lower post treatment in Chitate though this was not significantly different. IgE was higher in all circumstances after treatment. This also shows the effect of praziquantel even to schistosomiasis cercariae stage. Praziquantel has strong anti cercariae effect. IgG4 levels were not much changed. This could have had a bearing on the higher levels of IgE that was shown against the cercariae antigen. The pattern was also investigated against the egg antigen for the same population and the result was tabulated in the figure below,

Figure 3. 25: A comparison of the Antibodies involved in allergic disorders between high and low transmission areas.

The low transmission and high transmission areas’ pattern was not very different. Although the levels of antibodies expressed before and post treatment varied significantly, they were
lower before treatment and highly significantly elevated post treatment. IgG4 were particularly highly elevated post treatment.

Below is a figure to compare of the ratios of the antibodies that are mainly involved in allergic disorders, at the same time playing an important role in anti-helminth immunity.

![Figure 3.26: A comparison of the ratio of the main antibodies involved in allergy and schistosomiasis response against the Derp1 Allergen](image)

The ratio IgE to IgG4 was very much close to 1 in Chitate than in Magaya post treatment. Chitate, the low transmission area was expected to present with high allergies and hypersensitive reactions. This was also supported in the skin prick test and ANA reactivity assays. The class switching of IgE to IgG4 is much expected to take place during the chronic schistosome infection which is expected to be the main case in Magaya, and to some extent in
Chitate. The lower level of IgE in Magaya post treatment could be due to the IL-10 mediated class switching between IgE and IgG4.

We observed that the ratio of IgE to IgG4 was almost 1:1 in the pre-treatment and post treatment stages. In Magaya, the post treatment numbers were slightly lower that in Chitate. The ratios could have an important role on the levels of autoimmune reactivity that was observed earlier- that the prevalence of autoimmune-reactivity was high in Chitate than in Magaya. Praziquantel treatment resulted in a significant increase in parasite specific IgE. Praziquantel showed no significant effect on atopic responses. The drug accelerated the development of schistosome-specific responses that are protective to re-infection.

3.8 Schistosome-specific immune responses in children

Blood samples collected from children were used to determine schistosome specific responses because in very young children that is those aged below one year, microscopic detection of eggs may be an unreliable measure of exposure to schistosome cercarie and of infection. The IgM response would give an indication of exposure to the different schistosome stages.

3.8.1 Pre-treatment schistosome specific responses

Initial analyses of the data show that levels of IgM directed against cercarie, adult worm and soluble egg antigens are present in children aged 5 years and below and that the IgM responses directed against the cercariae antigen show the highest titre which is not surprising since cercariae penetrating and getting stimulated at the skin are more than the
schistosomulae that develop to adult worms – conversely adult worm IgM levels show the lowest titres as shown in Figure 3.27 below.

**Figure 3.27:** Baseline schistosome-specific IgM responses in children aged 5 years and below

IgM anti adult worm shows low levels supporting immune evasion by established adult worms; while is children <5 years may mean very few worms established and recognised by the immune responses. The presence of IgM against the three antigens showed exposure to the parasite in the age groups 1-5 yr. IgM was increasingly being elicited against cercariae in (OD levels) with age probably due to increased frequency of exposure and immunological memory. The population are prone to re-infection. The lower sensitivity to the adult worm is probably due to the immune evasion by the worm.
3.8.2 Effect of treatment on schistosome-specific responses in children aged 5 years and below.

Previous studies have shown that treatment can alter schistosome-specific responses within 6 weeks of chemotherapy. Therefore, this effect could be observed in children under 5 years of age. This study showed a significant decrease in IgM levels reflecting a reduction in exposure to parasite antigens that is, fewer worms to stimulate the response due to worms being killed by treatment, as well as an increase in IgE responses which have been associated with protection to re-infection (Hagan et al., 1994).

3.10 Malaria parasitology results

There was no malaria infection observed in the whole study population during the period of the study. In the Mashonaland East region, malaria infection is sporadic, unlike in the Limpopo or Zambezi valleys, where the endemicity of malaria guarantees that there is an infection at any point in the human host. There was no parasite found on all slides observed under microscope. The results were further confirmed by running the blood on rapid malaria detection kit (Paracheck Pf™, Orchid Biomedical Systems, India).

The wide range of 200 million individuals frequently quoted as infected in the 300-500 cases per year is as a result of the lack of precise malaria statistics (Bell et al., 2005; Snow et al., 2005). Accuracy of a clinical diagnosis, though it is the most common method, varies with the endemicity and the malaria season, and age group (Dicko et al., 2005; Mwangi et al., 2005).

Giemsa microscopic examination is regarded as the most suitable diagnostic tool for malaria. It is able to differentiate the malaria species, quantify parasites and it is cheap. The laboratory accuracy detection threshold has been estimated to be 4-20 parasites/mcL, but under field conditions a threshold of about 50-100 parasites/mcL blood has been more realistic (Pampana, 1963; Dowling and Shute, 1966; Payne, 1988). Poor microscopy can be a
function of many factors which include: training and skills, maintenance, slide preparation techniques, workload, condition of the microscope and quality of laboratory supplies. False positive results can be obtained from a poor blood film of preparation which generates artefacts which may be mistaken for the malaria parasite. Blood platelets also confound diagnosis. Some of the common artefacts in malaria false positive diagnosis include the stain precipitation, dirt and cell debris (Houwen, 2002).

A false negative result can emerge when there is a low parasite density (McKenzie et al., 2003; Maguire et al., 2006). The recommended fields on a thick blood film required before declaring slide negative vary from 100-400. In this study we used 200 fields which were optimum. The microscopists that examined the slides are well trained and are always doing the examinations all year round. Quality control was done as well by some experienced personnel. The only limitation to accurately reliable malaria parasitology could have been the decreased parasite density given that the thick film was only examined for 200 fields.

Microscopy remains the golden standard, but in the developed world, efforts to improve malaria diagnosis are currently underway.

Rapid diagnostic test (RDT) for malaria detects malaria parasites’ presence in a small amount of blood. The method was employed to validate the microscopic result. The immuno-chromatographic method makes available monoclonal antibodies directed against the target parasite antigen. For the RDTs to be reliable, they must have an efficiency of greater that 95% (WHO, 2000) and this has been established in the RDT testing kit used for P. falciparum.
3.11 Anti-malaria immune responses in the general population

3.11.1 Anti-merozoite responses

The IgM that was noted in the population was not due to malaria. There was no relationship between the IgM directed against MSP 1-19 and MSP3 ($\chi^2=0.293$, df=1, p=0.588) and ($\chi^2=0.336$, df=1, p=0.562) respectively (Figs 3.32 and 3.33 index). In animal experimental models, parasitic helminth infections can protect the host from autoimmune diseases.

Schistosome infection level showed no relationship to levels of antibodies directed against an antigen from malaria parasites, the vaccine candidate merozoite surface protein (MSP) 1-19 and MSP3 ($\chi^2=0.293$, df=1, p=0.588) and ($\chi^2=0.336$, df=1, p=0.562) respectively, (Fig 3.30, Fig 3.31), indicating that this relationship was specific to the nuclear antigens. Hence, the results suggest that it is the presence of schistosomes which is important in modulating production of auto-antibodies, and in a manner related to the intensity of infection.

This was confirmed by following a subset of the population (n=102, schoolchildren aged 5-16 years, which is the WHO-target population for school-based mass chemotherapy treatment programmes) treated with the anti-helminthic drug praziquantel and demonstrating a statistically significant increase in auto-antibody reactivity 6 months later. The explanation for the negative association between schistosome infection and auto-antibody levels could be related to the so-called ‘hygiene hypothesis’ (Strachan, 1989). The hypothesis suggests that exposure to infectious agents, such as parasitic helminths, elicits immune deviation or dampening that serves to reduce incidence or severity of allergy and autoimmunity (Christen and von Herrath, 2005).

This may result from the need for helminths to manipulate host immune responses to enhance their survival (Matricardi et al., 2000). A recent study of 24 sufferers of the autoimmune condition multiple sclerosis (MS) showed that intestinal helminths can modulate MS-related T and B cell responses (Sanchez et al., 2000). The results of this study show a clear
relationship between schistosome infection levels and auto-reactivity. They also indicate the need for further investigation into the nature and aetiology of the association between helminth infections and autoimmune reactivity. This need has become urgent in the face of current large scale global helminth control initiatives whose long-term health consequences have yet to be fully understood.

3.11.2 IgM anti-malaria.

We investigated for malaria infection using the thick and thin smears for malaria examination as the standard method of malaria slide preparation (Greenwood and Mutabingwa, 2002) at baseline and at 6 weeks post-treatment, all slides were negative for active Plasmodium infection.

An immuno-assay was employed detecting IgM antibodies directed against *P. falciparum*-schizonts following the established ELISA protocol by (Mutapi and Mduluza et al., 2003) to determine if there were any people positive for current exposure to Plasmodium parasites. As a result focused in children aged 6 months to 6 years and the age distribution of this response is shown in Figure 3.29 below. This shows that children as young as 6 months had been recently exposed to malaria parasites. The presence of malaria-specific IgM and/or IgE in cord blood has been used as evidence of in utero activation (priming) of foetal B cells (Desowitz, 1988; Fievet et al., 1996).
The Fig. 3.28 below shows IgM responses against the *P. falciparum* schizont in children under 6 years of age.

**Figure 3.28**: Distribution of malaria exposure measured using IgM anti-schizont in children 6 years and below in Chitate Community.

Between the successive age groups, there was no significant difference in the level of IgM. Notably there was a significant difference in IgM levels between 0 and 1 year and except at 5 years, there was a significant difference of 0 years and other age groups. The beginning of lower levels of IgM may be due to lower exposure rates as the immune system tends to build
memory. This was evidence of exposure to *P. falciparum* malaria of the children under 6 years.

Furthermore, the study indicated that children as young as 1 year were already developing immune responses directed against malaria vaccine candidates, confirming observations that children gradually develop acquired resistance to malaria parasites (Desowitz *et al*., 1992). This is further illustrated in Fig. 3.35 showing immune responses of 6 month to 6 years old children from Chitate directed against MSP1-19, one of the leading malaria vaccine candidates. *Plasmodium falciparum* infection to the mother during pregnancy can lead to the transplacental passage of malarial antigens that are capable of inducing acquired immune responses in the foetus and subsequently in the newly born baby. In humans, the immune system of the foetus develops early in gestation and is able to respond to foreign antigens (Desowitz, 1988; Soboslay *et al*., 1999; Holt and Jones, 2000). By the 18th week of gestation, foetal splenic T cells can produce cytokines and express co-stimulatory molecules as well as induce Ig switching in B cells (Holt and Jones, 2000). B cells expressing CD19, CD20, CD21, CD22, IgM, and HLA-DR have been found in human foetuses as early as the 12th week of gestation, with approximately 90% of mature B cells being CD5+ B positive (B-1 B cells). In humans, IgM and IgG synthesis can start as early as the 10th week of gestation and increases with gestational age. At birth, foetal cord blood mononuclear cells (CBMC) are generally less responsive than those of adults (Splawski *et al*, 1991), but they can proliferate and produce cytokines and antibodies in an antigen-specific manner in vitro (Splawski and Lipsky, 1999, Ettinger, 2005). In new-born babies, it is mostly IgG that is thought to be acquired across the placenta (Holt *and Jones*, 2000). This acquisition of immunity may also be a confounder to the resistance to chronic morbidity in malaria exposed people.
Figure 3.29: Immune responses directed against malaria vaccine candidate in children also exposed to schistosomiasis from Chitate aged 6 years and below
The WHO increased the minimum threshold in recommending that cure rates should be at least 90% and preferably 95% for malaria at less than 28 days. With the artemisinin-based combination treatment, the levels of clinical malaria cases have fallen sharply, just as the mortality rate has declined. In lower transmission regions such as the Murewa region, the malaria incidence is likely to fall. Artemisinin and its derivatives mainly the one that is being used currently-Artemether-Lumefantrine (Coartem-Ether™) is very effective because of the high killing rates.

A three day treatment course with Coartem Ether exposes two asexual cycles therefore reduces the number of parasites in the body by approximately 100 million fold. Co-artem Ether comprises of artemether 120 mg plus lumefantrine 20 mg, given in four doses, provides effective antimalarial treatment for children in many sub-Saharan countries. The gametocidal activity of the artemisinin compounds is an added advantage in reducing transmission, thereby continuing to reduce malaria incidences in regions of low transmission (Prince et al., 1996).

3.11.3 Specific immune responses to diverse antigens in children under 5 years.

Antibodies (IgM, IgG4 and IgE) directed against the schistosome crude antigens cercariae (CAP), adult worm (SWAP) and egg (SEA) and Plasmodium crude, schizont, the malaria vaccine candidates MSP-1_19 and MSP-2 (CH150 and DD2) were measured by enzyme linked immunosorbent assay (ELISA).

All children investigated showed that they had previous exposure to schistosome antigens, as well as malarial antigens. The immune response mounted against the cercariae antigen, (CAP) was predominantly high in children 1-3 years.
Both IgE and IgG4 were high as compared to other age groups against the same antigen but IgM was predominantly lower supporting the theory of immune evasion by the cercariae. It is only IgG4 that was high against the soluble egg antigen (SEA). As for the worm antigen, a similar response was mounted in all the age groups by IgE, but IgG4 and IgM behaved differently.

The diagram (Fig. 3.30) below shows the responses of the children under 5 years to specific schistosomiasis antigens and the malaria antigens.

**Figure 3.30:** Different antibody responses to the antigens of schistosomiasis in the age groups below 5 years.
The children under 5 years exhibited exposure to schistosomiasis as evidenced by the diverse antibody responses to the schistosomiasis antigens (Figure 3.30), the cercariae, the egg and the worm antigen preparations. Only IgG antibody can be passed from the mother to the child, therefore, the presence of the other classed of antibodies, particularly IgM and IgE which are highly anti parasitic, are an evidence of schistosomiasis exposure. Since mothers carry out household chores at homesteads, they usually do in the company of the infants, whom they carry on their backs, and it is inevitable that when its bath time, these infants are also bathed in the river with infested water.

Figure 3.31: Different antibody responses to the malaria parasite antigens in the age groups below 5 years

Schistosome – specific IgM directed against all 3 life stages of the parasite and anti-egg IgE increased with age. Anti-worm IgE and IgG4 did not change significantly with age. Figure 3.31 shows that anti-Plasmodium schizont responses (IgM and IgG) and MSP-2 (CH150
serotype) declined with age. Different antibodies directed against schistosome antigens and *Plasmodium* antigens show distinct age-profiles. The picture displayed here shows that though malaria antibodies may have been passively inherited, the presence of IgM anti Pf schizont is clear evidence of the infants’ exposure to malaria.
Discussion
Discussion

A: Study area, Schistosomiasis epidemiology, knowledge attitudes and practices.

Questionnaires as well as direct participation of the study population, allowed the GPS mapping of water contact sites in the two villages and these showed different distribution patterns of homesteads along the rivers with homes in Magaya being closely associated with rivers as shown (Figure 3.1 & 3.2). The rivers flow downstream to Magaya, with Chitate acting as a catchment. Therefore it was expected that even in the dry season, rivers in Chitate would dry up, while those in Magaya will still be containing some water, hence the transmission difference.

The area of Murewa is a market gardening area. The area has a number of perennial rivers which is a transmission point and makes in conducive to complete malaria and schistosomiasis life cycles. In our administration of the questionnaire, assessing nearest water sources and water usage habits we noted that the proximity of a homestead to a river, given the unavailability of tapped water and poverty would see on involuntarily resorting to the use of river water for major household chores, also established elsewhere by Ugbomioko et al., 2010. The contact of water by individuals during basic household chores was very high. Due to the lack of proper potable water nearby, and the hive of market gardening, communities were found to be prone to new infections. The role of the community health worker and other health education methods cannot be underscored. It was noted that the community had no reliable source of information on neglected diseases. The school only catered for 35 %. Later in life as they turn to puberty and young adults 15-25 years,
protective immunity mounted by IgE antibodies progressively take over, resulting in the partial elimination of the resident worm burden and installation of a more steady state of resistance to re-infections (Hagan et al., 1991).

B: Allergic responses

All participants had detectable levels of parasite-specific antibodies particularly IgM reflecting that all participants had been exposed to schistosome infection. The population had highly prevalent Th2-like systemic responses with most people producing IgE antibodies directed against cercariae, adult and egg antigens.

Helminths are believed to induce a strong Th2 response in both humans and experimental models characterized by high-level tissue eosinophilia, mucosal mastocytosis and the production of IgE (Gurish et al., 2004).

Autoimmune diseases cause significant and chronic morbidity and disability (Bach, 2002). Their epidemiology remains poorly understood (Bach, 2002), but experimental studies in mice suggest that parasitic helminth infections can protect against autoimmune diseases such as Type I diabetes (Bodansky et al., 1992). All currently recognized autoimmune diseases are associated with circulating antibodies directed against self-antigens or auto-antibodies (Herz et al., 2000).

The results of this study showed that a fraction of individuals with high auto-antibody reactivity significantly decreased with increasing schistosome infection intensity. This relationship was robust to potential confounders, host age, sex, HIV status, and residential location. None of the participants in this study had evidence of active malaria infection detectable by microscopic examination of thin and thick smears prepared from venous blood;
therefore auto-antibodies in these populations could not be attributed to an active malaria infection as has been reported in other settings (Agyei-Frempong et al., 2008). This relationship was replicated across the two villages of significantly different schistosome infection levels; Chitate with low infection and Magaya with high infection prevalence of 14% and 68% and mean infection intensities of 15 and 58 eggs/10 ml urine, respectively.

Auto-antibody prevalence was significantly higher in Chitate (48%) compared to Magaya (22%). Consistent with results from other earlier studies, schistosome-specific immune responses develop earlier in residents of the higher infection area arguing that the reduced auto-reactivity of this population is not due to a general loss of antibody generating capacity (Mutapi et al., 2007). The following figure illustrates the mechanism of immune evolution during acute schistosome infection and allergic response, and the chronic stages.
Figure 3.2: The mechanism of immune evolution during acute schistosome infection and allergic response, and the chronic stages (Rujeni et al., 2011).
During primary infection with a low dose of schistosome infection, the antigen presenting cells and cytokines released orchestrate a Th2 response which is mainly IL-4 regulated. Degranulation of the eosinophils and mast cells results in allergic responses and the production of IgE antibodies. Upon a heavier dose infection, IL-4 and IL-10 which are responsible for directing the type of immunity coupled with Treg cells are then responsible for class switching between IgG4 and IgE. The observed ratio between IgG4 and IgE could explain the hygiene hypothesis.

C: Schistosomiasis antibody responses
The whole population was assayed for response to anti-schistosome antibodies, the cercariae, whole worm and egg antigen, and apparently all participants showed that they had at one point been exposed to schistosomiasis. The infection with schistosomiasis in such a set up where the means of earning income is market gardening is highly unavoidable. High levels of parasite specific IgE was noted in all age groups especially after treatment with a single dose of praziquantel. IgE has been associated with resistance to re-infection and rendering protection in chronic schistosomiasis infection although what triggers the high levels of IgE still remains elusive. The protective role of IgE has been reported in other field studies where the continued re-treatment of infected individuals saw a rise in the titres of schistosome specific IgE (Mwinzi et al., 2009).

Antibodies indicative of exposure to schistosome parasites increase with age, reflecting the increase in exposure to infective water. The total IgG-specific activity is not on its own, indicative against malaria, even though it has been found out that IgG3 is significantly associated with relative malaria protection. There was a notable trend of the antibody responses; response against the cercariae was pronounced by both IgM and IgE. This could
be because IgM is a pentamer and IgE is the ‘gate-keeper’ antibody which is the first one to be produced and the cercariae is the primary entry point antigen that is found in the human body. IgM is also seen to be pronounced against the egg antigen as well as the whole worm, for the same reason stated.

IgE was highly elicited against the cercariae and the egg, and to a lesser extent against the worm. IgG4 was in low volumes and was not significantly different in all age groups. IgE and IgG4 are mainly associated with protection to re-infection. They are desirable protective antibodies in the helminth fight.
D: Malaria immunological responses.

The anti-\textit{Plasmodium falciparum} responses were as a result of the previous exposure to the malaria antigen. The reduction in transmission of malaria has been attributed to better treatment regimen and efficacy of the ACT treatment (Mharakurwa et al., 2013). Erratic rains have also been perceived as a contribution factor, due to the declining water bodies that are essential for the breeding of the transmitting vector. The development of an adaptive immune response that has been observed in endemic population has been associated with the parasite burden and higher degrees of premunition.

Adaptive immunity is triggered when the innate line is evaded and generates a threshold dose of antigen (Janeway and Travers, 1996). This antigen is now responsible for the adaptive type of immune responses. We have observed that the anti-\textit{P. falciparum} schizont antibodies were proof that the individuals had the \textit{P. falciparum} schizont in their system.

This type of adaptive immunity is slow to mature and usually, an adaptive immune response clears all the antigens present from the body, and antibody levels are expected to start declining thereafter up to such a time when the individual is re-exposed. Sometimes that antigen is retained for longer, like in this case of slow-forming adaptive immune response; it is assumed that this residual antigen is responsible for the sustenance of cells that mediate immunological memory. Janeway and Travers, 1996).
Role of *Anopheles gambiae* in malaria transmission on sub Saharan Africa.

*Anopheles gambiae* and *Anopheles arabiensis* play an important part in malaria transmission in southern Africa (Munhenga et al., 2008). In Zimbabwe, *Anopheles arabiensis* was found to be the predominant member of the *Anopheles gambiae* complex responsible for malaria transmission (Munhenga et al., 2008).

In Malawi, it has been established that, *A. gambiae* has a significant contribution, 55.1% *A. gambiae sensu lato* and 44.9% were *A. funestus in a given mosquito population* (Mwangangi et al., 2013), whereas, Zambia has noted *A. gambiae* becoming more and more than *A. arabiensis* (Chanda et al., 2012). The recent WHO entomological mapping shows that the species that are predominant in southern Africa are *A. arabiensis* and *A. funesitus*. *Anopheles gambiae* is predominant in northern Zambia and the DRC (Kiszewksi et al., 2004). It is therefore, imperative that entomological surveys are done regularly to monitor the type of vector involved in malaria transmission in our country. Zambia and Mozambique, which have reported having *A. gambiae*, have serious malaria incidences and deaths.

E: Co-infections

Antibodies indicative of exposure to Plasmodium parasites decrease with age possibly due to isotype switching to protective immune responses. Antibody responses associated with immunity to both parasites are significantly correlated.

The geographical distribution of the two human parasites *Schistosoma haematobium* and *Plasmodium falciparum* overlaps and the prevalence of both parasites in exposed individuals rise with age, peaking in childhood. Cytokines are thought to contribute to the pathological processes resulting from infection with these parasites. There is much of growing body of evidence indicating that co-infection with these 2 parasites can significantly affect
development of protective immunity and pathology. However, there is a paucity of studies in young children in whom these interactions may be particularly dynamic.

Therefore we investigated the relationship between exposure to both parasites, development of parasite-specific antibody responses in Zimbabwean children up to 5 years of age. Generally the study population had a response to all of the schistosome antigens that were used. The total antibody levels directed against, the recombinant, the soluble, and the crude worm antigens were comparable in *S. haematobium* negative and positive individuals. It may be true that high antibody levels in the negative people are associated with previous infection with schistosomiasis as we observed that even the little children had antibodies against schistosomiasis, showing early child-hood infection (Hagi *et al.*, 1990).

Total levels of IgG were against all the antigens came out high as expected. This was anticipated since most of the IgG class antibodies are involved in anti-schistosomiasis fight. Some are highly protective, that is IgG3 and some have been reported to block resistance-related effector mechanisms (Hagan *et al.*, 1988; Butterworth *et al.*, 1991). The conflicting results of schistosomiasis co-infection with malaria have been reviewed by Nacher, 2011.
Chapter Four

Conclusions and recommendations
Chapter Four

4.0 Summary, conclusions and recommendations

4.1 Summary of key results

When ascertaining knowledge attitudes and practice the results indicated guardians were more informed about malaria than they were of schistosomiasis. Guardians did not know what bilharzia was and whether or not one could catch bilharzia after praziquantel treatment; but knew most of malaria symptoms and that they could contract malaria even after treatment. This indicates the difference in acuteness of infections and the immediate danger of such infections in the communities.

Urinary schistosomiasis infection prevalence in the study population was 56%, there were no soil transmitted helminths or *S. mansoni* present. The two areas of different infection prevalence emerged, that is schistosome infection levels were significantly higher in Magaya (prevalence = 69%, 95% CI 63% - 75%) than in Chitate (prevalence = 14%, 95% CI 11% - 18%) ($\chi^2 = 187$, df=1, $p<0.001$) with mean infection intensity of 58 eggs/10ml urine. Infection levels followed the typical age-prevalence/intensity infection pattern.

All participants investigated produced IgM antibodies directed against all the three life stages of the schistosome parasites (cercariae, adults worms and eggs).

Only 4.8% of the children treated with praziquantel tablets and interviewed reported side effects of headache, loss of appetite, stomach ache, and general weakness occurring within 24 hours. This was less than what was reported in older primary school children (Midzi *et al.*, 2008). Of the treated populations, there was a reduction in infection prevalence and intensity in both areas although this was significant only for Magaya. No haematuria was observed in the treated children 6 weeks post-treatment, including the 3% whose parents had reported macro-haematuria in the questionnaire. In all groups; weight and height increased 6 weeks following treatment.
The overall prevalence of skin prick reactivity was 19% and the most prevalent allergic response was directed against the house dust mite with 12% of the population reacting against it. The prevalence and intensity of atopic responses remained unchanged 6 weeks post-treatment with praziquantel.

Children aged 5 years and below had IgM responses directed against the cercariae antigen showing evidence of exposure to schistosomiasis. After treatment there was a significant decrease in IgM levels reflecting a reduction in exposure to parasite antigens as well as an increase in IgE responses which has been associated with protection to re-infection, as IgE is involved in ADCC immune responses.

In 613 people naturally exposed to schistosomiasis, the results show that autoimmune reactivity is inversely associated with current infection intensity but is independent of host age and sex status. Autoimmune reactivity increases 6 months after anti-helminthic treatment (Fig 3.20 & 3.21). Auto-antibody prevalence was significantly higher in Chitate (48%) compared to Magaya (22%). This showed the important part played by parasite transmission in the area toward autoimmune reactivity.

Children as young as 6 months had been recently exposed to malaria parasites, IgM antibodies directed against *P. falciparum* – schizont were observed even though no parasite could be detected. The current treatment regimen indicate a possibility to controlling *P. falciparum* transmission.
4.2 Conclusions

4.2.1 The epidemiology of Schistosomiasis and Soil Transmitted Helminths.

In school going children and adults, the pattern of schistosomiasis distribution in a population has been found from this study to be similar to what has often been reported where the peak transmission and peak age intensity/prevalence is within the early teens gradually decreasing in the early twenties thereafter. The majority of the guardians/parents of the under 5 years old children had very limited knowledge about schistosomiasis.

*Schistosoma mansoni* and soil transmitted helminths are none existent in this rural area, similar to reports by Midzi and co-workers in Zimbabwe (Midzi et al., 2007); where rural areas had no STH compared to farming areas. This is probably due to environmental conditions where there is massive irrigations in the farming areas making the soils moisture and conducive to STH survival. Treatment is effective and the communities are receptive to treatment even for the young children. Health education and availability of treatment could reduce the burden and morbidity from the infections. The study indicates that children aged 6 months-5 years were exposed to schistosome infection and that they should be included in treatment programmes. The current drug of choice, praziquantel, is safe and efficacious to use in this age group.

It has no severe immediate side effects and has no significant effect on atopic responses. Furthermore, the drug accelerates the development of schistosome-specific immune responses that have been associated with resistance to re-infection and therefore may have longer-term effects on reducing re-infection and transmission.
Praziquantel, acts by altering calcium (Ca\(^{2+}\)) homeostasis in adult schistosomes. It modulates the voltage-gated Ca\(^{2+}\). A few moments after administration of praziquantel, it causes vacuolisation and rapid contraction of the worm muscles and eventually disruption of the worm tegument (Becker et al., 1980). The success of control programmes in this area will be greatly increased by educating parents/guardians of children on bilharzia, what it is, its mode of transmission, and the available treatment. It also revealed that school-aged children are the most at risk to experience co-infections or heavy schistosome infections.

4.2.2 Immune responses to diverse parasite antigen exposure

In the whole population, the study observed prevalence of urinary schistosomiasis infections and the likelihood of \textit{Plasmodium falciparum} infection. Treatment using Coartem® is extremely effective in reducing transmission and even reservoirs in the community. The response to different blood stages of the \textit{P. falciparum} parasite indicates that the introduction of the treatment regimen enabled the immune responses to be elicited by the antigens. However, no major prominent immune response could be found besides that the population had immunological responses to different antigen of the life stages. Additionally, even the youngest children also responded to the antigens indicating that these were also exposed.
The adult population had high exposures to schistosome parasites and haematuria was prevalent prior to treatment with praziquantel, whereas, post treatment very few individuals had heavy infection as a result very few individuals had haematuria.

Most individuals from the area responded highly to cercariae antigens. This shows that there is high sensitization to cercariae; antigens which may be translated to individuals having high exposures to waters infested with the cercariae. A few individuals were successfully infected after exposure, and hence, developed infection as shown by the eggs in their urine samples post treatment.

These individuals had elevated anti-egg responses post treatment showing that treatment makes available schistosome egg antigens and the immune responses is increased.

Similarly prior to treatment the immune responses to worm antigens are low with elevation post treatment. Treatment kills the worms and makes available the antigens that were once masked. As such, there was an increased response to worm antigens after treatment of the infected individuals.

The types of antibodies produced show some interesting patterns, with generally an increased response shown by IgE against most parasite stages post treatment. IgE has been reported to be important in protective immunity through its involvement with mast cells, eosinophils and other cells of the ADCC effector mechanism. These results show that it may be important to administer treatment regularly in endemic areas that would enhance development of protective immunity.
4.2.3 Allergic responses

Most individuals that were infected with schistosomiasis did not have any marked allergenic responses to the testing antigens. However, post treatment only the uninfected were seen to respond to allergens showing that somehow the presence of schistosomiasis and treatment would greatly dampen the immune responses and avoid massive allergenic reactions in individuals resident in high transmission area.
4.3 Recommendations

There are several important implications arising from the study:

- There is urgent need for educating the parents/guardians on bilharzia, its transmission, and symptoms and how it is treated. If the parents do not recognize bilharzia for what it is, then they will not seek treatment. We recommend that in the primary school educational curriculum be incorporated information about the diseases: bilharzias, malaria and the soil transmitted helminths.

- Pre-school-age children need treatment where we find over 25% of the children resident in transmission areas infected with schistosomes. The study has shown that praziquantel treatment is safe and a risk worth taking in children aged 6 months to 5 years of age. Praziquantel treatment at the recommended dose of 40 mg/kg body weight is efficacious in children aged 6 months - 5 years of age and also beneficial to all ages. Praziquantel treatment did not result in an increase in atopic responses 6 weeks and 6 months after treatment in children aged 6 months - 5 years of age.

- Regular school de-worming programmes should continue being implemented. Praziquantel treatment resulted in a significant increase in parasite-specific IgE, which has been associated with resistance to re-infection.
References
References


33. Chimbari MJ., Enhancing Schistosomiasis Control Strategy for Zimbabwe: Building on past experiences, University of Botswana, Okavango Research Institute, P. Bag 285, Maun, Botswana. [online]

    S23-S38.
    that cytokine-mediated immune interactions induced by *Schistosoma mansoni* alter 
    disease outcome in mice concurrently infected with *Trichuris muris*. *J. Exp. Med.* 
    181:769-774.
41. da Frota SM., Carneiro TR., Queiroz JA., Alencar LM., Heukelbach J., Bezerra FS. 
    2010. Combination of Kato-Katz faecal examinations and ELISA to improve accuracy 
    of diagnosis of intestinal schistosomiasis in a low-endemic setting in Brazil. *Acta 
    Trop:* in press.
42. David T., Thomas C., Zaccone P., Dunne DW., Cooke A., 2004. The impact of 
    Resistance to *Schistosoma mansoni* in humans. Influence of IgE/IgG4 balance and 


53. Dunne DW., Butterworth AE., Fulford AJ., Kariuki HC., Langley JG., Ouma JH.,
schistosomiasis: association between IgE antibodies to adult worm antigens and

responses to *Schistosoma mansoni* and resistance to reinfection. *Mem. Inst. Oswaldo
Cruz*, 87: 99-103.

55. Dunne DW., Butterworth AE., Fulford AJC., Kariuki HC., Langley, JG., Ouma JH.,
schistosomiasis: association between IgE antibodies to adult worm antigens and


influence of epitopes shared between different lifecycle stages on the response of

58. Ellis MK., Raso G., Li YS., Rong Z., Chen HG., McManus DP., 2007. Familial
aggregation of human susceptibility to co- and multiple helminth infections in a
population from the Poyang Lake region, China. *Int. J. Parasitol.* 37:1153-1161.

Leonard, P. E. Lipsky. 2005. IL-21 induces differentiation of human naive and

60. Fallon PG., Mangan NE., 2007; Suppression of Th2-type allergic reactions by


73. Greenberg RM., Ca2+ signaling, voltage-gated Ca2+ channels, and praziquantel in flatworm neuromusculature Marine Biological Laboratory 7 MBL Street Woods Hole MA 02543 USA [online] accessed October 2013.


86. Hoffmann K.F., Caspar P., Cheever AW., Wynn TA., 1998. IFN-gamma, IL-12, and TNF-alpha are required to maintain reduced liver pathology in mice vaccinated with Schistosoma mansoni eggs and IL-12. J. Immunol. 161:4201-4210.


119. Mharakurwa S., Mutambu SL., Mberikunashe J., Thuma PE., MossWJ., MasonPR., andfortheSouthernAfricaICEMRTeam. 2013. Changes in the burden of malaria following scale up of malaria control interventions in Mutasa District, Zimbabwe


121. MJ Chimbari 2012. Enhancing Schistosomiasis Control Strategy for Zimbabwe: Building on past experiences


137. Package insert, Biltricide (Miles), Rev 7/85, Rec 4/89.


5.2 A: Appendix of protocols

Blocking/ Dilution Buffer

Add 100 ml of 10X Dulbecco’s PBS Buffer + 900 ml distilled water + 300 µl Tween 20 = 50 mg Marvel and give a good stir on the magnetic stirrer.

This same buffer without Tween is used to dilute Streptoavidin Horse Radish Peroxidase.

TMB substrate

Reagents:

3’-3’.5’-5’- tetramethylenzidine dihydrochloride (TMB)

1:1 Acetic acid :dH₂O

Na₂PO₄

Citric Acid

H₂O₂

10X TMB Buffer (Phosphate citrate buffer 0.5M pH 5.0)

Add 25.5 g Citric acid to 45.7 g Na₂PO₄ and dissolve in a total of 500 ml distilled water.

Filter sterilize and store at 4 °C. To make a 1X, dilute 10X with distilled water.
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<th>TMB /g</th>
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<th>1X P04/citric acid Buffer/ ml</th>
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Table 1: The table below details the amount of TMB to add to different volumes of the 1X phosphate citrate buffer

The TMB was prepared and stored in polyethylene beakers and tubes and it was used still fresh.

5.3 Appendix B: Other maps and figures
Figure 3.33: GPRS mapping of the distribution settlement pattern in the two communities. Blue circles = rivers, triangle = homestead
Publications by the Author