COMPARISON OF PROTEINS OF PARASITE STAGES OF SCHISTOSOMA MANSONI AND THOSE OF THE TISSUES OF THE SNAIL VECTOR BIOMPHALARIA PFEIFFERI

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DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been presented for a degree in any other University or any other award.

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DEDICATION

This work is dedicated to my dear parents Mr. and Mrs. Opondo, my husband Moses Nyangiye and lovely sons, Larry and Randy for their understanding, love and support.
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ABSTRACT

Antigen sharing between a parasite and its host is a stimulating facet of host-parasite relationship which is currently receiving considerable attention. The aim of the study was to investigate presence of common proteins in various stages of the parasite, *Schistosoma mansoni* and its intermediate host, *Biomphalaria pfeifferi*. Comparison of protein profiles of extracts of uninfected and infected snail digestive gland, foot, soluble cercarial antigens (SCA) and soluble worm antigen preparation (SWAP) was achieved by native and SDS-PAGE. The study also compared *S. mansoni* infected snail tissues (day 8 foot, day 15 digestive gland and day 32 foot) with the uninfected tissues using native and SDS-PAGE. Immune cross-reactivity between the tissue proteins and antibodies raised against SWAP and SCA was also investigated in Western blots. Five proteins of approximate molecular weights 66 kDa, 46kDa, 26kDa, 16kDa, and 12kDa were found to be common in uninfected, infected samples, SCA and SWAP in SDS-PAGE while 178kDa, 50kDa, 25kDa and 14kDa proteins were found to be common in the samples by native PAGE. When infected and uninfected tissues were compared, a decrease in the intensity of proteins with Mr~178kDa and 44kDa was observed in the digestive gland, 120kDa and 30kDa in the foot. The proteins that cross reacted with anti-SWAP included Mr~220kDa, 180kDa, 89kDa, 38kDa and 12kDa in the foot, 76kDa, 46kDa, 22kDa, and 16kDa in the digestive glands. Proteins with approximate molecular weights of 220kDa and 16 kDa in the digestive gland cross reacted with anti-SCA. The study has revealed common proteins and immune cross reactivity between *S. mansoni* and its intermediate host *B. pfeifferi* antigens. These proteins could be useful as potential vaccine candidates and in the diagnosis of Schistosomiasis.

Key Words: *B. pfeifferi, S. mansoni*, protein profiles and immunocross-reactivity
CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

Schistosomiasis also known as bilharzia is a widespread infectious disease with a relatively low mortality rate but a high morbidity rate causing severe debilitating illness in millions of people. Schistosomiasis ranks high among parasitic diseases in terms of socio-economic and public health importance in tropical and sub tropical areas. The disease is caused by a digenetic blood trematode of the genus *Schistosoma* with the three main species infecting humans being *Schistosoma haematobium*, *S. japonicum* and *S. mansoni*. Two other species, more localized geographically are *S. mekongi* and *S. intercalatum*. In addition, other species of schistosomes, which parasitize birds and mammals, can cause cercarial dermatitis in humans. Two hundred million people in 74 countries have this disease, 120 million of them have symptoms, and 20 million have severe illness (Chitsulo *et al.*, 2000).

*Schistosoma haematobium* responsible for urinary schistosomiasis is the most prevalent and widespread species. It is found in much of the sub-tropical and tropical countries, in Iran, Iraq, Saudi Arabia, Yemen, Syria, India, Madagascar, Mauritius, Pemba and Zanzibar while *S. mansoni* is found in Africa and is the only species seen in Latin America. *S. japonicum* is restricted to the Pacific region including China and the Philippines. *S. intercalatum* occurs in 10 countries in the rainforest belt of Africa. *S. mekongi* is found in limited areas of Laos, Cambodia and Thailand (Chitsulo *et al.*, 2000).
As with other tropical diseases, population movements and refugees in unstable regions contribute to the transmission of the disease. Rapid urbanization has been accompanied by new foci of transmission (Patz et al., 2000). The large fresh water reservoirs associated with dams such as Akosombo Dam in Ghana, the Kainji Dam in Nigeria and the Kariba Dam in Zimbabwe as well as smaller reservoirs in the Sahel and irrigation systems throughout Africa are major transmission foci and thus endemic areas for schistosomiasis (Patz et al., 2000).

The disease mainly affects adult workers in rural areas, employed either in agriculture or the freshwater fishing sector. In many such areas, a high proportion of children between the ages of 10 and 14 are infected. Infection is by contact with contaminated water used in normal daily activities such as personal or domestic hygiene and swimming, or by professional activities such as fishing, rice cultivation, and irrigation.

Sustained heavy infection leads to morbidity, contributes to anaemia, and often results in retarded growth and reduced physical and cognitive function in children. Recent estimates suggest that the yearly death rate due to schistosomiasis in sub-Saharan Africa exceeds 200 000, which is largely attributable to renal failure or haematemesis (Patz et al., 2000).

The only effective control measure for the disease is praziquantel which has a limitation of potential development of resistance. Therefore there is a need for an alternative method of preventing the disease especially development of a vaccine for long-term prevention.
Evidence supporting the feasibility of a vaccine for schistosomiasis has been provided by successful vaccination/challenge experiments using attenuated cercariae in animals (Bickle et al., 1985; Maloney et al., 1985). However, due to the difficulty of producing large-scale, quality controlled, reproducible batches of these vaccines, and the associated safety considerations, they are clearly not suitable for use in humans. So far there are no intermediate host antigens tested as vaccine candidates. This study therefore aimed at finding common proteins in the parasite and its intermediate host and checking for immune cross reactivity between the proteins and the parasite's antibodies since these proteins could be useful as potential vaccine candidates and in the diagnosis of schistosomiasis.

1.1.1 Problem statement

Schistosomiasis has high morbidity rate and cause severe debilitating illness in millions of people in tropics. Currently, the only effective control measure for the disease is praziquantel which has a limitation of potential development of resistance. Therefore there is a need for an alternative method of preventing the disease especially development of a vaccine for long-term prevention.

1.1.2 Justification

Schistosomiasis remains endemic in 74 countries, putting an estimated 600 million people at risk of acquiring infection. Globally, Over 200 million people are estimated to be infected, 120 million of whom have symptoms and 20 million have severe illness.
Emphasis has, however, been placed on chemotherapy as the preferred method of treatment for schistosomiasis.

Although Praziquantel (PZQ) which is the current drug of choice for schistosomiasis has few and mild side effects and has high activity against all five species of the parasite capable of producing disease in humans, resistance of *S. mansoni* to praziquantel has been reported in some endemic regions (Liang *et al.*, 2002). Further, the control programs based on chemotherapy are complicated by the rapidity and frequency of reinfection and the difficulties and expense involved in maintaining these programs over the long term are overwhelming. Integrated control programs aimed at limiting schistosomiasis by improving education, sanitation, molluscicide treatment programs to reduce the population of the intermediate snail host, and chemotherapy have had limited success.

Vaccine strategies represent, therefore, an essential component of the control of this major helminth disease. The search for an effective vaccine has been hindered by the fact that not sufficient amount of the vaccine can be obtained from the parasite due to its minute size and maintenance of the parasite is also expensive. This study therefore aims at searching for common proteins between the parasite and the intermediate host with the aim of finding more antigens that can be used as potential vaccine candidates.

Since it is cheaper to maintain the snail hosts in the laboratory than the parasite, the snail host is larger in size and the antigen concentration is higher in the intermediate host. It will therefore be economical and easier to work with the snail.
1.1.3 Hypothesis

There exists a number of common proteins found in the digestive gland and foot of *B. pfeifferi* and *S. mansoni* and there is immune cross reactivity between antibodies raised against *S. mansoni* and protein extracts from the snail tissues.

1.1.4 Study objectives

1.1.4.1 General objective

The general objective of the study is to investigate the presence of common antigens between *S. mansoni* and its vector, *B. pfeifferi* since cross-reactive proteins may be important as possible candidates for vaccine and diagnosis of schistosomiasis.

1.1.4.2 Specific objectives

- To compare *S. mansoni* Soluble Cercariae Antigens (SCA) and Soluble Worm Antigen Preparation (SWAP) with proteins extracted from the foot and digestive gland of infected and uninfected *B. pfeifferi*.
- To compare protein extracted from the digestive gland and the foot of infected with those from uninfected *B. pfeifferi*.
- To investigate immune cross reactivity between *S. mansoni* (SCA and SWAP) antisera with *B. pfeifferi* antigens.
1.2 LITERATURE REVIEW

1.2.1 Global disease burden and Epidemiology

Schistosomiasis is second only to malaria in public health importance. It is estimated that 200 million people worldwide are infected with the snail-transmitted, water-borne parasitic helminth, and that 20,000 deaths per year are associated with the severe consequences of infection. These include bladder cancer and renal failure due to urinary schistosomiasis caused by *S. haematobium*; liver fibrosis and portal hypertension in intestinal schistosomiasis caused by *S. mansoni* (WHO, 2000). In sub-Saharan Africa where schistosomiasis constitutes an important public health problem, a survey by WHO (2000) of disease-specific mortality reported that 70 million individuals out of 682 million afflicted by the disease had experienced haematuria and 32 million dysuria associated with *S. haematobium* infection. It was estimated that 18 million suffered bladder wall pathology and 10 million hydronephrosis. Infection with *S. mansoni* was estimated to cause diarrhoea in 0.78 million individuals, blood in stool in 4.4 million and hepatomegaly in 8.5 million. Mortality rates due to non-functioning kidney (from *S. haematobium*) and haematemesis (from *S. mansoni*) have been estimated at 150 000 and 130 000 per year respectively, though data is limited (WHO, 2000). Although these are global estimates of the schistosomiasis disease burden, the public health impact of schistosomiasis in the field has been poorly evaluated and is still subject to controversy. Apart from a few situations where schistosomiasis is or was recognized as an obvious public health problem, as in Brazil, China, Egypt, the Philippines, northern Senegal and Uganda, the disease is often not a priority for health authorities and hence the high prevalence rates in areas where the disease is endemic.
1.2.2 Epidemiology and Disease burden in Kenya

According to a report by WHO (2006) on schistosomiasis and soil transmitted helminthes country profile, the endemicity of schistosomiasis in Kenya is well documented. Schistosomiasis (S. mansoni and/or S. haematobium) is found in 41 out of 70 districts, the southern half of the country being most affected. The two forms of the disease overlap in Machakos, Kitui, Taita-Taveta districts and in the Lake Victoria region. S. mansoni and S. haematobium are both widespread, however, surveys indicate that the prevalence varies considerably in different districts. Three separate endemic areas have been identified. These are coastal region, central and the lake regions.

In the coastal region, S. haematobium is widespread with a prevalence of more than 50% among school age children in Kilifi, Kwale, Malindi, Tana River, Taita-Taveta districts and also in Garissa district (WHO, 2006). In the North Eastern Province, a survey around the Hola Irrigation Scheme found an infection rate of more than 90% in 5 out of 9 schools in the area indicating the high infection rates in school going children especially those living around irrigation schemes where there is high transmission rate. In other districts prevalence rates below 25% were reported (WHO, 2006).

In the Central region, S. mansoni is widespread, but generally of lower prevalence (less than 25%). However, higher prevalence rates are found in parts of Machakos district and in the Mwea irrigation area of Kirinyaga district of the Central Province. S. haematobium is also found in Machakos and Kitui districts (WHO, 2006).
Schistosoma mansoni is also widespread in the lake region although the prevalence is less than 45% in most parts of Busia (Western Province), in South Nyanza, Kisumu and Siaya districts of Nyanza Province. Schistosoma haematobium prevalence rates of more than 50% are found in South Nyanza and slightly lower rates in Isuzu district.

Currently it is estimated that more than 5 million Kenyans are infected and less than 10 million people living in the rural areas are at risk of getting infected (WHO, 2006). In 1990, schistosomiasis was the 4th most frequently notified infectious disease in Coast Province, the 5th in Eastern and Nyanza Provinces, 6th in North Eastern Province and 10th in Central Province. A total of nearly 17,000 cases were reported. Chronic mild morbidity due to both S. mansoni and S. haematobium is common. Severe morbidity and mortality are focal and less common, but sufficient to be a public health concern (WHO, 2006 and Chitsulo, 2000).

1.3 Parasite Biology

1.3.1 Schistosomes host preference

Schistosomes require snails as intermediate host for completion of their life cycle. There are three genera of freshwater snail intermediate hosts of the important human schistosomes. These are: - Bulinus (S. haematobium), Biomphalaria (S. mansoni) and Oncomelania (S. japonicum). Each species of schistosome can only infect a single genus of snails. Geographically distinct strains have different abilities to infect a given species of snails (WHO, 1985).
The transmission of each strain of schistosomiasis is thus dependent on the presence of a suitable snail host. *S. mansoni* is transmitted by aquatic hermaphroditic snail species of family Plamobidae which are found among vegetation in lightly-shaded, slow to moderate flowing shallow waters with Biomphalaria as the most important intermediate host in Africa.

The four species of importance are *Biomphalaria pfeifferi* found in streams, seepages, water channels, dams and swimming pools; *B. sudanica* exist in swamps both in East and West Africa (Mandahl-Bath, 1958); *B. chaonomphala* species occurs in lakes and forms the main vector along lake Victoria while *B. alexandria* occurs in moderate flowing shallow water in the Nile valley in Africa.

*Schistosoma haematobium* is transmitted by aquatic hermaphroditic snail of the genus *Bulinus*, found among vegetation in stagnant or slow moving fresh water in irrigation channels, natural and man-made lakes and small water collections. *B. africanus* is the principal vector of schistosomiasis in Africa south of the Sahara but *B. globosus*, *B. nasutus*, *B. productus* and *B. ugandae* are also important vectors.

*Schistosoma japonicum* is transmitted by amphibious snail of genus *Oncomelania*, found in shallow water, marshland, seepage water in caves and moist soil along the banks of water courses and edges of irrigation channels. Three important species are involved in transmission, *O. nosophara* in South West China, *O. hupensis* in China mainland and *O. quadrasin* in the Philippines (WHO, 1993).
Though different species have varying tolerance to water temperature, current and turbidity, they seem to thrive best at optimum temperature range of 22-23°C (Malek, 1958). A common factor in population dynamics of many vector species is that they thrive on organic material and firm mud substrates. Usually they live in abundant aquatic vegetation that increases dissolved oxygen, and provide food through green algae encrusting the submerged portion of the plants.

Decayed vegetation also provide food and a suitable surface for depositing egg masses (Malek, 1958). Humans, especially children are the most important definitive host of *S. mansoni*. However, rodents and baboons are rare hosts (Cheesbrough, 1987). *Schistosoma japonicum* infects not only humans but also cattle, pigs, sheep, goats, water buffaloes, dogs, cats and wild rodent. Man is the most important definitive host of *S. haematobium* while *S. mansoni* can develop to maturity in a number of experimental animals: mice, velvet monkeys, baboons, hamsters and Rhesus monkey, often referred as permissive hosts.

### 1.3.2 Life cycle of *S. mansoni*

The life cycle of *S. mansoni* includes two hosts: a definitive host e.g. man where the parasite undergoes sexual reproduction and a single intermediate host (*Biomphalaria sp.*) where there are a number of asexual reproduction (Fig 1).

#### 1.3.2.1 Development of *S. mansoni* in the definitive host

Schistosome infections follow direct contact with fresh water that harbors free-swimming larval forms of the parasite known as cercariae which are released from the snail host under suitable conditions of temperature (10-30°C).
Cercariae then penetrate the skin of humans using secretions from its special glands; they then shed the bifurcated tail resulting into a schistosomulum (Gordon, 1998). Within 48 hours the schistosomulum passes completely through the skin into the blood vessels and lymphatic vessels.

The parasites are transported to the right side of the heart and into the lungs within 5-7 days. After several days, the worms migrate to the portal venous system, where they mature and unite. Pairs of the worms then migrate to the superior mesenteric veins in the case of \textit{S. mansoni}. Egg production commences four to six weeks after infection and continues for the life of the worm-usually three to five years. Eggs pass from the lumen of the blood vessels into adjacent tissues, and many then pass through the intestinal mucosa and are shed in the faeces, in the case of \textit{S. mansoni}. The life cycle is completed when the eggs hatch; releasing miracidia that, in turn, infect specific fresh-water snails (Jordan \textit{et al.}, 1993).

13.2.2 Development of \textit{S. mansoni} in the intermediate host

Successful penetration of \textit{S. mansoni} miracidium into the snail host via foot of the snail, tentacle and edge of the mantle initiates the development of the parasite in this host. Usually one or two miracidia undergo intramolluscan development. The ciliated epidermis disappears and mother sporocyst develops near the entry site and can be seen on the second day after infection (Sommro \textit{et al.}, 2005). At 96 hours, the mother sporocyst is an elongated sac structure filled with germinal cells and small centrally located vacuoles. The mother sporocyst increases in size with the duration of infection and is generally found in the head/foot region of the host within 7-9 days.
By day 11, the mother sporocysts contain daughter sporocysts, a few of which are elongated and almost mature. No migrating daughter sporocysts are found in the head and foot region and no parasite can be detected in the digestive gland/ovotestis area on days 10–11. By day 15, some daughter sporocysts are found in the digestive gland and by day 25 daughter sporocysts are found throughout the tissues around the gonad.

All the available space between the digestive gland and the gonads is occupied by metasporocysts i.e. daughter sporocysts containing developing cercariae (Sommro et al., 2005). Fully developed cercariae have moved into the large blood vessels and are on their way to leave the snail by 35 days. There’s little damage to the visceral organs. An increase in amoebocytes during the development of the parasite occurs, especially when the cercariae emerge from the host, and some granuloma like structures are also noticed during the development process (Gordon, 1998 and Sommro et al., 2005).
Fig. 1. Life cycle of schistosomiasis (Courtesy of Centers for Disease Control).
1.4 Pathology of Schistosomiasis

1.4.1 Immediate manifestations.

A maculopapular eruption may arise at the site of penetration by the cercarial form of the parasite. In migrants and tourists who become infected, skin reactions may develop within a few hours after infection. However, a rash may appear up to one week later. The dermatitis is similar to, but less severe than swimmers' itch which develops in sensitized persons when they are reinfected by species of schistosomes that do not colonize humans (Warren et al., 1973).

1.4.2 The Acute Phase of Infection

The acute phase of infection is also called 'Katayama' fever. It is associated with the onset of the female parasite laying eggs, (approximately 5 weeks after infection). Symptoms are thought to be mediated by the immune complex, and the majority of cases begin with the deposition of an egg into host tissues, then granuloma formation around the trapped eggs in the liver and intestinal wall. It resembles 'serum sickness' (i.e. acute immune complex disease), tender hepatomegaly is usually present, and splenomegaly occurs in one third of the cases. Respiratory symptoms have been reported in up to 70 percent of persons infected with S. mansoni but less frequent in those infected with S. haematobium (Cooke et al., 1999). Aseptic meningitis is rare. There may be radiologic evidence of interstitial pneumonitis. Not all patients shed eggs, but all have eosinophilia and most have positive serologic tests.
This phase of the infection is often asymptomatic, but when symptoms do occur they include fever, nausea, headache, an irritating cough and in extreme cases diarrhoea accompanied with blood, mucus and necrotic material. These symptoms, if present, last from a few weeks, to several months (Cooke et al., 1999).

1.4.3 The Chronic Phase of Infection

This is the most important aspect of *S. mansoni* pathology, and may be divided into two areas, namely gastrointestinal and liver disease. This phase manifests a number of years after infection. It results from the host’s immune response to schistosome eggs and the granulomatous reaction evoked by the antigens they secrete (Boros et al., 1970). The intensity and duration of the infection determine the amount of antigen released and the severity of the chronic fibro-obstructive disease. The granulomas destroy the ova but result in fibrotic deposition in host tissues. Most granulomas develop at the site of maximal accumulation of eggs- the intestine and the liver. However, periovular granulomas have been found in many types of tissues, including the skin, lung, brain, adrenal glands, and skeletal muscles (King, 2001).

Eggs retained in the gut wall induce inflammation, hyperplasia, ulceration, microabscess formation, and polyposis. Colicky hypogastric pain or pain in the left iliac fossa is frequent. Diarrhea is particularly common and may alternate with constipation. Diarrhea is particularly common in children, and its presence correlates strongly with schistosomiasis (Zhou et al., 1998).
Occult (or sometimes visible) blood in the faeces is usual. Colonic polyposis may be manifested as a protein-losing enteropathy (Hussein et al., 1983). Inflammatory masses in the colon may mimic cancer.

Eggs embolize to the liver, where the granulomatous inflammatory response induces presinusoidal inflammation and periportal fibrosis. This is referred to as clay-pipe-stem fibrosis, a condition which occurs in four to eight percent of patients who have chronic infection (King, 2001). It takes many years to develop and is associated with heavy infection. Hepatomegaly reflects the presence of granulomatous inflammation and occurs early in the evolution of chronic disease. Periportal collagen deposits lead to the progressive obstruction of blood flow, portal hypertension, and ultimately varices, variceal bleeding, splenomegaly and hypersplenism. Hepatocellular synthetic function is preserved until the very late stages of disease. Lobular architecture is retained, and nodular structure is retained, and nodular regenerative hyperplasia does not occur. Pathogenic reaction is a cellular, granulomatous inflammation around eggs trapped in the tissues, with subsequent fibrosis (Warren, 1973 and Allen et al., 2002).

1.5 Schistosomiasis Control

Schistosomiasis has been successfully controlled in many countries but still remains a major public health problem, with an estimated 200 million people infected, mostly in Africa (Chitsulo, 2000). Few countries in this region have undertaken successful and sustainable control programmes. The construction of water schemes to meet the power and agricultural requirements for development have led to increasing transmission, especially of S. mansoni (Chitsulo, 2000).
Increasing population and population movement have contributed to increased transmission and introduction of schistosomiasis to new areas. Most endemic countries are among the least developed whose health systems face difficulties in providing basic care at the primary health level. Further constraints to control include the lack of political commitment and infrastructure for public health interventions. There is need for increased support for schistosomiasis control in the most severely affected countries. The disease is associated with poverty and poor living conditions, inadequate sanitation and water supply as well as unplanned water resource development. The methods currently being used in schistosomiasis management include; early diagnosis and chemotherapy, improved and adequate sanitation, health education and snail control. Integration of these methods could be highly effective but is not feasible due to cost implications. It is therefore necessary to select an appropriate measure for each endemic area (WHO, 1993).

1.5.1 Chemotherapy

WHO has proposed a dual strategy which rests on morbidity control in high-burden regions and consolidation of control measures where the endemicity has been greatly reduced (Engels et al., 2000 and WHO, 2002). Safe, effective, single-dose antischistosomal drugs for example, praziquantel have been available for 25 years. The large reduction in cost to less than US$0.30 per treatment has been the leverage for chemotherapy-based morbidity control (WHO, 2002).
For example, biennial treatment with praziquantel in school-age children yielded a significant and sustained reduction in *S. haematobium* in Burkina Faso (Seydou *et al.*, 2008).

A serious limitation of using chemotherapy alone is however its indefinite dependence on praziquantel, potentially reducing the useful life-span of this drug due to drug resistance. In addition, praziquantel has no or very little activity against schistosomula but this can be overcome by drug administration twice a few weeks apart (N’Goran *et al.*, 2003). Laboratory studies and clinical trials suggest that combination chemotherapy with praziquantel and artemisinin derivatives is beneficial over praziquantel administration alone (Utzinger *et al.*, 2003). This is because artemisinins have strong activity against schistosomula and can therefore complement the stage-specific efficacy of praziquantel. Consequently, artemisinins can be recommended to prevent acute cases of schistosomiasis in specific high-risk groups for example flood-relief workers. Praziquantel-artemisinin combinations can be considered in regions where schistosomiasis is endemic but does not overlap with malaria for example in Egypt, large parts of northern and southern Africa, and most regions outside of Africa (Bergquist, 2002).

1.5.2 Sanitation and health education

Preventive measures, focused on clean water, adequate sanitation, and health education, are essential features of any long-term strategy for reduction and elimination of schistosomiasis (Katz, 1998 and Asaolu *et al.*, 2003). Basic sanitation and clean water supply combined with health education potentially constitute the most effective approach to schistosomiasis control but only in the mid-to-long term.
The classic solution involving only basic sanitation and clean water supply would, in theory be the most effective method, but it is very difficult and expensive to achieve such a solution in large endemic areas.

More simple methods of water supply and sanitation can reduce the prevalence and morbidity of the disease, but only in the long term and if associated with permanent health education programmes.

Effective and permanent control of schistosomiasis will only be possible with changes in human behavior through health education, with the improvement of the basic social and economic standards of the communities and with the enforcement by public health services.

1.5.3 Chemical control

Molluscsicides have been used to control schistosomiasis since the 1950s and their use is one of the procedures recognized (WHO, 1998). However, this strategy has been criticized for its high cost and the damage that molluscsicides cause to non-target organisms. Bayluscide, the most used synthetic molluscsicide is effective against all developmental stages of snails and schistosomes (Abu-Elyazeed et al., 1993 and Lowe et al., 2005).

At molluscsidial concentrations, it is lethal to fish (Goll et al., 1984). Other molluscsicides used for the control of schistosomiasis include trifenmorp, sodium pentachlorophenate, copper sulphate, yurimin, bis (tri-n-butyltin) oxide and nicotinanilide.
1.6 Vaccine Strategy

The control of schistosomiasis requires large-scale population-based chemotherapy in addition to environmental and behavioral modification. It is difficult and costly to sustain such a program (Morel, 2000).

There is a need for a vaccine for long-term prevention. Mathematical models indicate that drug treatment in combination with a vaccine would be more beneficial, and would contribute to savings for the health care system as a whole, even if the protection afforded by the vaccine were not absolute (Bergquist, 2004). Schistosomula appear to be the primary source of the target antigens that are vaccine candidates. A high level of protection against *S. mansoni* infection has been attained in mice and a similar level of protection against *S. japonicum* infection has been attained in mice, buffaloes, and pigs when the animals were immunized with irradiated cercariae (Maloney *et al*., 1985 and Shi *et al*., 1990).

Both type 1 and type 2 helper-T-cell responses may contribute to protection (McManus, 1999). The best protection using *S. mansoni* irradiated vaccine has been obtained in the baboon (Yole *et al*., 1996).

Efforts have been made to identify relevant schistosome antigens that may be involved in inducing protective immune responses, with a view to developing a recombinant-protein, synthetic-peptide, or DNA vaccine (McManus, 1999).

Coordinated laboratory and field research has identified *S. mansoni* molecules with protective potential (McManus, 1999). The antigenic identities of the biologically active molecules currently selected as candidates for schistosomiasis are paramyosin (Pearce *et al*., 1988); IrV-5, a myosin-like molecule implicated in the irradiated cercariae model
(Soisson et al., 1992); and Sm14, a protein characterized by its ability to bind fatty acids (Moser et al., 1991; Tendler et al., 1996). Glutathione-S-transferase (GST), (Balloul et al., 1987; Trottein, 1992 and Capron et al., 1995) is of particular interest due to its effect on worm fecundity resulting in suppressed egg production in addition to its anti-infection activity. Triose-phosphate isomerase (TPI) (Shoemaker et al., 1992) and Sm23 (Reynolds et al., 1992 and Koster et al., 1993) which represent antigens, from which ‘mimeotopes’ of the relevant epitopes of the full-length molecule, MAP3 and MAP4, respectively, have been constructed using synthetic peptides (Reynolds et al., 1994).

Exposure of vervet monkey Cercopithecus aethiops to cercariae attenuated with 30 krad of gamma radiation induced significant resistance to a challenge with normal parasites. Three vaccinations gave greater protection (48%) than a single exposure (26%) however, five vaccinations only achieved (39%) protection. Increasing the number of vaccinations beyond a certain point, instead of protection, may cause diminished level of protection due to induction of immunoregulatory mechanisms (Yole et al., 1996).

The development of multivalent vaccines consisting of several antigens has been shown to be a novel approach to creating broad-range protection against different parasite strains and parasite life cycle stages. Mice vaccinated with such a vaccine showed a significant level of protection (56%), and a decrease in the number and size, and change in the cellular profile of granulomas. Egg load in liver and intestine was reduced by 41.5% and 55.6% respectively (Mahmoud et al., 2008).
In recent Phase 1 and 11 clinical trials, the 28kD *S. haematobium* GST (Sh28GST) developed by Institut Pasteur de Lille (France), was safe and showed good immunogenecity in human volunteers in France, Niger and Senegal.

The schistosomiasis Vaccine Development Programme (SVDP), based in Egypt and supported by USAID, has focused on two *S. mansoni* antigens: paramyosin and a synthetic peptide construct containing multiple antigen epitopes (MAP) from the triose phosphate isomerase (Bachem Company, Los Angeles, USA). Another candidate vaccine, which is developed by FIOCRUZ (Rio de Janeiro, Brazil), is based on the use of Sm14, a 14 kDa fatty acid-binding *S. mansoni* protein with cross-reactivity with *Fasciola hepatica*.

In mice, Sm14 provided a 67% protection against challenge with *S. mansoni* cercariae and full protection against *F. hepatica* metacercariae (WHO, 2002).

Unfortunately, none of the candidate vaccines has moved from field trials to normal use in humans due to safety concerns and the inability to produce the vaccines in large amounts. There is therefore need for search of new vaccine candidate using other approaches. This study therefore aims at looking for common antigens between the parasite and the intermediate host as step to finding more antigens that are potential vaccine candidates. Since the intermediate host is larger in size, is easier to rear and has higher antigens concentration, it will be easier to extract the antigens from it.
1.7 Antigen sharing between *S. mansoni* and the snail host

The phenomenon of antigen sharing between a parasite and its host is a stimulating facet of the host-parasite relationship which is currently receiving considerable attention.

This is particularly evident in the area of schistosomiasis, where common antigens between schistosomes and their intermediate host (Dissous *et al.*, 1986; Iwanaga 1994; Chacón *et al.*, 2000) and between schistosomes and their definitive hosts have been described (Clegg *et al.* 1970 and Clegg *et al.*, 1971). The origins of common antigens in schistosomes are being elucidated, and cases of both antigen acquisition (Clegg *et al.* 1971) and antigen synthesis (Damian *et al.*, 1973) are now known. Although questions concerning the exact nature of the biological significance of common antigens remain to be answered, the concept that their presence is advantageous to the parasite appears to be gaining favor.

Common antigens between different species of *Schistosoma* and their intermediate hosts have been reported (Dissous *et al.*, 1986; Iwanaga 1994; Chacón *et al.*, 2000). Alarcón *et al.* (1989) demonstrated that sera from schistosome-infected persons reacted against soluble crude *B. glabrata* antigen (SBgA) by ELISA (100% of sensitivity) and that sera from mice immunized with SBgA recognized several homologous snail molecules by Western-blot (Chacón *et al.*, 2000). Studies using baboons infected with *S. mansoni* and rabbits immunized with antigens prepared from uninfected *B. pfeifferi* hepatopancreases demonstrated a complex group of antigens, some of which are shared between the snail and the cercariae or between the cercariae and the adult (Kempt *et al.*, 1974).
Similarly, Monoclonal antibodies (mAb) directed against *S. mansoni* tropomyosin isoform, SMTM (Xu et al., 1989) were used to test for cross-reactivity with *B. glabrata* antigens. Another mAb (1F10) recognized antigens of 39, 41, and 80 kDa in a snail head/foot antigen preparation but not a hepatopancreas antigen preparation.

Immunoelectrophoretic studies on common antigenicities using sera from rabbits immunized with the Bela Horizonte strain and Puerto Rican strains of *S. mansoni* adult worms or eggs, respectively, and antigens of several adult *Biomphalaria* snails revealed that *S. mansoni* adult worm extracts produced 8 bands both with extracts of *B. glabrata* pigmentation and *B. glabrata* pigmentado, 3 to 4 bands with those of *B. glabrata* albino and 1 to 2 bands with those of *B. straminea*. On the other hand, *S. mansoni* egg extracts produced 5 bands with extracts of *B. glabrata* pigmentation, 4 bands with those of *B. glabrata* pigmentado, 2 bands with those of *B. glabrata* albino and 1 band with those of *B. straminea*.

In the experimental infection of adult *Biomphalaria glabrata* snails with five *S. mansoni* miracidia, the infection rate in *B. glabrata* pigmentation was 78.8%, and *B. glabrata* 71.2% pigmentado, whereas the infection rate in *B. glabrata* albino was 10.3%, and *B. straminea* was not susceptible to *S. mansoni*. The infectivity of each snail corresponded with the number of bands representing common antigenicities between host and parasite (Iwanaga et al., 1992 and Iwanaga, 1994) suggesting co-evolution of the parasite and the host.
2.0 MATERIALS AND METHODS

2.1 Experimental Animals and parasites

A strain of *S. mansoni* originally obtained from humans and maintained in the Olive baboon (*Papio anubis*) at the Institute of Primate Research (IPR), Nairobi, Kenya was used for all the work involving preparation of antigens and to obtain miracidia for infection of snails. Laboratory bred snails (*Biomphalaria pfeifferi*) maintained at the IPR were used for the extraction of infected, naïve tissues and for production of cercariae for preparation of SCA. Two New Zealand white rabbits obtained from the Department of Biochemistry, University of Nairobi and maintained on normal pellet diet throughout the course of experiment were used for preparation of anti-sera for both the worm and cercariae antigens.

2.2 Maintenance of the snail hosts.

Maintenance of the snail hosts was done at the Institute of Primate Research as described by Yole *et al.*, (1996). Water was changed twice a week while soft lettuce (dipped in hot water and cooled) was added as feed.
2.3 Harvest of *S. mansoni* eggs from the baboons' faeces and hatching of miracidiae

Faecal samples from infected baboons ascertained by microscopic examination of the faeces was collected, eggs harvested and miracidiae hatched by the method of Yole *et al.*, (1996). Faecal samples were placed in a plastic beaker and a litre of normal saline added to completely cover the sample and the mixture stirred with a wooden spatula to obtain a thin suspension. The sample was then sieved through two sieves (mesh size 600µm and 250µm) into a collecting tray and the filtrate transferred into urine jars. The sieve was sprayed with saline from a wash bottle to ensure that no eggs remained on the mesh. The urine jars were then filled with saline and left for 30 min in the dark to prevent the eggs from hatching. The supernatant was poured off without disturbing the sediment.
The above procedure was then repeated thrice until the supernatant was clear and the sediments together with the eggs transferred into a glass Petri dish.

The urine jars were thoroughly sprayed with water using a wash bottle to ensure the transfer of all eggs to the Petri dish containing fresh water. The Petri dish was then placed under a lamp of 100 watts for 30 min. Emergence of miracidia was observed using a dissecting microscope with × 40 magnification.

2.4 Infection of snails with *S. mansoni*

Snails were infected with *S. mansoni* using infecting wells described by Yole *et al.*, 1996. A Pasteur pipette with a rubber bulb (teat) was used to pick 5-6 miracidia from the glass Petri dish under the dissecting microscope. The miracidia were dispensed into each of the wells of a 24 well culture plate containing a snail. The setup was then left for 30 min to allow miracidial penetration, after which the snails were transferred into newly prepared tanks as described in section 2.2. The prepatent period for *S. mansoni* is 5 weeks. At four weeks post-infection, the tanks were covered with a dark cloth to prevent trickle shedding of cercariae. These snails were used as a source of infected snail material, cercariae for antigen preparation and a source of cercariae to infect mice for SWAP.
2.5 Snail dissection and preparation of proteins

Snail dissection was done by gently cleaning the shell surface with 70% alcohol then placing the snails in a clean Petri dish under a dissecting microscope. The shell was then carefully crushed and the snail removed from it. Using a dissecting microscope, the digestive gland and the foot were located and removed using a sharp forcep. The digestive gland and the foot were then kept in different eppendorf tubes containing 500μl of phosphate buffer saline (PBS; consisting of 0.9% NaCl, 0.02% KCl, 0.115% Na₂HPO₄, 0.02% KH₂PO₄ and a pH of 7.3). A total of 50 clean snails were dissected. The above procedure was repeated for the infected snails on day 8, 15, and 32 post-infection to get the infected tissue samples. The tissues were homogenized completely in the presence of 1% Sodium Dodecyl Sulphate (SDS), centrifuged at 10,000g at 4°C for 30 min. The soluble protein supernatant was aliquoted, protein concentration was determined as described in section 2.8 and the samples stored at -80°C for proteomic assays.

2.6 Shedding of parasite by infected snails

Snails were artificially stimulated to release cercariae by the procedure described by Yole et al., (1996). Five weeks post infection snails were removed from the tanks in the dark and placed in a 100ml beaker with 20mls of unchlorinated water. They were then exposed to light (100 Watts bulb shielded with glass to safeguard the snails from the heat) for 1-2 hours to shed cercariae. The cercariae were then used for preparation of antigens.
2.7 Preparation of Soluble Cercariae Antigen (SCA)

Infected snails were allowed to shed cercariae for 2 hrs. Cercariae were chilled for 2 hrs at 4°C to allow them to settle at the bottom of the beaker. Excess water from the chilled cercariae was aspirated using a suction pump and cercariae made into a suspension by gently sucking in and out using a Pasteur pipette. The suspension was then placed in chilled glass tube and centrifuged at 450g for 10min. The supernatant was discarded and chilled distilled water added. The procedure of centrifuging and discarding was repeated twice.

The pellet consisting of cercariae was washed and resuspended in sterile PBS. The suspension was then sonicated using a (Soniprep 150 UK) at 923kHz, 16μm amplitude for a minute, 10 times. The homogenate was then centrifuged for 1 hr at 10,000g at 4°C. Soluble protein supernatant was sucked out and pellet discarded. Protein concentration was determined using Lowry et al., (1951) as described below and the proteins stored at -20 °C for further analysis.

2.8 Protein concentration estimation

Protein concentration was determined using the Lowry et al., (1951) method with bovine serum albumin (BSA) as the protein standard. Dilutions of BSA (30-300μg/ml) and samples were incubated at room temperature for 15 min.

This was followed by a further incubation with Folin-phenol for 45 min. Absorbances were then read at 650nm using a Beckman Coulter DU 530R life Science UV/V Spectrophotometer and a standard curve drawn.
Optical densities of dilutions of the different samples were obtained at the same wavelength as BSA dilutions. The concentration of the samples was determined using the standard curve.

2.9 Soluble Worm Antigen Preparation (SWAP)

Adult *S. mansoni* worms were obtained by perfusion at end of the 5th week from mice infected with *S. mansoni* cercariae. The mice were perfused to recover worms from the liver and the mesenteric veins. The perfusion fluid was pumped mechanically through the left ventricles so that it passed through the arterial system. Since the hepatic portal vein had been incised, the fluid flushed out the worms in the liver and mesenteries. The worms were washed in sterilized PBS containing 1% SDS. Sonication, centrifugation and protein determination was done.

2.10 Proteomic studies

2.10.1 Polyacrylamide gel electrophoresis (PAGE) and SDS-Polyacrylamide gel electrophoresis

Both native Polyacrylamide and SDS- polyacrylamide gel electrophoreses slab gels were used for analysis of the native and subunit composition of the various sample extracts.

2.10.1.1 Native Polyacrylamide Gel Electrophoresis

Native polyacrylamide gel (PAGE) was used to determine the native molecular weight of the samples. Samples (100µg) were mixed with 10µl of sample buffer (0.125M Tris/HCl pH 6.8, 10% glycerol and 0.002% Bromophenol blue) and applied onto 5-20% native-PAGE gels. Molecular weight standards (Sigma) were run alongside the samples.
The gels were run at a constant voltage (70V) in 25mM Tris-glycine buffer, pH 8 until the Bromophenol tracking dye reached the end of the gel.

Three gels were run containing the samples as shown below:

I. Uninfected digestive gland (DG), uninfected foot (FT), SCA and SWAP
II. Day8 foot, day15 digestive gland (DG), day32 foot (FT), SCA and SWAP
III. Uninfected DG, uninfected FT, day8 FT, day15 DG, day32 FT.

The proteins were then visualized after staining the gel with Silver stain according to Wray *et al.*, (1981).

2.10.1.2 Sodium Dodecyl Sulphate- Poly Acryl amide Gel Electophoresis (SDS-PAGE)

SDS-PAGE was used for determination of sub unit composition of the samples. This was carried out using the method described by Leammli (1970) in a vertical electrophoretic unit (Bio-Rad). Gradient gels (5-20% acrylamide) containing 1.875M Tris-HCl pH 8.8 and 0.1% SDS was used as the separating gel while 3.5% acrylamide gel was used as stacking gel.

The samples were prepared by mixing 100μg of the sample with 10μl of the sample buffer (0.125M Tris/HCl pH 6.8, 5% SDS, 10% β-mercaptoethanol, 10% glycerol and 0.002% Bromophenol blue) and heated at 94-100°C for five minutes to denature the proteins prior to loading onto the gel. The gels were run at constant current of 20 mA until the Bromophenol tracking dye reached the end of the stacking dye then increased to 25mA for about an hour until the dye reached the end of the separating gel. The proteins were then visualized after staining the gel with Silver stain.
2.10.4 Silver Staining

Detection of proteins was by staining the gel with Silver stain according to Wray et al., (1981). All the steps were carried out with gentle agitation on an orbital shaker. Staining was done according to the following protocol.

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Protein Fixation</strong></td>
<td></td>
</tr>
<tr>
<td>10% Acetic acid, 50% Methanol</td>
<td>-1-2 Minutes</td>
</tr>
<tr>
<td>-50% Methanol</td>
<td>-5 min</td>
</tr>
<tr>
<td>-Distilled water</td>
<td>-20 Min</td>
</tr>
<tr>
<td>-50% Methanol</td>
<td>-5 Min</td>
</tr>
<tr>
<td>-2.5% Glutaraldehyde</td>
<td>-30 Min</td>
</tr>
<tr>
<td>-Washed severally in distilled water</td>
<td>-10 Min</td>
</tr>
<tr>
<td>-50% Methanol</td>
<td>-20 Min</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Incubation of gel for 15 Min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Soln. A</strong> (0.4g AgNO₃ in 1.2ml of distilled water)</td>
<td></td>
</tr>
<tr>
<td><strong>Soln. B</strong> (1.0ml of 2M NaOH, 0.8ml NH₄OH adjusted to 20ml with distilled water)</td>
<td></td>
</tr>
<tr>
<td>-Titrate soln A to B</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Development</th>
<th>Incubate gel to desired coloration</th>
</tr>
</thead>
<tbody>
<tr>
<td>-1.25ml of 1% citrate, 62.5µl of 38% formalin adjusted to 125ml with distilled water.</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>Stopping Reaction</th>
<th>5 Minutes</th>
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<tbody>
<tr>
<td>-5% Acetic acid.</td>
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<table>
<thead>
<tr>
<th>Storage</th>
<th>Indefinite</th>
</tr>
</thead>
<tbody>
<tr>
<td>-7% Acetic acid.</td>
<td></td>
</tr>
</tbody>
</table>
2.11. Immunological studies

2.11.1 Raising antibodies in rabbits

Two New Zealand white rabbits were used for raising antisera for both the worm and cercariae antigens. Fifty micrograms per milliliter of antigen preparation in 0.5ml Freund's complete adjuvant was injected intramuscularly at the initial dose. Boosting was done once after every two weeks for a period of eight weeks with 25µg per ml of antigen in 0.5ml Freund's incomplete adjuvant for each rabbit.

2.11.2 Detection of antibody

Detection of antibody was carried out to facilitate visual observation of antigen-antibody interactions in a gel matrix. It is based on the theoretical principle that antigen-antibody in the correct proportion will result in a precipitate.

A clean microscopic slide was placed on a level surface. Four milliliters of molten 2% agar was quickly but gently poured onto the slide so that it spreads over the slide forming a uniform thickness. The gel was left to set and wells punched approximately 5mm apart. The number of wells punched per gel depended on the number of samples to be loaded but a maximum of six. The antigens (SWAP or SCA) were introduced into the central well and the test sera collected at different time points during the immunization regime, placed in the peripheral wells. The slide was kept in a moist chamber overnight. It was then washed gently in distilled water for 1-2 hours, then in PBS/Azide for 3 hours. The slide was then placed on a paper towel and left overnight, it was then stained with Coomassie Brilliant Blue for 25 minutes. Coomassie Brilliant Blue was prepared by mixing 0.1% Coomassie Brilliant Blue R250 in 50% methanol and glacial acetic acid,
The stain was replaced with a destain (10% methanol, 7% glacial acetic acid) and washed several times. The presence of a precipitin line indicated the presence of SWAP antibodies in the test serum.

2.11.3 Western blotting analysis

This was done according to the method by Burnette (1981). The protein samples were separated under denaturing conditions on 5-20% gradient SDS-PAGE mini gel as described in section 2.10.3 and transferred onto a nitrocellulose membrane by semi dry blotting using a Bio-Rad transblot apparatus. The transfer was carried out at a current 3mA/cm² of the membrane for 2 hours. To ascertain the transfer, the blot was stained using Rouge Ponceau S solution [5 %/( w/v) in 1% acetic acid] and destained with distilled water until the protein bands cleared. Further washes with distilled water were performed to wash off all the ponceau. The nitrocellulose was then incubated overnight with the blocking solution (3% BSA in PBS containing 0.05% Nondiet NP-40, 0.05% Tween80). It was then incubated in a 1:100 dilution of immune sera in blocking buffer for 1 hour and washed (3×10 min) in PBS-Tween (0.05%Tween 80 in PBS, pH 7.4) and washed as above before incubating with horse radish peroxidase-conjugated goat anti-rabbit secondary antibody (1:1000 dilution in coating buffer) for 45 minutes on a rocking shaker. The membrane was re-blocked in 10% non-fat dry milk in PBS –Tween 80 for 10 minutes. To reveal the bands, the sheet was incubated with the substrate, 4-chloro-1-naphthol (1 part diluted in 6 parts of 0.05M Tris-HCl, pH 7.4) from a stock solution containing 3mg/ml of 4-chloro-1-naphthol in absolute methanol and 10μl of 30% Hydrogen peroxide. Further colour development was stopped by rinsing the sheet in distilled water.
CHAPTER THREE

3.0 RESULTS

3.1 Protein concentration determination

Protein concentration in various extracts was determined from a standard curve of BSA concentrations between 30-300μg/ml (Fig 3). A comparison of the overall protein yield in the protein extracts from the snail host was found to be higher than those in the parasite stages. However, because of the small sample size the significance of the difference was not tested. (Fig 4).
PROTEIN CONCENTRATION DETERMINATION

Fig 3. Protein standard curve
Fig 4. Total protein recovered in the samples

KEY:

- Naïve DG- Uninfected digestive gland
- FT-Foot
- DY8ft-Day 8 foot
- Dy15DG-Day 15 digestive gland
- Dy32FT-Day 32 foot
- SCA-Schistosomal cercariae antigen
- SWAP-Soluble worm antigen preparation
3.2 Electrophoretic analysis of snail tissues and parasite proteins

A 5-20% native PAGE analysis of protein extracts from uninfected snail foot, digestive gland, SCA and SWAP revealed five proteins of Mr~ 178kDa(a), 50kDa(b), 25kDa(c) and 14kDa(d) (Plate 1a) which were common in all the samples while a 5-20% SDS-PAGE of the same samples revealed 5 peptides common in all the samples (Plate 1b). Their molecular weights were estimated to be 66kDa (a), 46kDa (b), 26kDa (c), 16kDa (d) and 12kDa (e). Proteins of Mr~ 55kDa (f) and 42kDa (g) were found to be common in SCA and the tissues. There were fewer bands observed in the parasite stages as compared to the tissues. A 22kDa (h) protein was observed only in SWAP and was present in both tissues.

Similarly, native gel analysis of infected tissues and parasite stages mentioned above showed the common proteins to be of similar molecular weights as in the uninfected tissues i.e. Mr~ 178kDa (a), 50kDa (b), 25kDa(c) and 14kDa (d) (Plate 2a) while the same samples when separated on a 5-20% SDS-PAGE showed shared proteins which had similar molecular weights as those in plate 1b. The common proteins observed included those of Mr~66kDa (a), 46kDa (b), 26kDa (c), 16kDa (d) and 12kDa (e). Proteins of Mr~ 55kDa (f) and 42kDa (g) were found to be common in SCA and the tissues but absent in SWAP. A 22kDa (h) protein was observed only in SWAP and the infected snail tissues.
Plate 1a. Comparison of protein profiles in the tissue protein extracts of uninfected tissues of *B. pfeifferi* and *S. mansoni* developmental stages on a 5-20% Native-PAGE.

Lane 1- Molecular weight marker (Sigma)-5μl
Lane 2- Uninfected snail digestive gland (100μg)
Lane 3- Uninfected snail foot (100μg)
Lane 4- SCA (100μg)
Lane 5- SWAP (100μg)

*a, b, c and d* - common proteins
Plate 1b. Comparison of protein profiles in the tissue protein extracts of uninfected tissues of *B. pfeifferi* and *S. mansoni* developmental stages on a 5-20% SDS-PAGE.

Lane 1- Broad range molecular weight marker (Promega) (20μg)

Lane 2- Uninfected snail digestive gland (100μg)

Lane 3- Uninfected snail foot (100μg)

Lane 4- SCA (100μg)

Lane 5- SWAP (100μg)

*a, b, c, d and e* – Proteins common in all the samples

*f and g* – Proteins common between SCA and the snail tissues

*h* – Protein common between SWAP and the snail tissues
Plate 2a. Comparison of protein profiles in the tissue protein extracts of infected *B. pfeifferi* and different *S. mansoni* developmental stages on a 5-20% Native-PAGE.

Lane 1- Molecular weight marker (Sigma)-5μl
Lane 2- Day 15 snail digestive gland (100μg)
Lane 3- Day 8 snail foot (100μg)
Lane 4- Day 32 snail foot (100μg)
Lane 5- SCA (100μg)
Lane 6 -SWAP (100μg)

*a, b, c and d* - common proteins
Plate 2b. Comparison of protein profiles in the tissue protein extracts of infected *B. pfeifferi* and different *S. mansoni* developmental stages on a 5-20% SDS-PAGE.

Lane 1 - Broad range molecular weight marker (Promega) - (20 µg)

Lane 2 - Day 15 snail digestive gland (100 µg)

Lane 3 - Day 8 snail foot (100 µg)

Lane 4 - Day 32 snail foot (100 µg)

Lane 5 - SCA (100 µg)

Lane 6 - SWAP (100 µg)

*a, b, c, d and e* – Proteins common in all the samples

*f and g* – Proteins common between SCA and the snail tissues

*h* – Protein common between SWAP and the snail tissues
A comparison of uninfected and infected *B. pfeifferi* tissues in 5-20% native gel revealed an increase in intensity of a 112 kDa (a) protein in the digestive gland. And a decrease in intensity of proteins of Mr~23kDa (b) and 17kDa (c) in the foot (Plate 3a). A 5-20% SDS-PAGE analysis of the same tissues revealed differences in various proteins; between uninfected and infected digestive gland at day 15, a decrease in the intensity in the proteins of Mr~ 178kDa (a) and 44kDa (c) was noted. Similarly, when the foot extracts were compared between uninfected and infected (day 8 and 32) tissues, a decrease in intensity in the proteins with Mr~ 120kDa (b) and 30kDa (e) was noted (Plate 3b).
Plate 3a. Comparison of protein profiles in the uninfected and infected tissue protein extracts of infected *B. pfeifferi* on a 5-20% Native-PAGE.

Lane 1- Molecular weight marker (Sigma)-5μl
Lane 2- Uninfected snail digestive gland (100μg)
Lane 3- Day 15 digestive gland (100μg)
Lane 4- Uninfected snail foot (100μg)
Lane 5- Day 8 foot (100μg)
Lane 6- Day 32 foot (100μg)

*a* - a protein increasing in intensity in the digestive gland.

*b* and *c* – proteins decreasing in intensity in the foot.
Plate 3b. Comparison of protein profiles in the uninfected and infected tissue protein extracts of infected *B. pfeifferi* on a 5-20% SDS-PAGE.

Lane 1- Broad range molecular weight marker (Promega) (20µg)

Lane 2- Uninfected snail digestive gland (100µg)

Lane 3- Day 15 digestive gland (100µg)

Lane 4- Uninfected snail foot (100µg)

Lane 5- Day 8 foot (100µg)

Lane 6- Day 32 foot (100µg)

*a* and *c* – proteins decreasing in intensity in the digestive.

*d* and *e* – proteins decreasing in intensity in the foot upon infection by the parasite.
3.3 Immunological studies

3.3.1 Antibody detection

Upon carrying out gel immune-diffusion assay, it was noted that the rabbit immunized with cercariae antigen (SCA) developed the antibodies on the 8th week after the immunization, plate 4(a) while the one immunized with the worm antigen (SWAP) developed the antibodies two weeks after immunization plate (4b).
Plate 4a. Double radial immunodiffusion analysis of antiSCA serum

The central well contained 50μl of SCA while the peripheral wells contained 50μl anti-SCA serum obtained at different times after immunization.

A- 2nd week
B- 4th week
C- 6th week
D- 8th week
E- Shows a precipitin line of the Ag-Ab reaction.
F- Central well containing 50μl SCA (antigen)
Plate 4b. Double radial immunodiffusion analysis of anti-SWAP serum

The central well contained 50μl of SWAP while the peripheral wells contained 50 μl anti-SWAP serum obtained at different times after immunization.

A- 2nd week
B- 4th week
C- 6th week
D- 8th week
E- Shows a precipitin line of the Ag-Ab reaction
F- Central well containing 50μl of SWAP (antigen)
3.3.2 Western blotting

Nitrocellulose membrane blotted with soluble uninfected *B. pfeifferi*, SCA, SWAP antigens incubated with the antiserum from a rabbit immunized with SWAP showed cross-reactions with several protein in the various tissues and the parasite proteins.

Out of the five common proteins detected by SDS-PAGE, two proteins of molecular weight Mr~ 46kDa(e) and 16kDa(g) in the digestive gland and a 12kDa(h) protein in the foot cross-reacted with antibodies raised against SWAP while proteins of Mr~ 76kDa(d) and 22kDa(f) in the digestive gland, and those with Mr~ 220kDa(a), 180kDa(b), 89kDa(c) and 38kDa(i) in the foot cross-reacted in western blot but were not shown to be common in SDS-PAGE (Plate 5b). Similarly, when nitrocellulose membrane blotted with uninfected *B. pfeifferi* tissues was incubated with the antiserum from a rabbit immunized with SCA, cross-reaction was noted with two proteins with Mr~ 220kDa(a) and 16 kDa(b) in the digestive gland but there was no cross-reaction observed in the foot (Plate 6b).

A Western blot of infected snail tissues with antibodies raised against SWAP cross-reacted with proteins of Mr~ 46kDa(e) and 16kDa(g) in the digestive gland shown to be common by SDS-PAGE while Mr~76kDa(d) and 22kDa(g) proteins in the digestive gland cross-reacted but were not shown to be common in SDS-PAGE. In day 8 and day32 foot, the proteins which cross-reacted but were not shown to be common by SDS-PAGE included those with Mr~ 220kDa(a), 180 kDa(b), 89kDa(c) and 38kDa(f) (Plate 7b). A western blot of infected snail tissues with anti-SCA resulted in cross-reactions with proteins of Mr~ 220kDa(a) and 16 kDa(b) in the digestive gland. There was no cross-reaction in the infected snail foot (Plate 8b).
Plate 5a. Protein profiles of parasite stages of *S. mansoni* and snail tissues after transfer into nitrocellulose membrane and staining with ponceau S stain.

Lane 1- Molecular weight marker- (Promega-20μg)

Lane 2-Uninfected digestive gland (100μg)

Lane 3-Uninfected foot (100μg)

Lane 4- SCA (100μg)

Lane 5-SWAP (100μg) – antigen (positive control)
Plate 5b. Western blot of proteins using anti-SWAP

Lane 1- Molecular weight marker- (Promega-20μg)
Lane 2-Uninfected digestive gland (100μg)
Lane 3-Uninfected foot (100μg)
Lane 4- SCA (100μg)
Lane 5- SWAP

a, b, c, h and I – cross reacting antigens in the uninfected snail foot

d, e, f and g – cross reacting antigens in the uninfected snail foot
Plate 6a. Protein profiles of uninfected snail tissues and *S. mansoni* parasite stages after their transfer into nitrocellulose membrane and staining with ponceau S.

Lane 1 - Molecular weight marker- (Promega-20μg)

Lane 2 - Uninfected digestive gland (100μg)

Lane 3 - Uninfected foot (100μg)

Lane 4 - SCA (100μg) - antigen (positive control)

Lane 5 - SWAP (100μg).
Plate 6b. Western blot of proteins using anti-SCA.

Lane 1- Molecular weight marker (Promega) (20μg)
Lane 2- Naïve digestive gland (100μg)
Lane 3- Naïve foot (100μg)
Lane 4- SCA (100μg) - antigen (positive control)
Lane 5- SWAP (100μg).

a and b-cross reacting proteins in the digestive gland
Plate 7a. Infected snail tissues and parasite proteins after their transfer into nitrocellulose membrane and staining with Ponceau S.

Lane 1- Broad range molecular weight marker- (Promega) (20μg)

Lane 2- Day 15 snail digestive gland (100μg)

Lane 3- Day 8 snail foot (100μg)

Lane 4- Day 32 snail foot (100μg)

Lane 5- SCA (100μg)

Lane 6 - SWAP (100μg)- antigen (positive control)
Plate 7b. Western blot of infected snail tissues using anti-SWAP

Lane 1 - Broad range molecular weight marker- (Promega) (20µg)
Lane 2 - Day 15 snail digestive gland (100µg)
Lane 3 - Day 8 snail foot (100µg)
Lane 4 - Day 32 snail foot (100µg)
Lane 5 - SCA (100µg)
Lane 6 - SWAP (100µg)-antigen (positive control)

*a, b, c, h* and *l* - cross reacting antigens in the infected snail foot

*d, e, f and g* - cross reacting antigens in the infected snail foot
Plate 8a. Protein profiles of infected tissues and parasite proteins after their transfer and staining with Ponceau S stain.

Lane 1- Broad range molecular weight marker- (Promega) (20µg)
Lane 2- Day 15 snail digestive gland (100µg)
Lane 3- Day 8 snail foot (100µg)
Lane 4- Day 32 snail foot (100µg)
Lane 5- SCA (100µg)-the antigen (positive control)
Lane 6 -SWAP (100µg)
Plate 8b. Western blot of infected snail tissues using anti-SCA.

Lane 1- Broad range molecular weight marker- (Promega) (20μg)
Lane 2- Day 15 snail digestive gland (100μg)
Lane 3- Day 8 snail foot (100μg)
Lane 4- Day 32 snail foot (100μg)
Lane 5- SCA (100μg)-antigen (positive control)
Lane 6 - SWAP (100μg)

a and b-cross reacting proteins in the digestive gland
4.0 DISCUSSION

The phenomenon of antigen sharing between a parasite and its host is a stimulating facet of the host-parasite relationship which is currently receiving considerable attention. This is particularly evident in the area of schistosomiasis, where common antigens between schistosomes and their intermediate host (Dissous et al., 1986; Iwanaga 1994; Chacón et al., 2000) and between schistosomes and their definitive hosts have been described (Clegg et al. 1970 and Clegg et al., 1971).

The results of this study revealed the presence of common proteins both by native and SDS-PAGE between *S. mansoni* and the uninfected digestive gland and foot of its intermediate host, *B. pfeifferi*. Analysis by native-PAGE revealed four proteins common in all the samples while SDS-PAGE analysis of the same samples showed five common proteins. Two proteins with Mr~ 55kDa and 42kDa were shown to be common in SCA and the tissues, while a 22kDa protein was found in SWAP and the tissues. The existence of antigenic community between *S. mansoni* and *Biomphalaria sp* has been demonstrated in past studies (Dissous et al., 1986, 1990; Iwanaga et al., 1992; Iwanaga, 1994; Weston et al., 1994). Earlier studies involving comparison of protein extracts of *B. pfeifferi* hepatopancrease, SCA and worm revealed the presence of a complex group of antigens, some of which are shared between the snail, cercariae, and adult (Kemp et al., 1974). In another study, shared antigens were demonstrated between *B. glabra* hepatopancrease and *S. mansoni* SCA (Kathleen et al., 1985).
Analysis of infected snail tissues—day 8 foot, day 15 digestive gland, day 32 foot and *S. mansoni* parasite stages revealed common proteins of similar molecular weights as those found with the uninfected tissues. The possible role of these shared antigens in immune evasion in the host or in the maintenance of antigenic parasite polymorphism has been elucidated. It has been suggested that at least some of these shared antigens reflect molecular mimicry between the host and the parasite as a result of selection pressure (Damian, 1987).

The present study further revealed differences in tissue proteins of uninfected and *S. mansoni*-infected *B. pfeifferi* snails. The protein profiles in SDS-PAGE demonstrated a decrease in intensity in the proteins with Mr~ of 178 kDa and 44 kDa in the digestive gland while a native-PAGE analysis of the proteins showed a decrease in intensity of a 112 kDa in the digestive gland and a decrease in proteins with Mr~ 23 kDa and 17 kDa in the foot. Changes in intensity of different proteins in *Biomphalaria* tissues after infection with *S. mansoni* was thus noted in this study. An increase in intensity of Proteins of Mr~ 44, 56, 65, 144 kDa and 36 kDa has previously been demonstrated in *S. mansoni*-infected *B. alexandrina* tissues (El-Ansary *et al.*, 2000). The differences in the different proteins had no effect on the proteins found to be common in the parasite and the intermediate host. These changes could be due to the intermediate host defending itself from the parasite or it could be parasite initiated down regulation of the proteins to make the snail host environment conducive for its existence (Walker, 2006; Nelson *et al.*, 2008).

Further analysis of proteins from uninfected digestive gland, uninfected foot, SCA and SWAP by Western blot using anti-SWAP antiserum showed cross-reactions with four proteins in the digestive gland while five proteins cross reacted in the foot.
Cross-reactions with similar proteins were observed in the infected snail tissues and anti-SWAP antiserum.

Immune cross-reaction between anti-SCA antibodies and uninfected snail tissues showed cross reaction with two proteins in the digestive gland but no cross-reaction in the foot. While proteins of similar molecular weights as those in uninfected tissues cross-reacted in the digestive gland against anti-SCA.

Immune cross-reactivity between schistosome proteins and *Biomphalaria* has been shown in previous studies between proteins in both infected and uninfected snail hemolymph and tissues as previously demonstrated (Yoshino *et al.*, 1983 and El-Dafrawy *et al.*, 2007). Similarly, cross reactions have also been demonstrated in hepatopancrease proteins obtained from uninfected *B. pfeifferi* (Kemp *et al.*, 1974 and Carlos *et al.*, 1985) and *B. glabrata* tissues (Chacón *et al.* 2000; 2002). Cross-reactive antigens may probably result from the adaptation of parasites to their invertebrate and vertebrate hosts and in consequence, could prevent immune recognition in the latter.

The present study has therefore demonstrated the presence of common proteins between *S. mansoni* (SCA and SWAP) and tissues (foot and digestive gland) of its intermediate host *B. pfeifferi*. Immune cross reactivity between different proteins has also been demonstrated in the study. It has therefore revealed a number of proteins that require further studies to evaluate their potential in search of vaccine candidates and diagnostic targets, considering the abundance of antigenic material that can be obtained from the snail host.
CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

The study has shown the presence of five proteins to be shared between S. mansoni and B. pfeifferi uninfected and infected tissues by SDS-PAGE. While four proteins were shown to be common by native PAGE.

Four proteins in the digestive gland and five proteins in the foot cross-reacted with antibodies raised against the parasite stages implying further similarities in their composition and structure of their epitopes.

5.2 RECOMMENDATIONS

There is need for further studies of these proteins to ascertain their use as potential vaccine candidates in animal models.

The possibilities of the shared antigens to be used for the diagnosis of schistosomiasis should also be studied. This could then assist in developing a diagnostic kit to help in early diagnosis of the infection and thus prompt treatment.

Lastly, analysis of the identified proteins as targets for development of new drugs is recommended since they could be targets for other new drugs.
REFERENCES


