PROFILING OF ANTI-SCHISTOSOMA ANTIBODIES PRE AND POST PRAZIQUANTEL TREATMENT IN PERSONS LIVING IN A SCHISTOSOMIASIS ENDEMIC AREA IN GHANA

By

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DECLARATION:

I hereby declare that this submission is my own towards an MPhil and that to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

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ABSTRACT

Schistosomiasis, a water borne parasitic disease where 240 million people in 78 countries are affected, majority occurring in Africa. Sensitive diagnostic tools for detection of infections, pre and post treatment is the foundation of the success of mass drug administration. Detection of antibodies in the serum gives knowledge on therapeutic responses and persistence of infection. ELISA was used to measure the sero-prevalence of anti- *Schistosoma mansoni* and *Schistosoma haematobium* IgM, IgG and IgE to soluble egg antigen (SEA) and adult worm antigen (AWA) among participants. Blood samples were collected from participants aged between 6 and 76 years 2 weeks before treatment and 8 weeks after treatment with praziquantel.

At pre-treatment, the sero-prevalence of anti-*Schistosoma mansoni* IgM to SEA and IgE to AWA was higher in participants. At 8 weeks post-praziquantel treatment, there was no statistically significant decrease in sero-prevalence of participants having IgM against SEA. Participants having IgE against SEA was reduced significantly and IgG increase was statistically significant. A significant decrease was observed in participants having IgM and IgG against AWA whiles IgE increase was not statistically significant. For *Schistosoma haematobium* at pre-treatment, participants had a higher sero-prevalence of IgE against SEA and IgG against AWA. At post treatment, there was a statistically significant increase of participants having IgM and IgG and a reduction of IgE against SEA. IgE against AWA tend to increase whiles IgG reduced and IgM sero-prevalence remained the same.

Results from this study showed history of individuals that have recent or past infections and an individuals immune status before an infection and after chemotherapy. Thus antibody profiling can be a useful tool in determining therapeutic response and persistence of infection.

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LIST OF ABBREVIATIONS

ABTS	2, 2-azinobis 3-ethylbenzothiazoline-6-sulfonic acid
ADCC	Antibody dependent cell mediated cytotoxicity
BSA	Bovine Serum Albumin
CDC	Centres for Disease Control
°C	Degree Celcius
DNA	Deoxyribonucleic acid
ELISA	Enzyme Linked Immunosorbent Assay
g	Gram
IgA	Immunoglobin A
IgD	Immunoglobin D
IgE	Immunoglobin E
IgG	Immunoglobin G
IgM	Immunoglobin M
IL4 and IL13	Interleukin 4 and 13
MDA	Mass drug administration
µg	Microgram
µl	Microlitre
µM	Micro molar
nM	Nano molar
mg	Milligram
ml	Millilitre
PBS	Phospahte buffered saline
PBST	Phosphate buffer saline with Tween 20
PCR	Polymerase chain reaction

PCT	Preventive chemotherapy
pH	Hydrogen ion concentration
RPM	Revolution per minute
Sm SEA	*Schistosoma mansoni* soluble egg antigen
Sm AWA	*Schistosoma mansoni* adult worm antigen
Sh SEA	*Schistosoma haematobium* soluble egg antigen
Sh AWA	*Schistosoma haematobium* adult worm antigen
Th1 and Th2	Type 1 and 2 T helper cell
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.0 Background

Schistosomiasis is the second most predominant and devastating tropical disease in the world, particularly in tropical and subtropical areas after malaria in terms of socioeconomic and public health importance (The Carter Centre, 2016).It is a major cause of morbidity and mortality in developing countries in Africa, Asia, the Caribbean, South America and Middle East (WHO, 2014). It is considered as the most important helminth infection in terms of morbidity. It is a major health problem due to its ability to manifest severe clinical forms and its severity in endemic areas particularly where the health resources are limited (Montresor *et al*, 1997). About 207 million people have been estimated to be infected with one of the major schistosomes with more than 90% of the cases occurring in sub-Saharan Africa and almost 200,000 people dying annually from schistosomiasis in Africa (Hotez and Fenwick, 2009). It is estimated that about 7million school children in Ghana are at risk of infection and around 15million infections occur annually in the country (Medicine on the move, 2015). The large-scale administration of praziquantel to persons in a schistosomiasis community is the mainstay of current programs focusing on morbidity control (Fenwick *et al*; 2009).

Schistosomiasis is also developing in non-endemic areas due to migration of travelers and the increase in the distribution of vectors snail. There have been recent reports of urinary schistosomiasis infections among travelers and inhabitants in South Portugal, France and Spain and these could be possibly be autochthonous infections since there was evidence of the snail vectors present in these areas (CDC, 2014). These new findings therefore necessitates the development of effective preventive and control mechanism against schistosomiasis. Effective

control of infection depends on accurate, sensitive, specific and reliable diagnostic methods. Furthermore, monitoring and evaluation of chemotherapy programmes are largely dependent on accurate and early identification of infection for the effective execution of these control programs. Laboratory diagnosis of the parasites for urinary and intestinal schistosomiasis for many years rely on the traditional microscopic examination of stool and urine samples whiles serology and polymerase chain reaction (PCR) methods have been used as a confirmatory diagnostic tools. (Pontes *et al*, 2002). Microscopy, which relies on the presence of eggs for the detection and identification of species and estimation of infection intensity, is regarded as the gold standard for schistosomiasis diagnosis (Weiss, 1995). Nonetheless, the challenges in meeting the various sampling requirements for microscopic diagnosis frequently produce imperfect results (McCarthy *et al*, 2012). Samples also need to be collected at suitable or specific times for a good recovery of parasite eggs (Gryseels and Sake de Vlas, 1996). Samples will also have to be processed within 24 hours with careful examination to give high sensitivity particularly when infections are light (Gryseels and Sake de Vlas, 1996). Microscopic diagnosis is labour intensive and time consuming process. These can lead to technical errors resulting in reduced sensitivity and precision of results (Wang *et al*, 2011).

Molecular diagnostic methods employing the use of polymerase chain reaction (PCR) are currently a confirmatory diagnostic tools used in areas with medium and low intensity of infection (Pontes *et al*, 2002). PCR technique has high sensitivity and specificity and it is used in the detection of schistosome DNA in biological specimen such as feces, urine and blood. Its application in wide epidemiological surveys and field applicability is currently limited, as it requires significant infrastructure, financial resources, and skilled personnel.

Immunodiagnostic techniques which are more sensitive than microscopy remain the best accessible techniques for diagnosis in areas of low intensity of infection. Serological tests based on the detection of host antibodies directed against schistosome antigens have therefore been optimized in diagnosing schistosomiasis (Kanamura *et al*, 1979). Antibody detection has shown to be more sensitive than microscopy stool examination in areas characterized by low level of transmission, low prevalence and particularly low intensity (Xu *et al*, 2011). Antibodies can persist for a long time after elimination of the parasites, thus a particular antibody(IgG) maybe present in a patient in whom there are no egss for example those with Katayama fever (Ross *et al*, 2002). In human schistosomiasis, the mechanism by which parasites(schistosomula larvae) are killed is through antibody dependent, cell mediated cytotoxicity. Some immunoglobulins are indications of infection while others serve as blocking or protective isotypes (Naus *et al*, 1998). Immuno-epidemiological studies have also indicated that high or low level of specific anti-*Schistosoma* IgE and IgG_4 antibodies in a human serum indicate resistance or susceptibility to reinfection (Kleij, 1999).

Thus, this study aimed at determining the sero-prevalence of anti- *Schistosoma* antibodies among participants and how they are expressed before and after schistosomiasis treatment.

1.1 Problem Statement

Neglected Tropical Diseases programmes in Ghana have employed preventive chemotherapy (PCT) as an effective way to control schistosomiasis by focusing primarily on mass drug administration (MDA) (Seddoh *et al*, 2013). Communities along the Vea dam in the Upper East Region of Ghana are known to have high schistosomiasis burden because of the lack of access to

potable water thus the use of the dam water, good toilet facilities and the presence of intermediate host snails around the banks of the dam. This therefore necessitated an annual MDA programme by the Ghana Health Service in the area to help reduce the disease burden. Nonetheless, monitoring and evaluation of these programs is largely dependent on the prevalence of infection by microscopy. Microscopy lacks sensitivity in post treatment situations, in the control of transmission in low endemic areas (Pontes *et al*, 2003). Microscopy often fails to diagnose cases accurately leading to false negative results in areas that have a reduction in intensity and infection prevalence after treatment. Serology, based on the detection of specific anti-*Schistosoma* antibodies to specific schistosome antigens has been shown to be more sensitive than microscopy in such situations (Coon, 2005). It is perceived that it is helpful in field studies for characterizing regions of low- level endemicity where individual patients have low egg burdens.

Thus, there is a necessity to determine specific anti-*Schistosoma* antibody sero-prevalence pre and post treatment since they are good indicators of an individual's immune status to re-infection after chemotherapy.

1.2 Rationale of study

The mainstay of current schistosomiasis control programs focuses on morbidity control by large-scale administration of praziquantel to persons in schistosomiasis endemic communities using the community or school based platforms (Fenwick *et al*, 2009). The measure of success of the mass drug administration program depends on sensitive methods of diagnosis to detect infection pre and post treatment.

Profiling of anti- *Schistosoma* antibodies serves as a useful tool to determine therapeutic responses, the persistence of infection and intermittent control of transmission of infection.

There has not been any known report in Ghana on the comprehensive anti-*Schistosoma* antibody profiling of persons pre and post treatment to know which antibodies are indicators of resistance to reinfection and susceptibility to infection after treatment. This study therefore is aimed at measuring the sero prevalence of specific anti-*Schistosoma* antibodies (IgM, IgG and IgE) of persons living in a schistosomiasis endemic region in the upper east region of Ghana pre and post treatment.

1.2.1 General Objective

The main objective of the study was to measure the serum anti-*Schistosoma* antibodies of people living in a schistosomiasis endemic area pre and post praziquantel treatment.

1.2.2 Specific objectives

- To measure the anti- *S. mansoni* and *S. haematobium* IgG, IgM and IgE sero-prevalence among participants pre praziquantel treatment using ELISA.
- To measure the anti-*S. mansoni* and *S. haematobium* IgG, IgM and IgE sero-prevalence among participants post praziquantel treatment using ELISA.
- To compare the sero-prevalence of anti-*S. mansoni* and *S. haematobium* IgG, IgM and IgE pre and post praziquantel treatment.

CHAPTER TWO

LITERATURE REVIEW

2.1 Epidemiology and Distribution

Schistosomiasis, also called bilharzia or snail fever is a water borne parasitic disease caused by trematode blood flukes, of the genus *Schistosoma* which is carried by fresh water snails. It is a disease commonly associated with poverty in most rural settings and it affects approximately 200million people worldwide in 76 endemic countries with 650million at risk of infection and 85% of them living on the African continent (Engels *et al,* 2002). An incease in transmission is due to high population size and infected fresh water bodies among those who live near lakes or rivers thus high risk of infection is observed in these areas (Ahmed, 2016).

Schistosomiasis is commonly found in areas with poor sanitation and school age children who live in these regions are often the most at risked group because they spend time wading or swimming in ponds, lakes and other water bodies that are infested with the snails that are the natural repositories of the *Schistosoma* pathogen. Other high risk groups include fishermen, farmers and people using unclean water for their daily chores. Although it has a low mortality rate, chronic schistosomiasis can damage internal organs, weaken the body's resistance to other infections and sometimes cause premature death and in children, it can impair growth and cognitive development. (The Carter Centre, 2016).

Three main species are known to cause Schistosomiasis in humans. These are *Schistosoma mansoni, Schistosoma haematobium* and *Schistosoma japonicum* with other less prevalent species of *S. mekongi and S. intercalatum* which may also cause systematic human disease.

S. mansoni, an intestinal schistosomiasis is found in Africa, the Caribbean, Middle East and South America and affects 83.31million people worldwide (Crompton, 1999). It is the most

widespread of the human infecting schistosomiasis which is present in 54 countries. The snail intermediate host of *S. mansoni* is *Biomphalaria spp.*

S. haematobium is associated with urinary schistosomiasis and is endemic in about 50 countries in Africa and the Middle East, where the intermediate snail host, Bulinus resides (Roberts and Janovy, 2000).

S. Japonicum is endemic in Indonesia, China and Phillippines and Thailand, *S. mekongi* is found in several districts of Cambodia and the Lao People's Democratic Republic and *S. intercalatum* rain forest areas of central Africa (WHO, 2014).Other schistosomes like avian or mammalian primary host can bring about extreme dermatitis in humans eg swimmer's itch secondary to *Tricobilharzia ocellata.* In Ghana, the disease is known to be caused by two species, *S. mansoni* and *S. haematobium* with the former causing intestinal schistosomiasis and the latter urinary schistosomiasis (Bosompem *et al,* 2004).

Infection occurs when the skin of human or susceptible mammal host comes in contact with fresh water in which certain types of snail intermediate hosts of the family Planorbidae that carry schistosomes are living.

2.2 Clinical manifestation

Schistosomiasis is a chronic disease and numerous infections are sub-clinically symptomatic, with malnutrition and mild anemia being common in endemic areas. Symptoms are caused by the body's response to the worm eggs. Signs and symptoms depend on the type of infection and the location of the parasite inside the body.

Each of the schistosome species gives rise to different disease spectra of different pathologies and severities. The range of clinical manifestation and complications depends on the magnitude

of the host immune response and intensity of infection. A disease in an advanced stage can result in life- long disabilities or extreme complications which can result in death. The disease status of schistosomiasis can be classified into acute, chronic and advanced.

2.2.1 Acute schistosomiasis

Acute schistosomiasis, sometimes known as Katayma fever (syndrome) occurs as a result of the host immune responses to egg production, migrating worm maturation, and the release of egg antigens (Weerakoon *et al*, 2015). It is often asymptomatic, characterized by allergic responses and may occur within 4 to 8 weeks after contact with infested water (Ahmed, 2014). It has an incubation period of 14 to 84 days. Symptoms occur 2 to 8 weeks after exposure to *S. mansoni* and *S. japonicum.* Katayama fever is seldomly found in endemic areas but mainly found in non-immune individuals after their first exposure to schistosomes, hence an essential differential diagnosis in returning travelers with fever. Despite the syndrome's name, fever is absent in at least one-third of symptomatic patients. Dry cough, abdominal pain, general fatigue, myalgia, headache and diarrhea or abdominal tenderness are commonly seen and often accompanied by a peripheral blood eosinophilia. Severe, possibly life-threatening complications are rare and include cardiac and central nervous involvement.

2.2.2 Chronic schistosomiasis

Chronic infection develops months to years after initial exposure to infection and its endemic in poor rural areas. It gives a clinical presentation depending on the species of schistosoma and the location of worms in the body (NHS, 2016). Symptoms are caused by a granulomatous inflammatory reaction against the schistosome eggs, which are held up in the intestine or bladder, bringing about inflammation or scarring. In children, infection can cause malnutrition, anemia and learning difficulties (USAID, 2015). In an untreated schistosomiasis, eggs that get into the bloodstream lodge in various vital organs, resulting in lungs, heart, intestine and central

nervous system damage. Recurrent infections over a period of years can also result in serious damage to vital organs in the body (Medical news today, 2009).

In intestinal schistosomiasis, eggs are lodged basically in the liver and the intestinal wall and this can prompt various granuloma formation and tissue injuries in these organs. This causes intestinal mucosal hyperplasia, ulceration, polyposis , and abscess formation, and this manifest clinically predominantly as abdominal pain, chronic diarrhea, and rectal bleeding (Issa *et al*, 2014). Granuloma formation caused by the lodging of eggs in the liver results in a periportal fibrosis extending to advanced disease, with portal hypertension and hepatosplenomegaly. Ascites and variceal bleeding are serious and basic complications at this stage, which can bring about death (Weerakoon *et al*, 2015). In rare instances, the central nervous system is affected.

Chronic urinary schistosomiasis happens after egg deposition and granuloma formation, for the most part in the urinary bladder wall, bringigng about abnormalities in the mucosa (Weerakoon *et al*, 2015). The disease manifests with lower urinary tract symptoms, for example hematuria, dysuria and frequency in urinating and in advanced stages, bladder calcification, urinary tract fibrosis bringing about obstructive uropathy, and bladder malignancies (WHO, 2015). Urogenital schistosomiasis may bring about genital sores, vaginal bleeding, infertility, pain during sexual intercourse and nodules in the vulva of women affected with female genital schistosomiasis. In men, it can impel pathology of the seminal vesicles, prostate and other organs. This disease may likewise have other long-term irreversible results, including infertility (WHO, 2015). Infection with *S. haematobium* can sometimes bring about hepatic complications as well.

2.3 Life cycle and transmission of Schistosomiasis

Schistosomes have digenetic life cycles involving, asexual reproduction in snail intermediate host depending on the species and sexual reproduction in the vertebrate definitive host. The life cycles of all the different species of schistosomes are broadly similar (Figure 1). Infection occurs when the skin of human or susceptible mammal host comes in contact with cercariae in fresh water in which snail intermediate hosts of the family Planorbidae that carry schistosomes are living. Every schistosoma species has a confined scope of snail host. Eggs of schistosomes released in faeces or urine of an infected individual hatches on contact with the fresh water to discharge free swimming miracidium which survive for about 8-12 hours. The miracidium penetrates the tissue of the snail and transforms into a primary sporocyst close to the site of penetration. The germ cells within the primary sporocyst further divides into a secondary or daughter sporocyst 2-6 weeks after infection. The secondary sporocyst migrates to the snails' hepatopancreas were the gem cells divides and produces thousands of cercariae which is the infective larval stage within 4 to 6 weeks (New World Encyclopedia, 2015). Cercariae are excreted daily by the snail in a circadian rhythm depending on light and ambient temperature into the water where they survive for up to 48hours which they should attach to a human skin or another susceptible mammalian host or die. Enzymes discharged by the parasite breaks down the skin's protein to empower entrance of the cercarial head through the skin. Cercariae attach to humans by suckers and move through intact skin to reach the pulmonary vessels over some few days. During migration, cercariae metamorphose into a schistosomula larval stage and become exceptionallyimpervious to human immune responses. The schistosomulum which is recently transformed may stay in the skin for two days before finding a post-capillary venule. The schistosomula assimilate host proteins, including histocompatibility complex and blood group antigens. The worms relocate through the pulmonary capillaries to the systematic circulation and

portal veins where they develop within six to eight weeks and the female begin to produce eggs. The matured male and female worm pair up within the portal vasculature and they migrate to the veins surrounding the intestines or bladder depending on the type of worm species. Worm pairs of *S. mansoni* relocate from the liver sinusoids to the mesenteric or rectal veins whiles *S. haematobium* schistosomula relocate to the perivesical venous plexus of the bladder, ureters, and kidneys through the hemorrhoidal plexus from the liver sinusoids. About 300 to 3000 eggs are produced on a daily basis. (Parasites in humans, 2015). Eggs of *S. mansoni* and *S. haematobium* relocate through the bowel or bladder wall respectively to be shed in faeces or urine to finish the cycle. Eggs that are not shed may stay in the tissues or might be cleared back to the portal circulation or into the pulmonary circulation, which may live in the body for an average of four and half years and may persist for longer (Ahmed, 2014).

The eggs are exceedingly antigenic and incite an extraordinarily granulomatous rection which is the primary cause of morbidity.

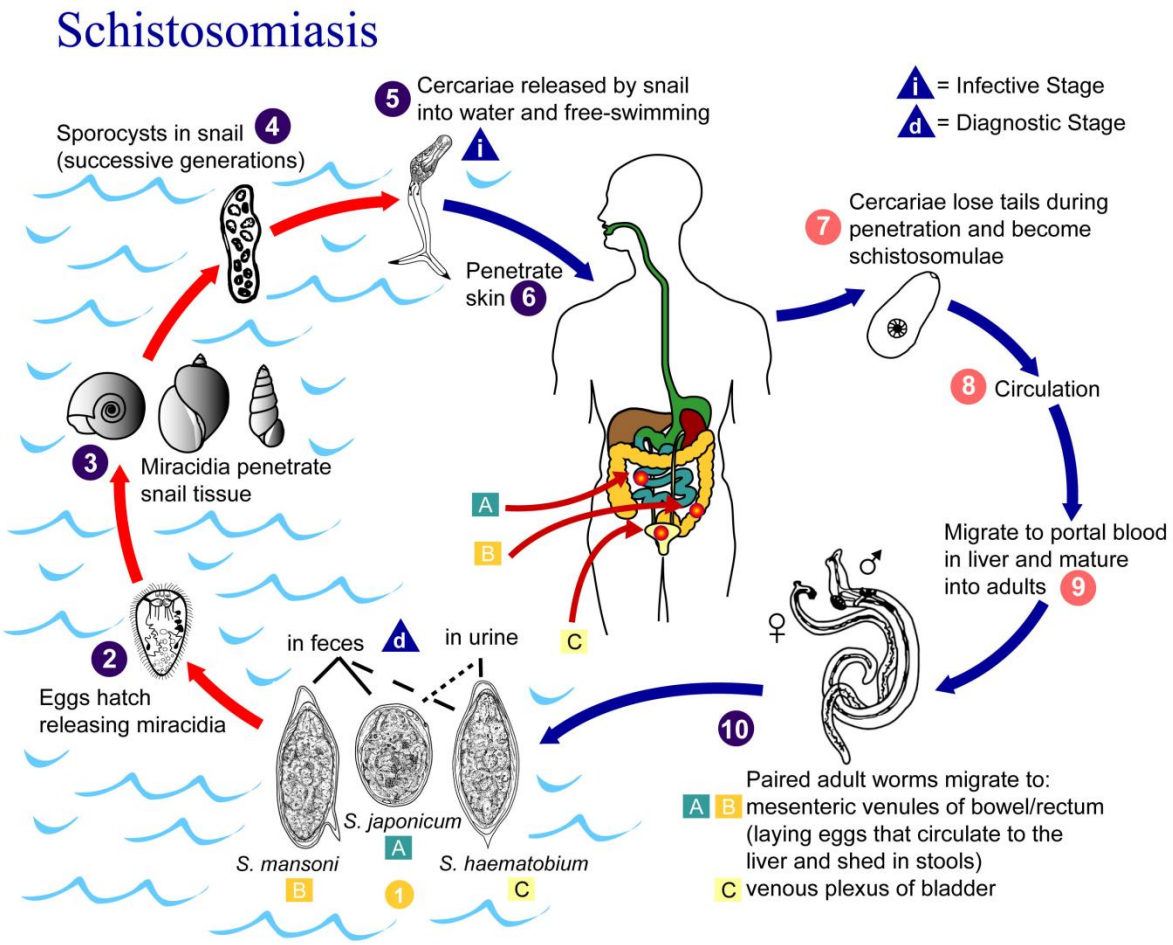


Figure 1: Life cycle of Schistosoma species (Source: http:// www.dpd.cdc.gov/dpdx)

2.4 Diagnosis of Schistosomiasis

Diagnostic approach to schistosomiasis depends on the epidemiological situation. Methods for diagnosing schistosomiasis include parasitological, immunological molecular procedures and the use of metabolites, cytokines and other *Schistosoma* molecules as biomarkers. Diagnosis can be divided into direct parasitological procedures which includes the identification of parasite eggs and indirect methodologies including detection of antibody or circulating antigens in serum.

2.4.1 Parasitological diagnosis

Microscopy, which relies on the presence of eggs for the detection and identification of species and estimation of infection intensity in stool, on account of intestinal schistosomiasis or urine for urinary schistosomiasis is regarded as the gold standard for Schistosomiasis diagnosis (Weiss, 1995). It is the most specific and direct means by which the presence of a schistosome infection can be established. The absence of eggs in urine or stool does not necessarily exclude the possibility of infection since studies have shown that eggs may be lodged in various host tissues such as bladder, liver, lung and occasionally the central nervous system (Stothard *et al*, 2014). Thus, this technique is associated with poor sensitivity, which limits both the diagnosis of individuals with early or low level infections and in the assessment of chemotherapy (Wang *et al*, 2011).

There are various methods for diagnosing intestinal schistosomiasis which includes Kato-Katz technique, direct smear examination, and formalin-ethyl acetate method. Kato- Katz thick stool smear method is the most used standard method recommended by the World Health Organization (WHO) for both qualitative and quantitative diagnosis of intestinal schistosomiasis. It is highly specific, inexpensive, relatively simple and it creates a semi-quantitative egg counts that can be utilized as a proxy of infection intensity which is used for field surveys and investigation purposes (McCarthy *et al*, 2012). Thus, single Kato-Katz slide preparation from a single stool specimen results in reduced sensitivity, particularly in light infections. Increase in sensitivity of the parasitological techniques (urine filtration and Kato-Katz technique) in circumstances of low worm burden requires screening of larger number of urine or stool samples, or varoius of Kato-Katz slides prepared from a single or successive stool sample. However, this builds costs and may hinder the overview of repeated samples, and would entangle control procedures taking into account screen and treat (Rabello and Enk, 2006). The

advancement of other parasitological procedures in light of sedimentation, centrifugation, fluctuation and miracidium hatching, may constitute other options to the Kato Katz method (Gomes *et al*, 2013). These techniques give better sensitivity however are more laborious, more helpful for research, schistosomiasis diagnosis in travellers and as an extra diagnostic tools for the profundity investigation of infection rates before and after treatment.

Urine filtration and concentration microscopic techniques, is mainly used in diagnosing urinary schistosomiasis which is simple and used in areas with high schistosome transmission levels. *S. haematobium* infection is detected by the presence of micro- or macrohaematuria in urine using a number of indirect diagnostic tests such as dipstick methods useful for epidemiological mapping of prevalence (McCarthy *et al*, 2012).

2.4.2 Immunological diagnosis

Immunological diagnosis detects circulating *Schistosomal* antigens or anti *Schistosomal* antibodies in serum, plasma, urine or sputum to determine therapeutic responses and persistence of infection. Immunological methods are normally useful in areas where parasitological tests are negative for individuals with light infections (Weerakoon *et al* 2015). Patent schistosome infection is highly immunogenic, and specific anti-schistosome antibodies can be easily detected using a number of immunodiagnostic techniques with Enzyme linked immunosorbent assay being the most widely used (McCarthy *et al*, 2012). Immunodiagnostic techniques are used in occasional control of transmission of infection after it has been dispensed in an area. Parasitological methods often fail to diagnose cases precisely leading to false negative results in areas that have a reduction in intensity and infection prevalence after treatment. However, immunodiagnostic techniques is best used in such situations because it has a higher sensitivity and thus it remain the best accessible technique for diagnosis in areas of low intensity of

infection. Sero-diagnosis of schistosomiasis has a number of limitations in antibody detection; including the inability to measure infection intensity, difficulty distinguishing active from past infection since antibodies remaining in the body for long after cure, inability of discriminate between the different species of *Schistosoma* and mixed infections. It is an expensive technique for use in the developing world and may cross react with other helminthic infections (Valli *et al*, 1997).

Antigen detection in serum is less delicate in diagnosing light infections hence it is not valuable for clinical applications but rather a helpful research device for epidemiological and therapeutic studies (Lieshout *et al* 1997).

2.4.3 Molecular methods

Molecular methods are Deoxyribonucleic acid (DNA) and Ribonucleic acid (RNA) detection based method using Polymerase chain reaction (PCR) technique. This method detects schistosome DNA in urine, stool and organ biopsy samples whiles in RNA schistosome detection employs the use of blood, plasma or serum samples. The PCR technique for detecting schistosomiasis has been investigated for use in areas of medium and low intensity of infection. PCR application is currently limited but highly sensitive and specific, it requires expensive laboratory infrastructure, financial resources and trained personnel (McCarthy *et al*, 2012).

2.5 Immunity to schistosomiasis

The immune systems of infected hosts have different life cycle stages of the parasite that are highly antigenic which invigorate solid and effectively recognized humoral and cellular immune response. During chronic infection, some of the responses increase continually whiles others are strongly down regulated (Colley and Secor, 2014). People who live in endemic regions have been known to obtain some form of immune resistance after years of exposure through various

proven clinical and epidemiological studies (Gryseels *et al* 2006). The immune response in schistosome infection advances in various stages relating with the time of infection. Evidences from various studies have shown that protective immunity against the development of schistosomula to adult worm progresses slowly and its quickened by the dying of the worm naturally or as they are being killed by praziquantel (Colley and Secor, 2014). In human schistosomiasis, antibody dependent, cell mediated cytotoxicity is the principle sytsem of killing parasite (schistosomula larvae). For maximum protection of a host infected with schistosomiasis, both antibodies and T cells are important in humoral and cellular immune response. Prevalence of schistosomiasis in an untreated population can be determined by the detection of IgG, or IgM. Immune response is basically Th1 in nature during the first 4-5weeks preceding exposure to cercariae when the host immune system is directed against worm antigens(Zhang and Mutapi, 2006). The immune response, thus becomes highly Th2 polarized when *Schistosoma* eggs are produced during an infection. IgE increment at about the time that the worms develop and egg production starts. Th2 delivers moderately abundant circulating IgE antibodies which are actuated by cytokines IL4 and IL13 which bind the particular receptors on mast cells, basophils and eosinophils. This trigger degranulation of the cells and the contents of the granules are released onto the surface of the worm thereby increasing permeability and killing the parasites(Zhang and Mutapi, 2006) .

It has been proposed from a few investigations of reinfection that there are blocking antibody isotypes such as IgG_2, IgG_4 and IgM that blocks the protective activity of IgE and protective antibody isotypes, such as IgE that mediates antibody- dependent cytotoxicity against schistosomula (Naus *et al*, 1998; Dunne *et al*,1992; Caldas *et al*,2000). There is a relation between IgM and IgG_2 responses to the parasite egg and susceptibility to reinfection. It has

likewise been shown that IgM, IgG_4 and IgG_2 can go about as blocking antibodies, preventing the expression of a strong defensive immunity. These antibodies seem, by all accounts, to be inspired because of egg polysaccharide antigens, and cross-react with glycosylated epitopes situated on the schistosomulum surface (Butterworth, 1998). Also, a few studies have demonstrated that higher IgG_4, IgG_2 and IgM levels are associated with higher risk of infection, whereas IgE and IgG_1 are protective (Naus *et al*, 1998;Odongo-Aginya *et al*, 2012). IgG_1 intercedes eosinophil killing of schistosomula as well as activates the complement system, which causes a course of response prompting the death of the parasite. However the binding of both IgE and IgG_1 can be blocked by IgG_4, and IgM as well as IgG_2. Various studies have thus shown that IgG_4 is associated with susceptibility to reinfetion and IgE resistance to infection due to the various roles they play during immunity to schistosomiasis (Zhang and Mutapi, 2006; Dam *et al*, 1996).

2.5.1 Antibodies and their role in Schistosomiasis

Different antibodies which are proteins made by the body's immune system to fight against diseases attach to the schistosoma during an infection so the immune system can destroy the parasite (About.com, 2016). The five major types antibodies include: Immunoglobin D (IgD), Immunoglobin (IgA), Immunoglobin (IgM), Immunoglobin (IgG) and Immunoglobin (IgE). IgA is delivered principally at the chronic granuloma formation stage.

IgM, the largest antibody is found mainly in the blood and lymph fluid and it is the first antibody produced in a first response to an infection thus useful in the basis for diagnosing infectious diseases. Hoisted levels can be an indication of recent infection or exposure to antigen. It is an extremely viable neutralizing agent in the early phases of infection. In light of its substantial

size, IgM does not diffuse well and accordingly is found in very low concentrations in the intercellular tissue fluids (Acharya, 2016).

IgG is the smallest and most plentiful kind of antibody located in all body fluids. It is important in battling bacterial and viral infections and it also activates the immune system complement system. It binds and neutralizes the body from infection of the body tissues. IgG is the main kind of antibody that can cross the placenta in a pregnant woman to help secure her baby (fetus). It is conveyed in a delayed response to an infection and can be held in the body for quite a while. Detection of IgG typically shows an earlier infection or vaccination. Clinical measured IgG antibody levels are for the most part thought to be an indication of an individual's immune status to particular pathogens. The life span in serum makes IgG most valuable for passive immunization by the transfer of this antibody (eBioscience, 2016).

There are four IgG subclasses that are distinguished by their amino acid sequences. IgG1, IgG3, and IgG4 promptly cross the placenta and assume an imperative part in protecting the developing fetus. IgG3 is the best effective complement activator, trailed by IgG1; IgG2 is less efficient, and IgG4 is not able to activate complement at all. IgG1 and IgG3 bind with high liking to Fc receptors a protein found on the surface of certain cells, including, among others, B lymphocytes, follicular dendritic cells, natural killer cells, macrophages, neutrophils, eosinophils, basophils and mast cells that add to the defensive elements of the immune system (eBioscience, 2016).

IgE is found in the lungs, skin, and mucous membranes of mammals. It is the least abundant in blood serum. It plays unparalled roles in allergy and parasitic infection (About.com, 2016). They are included in hypersensitive responses to milk, a few medicines, and some poisons. IgE

antibody levels are frequently high in individuals with allergies. They cause the body to respond against foreign substances. IgE's fundamental capacity is immunity to parasites.

2.6 Schistosomiasis related to Age

The development of immune response may affect by sex, age, Infection history, ethnicity and the immune system (Zhang and Mutapi, 2006).There is an age- dependency in the intensity of infection in a schistosomiasis endemic area where individuals of the older age group are less heavily infected and individual below puberty are prone to heavy infection (Pearce and MacDonald, 2002). Protective immunity against *Schistosoma* is developed gradually with the increase of age (Zhang and Mutapi, 2006). Several studies have shown that after treatment of an infected area, children typically turn up to be intensely re-infected, whereas older individuals might become reinfected, yet stay less heavily infected than they were before treatment. In endemic areas, older individuals are impervious to reinfection.

Children are thought to produce high amounts of IgG_4 which interferes with complement activation by IgG_1 and this blocks mast cell degranulation by competing with specific anti parasite IgE for antigenic worm antigen (Hagan and Frederico, 1992). Thus, the slow achievement of immunity reflects a delayed production of protective IgE and an early production of IgG_4 which blocks the activity of anti- parasite IgE.

2.7 Prevention and control

Eradication of schistosomiasis is targeted at reducing mortality in endemic areas by improvision of safe water supplies and sanitation facilities, snail control and preventive chemotherapy (Fenwick *et al*, 2009). A number of vaccines have been explored to prevent Schistosoma infection and reinfection by reducing worm fecundity.

2.7.1 Chemotherapy

Chemotherapy is the main tool used by WHO to treat schistosomiasis due to its safety and low cost delivering a rapid impact (Fenwick *et al*, 2009). School children are the highest group that are at high risk of *Schistosoma* infection thus prevention of morbidity is focused on this group by using preventive chemotherapy.Praziquantel is a drug of choice, effective against all *Schistosoma* species which depends on the timing of treatment due to its effectiveness against adult worm (CDC, 2012). Praziquantel (40mg/kg body weight, single dose by oral route),is used for population - based mass chemotherapy where about 80% is absorbed from the gastrointestinal tract and metabolized by the liver (Inobaya *et al* 2014). Advantages of this drug include easy administration, high efficacy, relatively safety and mild to moderate side effects. Treatment with praziquantel sometimes fails due to drug resistance and ineffectiveness in early stages treatment of *Schistosomes*. Poor compliance may also result in uncured and untreated cases. To achieve effectiveness, repeat treatment may be needed after 2 to 4weeks which depends on endemic areas according to the prevalence and intensity of infection (Stothard *et al*, 2013). Oxamniquine which also another drug used is rapidly absorbed and effective against *S. mansoni* invasive stages and adult worm. It is administered in a single dose of 60mg/kg body weight over 2 to 3 days and it is similar to praziquantel in terms of efficacy and safety.

2.7.2 Vector control

Intermediate snail host can be controlled and their breeding sites can be hindered at infected sites by chemical, environmental and biological means (Inobaya *et al*., 2014). Molluscicides which are chemical compounds used to control snail is an effective method used to complement chemotherapy against schistosomiasis. Effectiveness of molluscicides depends on its application twice a year, which leads to high cost and time consuming especially for large areas. Thus an

effective and economical method used is the focal and seasonal application of molluscicide (Inobaya *et al.*, 2014). Other disadvantages include its toxic effect on micro and macro organisms and environmental pollution. Environmental methods involve the burying of the habitat of the snails, digging ditches or water drainage tunnels to reduce the amount of vegetation in water, which snails depend on for food and shelter and flooding snails with water up to several meters in depth. Biological method involves the use of crayfish, duck, fish or competitor snails to control snail intermediate host population.

2.7.3 Education

Over the years, there have been more feasible and enduring efforts to prevent infection and reinfection which includes access to safe water and good sanitation and health education. Health education educates people on their role in transmission of infection. In this manner,education prompts the avoidance of contact with schistosome-invaded water and contamination of the environment with feces and urine by individual activities. The effectiveness of health education is complemented with a safe water supply and good sanitation. The blend of chemotherapy, snail control, procurement of consumable water, and enhanced sanitation results in less morbidity, prevalence, and severity of infection.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

The study communities with a total population of about 3,139comprised of Vea central, Zangongo and Gonga located near the Vea irrigation dam in the Bongo District of the Upper-East Region of Ghana. The vegetation of the District is the Guinea Savannah Woodland with a single rainy season for which maximum rainfall ranges between 600mm and 1400mm with a maximum and minimum temperature of 12°C and 40°C respectively (Adongo, 2014). The economic activity of the people is mainly agriculture involving crop production, animal rearing, fishing and agro-forestry. Inhabitants of these communities use water from the Vea dam for washing, bathing, fishing and for other recreational activities such as swimming (Bongo District Assembly, 2013). Along the shores of the dam are aquatic plants (mostly water hyacinth) which harbour schistosome host snail (*Bulinus globusus* and *Biomphalaria pfeifferi*) making the lake the main source of infection. Due to the high prevalence of schistosomiasis and the use of water from the dam, the communities were chosen for screening and Mass Drug Administration in the region by the Ghana Health Service.

3.2 Study design

This work was a cohort study that made use of archived serum sample from school children aged between 6 and 18 years and adults above 18 years resident in Vea communities of the Bongo District in the Upper East Region. The original study was conducted in 2013 and the objective was to assess eosinophil cationic proteins as possible biomarker for estimation of

Schistosoma infection intensity before and afrer praziquantel treatment administered by Ghana Health Service. Ethical clearance and informed consent had been sought. Profiling of serum anti-*Schistosoma spp* antibodies was to be done as part of the study to know the infection exposure status of participants whose urine and stool samples would be negative for parasite eggs by microscopy to enhance discussion or results. That part of the study could not be carried out then, mainly due to unavailability of parasite antigens. This study made use of the samples which were stored for analyses later. Briefly, in 2013, participants who gave their consent after explanation of the study were recruited and registered. After which they provided stool, urine and blood samples. Two weeks later, the whole community including the study participants were were treated with praziquantel by the Ghana Health Service. At 8 weeks post- treatment, participants were followed up for a second round sample collection for analyses. All stool and urine samples were analyses in 2013 whilse aliquots of sera obtained from the blood samples were stored at -20°C for further analyses later. This study assayed the sera for anti *Schistosoma mansoni and S. haematobium* antibodies by Enzyme Linked Immunosorbent Assay (ELISA).

3.2.1 Ethical Issues

Permission was obtained from the District Directorate of Health Service, Bongo and the District School Health Education Program (SHEP), coordinator of the Ghana Education Service (G.E.S.). Prior to the onset of the study, the staff of the school and parents were educated on the study. Informed consent for school aged children were obtained directly from their parents and informed assent for children was obtained from parents and teachers before sample collection.

3.2.2 Sample collection

A 5ml venous blood sample was collected from the cubital vein of each study participants 2 weeks before drug administration (212 samples) with praziquantel and a repeat sampling 8 weeks post-treatment (112) into sterile serum separator tubes by qualified medical personnel using a sterile hypodermic syringe. Blood samples were left on the bench for some time to clot completely after they have been inverted 3-5 times to activate the clotting process. The clotted blood samples were then transported in a cold box to the Regional hospital in Bolgatanga where sera were harvested by centrifugation of the blood samples at 14000rpm for 20mins. The sera were then aliquot into tubes and stored at -20^{o}C until they were transported in a cool box to the Parasitology Department, NMIMR. These samples were assayed for anti-*Schistosoma haematobium* and *S. mansoni* antibodies sero-prevalence by ELISA.

3.2.3 Drug administration

Drugs were administered by the Ghana Health Service. Praziquantel dosage was determined using height of each participant as recommended by WHO. The height–dosage relationship was 94–109 cm for 1 tablet, 110–124 cm for 1½ tablets, 125–137 cm for 2 tablets, 138–149 cm for 2½ tablets, 150–159 cm for 3 tablets, 160–177 cm for 4 tablets and ≥178 cm for 5 tablets. The inclusion or exclusion criteria were the same as the National Control Program (Gyapong et al, 2001), which excludes pregnant women, children 4years and younger and persons seriously ill. Pregnant women were not given praziquantel however it is presently allowable (WHO, 2002). Drug administration was a directly observed therapy (DOT) and all treatments were taken simultaneously.

3.3 Sample preparation

Refer to appendices for preparation of solutions

3.4 Serology analysis

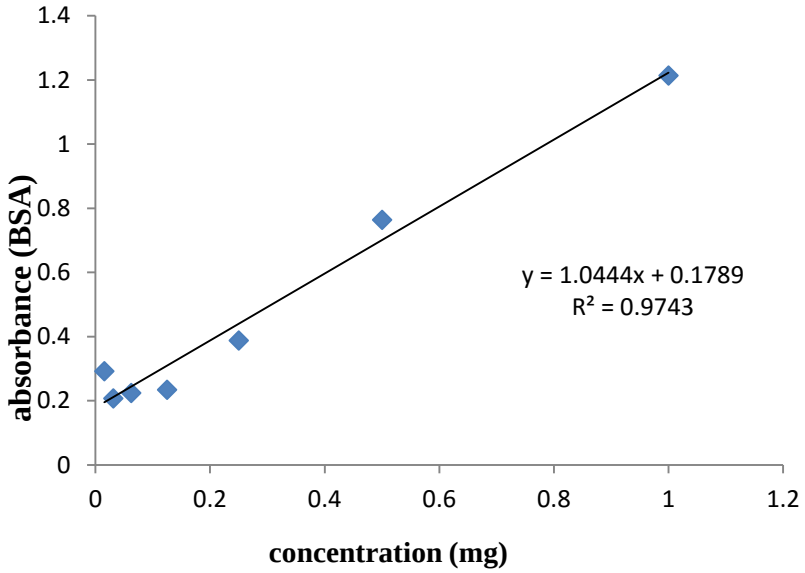
3.4.1 Soluble egg and adult worm antigen Preparation for *S. haematobium*

2g of mixed sex worms and eggs were suspended in Phosphate buffered saline (PBS) and homogenized using a manual homogenizer. When approximately 95% (or more) of the eggs and worm were disrupted, the crude mixture was centrifuged at 4°C at 1600 RPM for 20 minutes. The supernatant was collected and ultracentrifuged for 90 minutes at 1100 RPM at 4°C. The supernatant was then sterilized by passing it through a 0.2 µm filter.

3.4.2 Estimation of *S. mansoni and S. haematobium* egg and worm antigen concentration

Coomasie brilliant blue dye (Bio-rad) was prepared by diluting 8ml of a concentrated dye reagent to 32ml of double distilled water. Concentration of 1mg/ml of Soluble egg antigen and adult worm antigen *S. mansoni* and *S. haematobium* was prepared by adding 1mg to 1ml of double distilled water. Bovine serum albumin (BSA) was used as protein standard and was prepared by adding 1mg of BSA to 1 ml of double distilled water to get concentration of 1mg/ml. Seven serial dilutions of the protein standard solution (BSA) and antigen (representative to be tested) were done using PBS diluent on a dilution plate. 10µl of each BSA and antigen solution was pipetted into separate microtitre wells of the same plate. 200µl of the dye solution was added to each well using multichannel pipette and the sample and reagent were mixed thoroughly. The plate was incubated for 5minutes at a room temperature and absorbance was read at 595nM

using a microtitre plate reader. The absorbance of the protein standard (BSA) was plotted on the Y-axis against its corresponding concentrations on the X-axis.

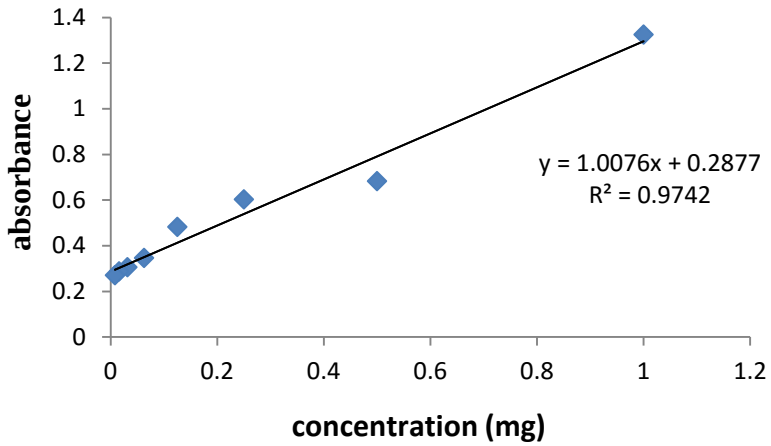


Graph of absorbance against concentration of protein standard (BSA) for soluble egg and worm antigen *S. mansoni*

The equation of the line of the graph was determined and used to extrapolate the concentration of the egg and worm antigen for *S. mansoni* as follows:

Equation of the line: absorbance(Y) =1.0444 concentration(X) +0.1789

→ Concentration (X) = {absorbance(Y) – 0.1789}/(1.0444)

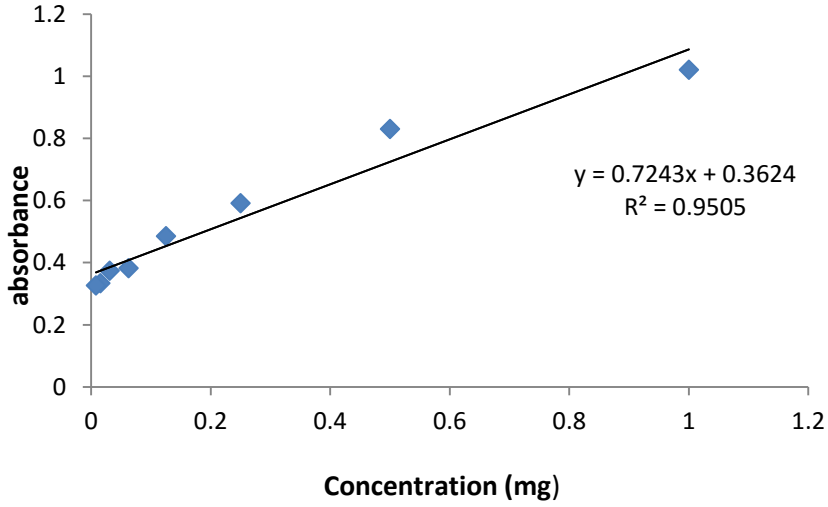


Graph of absorbance against concentration of protein standard (BSA) for soluble worm antigen *S. haematobium*

The equation of the line of the graph was determined and used to extrapolate the concentration of the adult worm antigen for *S. haematobium* as follows:

Equation of the line: absorbance(Y) =1.0076 concentration(X) +0.2877

→ Concentration (X) = {absorbance(Y) – 0.2877}/(1.0076)



Graph of absorbance against concentration of protein standard (BSA) for soluble egg antigen *S. haematobium*

The equation of the line of the graph was determined and used to extrapolate the concentration of the egg antigen for *S. haematobium* as follows:

Equation of the line: absorbance(Y) =0.724 concentration(X) +0.362

→ Concentration (X) = {absorbance(Y) – 0.362}/(0.724)

3.4.3 Determination of coating concentration of the antigen and conjugate working concentration

Soluble egg antigen, *S.mansoni* (Sm SEA), adult worm antigen *S. mansoni* (Sm AWA) and adult worm antigen *S. haematobium* (Sh AWA) were serially diluted starting from 5µg/50µl to 0.0000488µg/µl in a twofold dilution in carbonate, bicarbonate coating buffer and added to the wells across a microtitre plate and left to incubate overnight at 4^{o}C. Soluble egg antigen, *S. haematobium* (Sh SEA) was serially diluted starting from 2µg/1ml to 0.00098µg/ml The plate was then flipped and washed two times with 200µl of 0.05% tween 20 PBS (washing buffer). Horseradish peroxidase conjugated goat anti-human IgG, IgM and IgE dilutions were diluted serially in PBS. IgG was diluted in 1/5000, 1/10000 and 1/20000: IgM from 1/1000 to 1/8000 and IgE 1/500 to 1/3000. 50µl of each diluted conjugate was added to the wells in the same order as during dilution and incubated for 1hour. The plate was then flipped and washed three times. 100µl of 2, 2-azinobis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) solution (substrate) was added to the wells and incubated for 30 minutes in the dark. The plate was read at 405nm and the color intensities and absorbance was compared. An absorbance of 0.78µg/µl of Sm SEA, Sm

AWA and Sh AWA and 2µg/ml Sh SEA were found to be optimal concentrations and subsequently used to coat the plate for sample analysis.

3.4.4 Optimizations of ELISA procedure

Wells of a microtitre plate were coated with 50µl of Sh SEA at 2µg/ml with and Sm SEA, Sm AWA and Sh AWA at 0.78µg/ml in coating buffer and left overnight at 4°C. The plate was then flipped washed two times with 200µl 0·05% Tween-20 (PBST). 200µl of casein blocking buffer was used to block the plate which was coated with Sm SEA, Sm AWA and Sh AWA and bovine serum albumin Sh SEA was used to block each well and incubated for 1hour. The plate was flipped, emptied and washed two times with wash buffer. A positive and negative control sera was diluted in PBS at different concentrations in duplicates on a dilution plate and 50µl was added to each well and incubated for 90mins. Two columns containing PBS were left for blanks. The plate was flipped and washed three times with 200µl of wash buffer and completely dried after each wash. The antibodies, IgG, IgM and IgE were diluted in different concentrations in blocking buffer and 50µl was added to each well and incubated for 1hour. 200µl of wash buffer was used to wash the plate three times. 100µl of ABTS was added to the wells and incubated for 30 mins in the dark. The absorbance was then read at 405nm with an ELISA plate reader. The best concentration of the positive and negative which was comparable was chosen and used to analyse the sera samples.

3.4.5 Detection of anti-*Schistosoma* antibody by ELISA

An indirect ELISA method was used to detect anti-*Schistosoma* IgM, IgG and IgE antibodies in individual serum samples. A concentration of 2µg/ml of Sh SEA and 0.78µg/ml of Sm SEA, Sm AWA and Sh AWA were prepared and 50µl was used to coat a 96-well micro titre plate and kept overnight at 4°C. The plate was flipped emptied and washed two times with 200µl PBS plus 0·05% Tween-20 (wash buffer). 200µl blocking buffer was pipetted into the wells and incubated for 1hr at room temperature to block unspecific sites. The plate was then flipped emptied and washed two times with 200µl PBS plus 0·05% Tween-20 shaking briefly, flipped and banged on tissue paper after each wash. 50µl of serum samples and controls (known positive and negative sample) diluted in PBS was added to each well and incubated 90mins at room temperature. The plate was washed three times, flipped emptied and banged. The wells were then filled with 50µl goat antihuman antibody conjugate (IgG, IgM and IgE) diluted in blocking buffer and incubated for 1hr at room temperature. The plate was flip emptied, washed and banged. 100µl of substrate ABTS solution was then added and incubated at room temperature for 30mins. The plates were read for absorbances at 405nm with an ELISA plate reader.

3.5 Determination of the cutoff point value.

A cut - off point was calculated by taking the mean Optical density(OD) + 3 standard deviation (SD) of the negative control serum sample. Samples were considered positive or negative when their OD's were greater than or equal to or less than the cut off value.

3.6 Data analysis

Data was entered into Microsoft Excel worksheet and cleaned before it was imported into Statistical Package for Social Scientist (SPSS) version 20.0 for descriptive and inferential statistical analysis. Frequency distribution was the main tool for descriptive statistics and a Student t-test was used for inferential statistics with a set Confidence level of 95% P values less than 0.05 are considered statistically significant.

CHAPTER FOUR

RESULTS

4.1 Characteristics of Study Participants

A total of 224 participants was recruited for the study out of which 212 provided their blood samples for serum extraction at pre treatment (baseline), which this study made use of. The participants were aged 6 to 76years (mean:19 ± 15.07). They consisted of 113 (53%) females and 99 (46.7%) males. At 8 weeks post-praziquantel treatment (endline), a total of 112 out of the 212 persons aged 6 to 76years (mean:12.06± 4.62) were available for follow up comprising 62 (55.4%) females and 50 (44.6) males provided sera. Majority of the participants were children in the 10 to13 years age group (Table 3).

Table 1: Demographic Characteristics of Participants

Characteristics		**Pre treatment(%) N= 212**	**Post-treatment (%) N=112**
Sex	Male	99(46.7)	50 (44.6)
	Female	113 (53.3)	62 (55.4)
Age group (years)	6-9	47 (22.2)	43 (38.4)
	10-13	104 (49.1)	60 (53.6)
	14-17	15 (7.1)	4 (3.6)
	≥18	46 (21.7)	5 (4.5)

4.2 Sero-prevalence of anti-*Schistosoma spp* antibodies among participants at baseline

Prevalence of sero-positivity of anti- *Schistosoma mansoni* IgM, IgG and IgE to soluble egg antigen (SEA) and adult worm antigen (AWA) among participants is presented in Figure 1. For SEA, anti - *S. mansoni* IgM prevalence among participants was the highest (99.1%) whiles participants having anti - *S. mansoni* IgE prevalence was highest (75.5%) for AWA.

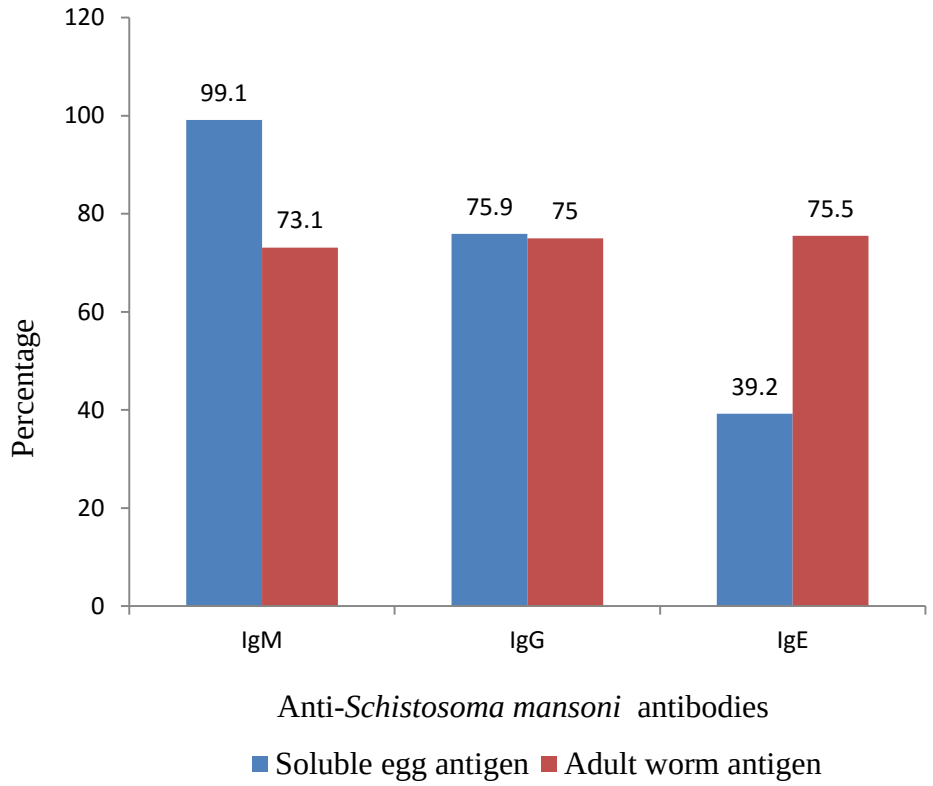


Figure 2: Sero- prevalence of anti-*Schistosoma mansoni* antibodies to soluble egg antigen and adult worm antigen among participants at pre praziquantel treatment

Sero-prevalence of anti- *Schistosoma haematobium* IgM, IgG and IgE to soluble egg antigen and adult worm antigen among participants is presented in Figures 3. For SEA elicited antibodies showed a higher IgE sero-prevalence whilst IgG prevalence against AWA was high.

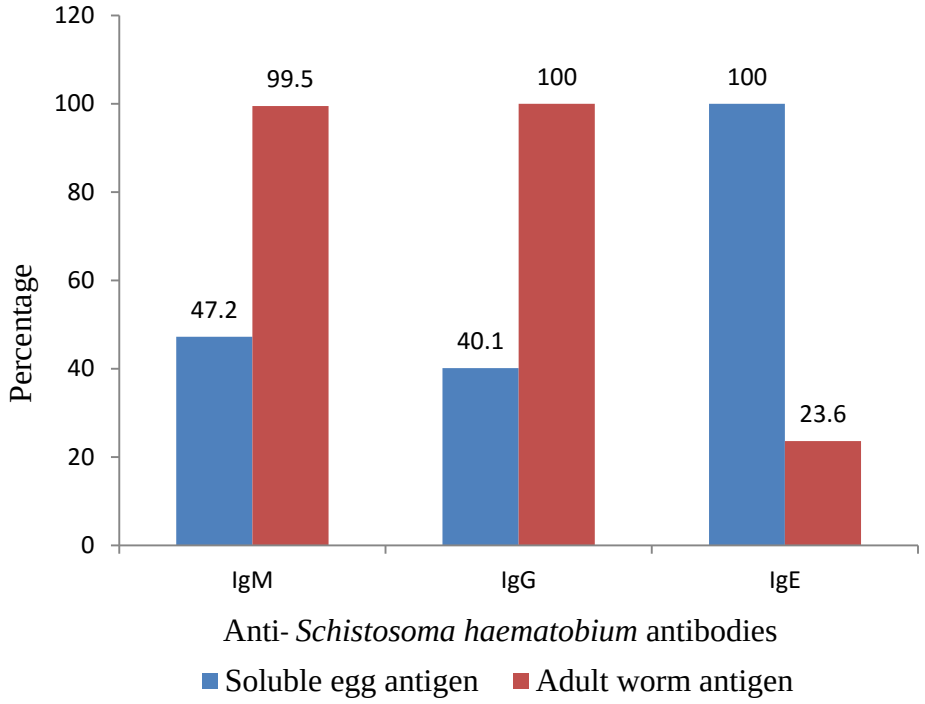


Figure 3: Sero- prevalence of anti-*Schistosoma haematobium* antibodies to soluble egg antigen and adult worm antigen among participants at pre praziquantel treatment

4.3 Sero- prevalence of anti-*Schistosoma spp* antibodies among matched participants at baseline and endline

Prevalence of serum anti-*Schistosoma spp* IgM, IgG and IgE to SEA and AWA of matched participants at baseline and endline are presented in Table 2. Proportion of participants having serum anti-*S. mansoni* IgM and IgE to SEA reduced after treatment whereas those with anti-*S. manoni* IgG recorded an increase with statistical significance ($p < 0.05$). The reduction in anti-*Schistosoma* IgM sero-prevalence to SEA was not statiscally significant ($p > 0.05$) whiles that in IgE was significant ($p < 0.05$). There was a reduction in sero-prevalence of anti- *S. mansoni* IgM among participants which was not statistically significant ($p > 0.05$) and IgG to AWA which was statistically significant ($p < 0.05$) whereas serum anti-*S mansoni* IgE prevalence recorded an increase but was not statistically significant ($p > 0.05$).

Sero-prevalence of anti –*S. haematobium* IgM and IgG to SEA, increased post treatment whiles IgE ser-prevalence reduced and was statistically significant ($p < 0.05$). There was a statistical significance ($p < 0.05$) in the increase of IgM and IgG ser-prevalence ($p < 0.05$) against SEA. Anti-*S. haematobium* IgM sero-prevalence remained the same post treatment ($p > 0.05$) whiles IgG sero-prevalence reduction was not statiscally significant after treatment ($p > 0.05$) and anti-*S. haematobium* IgE ser-prevalence increase ($p < 0.05$) against AWA was significant.

Table 2: Comparison of sero-prevalence of anti-*Schistosoma spp* antibodies from matched participants pre- and post-Praziquantel treatment

Anti-*Schistosoma* spp Antigen	Anti-*Schistosoma spp* Antibody	Anti-*S. mansoni* sero-positives (%), N=112			Anti-*S. haematobium* sero-positives (%), N=112		
		Pre-treatment	Post-treatment	*p* value	Pre-treatment	Post-treatment	*p* value
Soluble Egg Antigen (SEA)	IgM	110 (98.2)	104 (92.9)	0.30	64 (57.1)	79 (70.5)	**<0.001**
	IgG	82 (73.2)	96 (85.7)	**<0.001**	52 (46.4)	60 (53.6)	**0.002**
	IgE	112 (100)	80 (71.4)	**<0.001**	112 (100)	6 (5.4)	**<0.001**
Adult worm Antigen (AWA)	IgM	83 (74.1)	65 (58)	0.11	111(99.1)	111 (99.1)	
	IgG	85 (75.9)	59 (52.7)	**<0.001**	112 (100)	110 (98.2)	0.11
	IgE	93 (83)	112 (100)	0.95	28 (25)	99 (88.4)	**0.007**

p value was calculated by Student's t-test with confidence level (Cl) of 95% and 1 degree of freedom (df). *p* values less than 0.05 were considered statistically significant and are indicated in bold fonts

4.4 Comparison of matched anti-Schistosoma IgM and IgG sero- prevalence among participants at baseline and endline

The sero-positivity of both anti-*Schistosoma* IgM and IgG that was expressed in participants to *S. mansoni* SEA and *S. haematobium* SEA increased at endline whiles to *S. mansoni* AWA and *S. h*aematobium AWA reduced at endline. In the case where individuals elicted IgM and no IgG, prevalence for both anti- *S. mansoni* and anti- *S. haematobium* to SEA and AWA increased at endline with the exception of anti- *S. mansoni* IgM to SEA which reduced. In the absence of anti- *S. mansoni* IgM and presence of anti- *S. mansoni* IgG among participants, there was an increase in IgG to *S. mansoni* SEA and S. mansoni AWA at endline and a reduction in IgG to *S. haematobium* SEA whiles prevalence of anti- *S. haematobium* IgG to AWA was the same. Proportion of participants that expressed no anti- *S. mansoni* IgM and IgG to *S. mansoni* SEA and *S. mansoni* AWA increased and there was a reduction in anti-*S. haematobium* ser-prevalence to SEA whiles no change was recorded in anti- *S. haematobium* ser-prevalence to AWA at endline. For participants that had no IgM and IgG against SEA and AWA but had IgE, there was an increase at endline in anti- *S. mansoni* sero-prevalence SEA and reduction same to AWA whiles no change was recorded at endline in anti- S. *haematobium* IgE sero-prevalence to SEA and to AWA (Table 3).

Table 3: Status of participants ith respect to serum anti-*Schistosoma spp* antibodies to soluble egg antigen and adult worm antigen at baseline and endline

	Sero-prevalence (%)							
	Anti- Sm Ab SEA [3]		**Anti-Sm Ab AWA [4]**		**Anti- Sh Ab SEA [5]**		**Anti-Sh Ab AWA [6]**	
Anti- *Schistosoma spp* serum antibodies	**Baseline**	**Endline**	**Baseline**	**Endline**	**Baseline**	**Endline**	**Baseline**	**Endline**
IgM+ [1], IgG+	81(72.3)	92(82.1)	69(61.6)	32(28.6)	29(25.9)	41(36.6)	(99.1)111	109(97.3)
IgM+, IgG- [2]	29(25.9)	12(10.7)	14(12.5)	33(29.5)	35(31.3)	38(33.9)	0	2(1.8)
IgM -, IgG+	1(0.9)	4(3.6)	16(14.3)	27(24.1)	23(20.5)	19(17)	1(0.9)	1(0.9)
IgM-, IgG-	1(0.9)	4(3.6)	13(11.6)	20(17.9)	25(22.3)	14(12.5)	0	0
IgM -,IgG-, IgE +	0	3(2.7)	11(9.8)	20(17.9)	0	0	0	0
IgM -, IgG-, IgE -	1(0.9)	1(0.90	2(1.9)	0	23(20.5)	14(12.5)	0	0

1.Positive for anti- *Schistosoma spp* IgM or IgG or IgE
2.Negative for anti- *Schistosoma spp* IgM or IgG or IgE
3.Anti- *Schistosoma mansoni* antibodies to soluble egg antigen
4.Anti- *Schistosoma mansoni* antibodies to adult worm antigen
5. Anti- *Schistosoma haematobium* antibodies to souluble egg antigen
6. Anti- *Schistosoma haematobium* antibodies to adult worm antigen

4.5 Sero-prevalence of mixed anti- *Schistosoma spp* antibodies to soluble egg and adult worm antigen among participants at baseline and endline

Proportions of participants with mixed anti- *S.mansoni* and *S. haematobium* IgM to parasites SEA and AWA increased at endline, whiles ser-prevalence of anti-*Schistosoma spp* IgG to parasites' SEA and AWA reduced. There was statistical significance in the increase of sero-prevalence of anti-*Schistosoma* IgM to SEA ($p<0.05$) and reduction of anti- *Schistosoma* IgG to SEA ($p<0.05$). There was no statistical significance in the increase of sero-prevalence of anti-*Schistosoma* IgM to AWA ($p>0.05$) and the reduction in that for anti-*Schistosoma* IgG to AWA($p>0.05$).

Table 4: Sero-prevalence of mixed anti- *Schistosoma spp* antibodies to soluble egg and adult worm antigen among participants at baseline and endline

Anti- S*chistosoma Ig*	Antigen	Baseline	Endline	*p* value
IgM	SEA	64(57.1)	73(65.2)	**0.0002**
	AWA	39(34.8)	50(44.6)	0.51
IgG	SEA	83(74.1)	65(58)	**0.0006**
	AWA	85(75.9)	59(52.7)	0.13

p value was calculated by Student's t-test with confidence interval (CI) of 95% and 1 degree of freedom (df). *p* values less than 0.05 were considered statistically significant and are indicated in bold fonts

CHAPTER 5

DISCUSSION

Accurate and effective diagnosis of schistosomiasis is a very important aspect in the quest for effective treatment and elimination of the disease. Currently, the detection of ova in stool or urine by microscopy for *S. mansoni* and *S. haematobium* respectively presents as the main standard for diagnosis but it is fraught with challenges especially in large epidemiology studies and sub-clinical cases. Sero-prevalence of anti-*Schistosoma spp* antibody is useful in determining therapeutic response, periodic control of transmission and persistence of infection. In recent years, profiling of antibodies to determine therapeutic responses and the role of defensive and blocking antibodies in reinfection has been a subject of interest (Naus *et al*, 1998). During *Schistosoma* infection, IgE levels are elevated and there is an acquired immunity which is mediated by IgE but it is downregulated by excess production of blocking antibodies (IgM, IgG4 and IgG2) in susceptible individuals leading to the slow development of immunity (Gryseels, *et al* 2006). These blocking antibodies are triggered against the egg polysaccharide antigens expressed at or released from young schistosomulum surface which blocks the protective antibodies expression(Zhang & Mutapi, 2006).

This study measured anti- *Schistosoma mansoni* and *S. haematobium* IgM, IgG and IgE sero-prevalence to SEA and AWA at pre and post praziquantel treatment.

Results from this study at pre-praziquantel treatment showed a higher sero-prevalence of anti- *S. mansoni* IgM to SEA and anti- *S. mansoni* IgE to AWA in participants. High IgM against SEA in participants is indicative of high amount of eggs thus a recent infection to *Schistosoma* since it is the first antibody produced in response to an early infection and it also serves as a neutralizing agent in the early stages of infection (Goldsby & Kindt, 2007). High sero- prevalence of

participants having anti-*Schistosoma* IgE to AWA is due to the presence of high amount of worms in participants showing high rate of infection which is also justified by high IgM.

For *S. haematobium,* high prevalence of individuals showing IgG against AWA implies past infections in the community since IgG stays in the body for a long time even after treatment. The high number of people with anti-*Schistosoma haematobium* IgE to SEA is as a result of high amounts of egg thus high rate of infection.

Reduction in sero-prevalence following treatment with praziquantel in participants expressing anti-*S. mansoni* IgM and IgE to SEA shows that infection was reduced thus drug was effective. Increased anti-*Schistosoma mansoni* IgG to SEA might be due to an increase in one of the subclasses of IgG. Reduction in individuals expressing IgM and IgG against AWA shows a reduction in infection after treatment since praziquantel is effective against adult worm and IgE increase at endline against AWA might be due to the presence of other helminth infections. This correlates with study done by Odongo-Aginya et al., 2012 who reported IgG reduced in a ratito from 1:2003 to 1:1778, a study done in Uganda on *Schistosoma mansoni* infection and the related antibody immune response.

An increase was recorded in the sero-prevalence of the number of individuals showing anti-*Schistosoma haematobium* IgM and IgG to SEA whiles a reduction was recorded in IgE to SEA after treatment. High IgM and IgG (especially IgG_2 and IgG_4) might indicate reinfection after treatment. IgM and IgG are effective against eggs and are associated with a high risk of reinfection or susceptibility to reinfection (Naus *et al,* 1998). These antibodies are induced in response to egg antigens during an infection and they react with the *Schistosomes* glycoproteins thus blocking the effect of protective antibodies (IgE and IgG_1) mediating ADDC response against schistosomula (Cardoso, *et al* 2006). Anti-*Schistosoma haematobium* IgM to AWA

prevalence was the same implying no change in prevalence after treatment. There was a reduction in participants showing anti-*S. haematobium* IgG to AWA and IgE concentration was increased against AWA. This results shows the continuous presence of adult worms lodging in some parts of the body after treatment.

The expression of both anti-*Schistosoma* IgM and IgG in participants at baseline and endline, there was a reduction in sero-prevalence to *S. mansoni* AWA and *S.h*aematobium AWA and an increase against *S. mansoni* SEA and *S. haematobium* SEA. The increase of the antibodies against SEA shows the presence eggs and reduction in worms after treatment.

Participants having IgM and no IgG number against *S.mansoni* SEA was reduced and increased against *S. mansoni* AWA showing a reduction in egg concentration and an increase in adult worm thus treatment was not effective. An increase in *S. haematobium* AWA and *S. haematobium* SEA shows reinfection.

The presence of IgG and no IgM after treatment was increased in *S. mansoni* SEA and *S. mansoni* AWA showing past infections or the presence of subclasses of IgG. Reduction in *S. haematobium* SEA shows reduction in infection.

The percentage of participants that showed no IgM and IgG after treatment was slightly increased in *S. mansoni* SEA and *S. mansoni* AWA but reduced *S. haematobium* SEA. This result showed reinfection after treatment and this is evident when IgE increased against *S. mansoni* SEA and *S. mansoni* AWA.

Number of participants that had none of the three antibodies after treatment was slightly reduced in *S. mansoni* AWA and *S. haematobium* SEA.

Mixed infections of *Schistosoma mansoni* and *S. haematobium* was recorded in this study in which anti-*Schistosoma* IgM was increased after treatment in both SEA and AWA and in IgG reduced. This result suggest that more co-infections was recorded in participants having IgM after treatment.

CONCLUSION

Results from this study showed the immune status of individuals before and after chemotherapy and also whether treatment was effective or not. It can be concluded from this study that mass drug administration was effective in *Schistosoma mansoni* infections.

Thus antibody profiling can be a useful tool in determining therapeutic response and persistence of infection

RECOMMENDATION

More studies should be concentrated on the subclasses of IgG (IgG1, IgG2 and IgG4) to know the proportion of people that are susceptible or resistant to reinfection since these antibodies are indicators.

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APPENDICES

Preparation of solutions and working dilutions

Appendix 1: Preparation of phosphate Buffer Saline (PBS) p H 7.4

A litre of PBS was prepared by measuring 600ml of distilled water into a1litre beaker. Sodium chloride (NaCl) - 8.0g, Sodium hydrogen Phosphate (Na2HPO4) -1.4g, Potassium phosphate monobasic (KH_2PO_4) - 0.2g, Potassium chloride (KCl) - 0.2g was weighed with an electric balance and added. The beaker was then placed on a magnetic stirrer and magnetic stirring rod was gently slid into the beaker to help uniformly dissolve the salts. The rest of the measured distilled water (400ml) was added gently and the pH was adjusted to 7.4 using p H metre by adding HCl drop wise. The prepared solution was stored at room temperature.

Appendix 2: Preparation of washing buffer (PBS-Tween 20)

A litre of PBS was prepared following the procedure mentioned above and 0.5ml tween 20 was added while stirring. The solution was stored at room temperature after preparation.

Appendix 3: Preparation of coating buffer, p H 9.6

Distilled water (600ml) was measured and poured into a calibrated flask and a stirring rod was slid into it. Sodium hyrdrogen carbonate (NaHCO3) -2.93g and Sodium Carbonate (Na2CO3)– 1.5g were weighed using an electric balance and poured into the flask. More distilled was added to the level of the calibrated mark. The pH was adjusted to 9.6 using p H metre by adding HCl drop wise.

Appendix 4: Preparation of Casein Blocking Buffer

Preparation of 0.1N Sodium hydroxide (NaOH)

Calculations:

0.1N NaOH = 0.1M NaOH = 0.1mol/dm3 of NaOH

→0.1mol = amount of substance

$$\text{Molarity} = \frac{\text{mass}}{\text{Amount of substance}}$$

Mass = molarity x amount of substance

m = 0.1 × 40 (molar mass of NaOH)

=4g.

Sodium hydroxide, 4g was weighed and dissolved in 1000ml of distilled water.

In preparing 500ml of casein blocking buffer, 2.5g casein was weighed and suspended in 50ml of 0.1N NaOH and boiled. After the casein was dissolved, 450ml of PBS was slowly added.

Appendix 5: Preparation of Bovine serum albumin

In preparing 1% of BSA, 1g of BSA was dissolved in 100ml of phosphate buffered saline tween 20 (PBST).

Appendix 6: Tables for dilutions of positive and negative serum control and antibodies

Table 5: Dilutions for positive and negative serum control

	Sh SEA	Sm AWA	Sh SEA	Sh AWA
IgG	1/6400	1/6400	1/200	1/200
IgM	1/400	1/400	1/200	1/800
IgE	1/40	1/20	1/5	1/10

Table 6: Dilutions for each conjugate (IgE, IgM and IgG)

	Sh SEA	Sm AWA	Sh SEA	Sh AWA
IgG	1/10,000	1/5000	1/10,000	1/5000
IgM	1/3000	1/5000	1/6000	1/2000
IgE	1/1000	1/2000	1/2000	1/500

Appendix 7: Pictures of ELISA plates

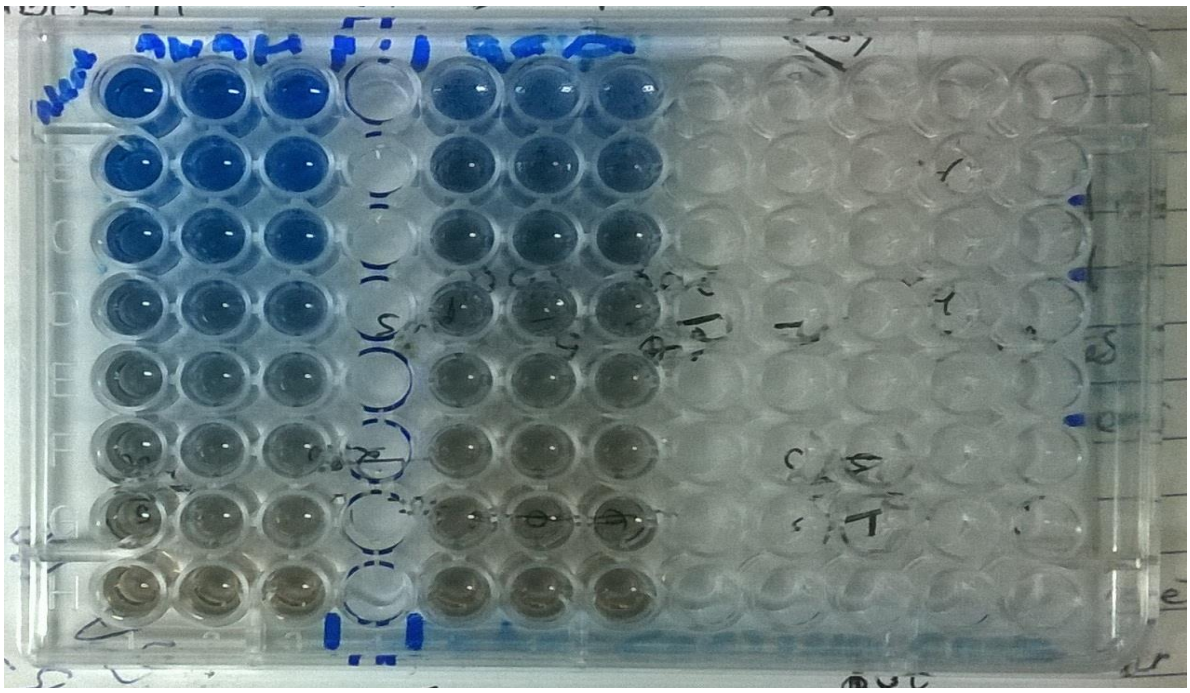

Plate 1: Proten assay to determine egg and adult worm antigen concentration

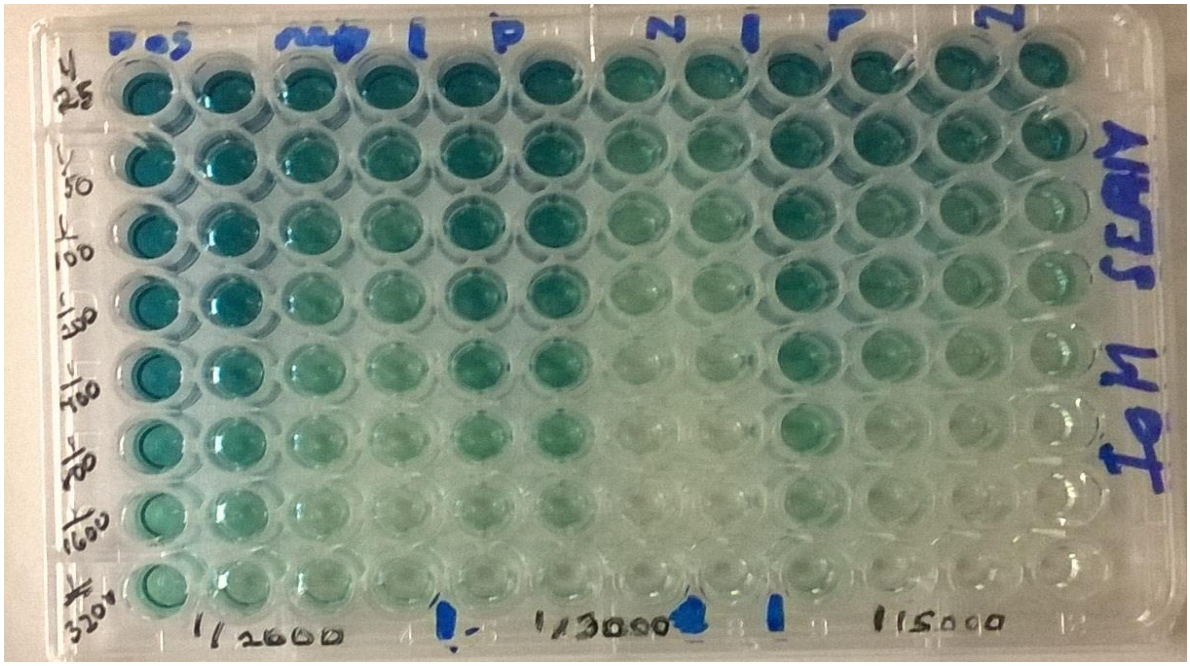

Plate 2: Picture determining coating concentration of antigen and conjugate working concentration

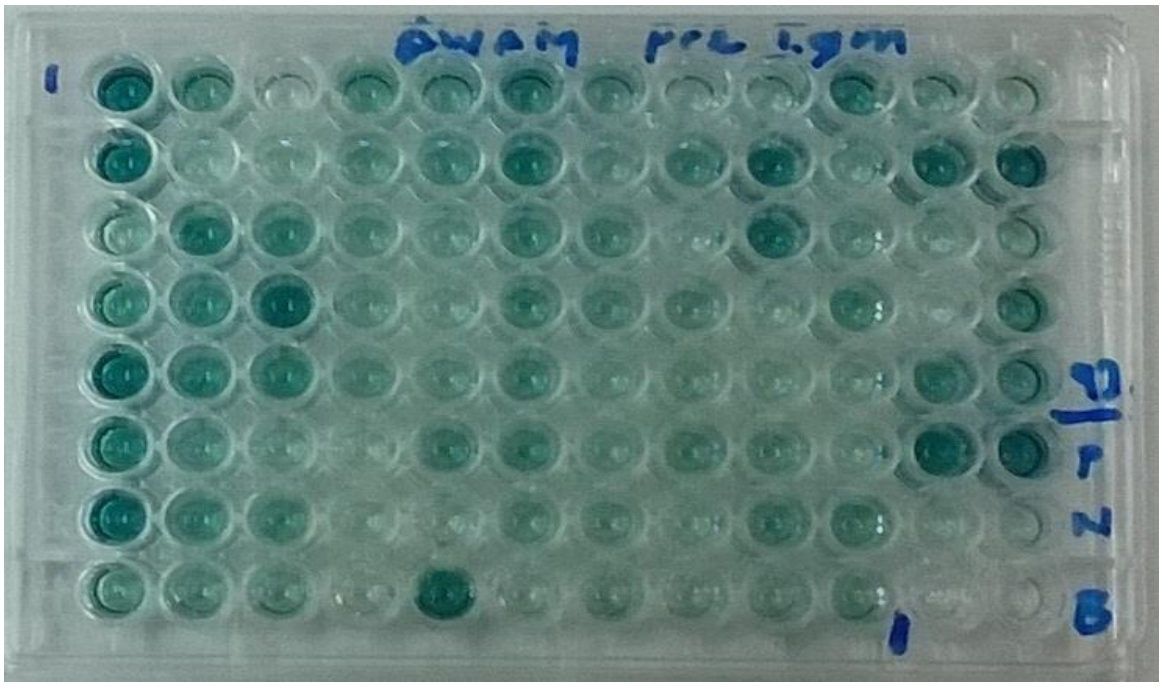

Plate 3: Picture of samples assayed