EVALUATION OF PASSIVE IMMUNITY TO SCHISTOSOMIASIS IN MICE TRANSFUSED WITH HYPER-IMMUNE BABOON SERUM

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DECLARATION

I Patrick Kiarie Nding'uri, duly declare that 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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Supervisors Approval

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DEDICATION

This thesis is dedicated to my dear parents: Mr. Stephen Nding'uri and Mrs. Mary Wangui Nding'uri. You are the pillars of my life.

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ABBREVIATIONS AND ACRONYMS

DALY : Disability adjusted life years

ELISA : Enzyme-linked immunosorbent assay

IFN-λ : Interferon-gamma

IL : Interleukin

IPR : Institute of Primate Research

PBMC : Peripheral blood mononuclear cells

PBS : Phosphate buffered saline

RA : Radiation attenuated

SEA : Soluble egg antigen

SWAP : Soluble adult worm preparation

Th : T helper

KLH : Keyhole Limpet Hemocyanin

ABSTRACT

Schistosomiasis is a tropical disease caused by worms in the genus Schistosoma. It remains one of the most prevalent parasitic infections and has significant economic and public health consequences. Despite having an effective chemotherapeutic drug, praziquantel, there is still need for a vaccine since the immediate manifestations in most people are negligible or indeterminate. Several antigens have been tested as vaccine candidates with largely disappointing results. Both rodents and primates exposed to cercariae larvae optimally attenuated with gamma radiation show a highly significant reduction in challenge worm burden. Vaccination of baboons with RA (Radiation attenuated) vaccine gave 54% protection after three exposures and 86% protection after five exposures. Protection due to RA vaccine is both humoral and cellular mediated with the IgG antibody levels thought to mediate the effector response declining over a relatively short period after the last vaccination. This study therefore aimed at evaluating the role of humoral immunity against migrating and or the pre-liver lung stage schistosomulae by passively immunizing mice with either 500 µl of hyper-immune serum or infection serum from baboons. In the first study, mice were immunized on day 7 post challenge and boosted on the 14^{th} day. In the second study, mice were immunized on the 3^{rd} day post challenge with no boost. A 24 hour egg count was carried out on the 5^{th}, 6^{th}, and 7^{th} week post challenge using Kato Katz thick smear technique after which perfusion was done seven weeks post challenge. Blood was collected at week 0, 2, and 4 in experiment two from the tail and through heart puncture during perfusion in both experiments for IgG ELISA assays. Sections of preserved livers were processed for histopathology and for eggs recovery. Immunization against the migrating schistosomes on the 3^{rd} day with the hyper-immune serum gave a 25.8 % protection but there was no protection obtained when pre-liver lung stage schistosomulae was targeted. There was no significant difference between the 24 hour faecal egg number, number of worms, liver eggs and in number and size of granulomas on both experiment. There was thus no significant protection that was conferred in mice against pre-liver-lung stage schistosomulae and or the migrating schistosome larvae by hyper-immune serum from RA vaccinated baboons probably due to the inability of the baboon serum to cooperate with the mouse effector system necessary in killing the schistosomulae. These results suggest that protective mechanism in baboons against schistosome worms could be different from those in mice and that though antibodies could be involved in protection, other factors such as cytokines could also play a major role. There is thus the need to further evaluate the mechanisms of protection in mice and experiment on homologous passive transfer of hyper-immune serum (baboon to baboon) .Other stages of the schistosome lifecycle should also be targeted during passive transfer experiments.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 Background

Schistosomiasis commonly known as bilharzia is a tropical parasitic disease caused by blood dwelling flukes of the genus *Schistosoma*. It is the most significant helminthic infection in humans because of its global prevalence, nature of its associated disease manifestations and the remarkable difficulties encountered in attempts to control its spread. Five species of *Schistosoma* are known to infect humans; *S. mansoni*, *S. japonicum*, *S. mekongi*, *S. haematobium*, and *S. intercalatum*. Of these, three species account for most of the human infection: *Schistosoma mansoni* that mainly causes hepatic and intestinal schistosomiasis, *S. haematobium* which causes the urinary form of the disease, and *S. japonicum* that results to hepatosplenic and intestinal schistosomiasis (Chitsulo *et al.*, 2000). The disease has been ranked second only to malaria and is one of the 10 tropical diseases that are targeted for control by the Special Program for Research and Training in Tropical Diseases of the United Nations Development Program, the World Bank, and the World Health Organization (Morel, 2000).

1.1.1 Geographic Distribution and Disease burden

Schistosomiasis being a water-borne disease that is transmitted by freshwater snails is endemic in 76 countries of the tropics and sub-tropics afflicting populations that live close to the water reservoirs (lakes, ponds, and irrigation canals) that are contaminated with the parasite (Chitsulo *et al*., 2000). WHO (2002) estimated that 200 million people are infected and 779 million at risk of infection with 85% of the latter living in Africa

alone (Steinmann *et al*., 2006). Of the estimated 200 million people that are infected, approximately 80% of them are in sub-Saharan Africa (WHO, 2002).

The distribution of the different species of schistosomes mainly depends on the ecology of the snail hosts. Natural streams, ponds, and lakes are typical sources of infection, but over the past few decades man-made reservoirs and irrigation systems have contributed to the spread of schistosomiasis (Oomen *et al*., 1990). Snail populations, cercarial density, and patterns of human water contact show strong temporal and spatial variations thus resulting in a focal distribution of the infection within countries, regions, and villages (Gryseels and Nkulikyinka, 1998). It has been observed that within a population and age-groups, schistosomes are over-dispersed thus implying that a small number of individuals carry most of the parasites (Gryseels and De Vlas, 1996). This could be attributed both to water-contact patterns as well as to innate and acquired immunity. Also observed to vary within a population is the sex-related patterns in relation to behavioural, professional, cultural, and religious factors (Jordan *et al*., 1993). Though the disease is largely a rural problem, urban foci can be found in many endemic areas (Mott *et al*., 1990).

Despite the high prevalence, morbidity associated with the disease is low and variable. Currently, the Global Burden of Disease Study attributes a disability weight of 0·06 and an annual mortality of 14, 000 deaths per year due to schistosomiasis. The total number of DALY (disability-adjusted life years) lost to schistosomiasis is estimated at 1·532 million per year, of which 77% are in sub-Saharan Africa (Lopez, *et al.*, *2006*). Schistosomiasis thus accounts for 0·1% of the total world global burden of disease and

0·4% of that in sub-Saharan Africa, which is of the same order as leishmaniasis and trypanosomiasis (Chitsulo *et al*., 2000).

Despite major advances in control and substantial decreases in morbidity and mortality, schistosomiasis continues to spread to new geographic areas. This spread has been shown to be facilitated by both the environmental changes that result from the development of water resources as well as the growth and migration of populations (Patz *et al.*, 2000). A good example is the construction of Diama Dam on the Senegal River which led to the introduction of *S. mansoni* into Mauritania and Senegal. The movement of refugees and the displacement of populations resulted in the introduction of *S. mansoni* into Somalia and Djibouti. The presence of the Aswan Dam in Egypt has also led to the virtual elimination of *S. haematobium* from the Nile Delta but has brought about the establishment of *S. mansoni* in upper Egypt (Ross *et al.*, 2001).

1.1.2 Biology and life cycle of the parasite

The main schistosomes infecting human beings are: *S. mansoni*, which is transmitted by *Biomphalaria* snails; *S. haematobium*, transmitted by *Bulinus* snails; and *S. japonicum*, transmitted by the amphibian snail *Oncomelania*. *S. intercalatum* and *S. mekongi* are of less importance while *S. japonicum* is a zoonotic parasite which infects a wide range of animals including cattle, dogs, pigs, and rodents. *S. mansoni* is also found in rodents and primates, but human beings are the main host. The main intermediate snail hosts of *S. mansoni* are species of *Biomphalaria (B)* with some of these being *B. pfeifferi*, *B. choanomphala, B. smithi, B. stanleyi, B.angulosa and B. sudanica* in East Africa. Schistosomes have no second intermediate host in their life-cycle and thus mature in the

blood vascular system of their definitive hosts. Adult schistosomes are white or greyish worms of 7–20 mm in length with a cylindrical body that has two terminal suckers, a complex tegument, a blind intestinal tract, and reproductive organs. Unlike other trematodes, schistosomes have separate male and female sexes. The male's body has a groove or gynaecophoric canal, in which it holds the longer and thinner female. They feed on blood and globulins through anaerobic glycolysis and the released debris is regurgitated in the host's blood (Jordan, 1993).

Schistosomes undergo two stages of development that involves multiplicative asexual stage of development in the snail intermediate host and the sexual stage in vertebrates, the definitive host (Fig. 1.1). The asexual stage starts when the eggs of the schistosomes are passed by the definitive hosts (vertebrates) in urine for *S. haematobium* or faeces for *S. mansoni, S. japonicum, S. mekongi,* and *S. intercalatum* into the water (Jourdane and Theron, 1987). The transmission cycle requires contamination of surface water by excreta, specific freshwater snails as intermediate hosts, and human-water contact. Under the guidance of light and chemical stimuli, these schistosome eggs from urine or faeces hatch in fresh water and release the *miracidium* (larva) that penetrates a snail of the appropriate species. The miracidia multiply asexually within the snail into multicellular sporocysts and later into cercarial larvae with embryonic suckers and a characteristic bifurcated tail. It is only after two generations — primary and then daughter sporocysts within the snail that cercariae are released (Jordan, 1993). Vast numbers of cercariae are released into the water 4-6 weeks after snail infection which through the secretion of cytolytic substances from the head glands can penetrate the definitive host where they

develop into schistosomula and eventually into adult worms (Jourdane and Theron, 1987).

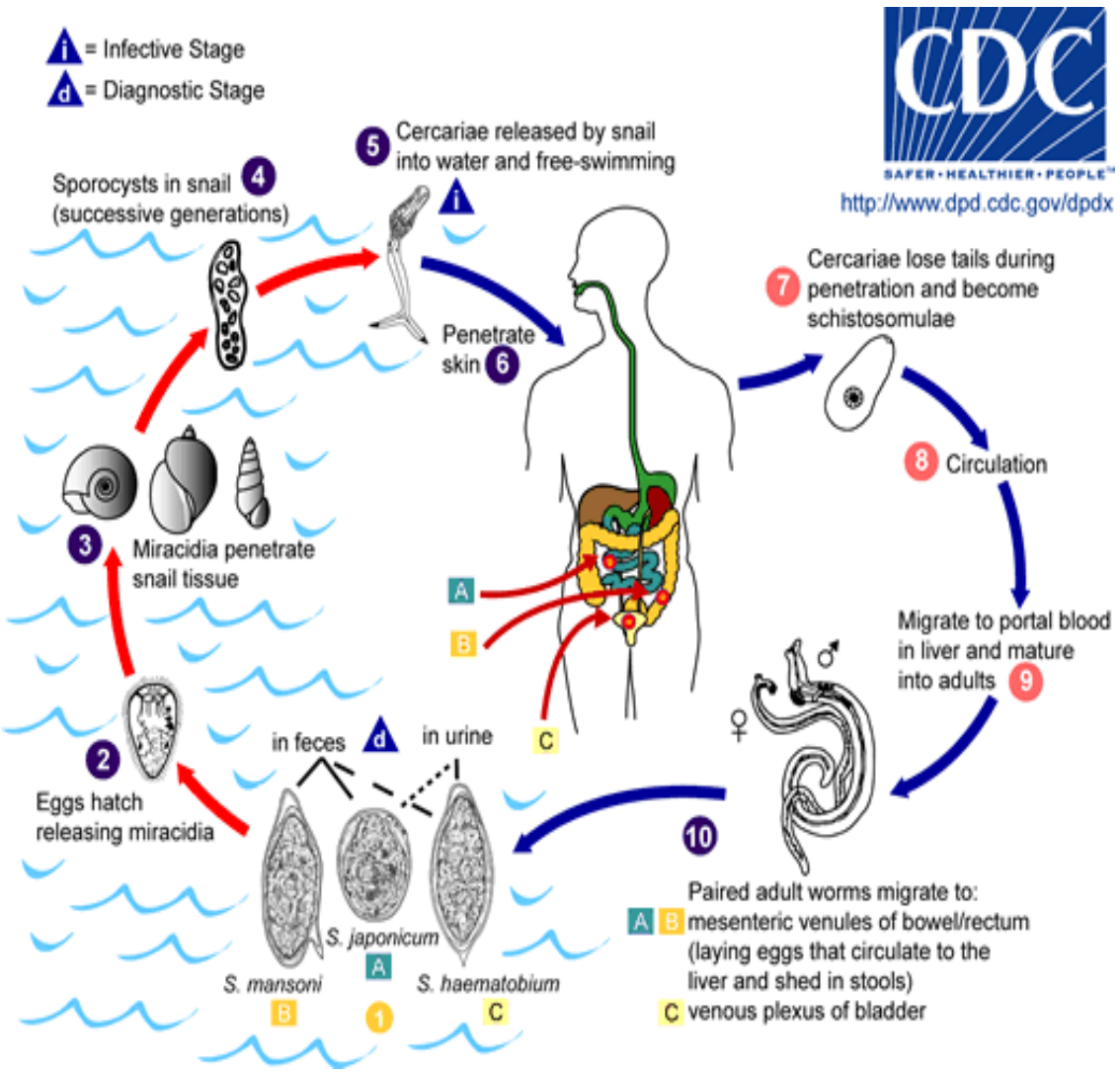

Figure 1.1: Schistosome Life-Cycle
(Center for Disease Control DPDX CD-ROM "*Laboratory identification of parasites of public health concern*" 2nd Edition, 2003.

The sexual stage occurs in vertebrates when they become infected with cercariae. Cercariae penetrate the skin of humans or, in the case of *S. japonicum*, humans and other

mammalian hosts that act as reservoirs for the infection. During infection, cercariae shed their bifurcated tails and the resulting schistosomula enter capillaries and lymphatic vessels and then move towards the lungs. After several days, the worms migrate to the portal venous system, where they mature and unite. Pairs of worms then migrate to the superior mesenteric veins (in the case of *S. mansoni*), the inferior mesenteric and superior hemorrhoidal veins (in the case of *S. japonicum*), or the vesical plexus and veins draining the ureters (in the case of *S. haematobium*) (Shiff, 2000). Egg production commences four to six weeks after infection and continues for the life of the worm — usually three to five years. Each ovum contains a ciliated miracidium larva, which secretes proteolytic enzymes that help the eggs to migrate into the lumen of the bladder (*S. haematobium*) or the intestine (other species). Eggs pass from the lumen of blood vessels into adjacent tissues, and most of them then pass through the intestinal or bladder mucosa and are shed in the feces (in the case of *S. mansoni* and *S. japonicum*) or urine (in the case of *S. haematobium*). The life cycle is completed when the eggs hatch releasing miracidia that, in turn, infect specific freshwater snails (Jourdane and Theron, 1987).

1.2 Disease patterns in man

1.2.1Cercarial Dermatitis

This is the condition that manifest itself as a temporary urticarial rash soon after the percutaneoues penetration of the cercariae. It can persist for days as papulopruriginous lesions especially after primary infection (Appleton, 1984). This condition is infrequent among those living in areas of endemicity, but migrants or visitors who are infected may develop it within a few hours of exposure. The resulting dermatitis is similar to

swimmer's itch, noted in persons sensitized and reexposed to avian or other nonhuman schistosomes found in freshwater bodies all over the world . It is not known if reexposure is a feature of cercarial dermatitis for *S. japonicum* (Warren, 1973).

1.2.2 Acute Schistosomiasis

Acute schistomiasis also known as Katayama fever is the systemic hypersensitivity reaction against the migrating schistosomulae occurring a few weeks to months after a primary infection (Lambertucci, 1993). It is common in areas of high transmission rates but seldom observed in endemic populations probably due to the early age (2-4 years) at which most of the children in the endemic areas are exposed to the parasite for the first time (Doherty, *et al.*, 1996). 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. Common symptoms include fever, headache, generalized myalgias, right-upper-quadrant pain, and bloody diarrhea. Respiratory symptoms have been reported in up to 70 percent of persons infected with *S. mansoni* but less frequently in those infected with *S. haematobium* (Bethlem, *et al*., 1997; Cooke *et al*., 1999). Other observed symptoms include tender hepatomegally and splenomegally in one third of the cases. Though most of the patients have eosinophilia and positive serologic tests not all patients shed eggs.

In humans, acute disease is associated with a massive dissemination of granulomas around the eggs, especially in the liver, lung, pancreas and lymph nodes. Acute, diffuse, severe hepatitis and enterocolitis have also been described. The humoral response (IgG, IgM and IgE) to egg and worm antigens has been shown to be equivalent in patients with acute and chronic schistosomiasis (Kanamura *et al*., 1979; Rabello *et al*., 1995). High

levels of IgG and IgM antibodies to Keyhole Limpet Hemocyanin (KLH) have also been detected in acute patients and this has thus become one of the simple diagnostic tools with high sensitivity and specificity, for differentiation between acute and chronic schistosomiasis (Alves-Brito *et al*., 1992; Rabello *et al*., 1995).

Cellular responses of acute patients clearly differ from those observed in most chronically infected patients (Gazzinelli *et al*., 1985). Acute patients express considerably higher *in vitro* responsiveness than do intestinal chronic patients, especially in regard to responses of their peripheral blood mononuclear cells (PBMC) to soluble schistosoma egg antigens (SEA). Katayama fever due to *S. mansoni* or *S. haematobium* is rarely seen in chronically exposed populations, possibly owing to under diagnosis or due to in-utero sensitization (Hatz, 2005). Acute schistosomiasis is however common, in tourists, travelers, and other people accidentally exposed to transmission (Jelinek *et al*., 1996). Katayama fever due to *S. japonicum* does however occur in people living in endemic areas and with a history of previous infections. Its manifestations can be severe with persistent fever, organomegally, and cachexia, which can evolve rapidly to hepatosplenic fibrosis and portal hypertension (Chen, 1993).

Treatment has mainly been with the use of Praziquantel since it works exclusively against the adult worms although Oxamniquine has been used in some instances (Sleigh, 1991; Chen and Mott, 1989). Most patients recover spontaneously after 2–10 weeks, but some develop persistent and more serious disease with weight loss, dyspnoea, diarrhoea, diffuse abdominal pain, toxaemia, hepatosplenomegaly and widespread rash.

1.2.3 Chronic Schistosomiasis

Chronic schistosomal disease mainly affects individuals with longstanding infections in poor rural areas. Immunopathological reactions against schistosome eggs trapped in the tissues lead to inflammatory and obstructive disease in the urinary system (*S. haematobium*) or intestinal disease, hepatosplenic inflammation, and liver fibrosis (*S. mansoni*, *S. japonicum*) (Gryseels *et al*., 2006). In urinary schistosomiasis, the eggs of *S haematobium* provoke granulomatous inflammation, ulceration, and pseudopolyposis of the vesical and ureteral walls (Cheever *et al*., 1978). Common early signs include dysuria, pollakisuria, proteinuria, and especially haematuria (Gryseels, 1989). Unfortunately this is sometime confused with menstruation in girls and even a coming of age in boys (Jordan, 2000). Some of these chronic lesions can develop to fibrosis or calcification of the bladder and lower ureters, resulting in hydroureter and hydronephrosis. Chronic compression can eventually lead to parenchymal damage and kidney failure (Gryseels *et al*., 2006). Borojevic (1992) regards the chronic phase of murine schistosomiasis as predominantly Th-1 mediated. Studies in murine schistosomiasis also demonstrate that the development of fibrosis requires the production of the profibrotic cytokines IL-2 and IL-4, and is suppressed by IL-12 and IFN-γ. (Cheever *et al.*, 1998; Correa-Oliveira *et al*., 1998).

Intestinal schistosomiasis is caused by Schistosome eggs migrating through the intestinal wall where they provoke mucosal granulomatous inflammation, pseudopolyposis, microulcerations, and superficial bleeding (Cheever *et al*., 1978). It is characterized by chronic or intermittent pain and discomfort, loss of appetite and diarrhea with or

sometime without blood (Gryseels, 1989). Inflammatory hepatic schistosomiasis is an early reaction to ova trapped in the presinusoidal periportal spaces of the liver. Granuloma formation is an inflammatory response against the parasite eggs that are trapped in the host tissues. The intensity of this response can either be modulated by the host (Goes *et al*., 1991) or by the parasite factors (Boulanger *et al*., 1992). There are several adult worm antigens that do cross react with the egg components and are thus able to modulate the immune responses against eggs (Dunne *et al*., 1988). Inflammatory hepatic schistosomiasis can either be caused by *S. mansoni*, *S. japonicum*, and *S .mekongi* (Gryseels *et al*., 2006). Bleeding from gastro-oesophageal varices is the most serious, commonly fatal, complication of fibrotic hepatic schistosomiasis. In *S. mansoni* infections, it tends to recur and grow more severe over time; in *S. japonicum*, bleeding is sudden and massive in many cases (Chen, 1993). Repeated or occult bleeding can lead to anaemia, hypoalbuminaemia, cachexia, and growth retardation (Gryseels *et al*., 2006).

1.3 Diagnosis

Detection of schistosomes eggs in the feces or urine is usually considered diagnostic of schistosomiasis and so far it remains the gold standard (Feldmeier and Poggensee, 1993). Quantitative egg counts after standardised urine filtration or in calibrated faecal thick smears are especially useful for epidemiological surveys and control since they correlate well with worm burdens and morbidity (Jordan and Webbe, 1993). Formalin-based techniques for sedimentation and concentration have been used to increase the diagnostic yield and incase of *S. mansoni* or *S. japonicum* eggs may be observed in stool specimens of 2 to 10 mg with or without suspension in saline (Garcia *et al*., 1999). However the

individual egg counts should not be over interpreted as a measure of disease since they have been shown to vary substantially within and between stool and urine samples (De Vlas *et al*., 1992). To rule out *S. japonicum*., miracidium-hatching test has also been extensively used by the public health workers in China (Cheever, 1978). For those patients who do show typical clinical presentation but have negative urine and feces specimens, a biopsy of bladder or rectal mucosa is usually done for diagnosis.

Antibody detection may also be carried out in some circumstances though it is limited due to the persistence of antibodies after parasitological cure. In such circumstances as Katayama fever where there are no eggs, a positive serologic test may be diagnostic (Tsang and Wilkins, 1997). Serologic testing is also applied in regions of low endemicity where the individual patients have low egg burdens (Al-Sherbiny *et al*., 1999). Though immunodignosis is still considered vital in some instances for diagnosis of schistosomiasis, the commercially available immunodiagnostic kits are not as sensitive as multiple fecal examinations and are also less specific (Tsang and Wilkins, 1997). Though quite sensitive, antibody-based assays cannot distinguish history of exposure from active infection and they have also been shown to cross-react with other helminths and are thus not easily applicable under field conditions (Feldmeier and Poggensee, 1993; Rabello, 1997). Immunodiagnosis in schistosomiasis is however important for diagnosis in travellers, migrants, and other occasionally exposed people as well as in incidence studies in children and in low-transmission or post-control settings (Tsang and Wilkins, 1997).

Most of these immunodiagnostic routine techniques detect IgG, IgM, or IgE against soluble worm antigen or crude egg antigen by ELISA, indirect haemagglutination, or

immunofluorescence. It has been noted that most of these assays have positive results for at least 2 years after cure and in many cases much longer (Rabello *et al*., 1997).

Detection of circulating adult worm and egg antigens in serum or urine of infected individuals may also be applied through the use of labeled monoclonal antibodies (Van Lieshout *et al*., 1997). This is however not appropriate in clinical applications as antigen detection in serum is not very sensitive with light infections. It is however a valuable research tool for epidemiological and therapeutic studies since it is a specific, direct and a stable measure of worm burdens (Polman, 2000). Even the less specific urine-based antigen detection assays have been shown to have the potential for the development of field-applicable reagent strips (van Dam *et al*., 2004). It is estimated that an immunoblot assay for the detection of adult-worm antigens have up to 95 percent sensitivity and 100 percent specificity (Wang *et al*., 1999).

Evidence of peripheral-blood eosinophilia, anemia (iron-deficiency anemia, anemia of chronic disease, or macrocytic anemia), hypoalbuminemia, elevated urea and creatinine levels, and hypergammaglobulinemia may also be used as an additional supportive laboratory evidence of schistosomiasis. In a hospital setup, cystoscopy and endoscopy are used to visualise bladder lesions and oesophageal varices (AbdelWahab *et al*., 1993). Laparoscopy and wedge biopsy can also be used to reveal the macroscopic and histological appearance of granulomatous inflammation or periportal fibrosis (Hayashi *et al*., 2000).

1.4 Control of Schistosomiasis

Control of schistosomiasis is a realistic option as endemic countries such as Brazil, China, the Philippines and Egypt, have been able to sustain national control programmes for a prolonged period and have succeeded in reducing morbidity to very low levels. Countries such as Caribbean Islands, the Islamic Republic of Iran, Mauritius, Morocco, Puerto Rico, Tunisia and Venezuela, are nearing elimination or have already achieved this goal (WHO, 2002) although there is almost non existent control measures in sub-Saharan Africa. Brazil, Egypt and Sudan have had less schistosomiasis related morbidity compared to sub-Saharan Africa where it has been suggested that deaths could be as high as 200,000 per year due to schistosomiasis associated ills (Gryseels, 1989). Different countries initiated control programs before or during the 1980s and some progress has been made. Unlike sub-Saharan Africa with a great number of people infected or at the risk of infection, formerly endemic countries such as Asia and America, have reduced the number of infected people and the risk of infection due to successful control programmes (Engels *et al.,* 1993).

Since the introduction of modern schistosomicides and particularly praziquantel, strategies employed in schistosomiasis control have been changing over the past few decades (Gryseels *et al*., 2006). WHO has recommended the control of early and late morbidity associated with schistosomiasis infection as the first objective (WHO, 2002). Improved access to sanitation and clean water, appropriate health education, and treatment, snail control measures as well as vaccine development are required for permanent solutions. As it has been shown in Japan, Schistosomiasis can in principle be

eliminated by behavioural changes, sanitation, and safe water supply (Minai *et al*., 2003). Behavior change has been observed to be difficult without other options of getting water but educational programmes can improve knowledge about the disease and health-care seeking (Engels *et al*., 1993; Sow *et al*., 2003).

1.4.1 Elimination of vector snails

The control of snails with molluscicides and toxic chemicals is expensive as well as complex. For its sufficient application, substantial human and material resources are needed as well as detailed epidemiological and malacological surveillance. Snail populations can be greatly reduced but rarely eliminated, so regular and long-term retreatment is necessary. In the past, the toxicity of molluscicides for other aquatic organisms, including fish has given rise to ecological and economic concerns. Large-scale chemical snail control is still used in Egypt and China, but owing to the success of population-based chemotherapy, its cost-effectiveness has been questioned (Zhang and Wong, 2003). Snail control can also be pursued by physical measures or biological competitors, but such methods are not easy to put into practice (Cowie, 2001; Laamrani and Boelee, 2002).

Today, the identification of early snail infection is considered the most relevant outcome for linking snail infection to water contamination (King *et al*., 2006). This is so because the mortality of infected snails can be higher after the cercarial shedding and thus measuring prepatent infection (incubation before shedding) has been considered more suitable than measuring patent infection for quantitative assessment of the impact of control programs on human-to-snail transmission (Woolhouse, 1989). Detection of snail

infection throughout prepatency has been done through PCR amplification of tandem repeated DNA sequences (Sm1–7 repeat of *Schistosoma mansoni* and DraI repeat of *S. haematobium*). Large-scale monitoring of field snails by DraI-PCR has been applied in Msambweni area of Kwale District, Coast Province, Kenya to detect prepatent *S. haematobium* infections in bulinid snails (Hamburger, 2004). In these kind of studies, 30–50% of field snails recovered were found to be infected with *S. haematobium* even when community-wide therapy was applied suggesting a high residue of water contamination by parasite eggs despite the fact that there were significant reductions in mean community infectious burden following treatment (Hamburger *et al.*, 2004). Thus, monitoring of continuing transmission potential (measured as snail prepatent infection rates) could help to select the most effective modes of snail control and chemotherapy timing. This could also enable rapid, performance-based decisions about applying additional snail control measures such as focal molluscicide application for prevention of recurring infection and disease (King *et al*., 2006).

Snails can also be controlled biologically using competitor snails. Competitor snails are voraciously feeding snails, species with higher fecundity rates, snails resistant to parasites, have a longer lifespan and are harmless to other animals and the surrounding crops (Giovanelli *et al*., 2003). *Biomphalaria glabrata* and *B. straminea* has been controlled by introducing *Thiara granifera* and *Melanoides tuberculata* (Pointier, 1993). The snail *Physa acuta* has also been used in biological control especially for *Bulinus truncates* (Bakry and Abd-el-Monem, 2005). The use of molluscivorous fish (predators of schistosome-transmitting snails) such as *Sargochromis codringtoni* and *Geophagus*

brasilensis has also been appreciated (Weinzettl and Jurberg, 1990; Slootweg *et al*., 1994; Makoni *et al*., 2005;). Other fish that can be considered to be molluscivorous include; North American crayfish *Procambarus clarkii* known to prey on *B. pfeifferi* and *B. glabrata*, and the African catfish, *Clarias gariepinus*, which is considered to be both molluscivorous and carnivorous (Makoni *et al*., 2005).

1.4.2 Chemotherapy

In the absence of a vaccine, water sanitation and efficient vector control mechanisms, treatment and control of schistosomiasis relies mostly on the single drug praziquantel (PZQ;2-(cyclohexylcarbonyl)-1,2,3,6,7,11b-hexahydro-4H-pyrazino(2,1-alpha) isoquinolin-4-one) (Doenhoff and Pica-Mattoccia, 2006). Though more expensive than praziquantel, oxamniquine (6-hydroxymethyl-2-isopropyl-aminomethyl-7-nitro-1,2,3,4-tetrahydroquinoline), is also available although it's bioactivity is restricted to *S. mansoni*, and has largely been replaced in favor of the more cost-effective PZQ (Beck *et al*., 2001). A single oral dose of 40-60 mg/kg has been shown to achieve cure rates of up to 60-90% (Doenhoff and Pica-Mattoccia, 2006) and it has been established as a safe and effective drug (Cioli and Pica-Mattoccia 2003). At either the community or individual level, PZQ is the recommended drug for the disease treatment, (WHO, 2002).

The exposure of the worms to PZQ causes a massive influx of calcium (Pax *et al*., 1978) contraction of the masulature (Fetterer *et al.*, 1980) and disruption of the tegument (Becker *et al.*, 1980). Though the precise molecular targets are not clear, there is accumulated evidence on the involvement of calcium channels which is supported by recent data with calcium-channel blockers and the actin depolymerization agent,

cytochalasin D (Pica-Mattoccia *et al*., 2007). PZQ has been found to have a decreasing efficacy against immature parasites relative to adult worms (Gonnert and Andrews 1977; Sabah *et al*., 1986). Thus a second treatment of PZQ, four to six weeks after the initial dose, is recommended in cases of clinical use to remove any parasites that have matured in the intervening period (Renganathan and Cioli, 1998). The greater use of PZQ through control programs such as the Schistosomiasis Control Initiative may quicken resistance to PZQ (Fenwick, 2006; Fenwick and Webster, 2006). PZQ has already become less effective with other fluke diseases (e.g. clonorchiasis in South East Asia) (Tinga *et al*., 1999; Keiser and Utzinger, 2004). The search for alternative chemotherapies for use either alone or, perhaps, in combination with PZQ are thus further encouraged.

1.4.3 Vaccination

The struggle for a schistosomiasis vaccine is considered a realistic aim since previous studies have shown that human populations in endemic areas invariably develop some degree of protection naturally (Butterworth *et al.,* 1985; Hagan *et al.,* 1991). Considerable effort has been devoted to the development of vaccines against schistosomiasis and some of the several antigens judged to be potential vaccine candidates have been tested in animals with varying results (Bergquist and Colley, 1998; Hewitson *et al*., 2005). The recombinant rShGST-28 (Bilhvax; Eurogentec, Herstal, Belgium) has already undergone phase I and II clinical trials though questions still remain about the feasibility, applicability, and relevance of schistosomiasis vaccines (Gryseels 2000; Capron *et al*., 2005). Vaccination with radiation-attenuated (RA) ceracarie is one of the most reproducible protocols (Coulson, 1997) resulting in 50 to 80% reduction in challenge worm burden. Due to safety reasons, this vaccine has not

been tested in human volunteers (Coulson, 1997) but it could form the basis for a recombinant vaccine if the relevant immune mechanisms and the protective antigens are known (Eberl *et al*., 1999). There is a great challenge in trying to mimic as well as accelerate the mechanisms that are observed in humans living in endemic areas in experimental animals (Bergquist *et al*., 2002).

Some of the antigens that have been isolated and tested as vaccine candidates include two different multiple antigenic pepetides (MAP), Sm23, an integrated membrane antigen, and triose phosphate isomerase (TPI) (Harn *et al*., 1995). Other antigens are full-length proteins: paramyosin (Pearce *et al.*, 1988; Flanigan *et al*., 1989; Sher *et al*., 1991), IrV5 (Soisson *et al*., 1993), GST (Capron *et al*., 2001) and Sm14, a fatty acid-binding protein (Brito *et al.*, 2000). Glyceraldehyde phosphate dehydrogenase (GAPDH) that was first reported by Goudot-Crozel *et al*., (1989) and then later on by Argiro *et al*., (2000) has also been researched on.

The golden standard for anti-schistosome vaccine development is the reduction in worm numbers. However since schistosome eggs are responsible for both pathology and transmission, a vaccine targeted on parasite fecundity and egg viability appears also entirely relevant as is *S. haematobium* GST vaccine, Bilhvax (Capron *et al*., 2005). Capron *et al*., (2001) has suggested that vaccination can be either targeted towards the prevention of infection or to the reduction of parasite fecundity. Previous immunization experiments of mice with one dose of irradiated cercariae results in 50–70% protection which can be increased to over 80% with two or three immunizations (Smythies *et al*., 1996). IL-12 has been shown to be a useful adjuvant for use in vaccination when used

together with an antibody that blocks the IL-10R. In these kind of experiments, sterile immunity against infection has almost been attained in mice that have either the gene for the immuno-regulatory cytokine IL-10 disrupted or in which IL-12 is used as a defined adjuvant by vaccination with radiation-attenuated cercariae (Wynn, 1996).

Unfortunately, the individual recombinant antigen vaccines tested in animal models have failed to achieve the protection levels predicted to be necessary for an efficacious and affordable human vaccine (Todd and Colley, 2002). DNA plasmids encoding single or multiple genes have several attractive attributes and have also been explored as vaccines for schistosomiasis (Da'dara *et al.*, 2001; Shalaby *et al*., 2003). Berghist *et al.*, 2002 noted that immunization of experimental animals with defined schistosome genes or recombinant antigens is consistently less effective in conferring protection than vaccination with attenuated cercariae regardless of the specific antigen under study or the laboratory performing the study. It has been demonstrated that the decreased efficacy of DNA vaccines, and probably recombinant proteins compared to irradiated cercariae vaccinated animals could largely be due to differences in the inflammatory responses they are capable of provoking (Shalaby *et al*., 2003).

1.4.3.1 Radiation attenuated (RA) vaccine

This is the most studied model of schistosome immunity induced by live attenuated parasites (Coulson, 1997). Immunity induced by the RA vaccine has been shown to have a strong T-cell component (James, 1999) and there is suggestive evidence that killing of the larval schistosomulae is mediated by the macrophages and the endotherial cells through the action of interferon λ (IFN-λ) (Coulson, 1997). However, it is not clear if the

killing in the lungs occurs as a result of nitric oxide (NO) release or whether migration is blocked by a focus of non-cytotoxic inflammatory cells. In animal models that have received multiple immunizations with attenuated parasites, antibodies have been shown to play an important role in protective immunity. This has been demonstaretd through passive transfer experiments in which significant levels of protection against challenge infection has been achived though passive immunization (Mangold and Dean, 1986).

In C57BL/6 mice vaccinated with RA vaccine, 60-70% protection is routinely achieved with a single exposure and the mechanism of immunity is thought to be cell mediated. The $CD4^+$ T cells of the Th1 subset play a central role in the pulmonary effector responses, which are orchestrated by the cytokine interferon gamma. Both IgG and IgM antibody isotypes specific to parasite antigens; RAP (material released during cercarial transformation into schistosomula from hours 0 to 3), SLAP (soluble proteins from lung schistosomula), and SEA (Soluble egg antigen), are detected after RA vaccination (Kariuki *et al.,* 2004). Their levels have been shown to be dependent on the amount of attenuating radiation as cercariae exposed to 20 kilo radiation (krad) have been shown to be better inducers of protection in 3 out of 4 experiments as compared to those exposed to 60 krad (Yole *et al.,* 1996). Simultaneous induction of both humoral and cell-mediated immunity is needed for effective vaccination against schistosome (Jankovic *et al.,* 1999).

In the RA vaccine trial by Kariuki *et al.,* (2004), antibody titers observed indicated an initial rise to a peak of IgM reactive with larval secretions RAP after two vaccinations before declining. IgM levels rose rapidly at 6 weeks post challenge with an observed

progressive rise of IgG levels. IgG levels in response to RAP at challenge gave the best correlation coefficient (0.43) between antibody response and protective immunity suggesting that this isotype mediates the effector response. Levels of IgG rapidly declined after vaccination with no boost provided by the challenge before egg deposition began.

1.5 Immune responses to schistosomiasis

There have been several observations that people living in endemic areas acquire some form of immune resistance after years of exposure (Butterworth, 1993). There is also the evidence in human population of both natural resistance (Viana *et al.,* 1995) as well as acquired immunity against schistosome (Hagan *et al*., 1991; Dunne *et al*., 1992). Since the parasite does not replicate in the definitive host, a partial reduction in the worm burden would be beneficial in reducing transmission and morbidity (Chan *et al.,* 1997). The identification of the relevant protective immune mechanisms in humans is a challenge due to the uncertainties of the worm burden acquired and also unlike experimental animals, they are continuously exposed to schistosome cercariae (Butterworth *et al.,* 1994). Humans are also highly susceptible to other parasite infection and due to their variation in the nutritional status and behavior, the immune mechanisms involved are further complicated (Marti *et al*., 1987; Gryseels, 2000). Some of the recent studies with infected humans have indicated that the response to schistosomes could be a Th2-cell response, high IL-10 and IgG4 antibody with a low IgE reactions (Maizels and Yazdanbakhsh, 2003).

Cellular immune responses induce most of the schsistosomiasis related pathology. The granulomatous reactions usually observed around the eggs are orchestrated by CD4-positive T cells and involve eosinophils, monocytes, and lymphocytes (Cheever *et al*., 2000). In a mice model, T-helper-1 reaction in the early stages of infection shifts to an egg-induced T-helper-2-biased profile, and the resulting imbalances between these responses lead to severe lesions (Pearce, 2005). These similar mechanisms are thus thought to be the reason behind fibrotic pathology in human beings (Abath *et al*., 2006).

Previous studies have demonstrated the role of the host's humoral response in the elimination mechanisms of *S. mansoni* from mice submitted to different immunization protocols (Mangold and Dean, 1986; Mangold and Dean, 1992). In various studies, it has been shown that there is a balance between the level of effective anti-schistosomula antibodies and the presence of blocking antibodies (Hagan *et al*., 1991). The latter (blocking antibodies) are sometimes most easily demonstrated as antibodies to egg antigens that cross-react with epitopes present in the schistosomula tegument (Butterworth *et al*., 1988). High levels of IgE against adult worm or larval antigens has been associated with resistance to reinfection while high levels of IgG4 and IgM antibodies against egg antigens generally parallel susceptibility (Hagan *et al*., 1991; Rihet *et al*., 1991). A correlation between resistance and elevated levels of IgA against a schistosome vaccine candidate (Sm 28 glutathione-S-transferase) has been reported (Grzych, *et al*., 1993) and further studies with other antigens have demonstrated that higher levels of IgE against a 22-kD schistosomula moiety (Dunne *et al*., 1992) and

higher levels of IgM against a 68-kD adult schistosome antigen18 also correlate with resistance to infection.

In mice, acute phase response has been shown to be mediated by the release of certain cytokines. In murine intestinal schistosomiasis, there is a type I (Th-1 type) immune response where increased interferon-gamma (IFN-γ) predominates in early infection, but this type of immune response changes as egg production and tissue reaction begins (Correa-Oliveira *et al*., 1998). As infection progresses, there is a decrease in IFN- γ which is accompanied by an increase in IL-10 and granuloma formations. The earliest hepatic granulomas, therefore, form in a Th-1 environment, with down-regulation of Th-1 and up-regulation of Th-2 responses six weeks after infection (Todt *et al*., 2000). The granuloma is conceptualized as a Th-2 dominant reaction, but under some conditions, Th-1 granulomatous response may be predominant and damaging (Rutitzkky *et al*., 2001). However, in chronic infections which are considered as 20 weeks and over, these responses are less marked (Henderson *et al*., 1992).

1.6 Protective Immunity

The acquisition of effective immunity against schistosomes has so far been difficult to prove, since the decrease in infection rates observed after adolescence can also be explained by reduced water contact. Comparative studies of re-infection after curative treatment have shown that children are far more susceptible than adults and that these differences cannot be explained by differing water-contact patterns (Gryseels, 1996; Woolhouse and Hagan, 1999). Other observations in people and animals suggest that

acquired immunity is mediated by IgE against antigens of larvae and adult worms, which trigger eosinophils to release cytotoxins targeting schistosomulae (Butterworth, 1993). Thus the observed slow development of acquired immunity is thought to be due to blockage of the IgE receptors by excess anti-schistosome IgG_4 and possibly other immunoglobulin isotypes in the first years of infection.

Humans have shown acquired immunity to schistosome infection and individuals are usually classified as either being susceptible or resistant to natural infection by schistosomes (Butterworth *et al*., 1994). Though the host responds to *S. mansoni* infection with an immune response that does limit subsequent challenge infection, there is little or no effect on established adult worms. This is so because the schistosome parasite is most susceptible to immune elimination during the skin and lung stages of development and least susceptible as an adult parasite (James, 1992). However, through haptenation (McCormick and Damian, 1987) or through treatment with praziquantel, adult schistosomes can be made vulnerable to immune elimination both *in vitro* and *in vivo*. Studies done on cellular and humoral immune responses to *Schistosoma mansoni* antigen preparations in individuals presumed to be susceptible or resistant to reinfection after chemotherapeutic cure have shown that resistance to reinfection is an age-related phenomenon, with most people in endemic areas becoming resistant, or expressing their resistance during their second decade of life (Caldas *et al*., 2000). This resistance is usually attributed to immunity rather than physiologic or behavioral changes with age as it appears to be unrelated to the degree of contact with cercariae containing water (Barbour, 1985).

Killing of the schistosome parasite after primary infection that is asscociated with IgE does mostly occur after the schistosomula has passed through the lungs since induction of a primary IgE response normally requires 5–10 days. This however still remains suggestive as the actual target and site for parasite attrition are speculative (King *et al*., 1997). In a rat model which is a non-permissive host, the killing of schistosomulae of challenge infection is by antibody-dependent cell-mediated cytotoxic (ADCC) response (Capron and Capron, 1986). In baboons and unlike mice, adult worm-specific IgE is uniquely associated with acquired immunity to *S. mansoni* infection (Nyindo *et al*., 1999).

1.7 Animal models for human Schistosomiasis

There are several animal models that have been used to study the basic biology, immunology and pathogenesis of schistosomiasis (Sturrock, 1986). Susceptibility to primary infection with *Schistosoma mansoni* has been shown to vary among different species and strains of laboratory animals (Warren and Peters, 1967). Because of their ease of availability, fast breeding and development of disease on experimental infection, mice have been used in most experimental *Schistosoma mansoni* infection (Nyindo and Farah, 1999). The disadvantage with using mice is that extrapolation of data from mice to humans is associated with some difficult due to the anatomical, genetic and immunological differences that exist between the two species. In addition, murine studies have been performed in highly inbred strains of mice, and might not reflect the heterogeneity of response expected from human populations. However, since the disease

progress in mice is to some extent similar to that seen in humans, murine schistosomiasis remains the most studied experimental model (Cheever *et al*., 2002).

Baboons represent suitable models for the study of human schistosomiasis infection as evaluated by Nyindo and Farah (1999). This is for several reasons: (1) baboons are anatomically, genetically and immunologically more similar to humans than are rodents, (2) they acquire natural infections and are highly susceptible to experimental infections, (3) they develop hepatic and intestinal pathology, modulate this pathology and acquire protective immunity, as do humans, (4) baboons infected in the wild show an age-dependent prevalence, with high rates of infection in juveniles and young adults, (5) they adapt readily to changes in their environment, can give birth twice every 18 months, can live for 20 years and can attain up to 20 kilograms body weight. Their moderate size and the capacity to monitor disease at different time points in an individual animal by surgical manipulations, as well as ease of perfusion for adult worm recovery makes the baboon a good model.

Baboons like mice and humans develop resistance to infection with *S. mansoni* and this protection has been thought to be immunologically mediated and related to increased production in the serum of adult worm-specific IgG (Suzuki and Damian, 1981). Like mice, baboons can also be immunized with *S. mansoni* irradiated cercariae and the repeated exposure of baboons to irradiated cercariae stimulates from 30% to 90% protection to challenge infection with *S. mansoni* (Yole *et al*., 1996). There is also an association of parasite-specific IgE and protection among primates infected with

schistosomiasis, along with pathology, anatomy, and genetic make-up thus indicating that baboons provide an excellent permissive experimental model for better understanding the mechanisms of innate and acquired immunity to schistosomiasis in humans (Nyindo *et al*., 1999).

Some of the drawbacks associated with the use of baboon as a model for schistosomiasis infection includes; (1) baboons are expensive to buy and maintain in captivity; (2) it is necessary to obtain the services of qualified primate medicine veterinarians to look after the health of the animals and to perform procedures for specific experiments; (3) there is an urgent need to develop baboon or non-human primate specific reagents because some of the human reagents might not work in baboon assays (Nyindo and Farah, 1999). Despite these drawbacks, the baboon will probably continue to be the non-human primate model of *S. mansoni* infection. Chimpanzees which are much closer genetically and physiologically to humans do become infected in the wild with schistosome (Renquist *et al*., 1975; Abe *et al*., 1993) developing pathology that is indistinguishable from that in human patients and have thus also been used in some experiments (Sadun *et al*., 1966; von Lichtenberg *et al*., 1971).

1.8 Passive immunization

Mangold and Dean (1992) have previously shown that partial immunity against *Schistosoma manssoni* infection can be passively transferred to C57Bl/6 mice with serum from mice that have been multiply immunized with cercariae irradiated with 50 kilorads of gamma radiation. It has been demonstrated that serum from irradiated cercarie

immunized rabbits partially protects mice against a *S. mansoni* challenge (Bickle *et al*., 1985). Different animals have been used as the source of this serum for passive immnunisation experiments. Use of large animals such as rabbits and baboons has the advantage of providing large amount of serum from a relatively small number of animals that can be repeatedly boosted. These previous experiments have shown that 1) the Fc portion of the antibody molecule is necessary for passive protection, 2) the timing of injection is crucial and 3) IgG antibodies are specifically involved in protection (Mangold and Dean, 1992).

Role of humoral immunity to *S. mansoni* infection has been examined by employing passive transfer system (Ford *et al.,* 1984; Mangold and Dean, 1986; Jwo and Loverde, 1989) with either fractionated or non fractionated serum. Antibody specificity rather than quantity could be more relevant to protective immunity as no consistent association between antibody titer and level of resistance is apparent (Richter *et al.,* 1995). The IgG isotype particularly IgG1 seems to be protective and may be synergistically enhanced by the presence of IgM (Jwo and Loverde, 1989). The parasite stage that is the target for antibody mediated attack and the site of immune elimination are controversial (Coulson and Mountford, 1989; Richter *et al.,* 1995) although Sher *et al.,* (1984) demonstrated that the newly transformed schistosomula could be the target of antibody mediated attack. Serum administered one day pre-challenge, 4^{th} to 7^{th} day or day 15^{th} post-challenge have been shown to be less effective, most effective and totally ineffective respectively (Mangold and Dean, 1992). Both IgM & IgG isotypes of sera from humans with chronic

Schistosoma mansoni infections has been shown not to protect when administered greater than or equal to 24 days after challenge (Jwo and Loverde, 1989).

1.9 Statement of the problem

An effective vaccine will be able to sustain the protective immunity for a significant period of time. The vaccine would be required to induce a strong humoral response that can be sustained for long. Previous studies have shown that vaccination with Radiation Attenuated vaccine induces a strong humoral response but the antibody titer soon drops a few weeks after the last vaccination with a corresponding drop in the level of protection after challenge infection (Kariuki *et al.,* 2004). This could imply that there is an inability to sustain antibody titer for long. It could also imply that the role of antibodies in mediating protection is not yet understood.

1.10 Research question

Can hyper-immune serum from baboons confer protection in mice against pre-liver-lung stage schistosomulae and or the migrating schistosome larvae?

1.11 Null hypotheses

Ho_1: Hyper-immune serum from baboons does not protect mice against pre-liver-lung stage schistosomulae.

Ho_2: Hyper-immune serum from baboons does not protect mice against the migrating schistosome larvae.

1. 12 Objectives

1.12.1 Main objective

To determine the role of humoral immunity in mice infected with *Schistosoma mansoni.*

1.12.2 Specific objectives

i. To determine the effect of antibodies generated from RA vaccinated baboons on the number and nature of schistosome worms.
ii. To determine the effect of antibodies generated from RA vaccinated baboons on the faecal and trapped schistosome eggs in the liver.
iii. To determine the effect of antibodies generated from RA vaccinated baboons on the size and number of granulomas in the liver.
iv. To compare the levels of IgG antibodies against soluble egg antigen (SEA) and soluble adult worm antigen (SWAP) in mice.

1.13 Justification and expected outcome

Radiation attenuated (RA) vaccine evaluated in the olive baboon, *Papio anubis* (Kariuki *et al*., 2004), have shown 86% protection against challenge with 1,000 normal cercariae after vaccination, five times (5×), with 9,000 RA cercariae at 4 week intervals. Given the efficacy of RA vaccine in rodents and the protection levels it elicits in baboons, it can serve as an important paradigm for human vaccine against schistosomiasis (Coulson, 1997). In this vaccine trial in baboons, the difference in mean worm burdens between the 3-week challenge controls and the VC3 (vaccinated and challenged 3 weeks post last vaccination) group at perfusion amounted to 72% protection, while that between the 12-

week challenge controls and the VC12 (Vaccinated and challenged 12 weeks post last vaccination) was 53% (Kariuki *et al.,* 2004). If the protection due to this vaccine is antibody mediated, the reason protection is not sustained could be the inability to maintain antibody titers.

There is thus need to evaluate the role of the antibodies in mediating protection in order to formulate recombinant antigen vaccines that will maintain high antibody titers for long. Since antibodies could be involved in protection against cercariae challenge, a reduction in schistosome liver and faecal eggs, adult worms, size and number of granulomas after passive immunization, will demonstrate the active role of antibodies against cercariae challenge. Differences in level of protection due to different time points of serum injection, will give a better understanding on the schistosome life-cycle stage (migrating larvae or pre-liver lung stage schistosomulae) most susceptible to antibodies. This information is important when developing more effective vaccines that can maintain high antibody titers for long.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Experimental Design

There were two experiments each with 15 adult C57BL/6 mice aged 6-8 weeks at the start of the experiment. In each experiment, mice were challenged with 200 *S. mansoni* cercariae and then divided into three groups each of 5 mice. Group one received 500 µl of hyper-immune serum, group two 500 µl of infection serum and group three was not immunized. In experiment one, mice were immunized on day 7 and boosted on day 14 after challenge while in experiment two, mice were immunized on day 3 after challenge with no boost. The research protocols were reviewed and approved by the Ethical Review Board of Institute of Primate Research (IPR) (Nairobi, Kenya).

2.2 Intermediate host snails and parasites

Biomphalaria pfeifferi snails obtained from a schistosome free dam in Kibwezi district, Kenya were used in these experiments as the intermediate host for *Schistosoma mansoni*. They were screened for any pre-existing schistosome infection by exposing them to strong light (100 watts lamp) for two hours for six consecutive weeks (Frandsen and Christensen, 1984). Those snails that were negative (not shedding cercariae) were maintained in snail tanks within the snail colony at Institute of Primate Research (IPR) and water was changed twice a week. They were fed on soft lettuce and the temperature maintained at 25 ^{o}C -27 ^{o}C. The *S. mansoni* parasites used as a source of schistosome eggs in this experiment were maintained in chronically infected baboons, PAN (*Papio*

anubis) 1875 and PAN 1873, at IPR primate colony. They were originally from schistosome eggs collected in stools from children in Kibwezi district Kenya.

2.3 Definitive host C57BL/6 mice

Adult male and female C57BL/6 mice (n =30) aged (6-8) weeks at the start of the experiment and bred at International Livestock Research Institute, (ILRI, Nairobi Kenya) Animal House Breeding stock were used. The animals which were acclimatized for two weeks before experimental infection were housed in groups of five per a standard plastic cage with stainless steel cover lids and wood shavings as beddings. They were fed daily on commercial pellets (Rodents pellets®, Unga Feeds Ltd, Kenya) and water *ad libitum* within the rodents' animal house facility at IPR (Institute of Primate Research).

2.4 Source of serum for passive immunization

Serum tested in this experiment was from a previous baboon vaccination experiment performed at the Institute of Primate Research Karen Nairobi Kenya by Kariuki *et al.*, (2004). Juvenile Olive baboons (*Papio anubis*) were vaccinated five times with 9,000 RA cercariae at 4- week intervals and challenged 3 weeks after the last vaccination with 1,000 cercariae. Samples of 15 ml of blood obtained from vaccinated animals before challenge infection were incubated at 37 ^{o}C for 1-2 hours then stored at 4 ^{o}C overnight to allow complete coagulation. Serum was recovered by spinning the blood samples on a bench top centrifuge 1500 rpm for 15 minutes, and serum aliquots stored at -20 ^{o}C.

2.5 Passive immunization

Mice were immunized through an intravenous injection of the serum through the tail vein (Mangold, and Dean, 1986; Mangold and Dean, 1992). Group one was immunized with hyper-immune serum; five times vaccinated baboon serum (5× VBS) 0.5ml/mice and group two with 0.5 ml/mice of infection baboon serum (IBS) from a previous baboon vaccination experiment as explained in 2.4 above. Mice in the control group were not immunized. Immunization was done on the 7^{th} and 14^{th} day after challenge in the first experiment, and on the 3^{rd} day in the second experiment using. Mice to be immunized were isolated in a small cage and warmed with a 100 watt lamp to increase blood supply to the tail. Each mouse was then moved into the restraining devise and a portion of the tail (about 1.5 cm below the base) swabbed with alcohol. A 1.0 ml syringe fitted with a 26 gauge needle and containing either the immune serum or infection serum was guided into one of the tail veins and the serum slowly delivered. The needle was then removed after a few seconds and the immunized mouse returned to its cage.

2.6 Parasitological techniques

2.6.1 Infection of snails with miracidia

Biomphalaria pfeifferi snails were infected with miracidia hatched from *Schistosoma mansoni* eggs that were recovered from faecal material of chronically infected baboons. The faecal matter was homogenized with saline solution and the slurry sieved through 600µm and 125 µm test sieve (Arther Thomas CO. USA) into urine glass jars. The slurry was then left to sediment for 30 minutes in the dark after which the supernatant was

poured out and the pellet resuspneded in saline three times until the supernatant became clear. After the last wash, water was added and the supernatant poured. Sediments were then transferred into a section of a petridish and the remaining section gently filled with water. This petridish was then exposed to bright light (100 watts lamp) for one hour to facilitate the hatching of miracidia. Using a dissecting microscope, 3-5 miracidia were picked from the petridish using a pipette mounted onto a rubber bulb and dispensed into beakers containing approximately 1ml of water. A single snail was then placed into each beaker and left for 30 minutes to facilitate miracidia penetration. The snails were then transferred into a single snail tank for 5 weeks for cercariae production to take place and a week before scheduled cercarie shedding, the infected snails were kept in the dark (Stirewalt and Uy, 1969).

2.6.2 Infection of mice

After 5 weeks, cercariae were shed from infected snails (described in 2.5.1) by exposing the snails to bright light (100 watts lamp) for two hours. Using a dissecting microscope, 200 cercariae were counted and picked with a pipette mounted onto a rubber bulb and dispensed into 5ml beakers. Mice were percutaneously infected with 200 cercariae/animal following a procedure adapted from the ring method (Tendler & Pinto, 1981). Mice were anesthetized with 50µl of ketamine (100mg/ml; Agrar Holland BV, Soest, The Netherlands). After shaving the abdomen, an infection ring was attached on the abdomen into which cercariae suspension was then poured and left for 30 minutes to penetrate the mice skin. The mice were then maintained in the IPR rodents' house in groups of five for seven weeks.

2.7 Parasitological assays

2.7.1 Faecal eggs recovery through Kato katz method

Faecal schistosome eggs count was done on the 5th, 6th, and 7th, week post challenge in duplicates per mice using Katz *et al*., (1972) method. Mice were transferred into individual cages with no beddings in each of these tests where the 24 hour faecal material was collected, weighed and processed for egg counting using Kato Katz technique. Coverslips to be used were soaked in 50% glycerine malachite green solution 24 hours before use. After mixing the stool sample, about 1-2 grams of faecal material was forced through a 125µm mesh and the stool recovered at the back of the mesh. Using a template, a 50mg faecal specimen was transferred to a microscope slide and then covered with the cellophane coverslip soaked in the 50% glycerine malachite green solution. Slides were kept 24 hours in the dark then observed under the microscope at ×10 magnification. Eggs per gram were obtained by multiplying the average number of eggs per slide by 20.

2.7.2 Perfusion method

Infected mice were perfused 7 weeks post challenge to recover adult worms using a modified method of Smithers and Terry (1965). Each mouse was anaesthetized by injecting it with 100 µl of heparinized ketamine (1 ml of heparin into 5 ml of ketamine). A transverse mid-ventral cut was made on the skin of the abdomen and the mouse skin peeled round the ‘waist’ of the animal. Thoracic cavity and the abdomen wall were then opened up without cutting the viscera. Ribs on either side of the sternum were snipped off and the liver observed for any gross pathology. The hepatic portal vein was then located, incised and the area flushed with perfusion fluid (0.85% sodium chloride and 1.5%

sodium citrate) to remove any worms and prevent clotting. A 21 gauge needle connected to the tube with perfusion fluid was inserted into the left ventricle and perfusion fluid pumped through until the liver became pale in color and the mesenteric veins were clean. Recovered (normal and stunted) male and female worms were counted separately and the average found per each group. A male worm of between 6-13 mm in length and 0.8-1.0 mm in width and a female worm of 7-17 mm in length and 0.25 mm in width were considered normal while those worms that were smaller than this were classified as stunted (Loker, 1983).

2.7.3 Adult worm recovery

Adult worms were recovered using the method of Yole *et al*. (1996) where the perfusate containing the recovered worms in urine glass jars was topped up with phosphate buffered saline (PBS) and haemolyser DL-1 (Erma Inc. Japan) added to lyse the red blood cells. The supernatant was carefully sucked out after the worms had settled down and the washing procedure repeated three times. The recovered worms were then placed on a Petri dish with PBS and counted under a dissecting microscope. Resistance to challenge infection was expressed as the percentage reduction in the number of adult worms recovered from mice receiving hyper-immune serum or infection serum compared with non immunized control mice calculated as follows:

$$\text{Resistance} = (C-E)/C \times 100$$

Where C represents the mean recovery from challenge control mice and E represents the mean recovery from experimental mice (mice receiving hyper-immune serum or infection serum).

2.7.4 Tissue egg count

Frozen liver samples collected 7 weeks post infection during perfusion were finely chopped and put in 100ml vortex beaker with (20-30) ml of PBS and processed for eggs recovery using the method adapted from Liu *et al*., (1995). Samples were blended for 3-4 minutes and the emulsion transferred to conical flasks with 5% volume of 5% potassium hydroxide (KOH). Samples were then left overnight at room temperature and at 45 ^{o}C for 3 hours. Sample volume was then recorded and eggs randomized by bubbling air through. A counting slide was filled with randomized egg suspension in both chambers and eggs lying in the 24 grid squares counted with a dissecting microscope at × 40 magnifications. Number (N) of eggs in the original organ was calculated as follows;

$N = W/w \times V \times 24/n \times 1.05(r_1+r_2)/2$

Where;

W and w = the weights in grams of the whole organ and the sub sample respectively.

V= the volume in ml of the digest after incubation.

n= the number of grid squares counted.

r_1+r_2 = the number of eggs counted in each of the two chambers.

2.8 Bleeding of mice for serum

Blood samples were collected from tail veins of all mice prior to immunization in experiment two and thereafter at 2, 4 weeks interval and finally at 7 weeks post challenge during perfusion through heart puncture. For experiment one, blood samples were obtained during perfusion through heart puncture. Pooled serum samples for ELISA were prepared from each group by mixing an equal volume of serum from each group.

2.9 Pathological examination

2.9.1 Gross pathology

Gross pathological examination of the liver focused on its general appearance. The observations considered in the liver included the color, size and the presence of granulomas. The presence or absence of adhesions was also considered. An arbitrary score of 0 to 4 was used to categorize gross pathology (Dragana *et al*., 1998). A score of 0,1, 2, 3, 4 translated to no granulomas, few granulomas, moderate granulomas, severe and very severe pathology respectively.

2.9.2 Histopathology

Liver sections from sacrificed mice were collected 7 weeks post-infection during perfusion and fixed in 10% buffered formalin for two weeks according to the method of Bancroft and Stevens, (1982). Optimum dehydration was achieved by immersing the samples into 80%, 95% and 100% ethanol respectively. The tissues were then cleared in toluene and infiltrated in hot paraffin. They were embedded on tissue-embedding paraffin wax (Sherwood Medical co. USA) and then sectioned serially at 6 microns using a rotary microtome (Leitz, Germany). The tissue sections were mounted on glass slides and stained (Appendix I) with haematoxylin and eosin (H and E). Using a calibrated ocular micrometer, the tissue sections were observed under a light microscope at 100 × magnification and the number and size of granulomas recorded. Granuloma size was measured based on the vertical and horizontal diameters of granulomas with a visible centrally placed schistosome egg (Farah *et al*., 2000). The horizontal and vertical diameter average was taken as the diameter of the granulomas.

2.10 Immunological assay; Enzyme-linked immunosorbent assay (ELISA)

Soluble egg antigen (SEA) and Soluble adult worm preparation (SWAP) specific IgG responses in mice after challenge infection were determined by titration of the serum in different concentrations of these antigens. The ELISA technique employed is a modification of Hillyer and Gomez (1979) method. Coating concentrations of the antigens and the sera dilutions factor were determined after the titration of the serum against different concentrations of the antigens. The antigens were used at coating concentrations of 2µg/ml for SEA and 5µg/ml for SWAP while the serum was diluted as follows; SEA (1:400) and SWAP (1:200). The antigens were diluted with 1× phosphate buffered solution (1× PBS) from a stock of 1.4 mg/ml for SWAP and 1 mg/ml for SEA while serum was diluted in 1× PBS- Tween (0.05%). 96 well immunosorp ELISA plates (Dynex) were coated with the diluted antigen, 50 µl/well, and the plates incubated at 4^{o}C overnight. The plates were then washed three times with PBS-Tween (0.05%).

Non-specific binding sites were blocked by adding PBS-Tween (0.5%) 250 µl/well and then incubated for 2 hours at 37^{o}C. 50 µl of the diluted serum was added into each well and the plates incubated overnight at 4^{o}C. The plates were then washed six times with wash buffer and 50 µl of diluted anti-mouse IgG conjugated to HorseRadish Peroxidase (Bio-Rad Laboratory) added to each well at a working dilution of 1: 2000 in 1 × PBS-Tween (0.05%). The plates were then incubated for 1 hour at 37^{o}C and then washed 6 times. Detection was done by the addition of 50 µl of 3,3', 5,5'-tetra methylbenzidine (TMB microwell peroxidase substrate solution, Kirkegaard and Perry Labs, Gaithersburg,

Maryland, USA) into the wells. After a further 25 minutes incubation, absorbance was measured as optical density (OD) at 630 nm using an ELISA reader (Dynatech MR500).

2.11 Data analysis

Statistical analysis was done using Statistica software and Microsoft excel. Data were characterized by their means and standard error (mean ± Standard error). Analysis of variance (ANOVA) was used to compare the difference between means of variables. The *p*-values are one-tailed ($p > 0.05$ is not significant, $p < 0.05$ is significant). Resistance to challenge infection was expressed as the percentage reduction in the number of adult worms recovered from mice receiving hyper-immune serum or infection serum compared with non immunized control mice calculated as follows:

$$\text{Resistance} = (C-E)/C \times 100$$

Where C represents the mean recovery from challenge control mice and E represents the mean recovery from experimental mice receiving hyper-immune serum or infection serum (Mangold and Dean, 1986).

CHAPTER THREE

RESULTS

3. 1 Determination of the role of antibodies against the pre-liver-lung stage schistosomulae

Effect of immune serum generated from RA vaccinated baboons on the pre-liver-lung stage schistosomulae was determined by comparing the number and nature of schistosome worms recovered, number of faecal and trapped schistosome eggs in the liver and on the size and number of granulomas in the liver. The levels of IgG antibodies against soluble egg antigen (SEA) and soluble adult worm antigen (SWAP) were also compared in all the groups.

3.1.1 Effect of Immune serum from RA vaccinated baboons on the number and nature of schistosome worms.

The percentage worm reduction in this study is shown in Table 3.1. The mean number of *S. mansoni* worms recovered from mice immunized with hyper-immune serum, those immunized with infection serum and from the control was 49, 47.2 and 44.6 respectively. The difference in mean worm burdens between each of the immunized groups and that of control was however not statistically significant ($P > 0.05$). The table shows that mice receiving hyper-immune serum recorded the highest number of worms as compared to the control and infection serum recipients. In this study there were more male worms recovered compared to the female worms in all the groups after perfusion. Compared to the females, there were more stunted males in all the groups (Table 3.2). The difference in mean stunted worm between each of the immunized groups and that of control was statistically not significant.

Table 3.1: Mean Number of worms after passive immunization on the 7th and 14th day post-challenge

Group	Passive transfer	Worm recovery (mean ± S.E)	% Protection	*P*-value
Hyper-immune serum recipients	With hyper-immune serum	49 ± 9.7	-9.9	> 0.05
Infection serum recipients	With infection serum	47.2 ± 10.8	-5.8	> 0.05
Control	Without serum	44.6 ± 9.7	-	> 0.05

Table 3.2: Normal and stunted schistosome worms recovered after passively immunizing mice on the 7th and 14th day post challenge

Group	Female worms			Male worms		
	Normal	stunted	Total	Normal	Stunted	Total
Hyper-immune serum recipients	83	1	84	151	10	161
Infection serum recipients	90	0	90	141	5	146
Control	75	1	76	143	4	147

3.1.2 Effect of Immune serum from RA vaccinated baboons on the faecal and trapped schistosome eggs in the liver

The effect of hyper-immune serum from baboons vaccinated with RA vaccine against the pre-liver-lung stage schistosomulae was first determined by comparing the average number of 24 hour faecal eggs at weeks 5, 6 and 7 from both the immunized and the controls. There were no eggs detected in all the groups at week 5 and 6. At week 7, mice immunized with hyper-immune serum had the lowest mean number of eggs, (42.6) followed by mice immunized with infection serum with a mean of 74.8. The control group had the highest egg count with a mean of 86 though the differences between the means were not significantly different ($p > 0.05$, Fig. 3.1).

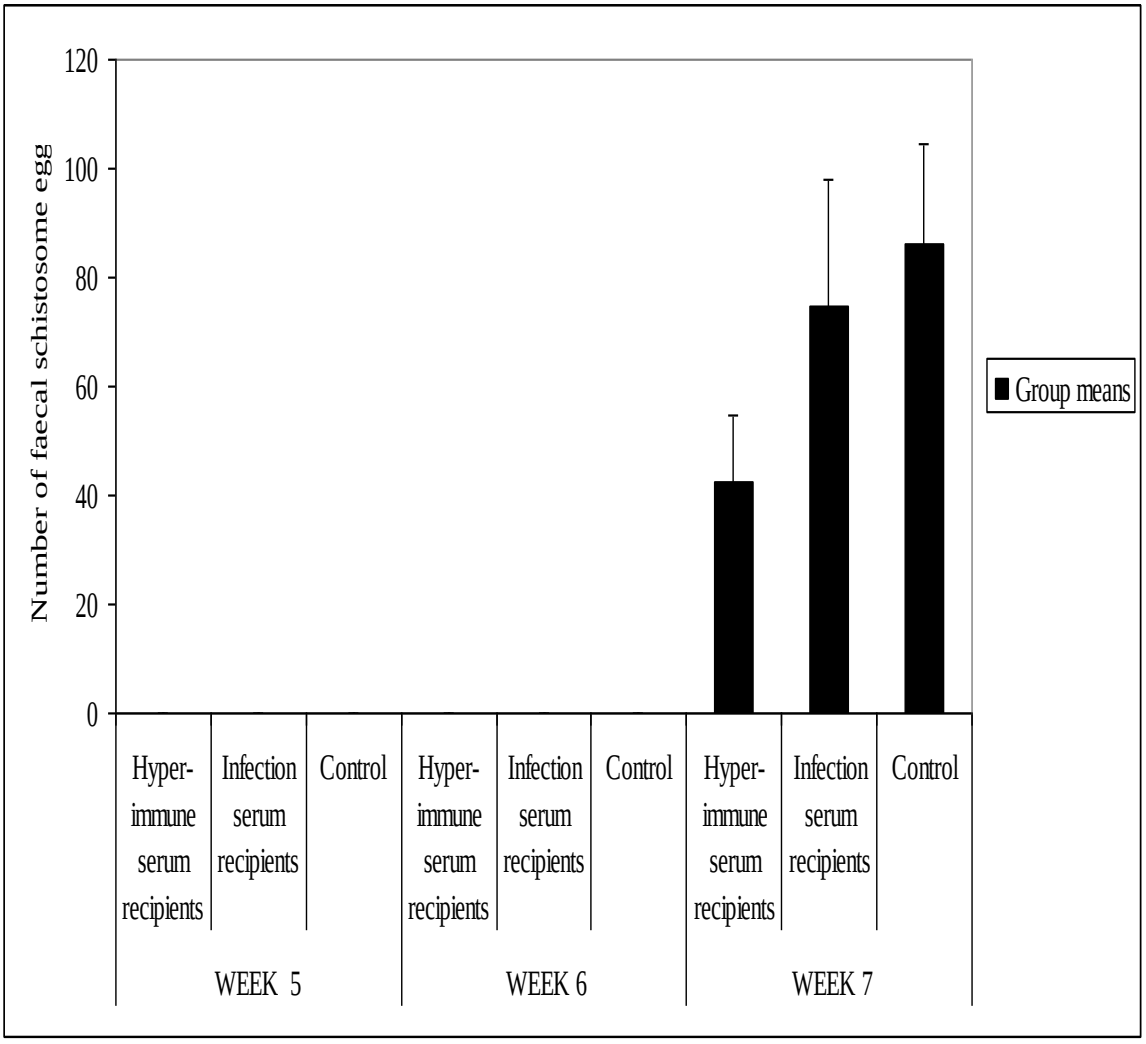

Figure 3.1: Mean number of schistosome faecal eggs (24 hour) in mice immunized on the 7th day and boosted on day 14 post challenge and the control group (means + SE).

The effect of immune serum from RA vaccinated baboons on the number of trapped schistosome eggs in the liver was also evaluated. There was a reduction in the mean number of eggs in the liver tissue of mice receiving hyper-immnune serum and infection serum. The control group had the highest number of liver eggs while the mice that received infection serum had the lowest though the difference between the immunized groups and the control was not statistically significant (P > 0.05, Figure 3.2).

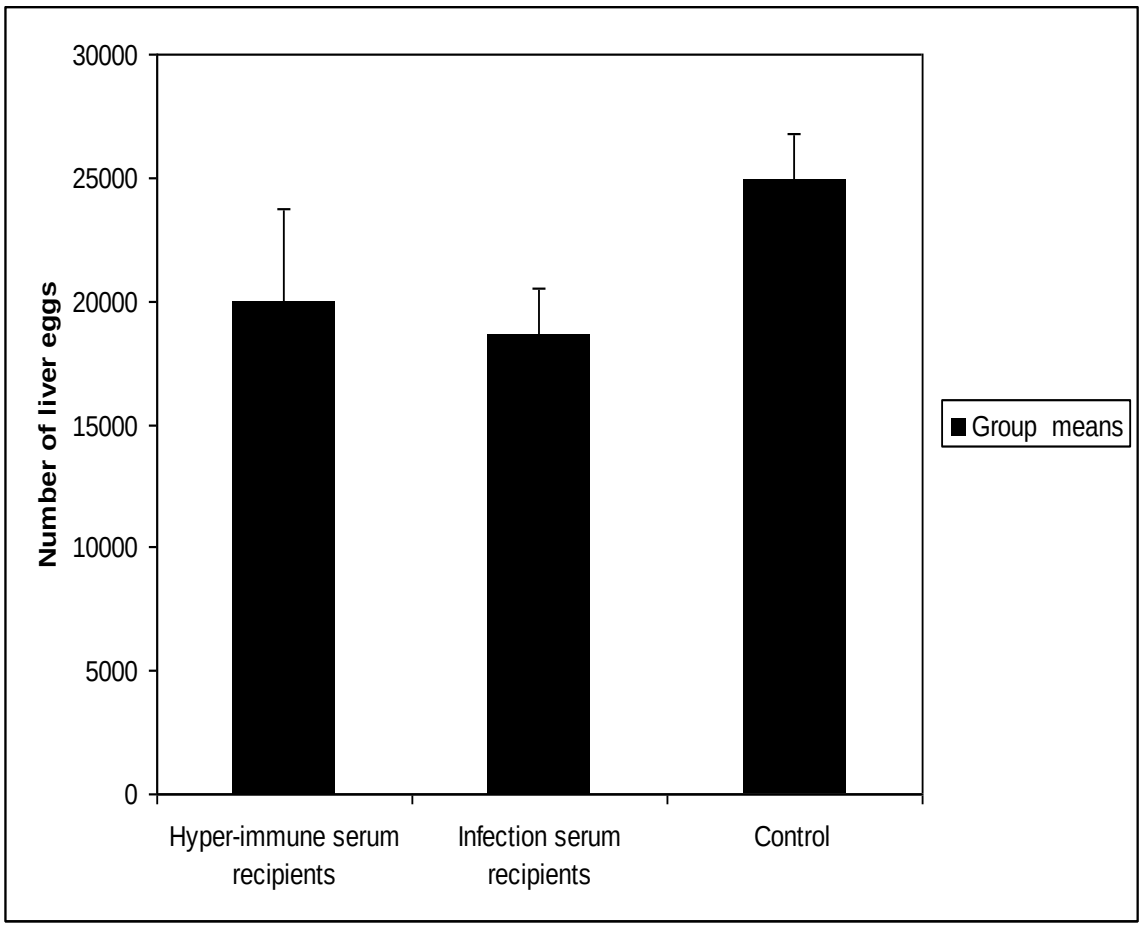

Figure 3.2: Mean number of liver schistosome eggs in mice immunized on the 7th day and boosted on day 14th post challenge (means + SE).

3.1.3 Effect of Immune serum from RA vaccinated baboons on the size and number of liver granulomas

The pathological findings in this study were divided into gross pathology and histopathology. Gross pathological examination of the liver showed that granulomas formed in all mice at week seven. Livers from both the immunized and the control had a whitish/creamy spots on the surface. Livers from all animals appeared pale compared to the red pink hue in the normal mice although the size of the liver lobes was not affected. As stated earlier on (Dragana *et al*., 1998), a score of 0 to 4 was used to categorize gross pathology. A score of 0,1, 2, 3, 4 translated to no granulomas, few granulomas, moderate granulomas, severe and very severe gross pathology respectively.

In the group immunized with hyper-immune serum, two mice had severe granulomas while the other three had very severe granulomas. In the group immunized with infection serum, only two mice had severe pathology while the other three had very severe granulomas. The most severe pathology was recorded in the non immunized group where four mice with very severe granulomas and one mouse with between severe to very-severe granulomas were observed. Adhesions were observed in all mice except one from the control group.

Scores of liver granulomas is as shown in Table 3.3 below. The mean granuloma size was calculated from the average measurement of the vertical and horizontal diameters of granulomas in each slide. Among the three different groups, there was no significant difference in the size of the granulomas. Mice immunized with the protective serum recorded the highest average size of granulomas while the lowest size was observed in

the control group though this difference was not statistically significant (P > 0.05). Mice receiving hyper-immune serum recorded the lowest number of granulomas as compared to the rest of the groups though not statistically significant (P> 0.05, Table 3.3). There was thus no significant difference between granuloma number and size in mice that received hyper-immune serum, infection serum and the control mice that were not immunized. Photomicrographs of schistosomal hepatic granulomas (Haematoxilin and eosin staining) in this experiment is as shown below in Figure 3.3.

Table 3.3: Number and size of liver granulomas after passive immunization on the 7th and 14th day post challenge

Group	**Granuloma Number (mean ± S.E)**	***P*-value**	**Granuloma Size (µm) (mean ± S.E)**	***P*-value**
Hyper-immune serum recipients	11 ± 1.55	> 0.05	37.56 ± 1.45	> 0.05
Infection serum recipients	18 ± 3.01	> 0.05	36.27 ± 0.81	> 0.05
Control	14 ± 3.81	> 0.05	34.34 ± 1.69	> 0.05

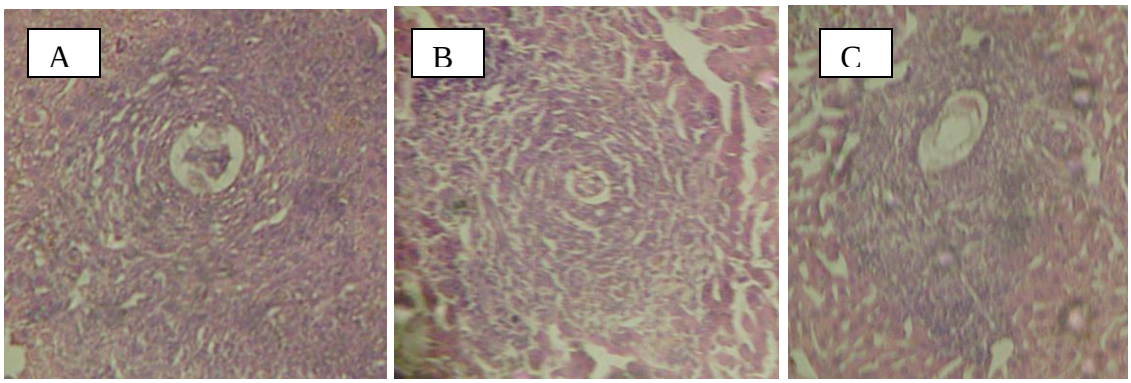

Figure 3.3: Photomicrographs of schistosomal hepatic granulomas (Haematoxilin and eosin staining). A (Hyper-immune serum recipients), B (Infection serum recipients), C (Control)

3.1.4 Levels of IgG antibodies against soluble egg antigen (SEA) and soluble adult worm antigen (SWAP)

The challenge infection with *S. mansoni* stimulated IgG antibody responses in C57Bl/6 mice. SWAP and SEA antigens were used to probe perfusion sera in the three groups of mice. Mice immunized with hyperimmune serum had the lowest response for both SWAP and SEA. The highest response to SWAP and SEA was recorded in mice transfused with infection serum and the control groups respectively (Figure 3.4). The differences in the means were significantly different for the IgG response to SWAP ($P < 0.05$) though not significantly different ($P > 0.05$) for the response to SEA.

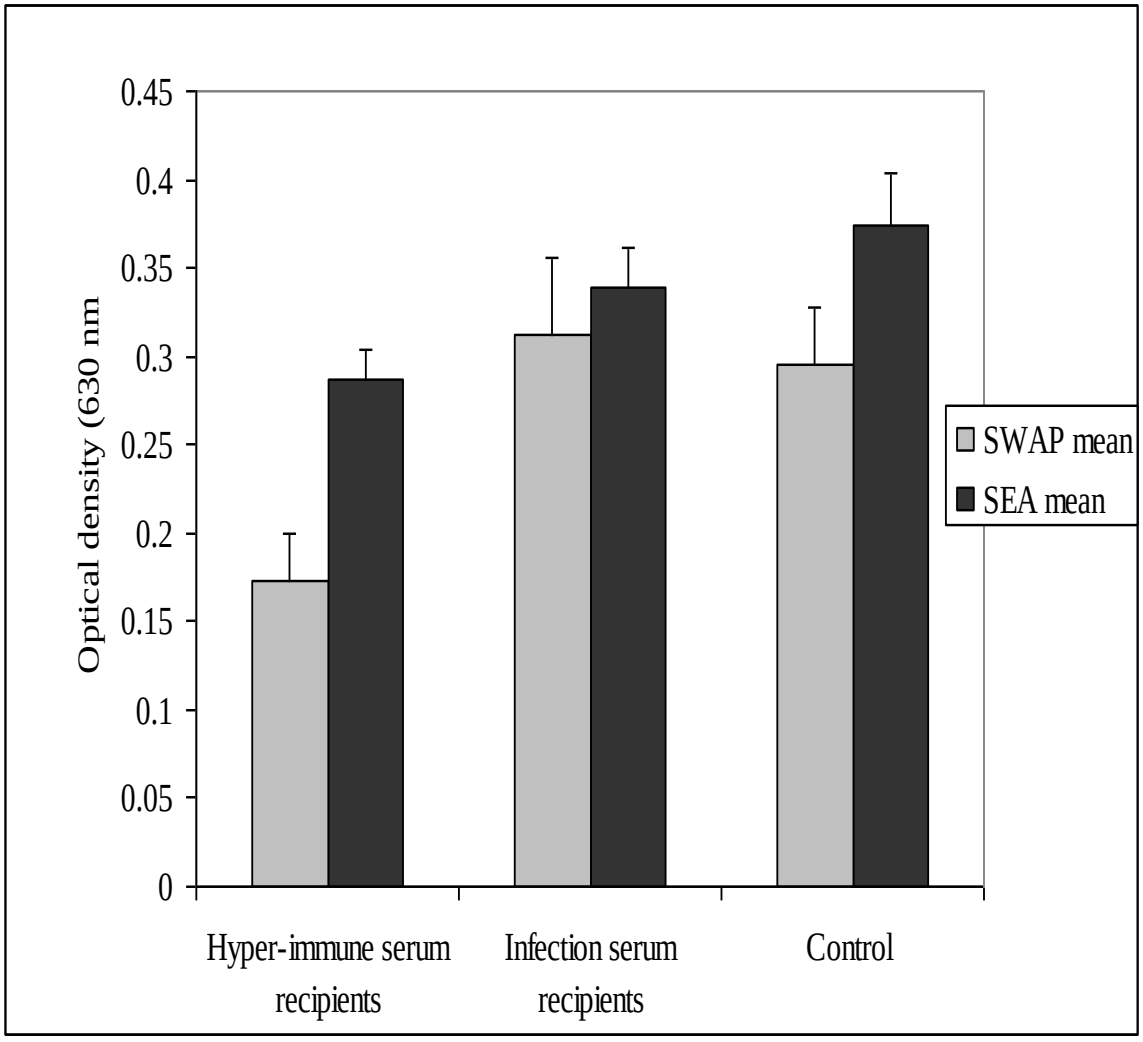

Figure 3.4: IgG response to SWAP and SEA at perfusion in mice immunized on the 7th day and boosted on day 14 post challenge.

3. 2 Determination of the role of antibodies against the migrating larvae

Effect of immune serum generated from RA vaccinated baboons on the migrating larvae was determined by comparing the number and nature of schistosome worms recovered, number of faecal and trapped schistosome eggs in the liver and on the size and number of granulomas in the liver. The levels of IgG antibodies against soluble egg antigen (SEA) and soluble adult worm antigen (SWAP) were also compared in all the groups.

3.2.1 Effect of Immune serum from RA vaccinated baboons on the number and nature of schistosome worms

Male and female worms recovered through portal perfusion at week 7 were counted and the results expressed as mean total worms (male + female). The percentage worm reduction in this study is as shown in Table 3.4.

Table 3.4: Worm recovery after passive immunization on the 3rd day post challenge

Group	Passive transfer	Worm recovery (mean ± S.E)	% Protection	*P*-value
Hyper-immune serum recipients	With hyper-immune serum	43.8 ± 8.6	25.8	> 0.05
Infection serum recipients	With infection serum	71 ± 20.8	-20.3	> 0.05
Control	Without serum	59 ± 14.5	-	> 0.05

The mean number of *S. mansoni* worms recovered were 43.8, 71 and 59 from the hyper-immune serum recipients, infection serum recipient and control, respectively (Table 3.4). This shows that mice receiving hyper-immune serum recorded the lowest number of worms as compared to the control and infection serum recipients. This translated into 25.8% protection in the hyper-immune serum recipient while mice receiving infection serum were not protected. The difference in mean worm burdens between each of the immunized groups and that of control was statistically not significant ($P > 0.05$). There were more total male worms recovered compared to the total female worms in all the groups at perfusion. In this experiment also, there were more stunted males in all the groups as compared to the females (Table 3.5). The difference in mean stunted worms between each of the immunized groups and that of control was statistically not significant.

Table3.5: Normal and stunted schistosome worms recovered after passively immunizing mice on the 3rd day post challenge

Group	Female worms			Male worms		
	Normal	stunted	Total	Normal	Stunted	Total
Hyper-immune serum recipients	65	0	65	135	19	154
Infection serum recipients	122	0	122	227	6	233
Control	94	2	96	191	8	199

3.2.2 Effect of Immune serum from RA vaccinated baboons on the faecal and trapped schistosome eggs in the liver

The efficacy of hyper-immune serum from baboons vaccinated with RA vaccine against the migrating *Schistosoma mansoni* was determined first by comparing the average number of 24 hour faecal eggs at weeks 5, 6 and 7 from both the immunized and the controls (Figure. 3.5).

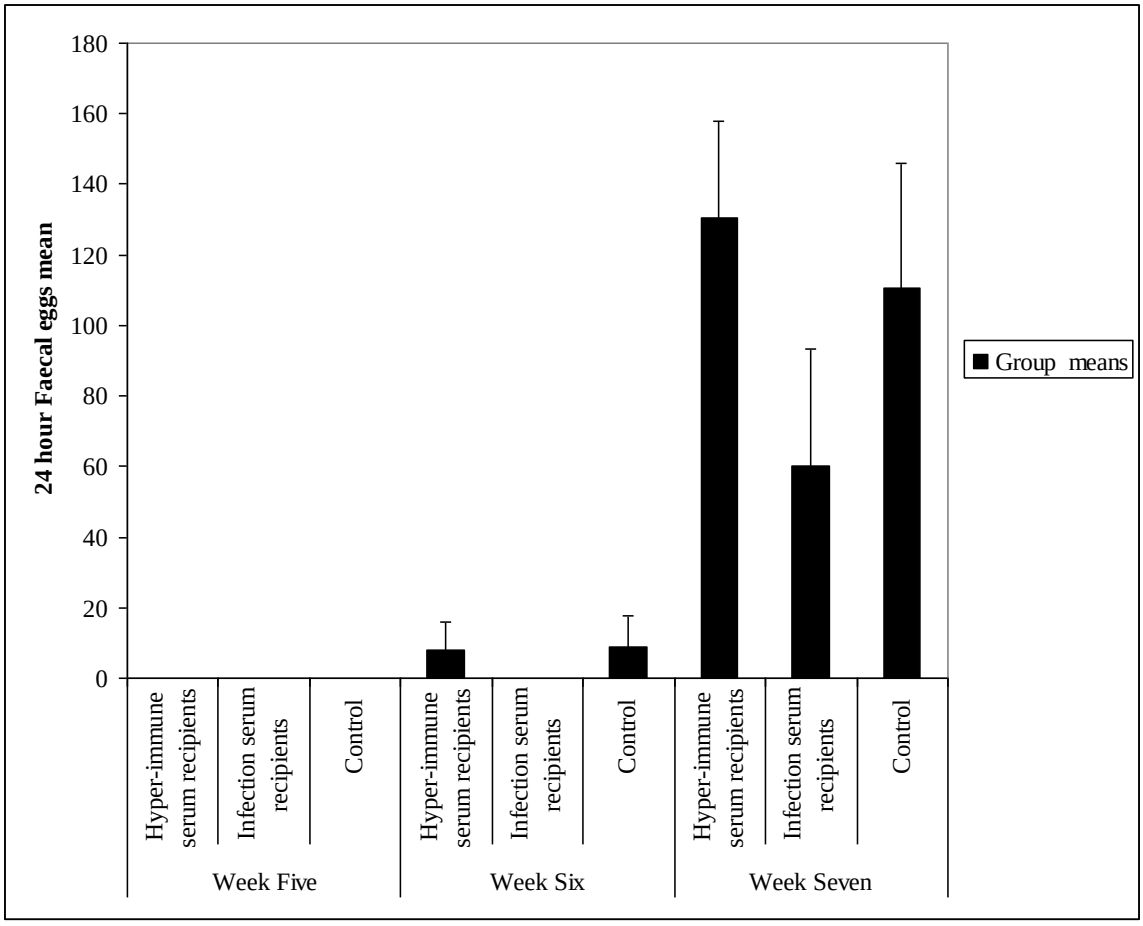

Figure 3.5: Mean number of schistosome faecal eggs in mice immunized on the 3rd day post challenge (means + SE).

There were no eggs detected in all the groups at week 5. Eggs were detected only in the hyper-immune serum recipient and the control group at week 6. At week 7, mice immunized with hyper-immune serum had the highest mean number of 24 hour egg count, 130.4 followed by the control group with a mean of 110.6. Mice immunized with infection serum had the lowest count with a mean of 60 though the differences between the means were not statistically significant ($P > 0.05$, Fig. 3.5).

The effect of immune serum from RA vaccinated baboons on the number of trapped schistosome eggs in the liver was also evaluated in this experiment. Mice receiving hyper-immune serum had the lowest egg count while the highest count was recorded in the control group. The differences between the group means were however not statistically significant ($P > 0.05$, Figure 3.6).

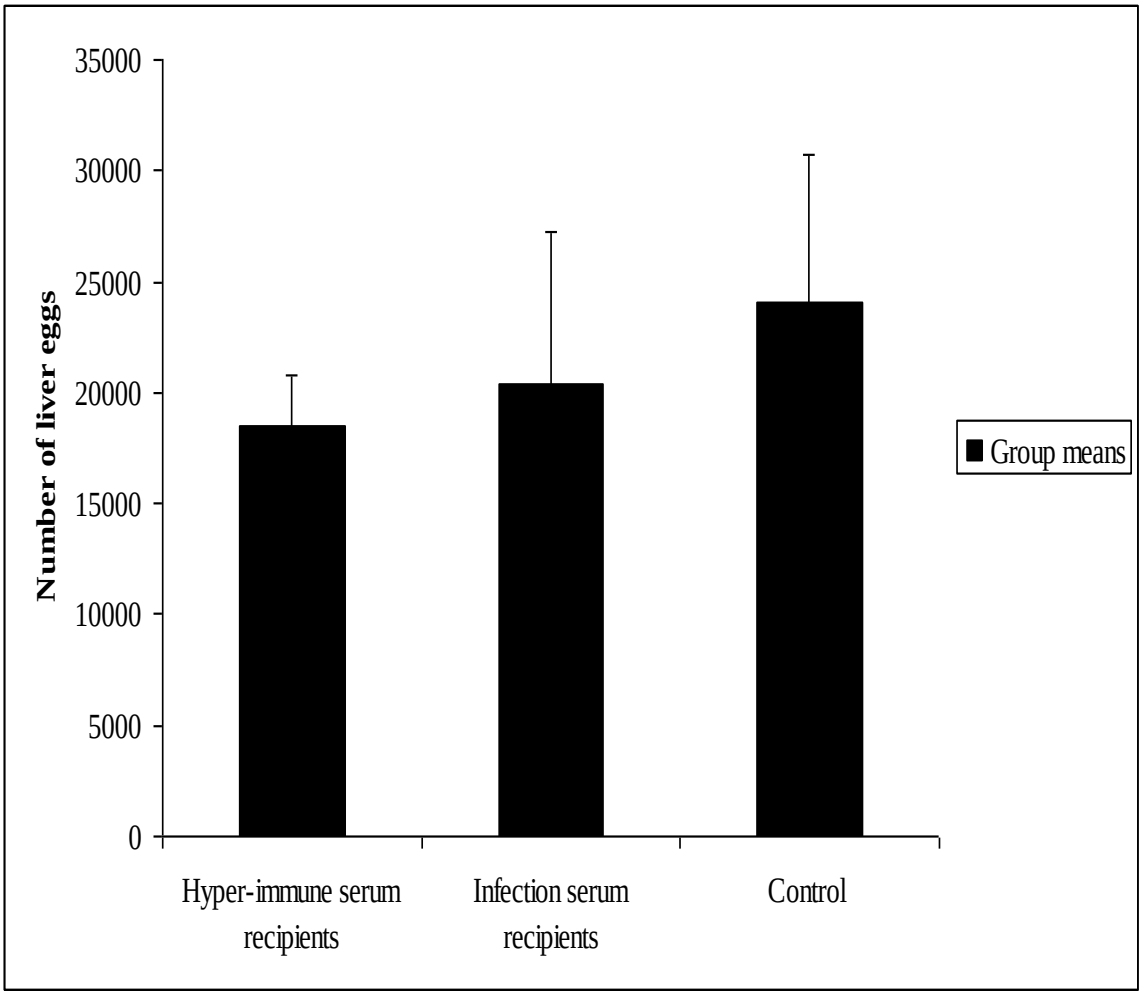

Figure 3.6: Mean number of liver schistosome eggs in mice immunized on the 3rd day post challenge (means + SE).

3.2.3 Effect of Immune serum from RA vaccinated baboons on the size and number of granulomas

Both the gross and the histopathological examination of the livers was carried out on the mice immunized on the 3rd day after infection. A score of 0 to 4 was used to categorize gross pathology (Dragana *et al*., 1998). A score of 0, 1, 2, 3, and 4 translated to no granulomas, few granulomas, moderate granulomas, severe and very severe gross pathology, respectively. Gross pathological examination of mice liver immunized on the

third day after challenge infection showed that granuloma had formed in all groups of mice except one mouse in the infection serum recipients group. In the hyper-immune serum recipient group, three mice had severe granulomas while the other two mice had very severe and moderate-severe granulomas, respectively.

Though there were no observed granulomas in one mice in the infection serum recipient group, two mice from this group had very severe granulomas while the other two had between moderately-severe granulomas. In the control group, one mice had very few granulomas, two mice had severe granulomas and one mice recorded an average of moderate to severe granulomas. In the control group, only one mice had very severe granulomas. Liver adhesions were detected in three, four, and three mice in the hyper-immune serum recipients, infection serum recipients, and control group, respectively. It was only in the infection serum recipients group that one mice had both the liver and lung adhesions.

Histopathological examination of the liver in the three different groups showed no significant difference in the size and number of the granulomas. Mice immunized with the infection serum recorded the lowest average number of granulomas while the control group had the largest average though this difference was statistically not significant ($P > 0.05$). The same was observed in the size of granulomas where mice immunized with infection serum had the lowest average size and control group recorded the highest average with no significant differences. There were no granulomas observed in one mice in the infection serum recipients group and this translates to a large standard error

recorded in this group especially in the size of granulomas (Table 3.6). Photomicrographs of schistosomal hepatic granulomas (Haematoxilin and eosin staining) in this experiment is as shown below in Figure 3.7.

Table 3. 6 Number and size of liver granulomas after passive immunization on the 3rd day post challenge

Group	**Granuloma Number (mean ± S.E)**	***P*-value**	**Granuloma Size (µm) (mean ± S.E)**	***P*-value**
Hyper-immune serum recipients	18 ± 4.33	> 0.05	38.46 ± 1.72	> 0.05
Infection serum recipients	15 ± 5.18	> 0.05	27.14 ± 6.87	> 0.05
Control	20 ± 5.87	> 0.05	41.95 ± 3.23	> 0.05

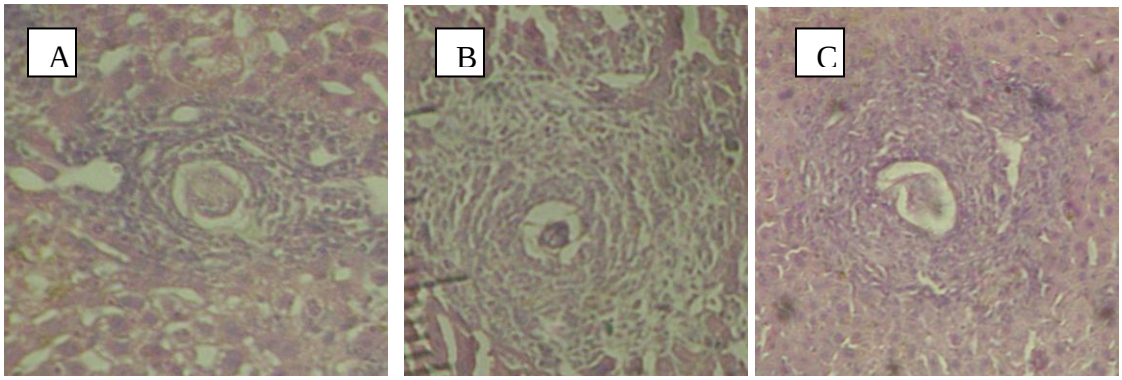

Figure 3.7: Photomicrographs of schistosomal hepatic granulomas (Haematoxilin and eosin staining). A (Hyper-immune serum recipients), B (Infection serum recipients), C (Control) in mice immunized on the 3rd day post challenge.

3.2.4 Levels of IgG antibodies against soluble egg antigen (SEA) and soluble adult worm antigen (SWAP).

To establish whether IgG antibody production differed significantly between the three groups, antibody responses to SWAP and SEA were measured at 0 day before challenge, 2 weeks after challenge, 4 weeks after challenge and at perfusion (7 weeks after challenge). The mean levels of IgG responses is shown in Figure 3.8 and 3.9 below for SWAP and SEA respectively.

The first two weeks were marked with a low or nearly no detectable response followed by a gradual increase in IgG antibodies for both SWAP and SEA antibodies throughout the infection period. This response to both SWAP and SEA was observed in all the groups at all time points without a significant difference between the groups at any of these assayed points. Mice immunized with infection serum recorded the lowest response in both assays but the observed difference as compared to the rest of the groups was not statistically significant. Immunization with either the hyper-immune serum or the infection serum did not affect the animals response to both SWAP and SEA as the observed gradual increase was recorded in all the groups.

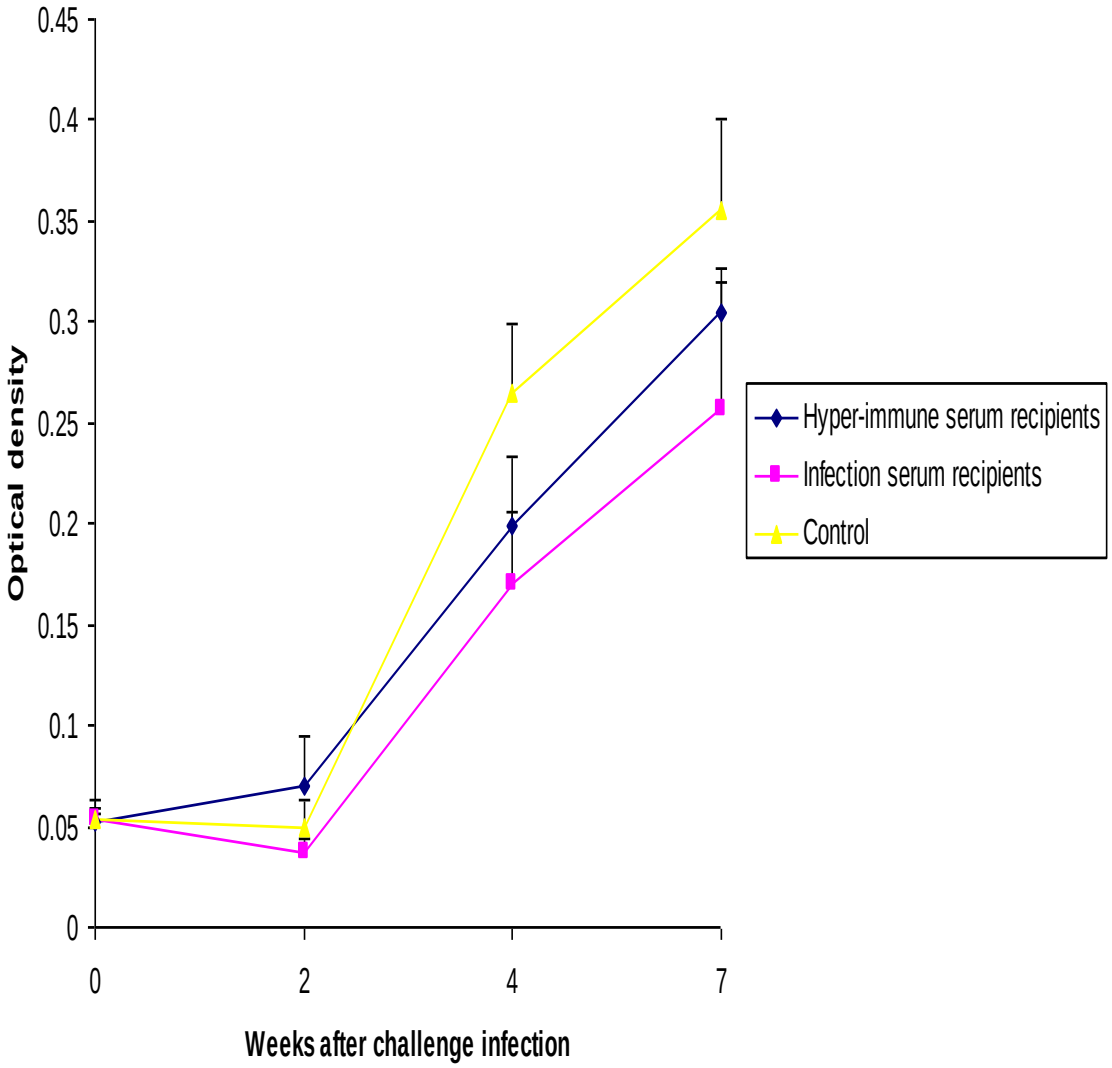

Figure 3.8: IgG response to SWAP in mice immunized on the 3rd day post challenge.

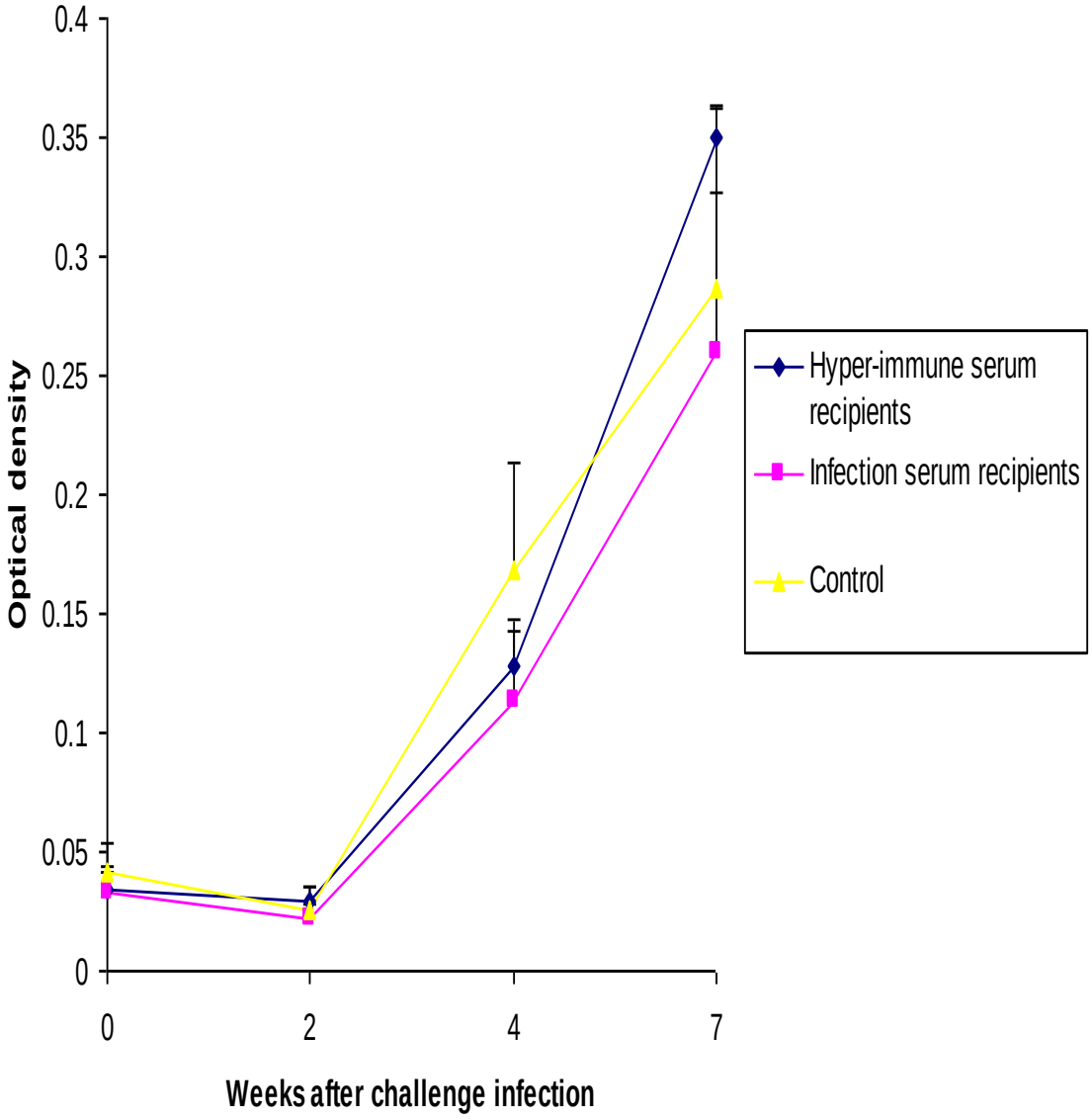

Figure 3.9: IgG response to SEA in mice immunized on the 3rd day post challenge.

CHAPTER FOUR

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

4.1 DISCUSSION

The role of humoral response induced after RA vaccination was studied by passively immunizing mice with either hyper-immune baboon serum from RA vaccinated baboons or with baboons infection serum. The results of this study demonstrate that passive immunization of mice with 500µl of hyper-immune serum from baboons vaccinated with RA vaccine did not provide significant protection against *Schistosoma mansoni* in C57Bl/6 mice contrary to the findings previously reported in the literature (Mangold and Dean,1986; 1992), This lack of significant protection was noted irrespective of the timing of serum injection after percutaneoues infection. In both studies, no eggs were detected in all the groups on the 5^{th} week after infection. Eggs were detected only in one mice immunized with hyper-immune serum and from one mice in the control at week 6 in the mice immunized on the 7^{th} day and boosted on the 14^{th} day post challenge. Similarly, there were no eggs detected at week 6 in the mice immunized on the 3^{rd} day post-challenge and this is probably due to the low sensitivity of kato katz technique (Kongs *et al*., 2008). The two experiments performed were of similar design varying only in the days of passive immunization.

Passive transfer of immunity by humoral factors has previously been studied by several investigators (Cook *et al*., 1972; Bickle *et al*., 1985; Mangold and Dean, 1992). In the present study where the role of humoral immunity against migrating schistosome was

evaluated by passively immunizing mice, a 25.8 % protection level was obtained though the mean worm burden in the immunized group compared to the control was not significant. This could suggest that the transferred hyper-immune serum could be involved in protection. There was no protection obtained when mice were immunized on the 7^{th} day and boosted on the 14^{th} day probably suggesting that the immune effector mechanism in this hyper-immune serum targets the migrating schistosomulae and not the pre-liver-lung stage schistosomulae. This is supported by findings of other workers who showed that the newly transformed schistosomulae is the most likely target of antibody mediated attack (Sher *et al*., 1984).

Heterologous transfer of immune serum from rats and rabbits to mice has been shown to afford significant protection in the recipients thus indicating that similar mechanisms of parasite elimination may be occurring in these species (Mangold and Dean, 1992; Ford *et al*., 1984). In this study, the diverging results between two similar immunization protocols could perhaps be explained by the use of serum from animals of greater genetic and physiologic difference to the serum recipients. The failure to transfer protection by passive immunization with the hyper-immune serum may also suggest that antibodies generated after vaccination with RA vaccine in baboons may not play a key role in mediating protection in mice. Failure to transfer resistance to mice with antibodies after vaccination has also been noted by Verity *et al*., (2001) who evaluated the efficacy of recombinant cathepsin D spartic protease from *Schistosoma japonicum*.

Sher *et al*. (1982) demonstaration that nude mice and mice treated from birth with anti-µ chain serum failed to develop anti-schistosomular surface antibodies or even manifest resistance following vaccination with irradiated cercariae is an indication that the humoral immunity is involved in this type of acquired resistance. Studies on species specificity of immunization and attempts to transfer resistance by Bickle *et al.* (1985) showed that though significant levels of resistance against *Schistosoma mansoni* challenge were developed in mice exposed to highly irradiated (20 krad.) ceracariae of the homologoues species (53-67%), their attempt to transfer this resistance to naive recipients by injection of serum and or spleen or lymph node cells from the vaccinated donor mice were largely unsuccessfull. In contrast, significant levels of resistance have been transferred to mice by injection with serum from rabbits exposed to irradiated cercariae (20 krad.). This protection translated to 34-69% when serum was injected at the time of challenge or 31-56% when injected 5-6 days later (Bickle *et al*., 1985).

These findings that sera from rabbits vaccinated with 20 krad. can confer significant protection to mice suggest that specific serum-borne factors stimulated by irradiated infections can kill schistosomula *in vivo* in mice. This inability of hyper-immune serum from baboons to transfer significant protection in this study could probably be due to differences in titre, isotype or specificity of the antibody responsible for protection (Verity *et al*., 2001). In the mice immunized on the 3rd day post challenge, one mice in the infection serum recipient group had only two worms that were recovered and this could account for the large standard error recorded in this group.

There was no protection achieved when mice were immunized with infection serum in the two studies. Infection serum used in this study coincided with egg production from the serum donors. It has been previously reported that schistosomes eggs stimulate the production of antibodies that are reactive with the antigens on the surface of skin-transformed schistosomulae but which fail to offer any significant protection (Ford *et al*., 1984). Early studies on homologues immune serum ware met with limited success thus making the understanding of the mechanisms underlying resistance to *Schistosoma mansoni* infection in either man or laboratory hosts to be more difficult. In humans, transfer of anti-schistosome gamma globuhin to children living in an endemic area failed to protect a significant proportion of the recipients from contracting the disease (Cook *et al*., 1972). Also an attempt to transfer homologous immune serum to rhesus monkeys failed to establish significant protection in recipients (Ogilive *et al*., 1966).

The time of serum injection appeared to be crucial in conferring protection against challenge infection as has also been shown by other researchers (Jwo and Loverde, 1989; Mangold and dean, 1992). Studies have shown that comparable levels of resistance could be transferred to mice with rabbit serum given at the time of challenge or 5-7 days later thus demonstrating that the challenge schistosomula remain susceptible up to or beyond the lung stage (Bickle *et al*., 1985). This is consistent with the results of experiment one since a 25.8% protection level was obtained when mice were immunized on the 3rd day. The site of parasite immune elimination still remain unclear since passive immunization on the 7th day and boosting on the 14th day post challenge failed to confer any significant protection (Coulson and Mountford, 1989; Richter *et al*., 1995).

Granuloma formation has been defined as the inflammatory response against parasite eggs that are trapped in host tissues. The intensity of this response is either modulated by the host (Goes *et al*., 1991) or by the parasite factors (Boulanger *et al*., 1992). Anti-schistosome monoclonal antibodies have been shown to confer resistance against challenge infection with *Schistosoma mansoni* resulting in a significant decrease in granuloma number and size (Attallah *et al*., 1999). This reduction in the size and number of granulomas was found by Attallah *et al*. (1999) to be consistent with an effective reduction in the number of schistosome worms *in vivo*.

For a reduction in the granuloma number to be experienced, it would be expected that the specific antigen would induce an immune response in the host which would then cause a severe damage to the essential life functions of the parasite leading to its death and consequently a reduction in the number of eggs produced (Grzych *et al*., 1993). This protection is provided during a passive immunization and if the target antigen in the developing worms are present, then a reduction in the number of worms and the eventual reduction in the number of egg granulomas would be expected. Lack of an eventual significant reduction in the number of worms in the immunized mice as compared to the control could thus account for the recorded high liver eggs in all the groups and the eventual lack of significant difference in the number and size of granulomas between the immunized and the controls (Attallah *et al*., 1999).

There were no granulomas observed in one mice in the group immunized with infection serum on the 3rd day and this could probably account for the low averages recorded in

number and size of granulomas in this group. This thus translates to a large standard error recorded in mice immunized with infection serum on the 3rd day especially in the size of granulomas (Table 3.6). This is consistent with the parasitological findings in this experiment as there were no eggs detected in the faecal matter or in the liver of this mouse.

There could be several reasons why baboon serum containing a high activity of anti-schistosomular antibody failed to protect the mice. It is possible that the humoral components from baboon may not be able to cooperate with the mouse effector system necessary in killing the schistosomulae. This is supported by findings of Lewis *et al*., (1977) who observed that plasma from humans infected with *Schistosoma mansoni* failed to protect mice from *Schistosoma mansoni* cercariae challenge. RA vaccine experiments in mice have shown that the secretion of abundant interferon-γ (IFN- γ) as part of a cell-mediated response in the lungs is crucial to the establishment of optimum levels (60–70%) of protective immunity (Coulson, 1997). Anti-parasite antibodies generated in mice after single vaccination with RA vaccine are thought not to contribute towards protection since antibody titers in different strains of vaccinated mice do not correlate with the level of immunity, neither does serum transfer to naive recipients confer passive protection (Richter and Harn, 1993; Coulson, 1997). It is thus possible that the mechanisms of protection in mice do not totally rely on humoral components a phenomenon that is yet to be proved.

4.2 CONCLUSIONS

The following conclusions can be made from the results of this study;

i. There was no effect of hyper-immune serum from RA vaccinated baboons on the number and nature of schistosome worms in C57Bl/6 mice.

ii. Passive immunization of C57Bl/6 mice with hyper-immune serum from RA vaccinated baboons did not affect the fecundity of schistosome worms irrespective of the timing of serum injection since there was no significant difference in the number of faecal and trapped schistosome eggs in the liver of all groups.

iii. There was no significant reduction of liver granulomas in both size and number after passive immunization irrespective of the timing of serum injection.

iv. There was no significant protection that was conferred in mice against pre-liver-lung stage schistosomulae and or the migrating schistosome larvae by hyper-immune serum from RA vaccinated baboons probably due to the inability of the baboon serum to cooperate with the mouse effector system necessary in killing the schistosomulae.

Therefore, the null hypotheses which stated that:

Ho_1: Hyper-immune serum from baboons does not protect mice against pre-liver-lung stage schistosomulae was accepted and the alternate hypotheses rejected.

Ho_2: Hyper-immune serum from baboons does not protect mice against the migrating schistosome larvae was accepted and the alternate hypothesis rejected.

4.3 RECOMMENDATIONS

i. Homologous passive transfer of hyper-immune serum (baboon to baboon) should be experimented on.

ii. Other stages of the schistosome lifecycle should also be targeted during passive transfer experiments.

iii. The mechanisms of protection in mice should be further evaluated.

REFERENCES

Abath, F.G.C., Morais, C.N.L., Montenegro, C.E.L., Wynn, T.A. and Montenegro, S.M.L. (2006). Immunopathogenic mechanisms in schistosomiasis: what can be learnt from human studies? *Trends in Parasitology*, **22**: 85–91.

AbdelWahab, M.F., Esmat, G., Farrag, A., Elboraey, Y. and Strickland, T. (1993). Ultrasonographic prediction of esophageal varices in schistosomiasis mansoni. *American Journal of Gastroenterology*, **88**: 560–563

Abe, K., Kagei, N., Teramura, Y. and Exima, H. (1993). Hepatocellular carcinoma associated with chronic Schistosoma mansoni infection in a chimpanzee. *Journal of Medical Primatology*, **22**: 237–239.

Al-Sherbiny, M.M., Osman, A., Hancock, K., Deelder, A.M. and Tsang, V.C. (1999). Application of immunodiagnostic assays: detection of antibodies and circulating antigens in human schistosomiasis and correlation with clinical findings. *American Journal of Tropical Medicine and Hygien*, **60**: 960-966.

Alves-Brito, C.F., Simpson, A.J., Bahia-Oliveira, L.M., Rabello, A.L., Rocha, R.S., Lambertucci, J.R., Gazzinelli, G., Katz, N. and Correa-Oliveira, R. (1992). Analysis of anti-keyhole limpet haemocyanin antibody in Brazilians supports its use for the diagnosis of acute schistosomiasis mansoni. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 86: 53–56.

Appleton, C.C. (1984). Schistosome dermatitis: an unrecognized problem in South Africa? *South African Medical Journal*, **65:** 467– 469.

Argiro, L.L., Kohlstadt, S.S., Henri, S.S., Dessein, H.H., Matabiau, V.V., Paris, P.P. Bourgois, A.A. and Dessein, A.J. (2000). Identification of a candidate vaccine peptide on the 37 kDa *Schistosoma mansoni* GAPDH. *Vaccine*, **18**: 2039–2048.

Attallah, A.M., Attia, H., El-Nashar, E. M., Nawar, A., Abdel Kadel, K., Ismail, H. and El Ebeidy, G. (1999). Induction of resistance against *Schistosoma mansoni* infection by passive transfer of an IgG2a monoclonal antibody. *Vaccine*, **17**: 306-310.

Bakry, F.A. and Abd-el-Monem, **S.** (2005). Effects of water plants and non-target snails on the infectivity of *Bulinus truncatus* with *Schistosoma haematobium*. *Journal of the Egyptian Society of Parasitology*, **35**: 859–874.

Bancroft J. D. and Stevens A. (1982). Theory and practice of histological techniques. London, New York: Churchill Livingstone.

Barbour, A.D. (1985). The importance of age and water contact patterns in relation to *Schistosoma haematobium* infection. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **79**: 151–157.

Beck, L., Favre, T.C., Pieri, O.S., Zani, L.C., Domas, G.G. and Barbosa, C.S. (2001). Replacing oxamniquine by praziquantel against *Schistosoma mansoni* infection in a rural community from the sugar-cane zone of Northeast Brazil: an epidemiological follow-up. *Memorias do Instituto Oswaldo Cruz*, **96**:165–167.

Becker, B., Mehlhorn, H., Andrews, P., Thomas, H. and Eckert, J. (1980). Light and electron microscopic studies on the effect of praziquantel on *Schistosoma mansoni*, *Dicrocoelium dendriticum*, and *Fasciola hepatica* (Trematoda) *in vitro*. *Zeitschriff fur Parasitenkunde*, **63**: 113–128.

Bergquist, R. N and Colley, D.G. (1998). Schistosomiasis vaccine: research to development. *Parasitology Today*, **14**: 99–104.

Bergquist, R., Al-Sherbiny, M., Barakat, R. and Olds, R. (2002). Blueprint for schistosomiasis vaccine development**.** *Acta Tropica*, **82**:183-192.

Bethlem, E.P., Schettino, G. and Carvalho, C.R. (1997). Pulmonary schistosomiasis. *Current Opinion in Pulmonary Medicine*, **3**:361-365.

Bickle, Q.D., Andrews, B.J., Doenhoff, M.J., Ford, M.J. and Taylor, M.G. (1985). Resistance against *Schistosoma mansoni* induced by highly irradiated infections: studies on species specificity of immunization and attempts to transfer resistance. *Parasitology*, **90**: 301-312.

Borojevic, R. (1992). Experimental murine schistosomiasis mansoni: establishment of the chronic phase of the disease. *Memorias do Instituto Oswaldo Cruz*, **87**: 171-174.

Boulanger, D., Reid, G.D.F., Sturrock, F.F., Wolowezuk, J., Balloul, J.M., Grezel, D., Pierce, R.J., Otieno, M.F., Guerret, S., Grimaud, J.A. and Butterworth, A.E. (1992). Vaccines against schistosomiasis: where do we stand? *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **86**: 1-2.

Brito, C.F., Fonseca, C.T., Goes, A.M., Azevedo, V., Simpson, A.J., and Oliveira, S.C. (2000). Human IgG1 and IgG3 recognition of *Schistosoma mansoni* 14 kDa fatty acid-binding recombinant protein. *Parasite Immunology*, **22**: 41–48.

Butterworth, A.E. (1993). Immunology of schistosomiasis. In: Jordan, P., Webbe, G. and Sturrock, F.S. (Eds), *Human schistosomiasis*, CAB International, Wallingford. pp. 331–366.

Butterworth, A.E., Capron, M., Cordingley, J.S., Dalton, P.R., Dunne, D.W., Kariuki, H.C., Kimani, G., Koech, D., Mugambi, M., Ouma, J.H., Richardson, B.A., Arap Siongok, T.K., Sturrock, R.F. and Taylor, D.W. (1985). Immunity after treatment of human Schistosomiasis. II. Identification of resistant individuals, and analysis of their immune responses. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **79**: 393–408.

Butterworth, A. E., Curry, A. J., Dunne, D. W., Fulford, A. J., Kimani, V., Kariuki, H. C., Klumpp, R., Koech, D., Mbugua, G., Ouma, J. H., Roberts, M., Thiongo, F. W., Capron, A. and Sturrock, R. F. (1994). Immunity and morbidity in human schistosomiasis mansoni. *Tropical and Geographical Medicine*, **46**:197–208.

Butterworth, A.E., Dunne, D.W. and Fulford, A. (1988). Immunity in human schistosomiasis mansoni: cross-reactive IgM and IgG2 antibodies block the expression of immunity. *Biochimie*, **70**: 1053–1063.

.

Caldas, I.R., Correa-Oliveira, R., Colosito E., Carvalho, O.S., Massara, C.L., Colley, D.G. and Gazzinelli, G. (2000). Susceptibility and resistance to *Schistosoma mansoni* reinfection: Parallel cellular and isotypic immunologic assessment**.** *American Journal of Tropical Medicine and Hygiene*, **62**: 57-64.

Capron, A., Capron, M., Dombrowicz, D. and Riveau, G. (2001). Vaccine strategies against schistosomiasis: from concepts to clinical trials. *International Archives of Allergy and Immunology*, **24**: 9–15.

Capron, A., Riveau, G., Capron, M. and Trottein, F. (2005). Schistosomes: the road from host-parasite interactions to vaccines in clinical trials. *Trends in Parasitology*, **21**: 143–149.

Capron, M. and Capron, A. (1986). Rats, mice and man models for immune effector mechanisms against schistosomiasis**.** *Parasitology Today*, **2**: 69–72.

Chan, M. S., Woolhouse, M. E. and Bundy, D. A. (1997). Human schistosomiasis: potential long-term consequences of vaccination programmes. *Vaccine*, **15**: 1545–1550.

Chen, M. and Mott, K. (1989). Progress in the assessment of morbidity due to Schistosoma japonicum infection: a review of recent literature. *Tropical Diseases Bulletin*, **85**:R1-R56.

Chen, M.G. (1993). *Schistosoma japonicum* and *S japonicum*-like infections: epidemiology, clinical and pathological aspects. In: Jordan P, Webbe G, Sturrock FS. Human schistosomiasis. Wallingford: CAB International, 1993: 237–270.

Cheever, A.W. (1978). Schistosomiasis and neoplasia. *Journal of the National Cancer Institute*, **61**:13-18.

Cheever, A.W., Dragana, J., Yap, G.S., Kullberg, M..C., Sher, A. and Wynn, T.A. (1998). Role of cytokines in the formation and downregulation of hepatic circumoval granulomas and hepatic fibrosis in *Schistosoma mansoni* infected mice. *Memorias do Instituto Oswaldo Cruz,* **93**: 25-32.

Cheever, A.W., Kamel, I.A., Elwi, A.M., Mosimann, J.E., Danner, R. and Sippel, J.E. (1978). *Schistosoma mansoni* and *S haematobium* infections in Egypt, III: extrahepatic pathology. *American Journal of Tropical Medicine and Hygiene*, 27: 55–75.

Cheever, A.W., Hoffmann, K.F. and Wynn, T.A. (2000). Immunopathology of schistosomiasis mansoni in mice and men. *Immunology Today,* **21**: 465–466.

Cheever, A.W., Lenzi, J.A., Lenzi, H.L. and Andrade, Z.A. (2002). Experimental models of Schistosoma mansoni infection. *Memorias do Instituto Oswaldo Cruz*, **97**: 917–940.

Chen, M.G. (1993). *Schistosoma japonicum* and *S japonicum*-like infections: epidemiology, clinical and pathological aspects. In: Jordan, P., Webbe, G. and Sturrock, F.S. Editors, *Human schistosomiasis,* CAB International, Wallingford. PP 237–270.

Chitsulo, L., Engels, D., Montresor, A. and Savioli, L. (2000). The global status of schistosomiasis and its control. *Acta Tropica*, **77** : 41–51.

Cioli, D. and Pica-Mattoccia, L. (2003). Praziquantel. *Parasitology Research*, **90**: S3–S9.

Cook, J. A., Warren, K. S., and Jordan, P. (1972). Passive transfer of immunity in human schistosomiasis mansoni: Attempt to prevent infection by repeated injections of hyperimmune antischistosome gamma globulin. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **66**: 777-780.

Cooke, G.S., Lalvani, A., Gleeson, F.V. and Conlon, C.P. (1999). Acute pulmonary schistosomiasis in travelers returning from Lake Malawi, sub-Saharan Africa. *Clinical Infectious Diseases*, **29**:836-839.

Correa-Oliveira R., Malaquias L.C., Falcao P.L., Viana I.R., Bahia-Oliveira L.M., Silveira A.M., Fraga L.A., Prata A., Coffman R.L., Lambertucci J.R., Cunha- Melo J.R., Martins-Filho O.A., Wilson R.A. and Gazzinelli G. (1998). Cytokines as determinants of resistance and pathology in human *Schistosoma mansoni* infection. *Brazilian Journal of Medical and Biological Research*, **31**: 171-177.

Coulson, P. S. (1997). The radiation-attenuated vaccine against schistosomes in animal models: paradigm for a human vaccine? *Advances in Parasitology,* 39: 272–384.

Coulson, P.S. and Mountford, A.P. (1989). Fate of attenuated schistosomula administered to mice by different routes, relative to the immunity induced against*Schistosoma mansoni*. *Parasitology*, **99** : 39-45.

Cowie, R.H. (2001). Can snails ever be effective and safe biocontrol agents? *International Journal of Pest Management*, **47**: 23–40.

Da'dara, A.A., Skelly, P.J., Wang, M. and Harn, D.A. (2001). Immunization with plasmid DNA encoding the integral membrane protein, Sm23, elicits a protective immune response against schistosome infection in mice. *Vaccine*, **20**: 359–369.

De Vlas, S.J., Gryseels, B., van Oortmarssen, G.J., Polderman, A.M. and Habbema, J.D. (1992). A model for variations in single and repeated egg counts in *Schistosoma mansoni* infections. *Parasitology*, **104**: 451–460.

Doenhoff, M.J. and Pica-Mattoccia, L. (2006). Praziquantel for the treatment of schistosomiasis: its use for control in areas with endemic disease and prospects for drug resistance. *Expert Review of Anti-Infective Therapy*, **4**: 199–210.

Doherty, J.F., Moody, A.H. and Wright, S.G. (1996). Katayama fever: an acute manifestation of schistosomiasis. *British Medical Journal*, **313**:1071-1072.

Dragana, J., Allen, W., Cheever, M.C., Kullberg, T. A., Wynn, G.Y., Patricia, C., Fred, A.L, Raphael, C., Jeffrey, V. R. and Alan S. (1998). $CD4^+$ T Cell-mediated Granulomatous Pathology in Schistosomiasis is Downregulated by a B Cell-dependent Mechanism Requiring Fc Receptor Signaling. *The Journal of Experimental medicine*, **187**: 619-629.

Dunne, D. W., Butterworth, A. E., Fulford, A. J., Kariuki, H. C., Langley, J. G., Ouma, J. H., Capron, A., Pierce, R. J. and Sturrock, R. F. (1992). Immunity after treatment of human schistosomiasis: association between IgE antibodies to adult worm antigens and resistance to reinfection. *European Journal of Immunology*, **22**: 1483–1494.

Dunne, D.W., Grabowska, A.M., Fulfordd, A.J.C., Butterworth, A.E., Sturrock, R.F., Koech, D. and Ouma, J.H.C. (1988). Human antibody responses to *Schistosoma mansoni*: the influence of epitopes shared between different life cycle stages on the response to the schistosomulum. *European Journal of Immunology*, **18**:123-7.

Eberl, M., Mountford, A. P., Jankovic, D. and Beck, E. (1999). Isolation of T cell antigens by using a recombinant protein library and its application to the identification of novel vaccine candidates against schistosomiasis. *Infection and Immunity*, **67**: 3383–3389.

Engels, D., Ndoricimpa, J. and Gryseels, B. (1993). Schistosomiasis mansoni in Burundi: progress in its control since 1985. *Bulletin of the World Health Organization*, **71**: 207–214.

Feldmeier, H. and Poggensee, G. (1993). Diagnostic techniques in schistosomiasis control: a review. *Acta Tropica*, **52**: 205–220.

Fenwick, A. (2006). Waterborne infectious diseases—could they be consigned to history? *Science*, **313**: 1077–1081.

Fenwick, A. and Webster, J.P. (2006). Schistosomiasis: challenges for control, treatment and drug resistance. *Current Opinion in Infectious Diseases*, **19**: 577–582.

Fetterer, R.H., Pax, R.A. and Bennett, J.L. (1980). Praziquantel, potassium and 2,4-dinitrophenol: analysis of their action on the musculature of *Schistosoma mansoni*. *European Journal of Pharmacology*, **64**: 31–38.

Flanigan, T.J. King, C.H., Lett, R.R., Nanaduri, J. and Mahmoud, A.A. (1989). Induction of resistance to *Schistosoma mansoni* infection in mice by purified parasite paramyosin. *Journal of Clinical Investigation*, **83**: 1010–1014.

Ford, M.J., Bickle, Q.D., Taylor, M.G. and Andrews, B.J. (1984). Passive transfer of resistance and the site of immune-dependent elimination of the challenge infection in rats vaccinated with highly irradiated cercariae of *Schistosoma mansoni. Parasitology*, **89**: 461-482.

Garcia, L.S., Shimizu, R.Y. and Palmer, J.C. (1999). Algorithms for detection and dentification of parasites. In: Murray, P.R., edition. Manual of clinsical microbiology. 7th edition. Washington, D.C.: American Society for Microbiology Press :1336-54.

Gazzinelli, G., Lambertucci, J.R., Katz, N., Rocha, R.S., Lima, M.S. and Colley, D.G. (1985). Immune responses during human schistosomiasis mansoni. XI. Immunological status of patients with acute infection after treatment. *The Journal of Immunology*, **135**: 2121–2127.

Giovanelli, A., Vieira, M.V. and Silva, **C.L.** (2003). Apparent competition through facilitation between *Melanoides tuberculata* and *Biomphalaria glabrata* and the control of schistosomiasis. *Memorias do Instituto Oswaldo Cruz*, **98**: 429–431.

Goes, A.M., Gazzinelli, G., Rocha, R.S., Katz, N. and Doughty, B.K. (1991). Granulomatous hypersensitivity to Schistosoma mansoni egg antigens in human schistosomiasis. III. In vitro granuloma modulation induced by immune complexes. *American Journal of Tropical Medicine and* Hygiene, **44**: 434-43.

Gonnert, R. and Andrews, P. (1977). Praziquantel, a new board-spectrum antischistosomal agent. *Zeitschriff fur Parasitenkunde*, **52**: 129–150.

Goudot-Crozel, V., Caillol, D., Djabali, M. and Dessein, A.J. (1989).The major parasite surface antigen associated with human resistance to Schistosomiasis is a 37 kDa glyceraldehyde-3P-dehydrogenase. *The Journal of Experimental Medicine,* **170**: 2065–2080.

Gryseels, B. (1989). The relevance of schistosomiasis for public health. *Tropical Medicine and Parasitology,* **40:** 134–42.

Gryseels, B. (1996). Uncertainties in the epidemiology and control of schistosomiasis. *American Journal of Tropical Medicine and Hygiene,* **55**: 103–108.

Gryseels, B. (2000). Schistosomiasis vaccines: a devils' advocate view. *Parasitology Today*, **16**: 46–48.

Gryseels, B. and De Vlas, S.J. (1996). Worm burdens in schistosome infections. *Parasitology Today*, **12**: 115–119.

Gryseels, B. and Nkulikyinka, L. (1998). The distribution of *Schistosoma mansoni* in the Rusizi plain (Burundi). *Annals of Tropical Medicine and Parasitology*, **82**: 581–590.

Gryseels, B., Polman, K., Clerinx, J. and Kenstens, L. (2006). Human schistosomiasis. *Lancet,* **368**: 1106-1118.

Grzych, J.M., Grezel, D., Xu, C.B., Neyrinck, J.L, Capron, M., Ouma, J.H., Butterowrth, A.E. and Capron, A. (1993). IgA antibodies to a protective antigen in human *Schistosoma mansoni*. *The Journal of Immunology*, **150**: 527-35.

Hagan, P., Blumenthal, U. J., Dunn, D., Simpson, A. J. and Wilkins, H. A. (1991). Human IgE, IgG4 and resistance to reinfection with *Schistosoma haematobium*. *Nature,* **349**: 243–245.

Hamburger, J. (2004). Large-scale, polymerase chain reaction-based surveillance of *Schistosoma haematobium* DNA in snails from transmission sites in coastal Kenya: a new tool for studying the dynamics of snail infection. *American Journal of Tropical Medicine and Hygiene*, **71**: 765–773.

Harn, D.A., Reynolds, S.R., Chikunguwo, S. Furlong, S. and Dahl, C. (1995). Synthetic peptide vaccines for Schistosomiasis. *Pharmaceutical Biotechnology*, **6**: 891–905.

Hatz, C. (2005). Schistosomiasis: an underestimated problem in industrialized countries? *Journal of Travel Medicine*, **12**:1–2.

Hayashi, S., Ohtake, H. and Koike, M. (2000). Laparoscopic diagnosis and clinical course of chronic Schistosomiasis *japonica*. *Acta Tropica*, **77**: 133–140.

Henderson, G.S., Lu, X., McCurley, T.L. and Colley D.G. (1992). *In vivo* molecular analysis of cytokines involved in the murine immune response during *Schistosoma mansoni* infection. *Journal of Immunology*, **147**: 2261-2269.

Hewitson, J.P., Hamblin, P.A. and Mountford, A.P. (2005). Immunity induced by the radiation-attenuated schistosome vaccine. *Parasite Immunology*, 27: 271–280.

Hillyer, G.V. and Gomez de Rios I. (1979). The enzyme-linked immunosorbent assay (ELISA) for the immunodiagnosis of schistosomiasis. *American Journal of Tropical Medicine and Hygiene*, **28**: 237-241.

James, S.L. (1992). Experimental models of immunization against schistosomes: lessons for vaccine development. *Immunological Investigations*, **21**: 477–493.

James, S.L., Cheever, A.W. and Wynn, T.A. (1999). Studies with double cytokine-deficient mice reveal that highly polarized Th1- and Th2-type cytokine and antibody responses contribute equally to vaccine-induced immunity to *Schistosoma mansoni*. *The Journal of Immunology,* **163:** 927–938.

Jankovic, D., Wynn, T.A., Kullberg, M.C., Hieny, S., Caspar, P., James, S.,Cheever, A. W. and Sher, A. (1999). Optimal Vaccination Against *Shistosoma mansoni* requires the Induction of Both B Cell- and IFN-Dependent Effector Mechanisms. *The Journal of Immunology, **162***: 345-351.

Jelinek, T., Nothdurft, H.D. and Loscher, T. (1996). Schistosomiasis in travelers and expatriates. *Journal of Travel Medicine*, **3**: 160–164.

Jordan, P. (2000). From Katayama to the Dakhla Oasis: the beginning of epidemiology and control of bilharzia. *Acta Tropica*, **77:** 9–40.

Jordan, P. and Webbe, G. (1993). Epidemiology In: Jordan, P., Webbe, G. and Sturrock, F.S. Editors, *Human schistosomiasis*, CAB International, Wallingford. pp 87–158.

Jordan, P., Webbe, G. and Sturrock, F.S. (1993). *Human schistosomiasis*, CAB International, Wallingford.

Jourdane, J. and Theron, A. (1987). Larval Development: Eggs to cercariae. In Rollinson, D and Simpson, A.J Eds (The Biology of Schistosomes): From Genes to Latrines., *Academic Press, London*. pp. 83-114.

Jwo, J. and LoVerde, P.T. (1989). Fractionated sera from *Schistosoma mansoni* infected patients confers passive protection in mice. *Journal of Parasitology, **75*:**252-260.

Kanamura, H.Y., Hoshino-Shimizu, S., Camargo, M.E. and da Silva, J.C. (1979). Class specific antibodies and fluorescent staining patterns in acute and chronic schistosomiasis mansoni. *American Journal of Tropical Medicine and Hygiene*, **28**: 242–248.

Kariuki, T.M., Farah, I.O., Yole, D.S., Mwenda, J.M., Van Dam, G. J., Deelder, A .M., Wilson, R.A. and Coulson, P.S. (2004). Parameters of the Attenuated Schistosome vaccine Evaluated in the Olive Baboon. *Infection and Immunity, **72***: 5526-5529.

Katz, N., Chavez, A. and Pellegrino, J. (1972). A simple device for quantitative stool thick-smear technique in schistosomiasis mansoni. *Revista do Instituto de Medicina Tropical de São Paulo*, **14**: 397-400.

Keiser, J. and Utzinger, J. (2004). Chemotherapy for major food-borne trematodes: a review. *Expert Opinion on Pharmacotherapy*, **5**:1711–1726.

King, C.H., Sturrock, R.F., Kariuki, H.C and Hamburger, J. (2006) Transmission control for schistosomiasis-why it matters now. *Trends in Parasitology*, **22**: 675-582.

King, C. L., Xianli, J., Malhotra, I., Liu, S., Mahmoud, A.A.F. and Oettgen, H. C. (1997). Mice with a Targeted Deletion of the IgE Gene Have Increased Worm Burdens and Reduced Granulomatous Inflammation Following Primary Infectin with *Schistosoma mansoni. The Journal of Immunology*, **158**: 294-300.

Kongs, A., Marks., G., Verlé., P. and Van der Stuyft., P. (2008). The unreliability of the Kato-Katz technique limits its usefulness for evaluating *S. mansoni* infections. *Tropical Medicine and International Health*, **6**: 163 - 169

Laamrani, H. and Boelee, E. (2002). The role of irrigation design and water management parameters in the ecology of transmission and control of schistosomiasis in central Morocco. *Cahiers d'études et de recherches francophones/Agricultures*, 11: 23–29.

Lambertucci, J.R. (1993). Acute schistosomiasis: clinical, diagnostic and therapeutic features. *Revista do Instituto de Medicina Tropical de Sao Paulo*, **35**:399–404.

Lewis, F.A., Sher, A and Colley, D.G. (1977). Failure of plasma from human schistosomiasis mansoni patients to protect mice from *Schistosoma mansoni* cercariae challenge. *American Journal of Tropical Medicine and Hygiene*, **26**: 723-726.

Liu, S., Song, G., Xu, Y., Yang, W. and McManus, D.P. (1995). Immunization of mice with recombinant Sjc26GST induces a pronounced anti-fecundity effect after experimental infection with Chinese Schistosoma japonicum. *Vaccine*, **13** (6): 603-7.

Loker, E.S. (1983). A comparative study of the life-histories of mammalian schistosomes. *Parasitology*, **111**: 167-175.

Lopez, A.D., Mathers, C.D., Ezzati, M., Jamison, D.T. and Murray, C.J.L. (2006). *Global burden of disease and risk factors*, Oxford University Press/The World Bank, New York/Washington.

Maizels, R. M. and Yazdanbakhsh, M. (2003). Immune regulation by helminth parasites: cellular and molecular mechanisms. *Nature Reviews Immunology*, **9**: 733–744.

Makoni, P., Chimbari, M.J. and Madsen, **H.** (2005).Interaction between fish and snails in a Zimbabwe pond, with particular reference to *Sargochromis codringtonii* (Pisces: cichlidae). *African Journal of Aquatic Science*, **30**: 45–48.

Mangold, B.L and Dean, D.A. (1986). Passive transfer with serum and IgG antibodies of irradiated cercariae-induced resistance against *Schistosoma mansoni* in mice. *The Journal of Immunology*, **136**: 2644-2648.

Mangold, B.L and Dean, D.A.(1992). The role of IgG antibodies from irradiated cercaria-immunized rabbits in the passive transfer of immunity to S*chistosoma mansoni*-infected mice. *American Journal of Tropical Medicine and Hygiene*, **46**(6):821-829.

Marti, R., Suter, M., Aellen, E., Ludin. and Degremont, A. A. (1987). Longitudinal study on the health status of children in a rural Tanzanian community: parasitoses and nutrition following control measures against intestinal parasites. *Acta Tropica*, **44**: 137–174.

McCormick, S.L. and Damian, R.T. (1987). Haptenation of adult *Schistosoma mansoni* and assessment of humorally mediated damage *in vitro*. *Journal of Parasitology*, **73**: 130–143.

Minai, M., Hosaka, Y. and Ohta, N. (2003). Historical view of schistosomiasis japonica in Japan: implementation and evaluation of disease-control strategies in Yamanashi Prefecture. *Parasitology International*, **52**: 321–326.

Morel, C. (2000). Reaching maturity- 25 years of the TDR. *Parasitology Today* **16**:522-528.

Mott, K.E., Desjeux, P., Moncayo, A., Ranque, P. and de Raadt, P. (1990). Parasitic diseases and urban development. *Bulletin of the World Health Organization*, **68**: 691–698.

Nyindo, M. and Farah, I.O. (1999). The baboon as non-human primate model of human schistosome infection. *Parasitology Today*, **15**: 478-482.

Nyindo, M., Kariuki, T.M., Mola, P.W., Farah, I.O., Elson, L., Blanton, R. E. and King, C.L. (1999). Role of adult worm antigens-specific immunoglobin E in acquired immunity to *Schistosoma mansoni* infection in baboons. *Infection and Immunity*. ***67 (2)*** : 636-642.

Ogilive, B. M., Smithers, S. R., and Terry, R. J.,1966. Reagin-like antibodies in experimental infections of *Schistosoma mansoni* and the passive transfer of resistance. *Nature*, **209**: 1221-1223.

Oomen, J.M.V., de Wolf, J. and Jobin, W.R. (1990). Health and irrigation. *ILRI publication*, **45**: ILRI, Wageningen.

Patz, J., Graczyk, T., Geller, N., Vittor, A. (2000). Effects of environmental change on emerging parasitic diseases. *International Journal for Parasitology*, **30**:1395-1405.

Pax, R., Bennett, J.L. and Fetterer, R. (1978). A benzodiazepine derivative and praziquantel: effects on musculature of *Schistosoma mansoni* and *Schistosoma japonicum*. *Naunyn-Schmiedeberg's Archives of Pharmacology*, **304**: 309–315.

Pearce, E.J. (2005). Priming of the immune response by schistosome eggs. *Parasite Immunology*, **27**: 265–270.

Pearce, E.J. James, S.L., Heing, S., Lanar, D. and Sher, A. (1988). Induction of protective immunity against *Schistosoma mansoni* by vaccination with schistosome paramyosin (Sm97), a non-surface parasite antigen. *Proceedings of the National Academy of Sciences, USA*, **85**: 678–5682.

Pica-Mattoccia, L., Valle, C., Basso, A., Troiani, A.R., Vigorosi, F., Liberti, P., Festucci, A. and Cioli, D. (2007). Cytochalasin D abolishes the schistosomicidal activity of praziquantel. *Experimental Parasitology*, **115**: 344–351.

Pointier, **J.P.** (1993). The introduction of *Melanoides tuberculata* (Mollusca: *Thiaridae*) to the island of Saint Lucia (West Indies) and its role in the decline of *Biomphalaria glabrata*, the snail intermediate hosts of *Schistosoma mansoni*. *Acta Tropica*, **54**: 13–18.

Polman, K. (2000). *Epidemiological application of circulating antigen detection in schistosomiasis [PhD dissertation]*, University of Leiden, Leiden.

Rabello, A. (1997). Diagnosing schistosomiasis. *Memorias do Instituto Oswaldo Cruz*, **92**: 669–676.

Rabello, A.L., Garcia, M.M., Pinto da Silva, R.A., Rocha, R.S. and Katz, N. (1997). Humoral immune responses in patients with acute *Schistosoma mansoni* infection who were followed up for two years after treatment. *Clinical Infectious Diseases*, **24**: 304–308.

Rabello, A.L.T., Garcia, M.M.A., Silva, R.A.P., Rocha, R.S., Chaves, A. and Katz, N. (1995). Humoral immune responses in acute schistosomiasis mansoni: relation to morbidity. *Clinical Infectious Diseases,* **21**: 608–615.

Renganathan and Cioli, D. (1998). An international initiative on praziquantel use. *Parasitology Today*, **14**: 390–391.

Renquist, D. M., Johnson, A. J., Lewis, J. C. and Johnson, D. J. (1975). A natural case of *Schistosoma mansoni* in the chimpanzee (Pan troglodytes versus). *Laboratory Animal Science*, ***25***: 763–768.

Richter, D. and Harn, D.A. (1993). Candidate vaccine antigens identified by antibodies from mice vaccinated with 15- or 50-kilorad-irradiated cercariae of Schistosoma mansoni. *Infection Immunology*, **61**: 146–154

Richter, D., Harn, D.A. and Matuschka, F.R. (1995). The Irradiated Cercariae Vaccine Model: Looking on the Bright Side of Radiation. *Parasitology Today*, ***8***: 288-293.

Rihet, P., Demeure, C.E., Bourgois, A., Prata, A. and Dessein, A.J. (1991). Evidence for an association between human resistance to *Schistosoma mansoni* and high anti-larval IgE levels. *European Journal of Immunology*, **21**: 2679–2686.

Ross, A.G.P., Li, Y., Williams, G., Jiang, Z. and McManus, D.P. (2001). Dam worms. *Biologist (London)*, **48**:121-124.

Rutitzky, L.I.,Hernandez, H..J. and Stadecker, M..J. (2001). Th-1 polarizing immunization with egg antigens correlates with severe exacerbation of immunopathology and death in schistosome infection. *Proceedings of the National Academy of Sciences*, **98**: 13243-13248.

Sabah, A.A., Fletcher, C., Webbe, G. and Doenhoff, M.J. (1986). *Schistosoma mansoni*: chemotherapy of infections of different ages. *Experimental Parasitology*, **61**: 294–303.

Sadun, E. H., von Lichtenberg, F., Hickman, R. L., Bruce, J. I., Smith, J. H. and Schoenbechler, M. J. (1966). Schistosomiasis mansoni in the chimpanzee: parasitologic, clinical, serologic, pathologic and radiologic observations. *American Journal of Tropical Medicine and Hygiene*, **15**: 496–506.

Shalaby, K.A., Yin, L., Thakur, A., Christen, L., Niles, E.G. and Lo-Verde, P.T. (2003). Protection against *Schistosoma mansoni* utilizing DNA vaccination with genes encoding Cu/Zn cytosolic superoxide dismutase, signal peptide-containing superoxide dismutase and glutathione peroxidase enzymes. *Vaccine*, **22**: 130–136.

Sher, A., Hieny, S. and James, S. L. (1982). Mechanisms of protective immunity against *S. mansoni* infection in mice vaccinated with irradiated cercariae. *Journal of Immunology,* **128**: 1800-84.

Sher, A., Hieny, S. and James, S. (1984). Mechanisms of protective immunity against *S. mansoni* infection in mice vaccinated with irradiated cercariae. Influence of the major histocompatibility complex. *Parasite Immunology,* **6**: 319-328.

Sher, A. Gazzinelli, R., Hakim, F., de la Cruz, V.F., Stover, V.F., James, S.L. and Pearce, E. (1991). Immunization against parasites: bridging the gap between attenuated and non-living vaccines. *Behring Institute Mitteilungen,* **88**: 244–248.

Shiff, C. (2000). Epidemiology of helminth infections. In: KE Nelson, C Masters Williams and NM Graham, Editors, *Infectious disease epidemiology: theory and practice,* Aspen Publishers, Gaithersburg .

Sleigh, A. (1991). WHO/WPRO consultant mission report. WHO-Ministry of Public Health National Workshop on Schistosomiasis Control in Lake Regions, Nanchang, Jiangxi Province, People's Republic of China, November 19–28, 1990. Manila, the Philippines: WHO Western Pacific Regional Office. (Report series no. RS/90/0293.)

Slootweg, R., Malek, E.A. and McCullough, **F.S.** (1994). The biological control of snail intermediate host of schistosomiasis by fish. *Reviews in Fish Biology and Fisheries*, **4**: 67–90.

Smithers, S. and Terry R.J. (1965). The infection of laboratory hosts with cercariae of *Schistosoma mansoni* and the recovery of adult worms. *Parasitology,* **86**: 695-700.

Smythies, L.E., Betts, C., Coulson, P.S., Dowling, M.A. and Wilson, R.A. (1996). Kinetics and mechanism of effector focus formation in the lungs of mice vaccinated with irradiated cercariae of *Schistosoma mansoni*. *Parasite Immunology,* **18**: 359–369.

Soisson, L.A., Reid, G.D., Farah, I.O., Nyindo, M. and Strand, M. (1993). Protective immunity in baboons vaccinated with a recombinant antigen or radiation-attenuated cercariae of *Schistosoma mansoni* is antibody-dependent. *The Journal of Immunology*, **151**: 4782–4789.

Sow, S., De Vlas, S.J., Mbaye, A., Polman, K. and Gryseels, B. (2003). Low awareness of intestinal schistosomiasis in northern Senegal after 7 years of health education as part of intense control and research activities. *Tropical Medicine &International Health,* **8**: 744–749.

Steinmann, P., Keiser, J., Bos, R., Tanner, M. and Utzinger, J. (2006). Schistosomiasis and water resources development: systematic review, meta-analysis, and estimates of people at risk. *The Lancet Infectious Diseases,* **6**: 411–425.

Stirewalt, M.A and Uy, A. (1969). *Schistosoma mansoni*: cercarial penetration and schistosomule collection in an in vitro system. *Experimental parasitology*, **26**: 17.

Sturrock, R.F. (1986). A review of the use of primates in studying human schistosomiasis. *Journal of Medical Primatology*, **15**: 267–279.

Suzuki, T. and Damian, R.T. (1981). *Schistosoma mansoni* in baboons. IV. Development of antibodies to *Schistosoma mansoni* adult worm, egg, and cercarial antigens during acute and chronic infections. *American Journal of Tropical Medicine and Hygiene*, **30**: 825–835.

Tendler, M. and Pinto, R. M. (1981). A simple device to immobilize mice for infection with *Schistosoma mansoni* cercariae. *Journal of Parasitology*, **67**: 583-584.

Tinga, N., De, N., Vien, H.V., Chau, L., Toan, N.D., Kager, P.A. and Vries, P.J. (1999). Little effect of praziquantel or artemisinin on clonorchiasis in Northern Vietnam. A pilot study. *Tropical Medicine & International Health*, **4**: 814–818.

Todd, C.W. and Colley, D.G. (2002). Practical and ethical issues in the development of a vaccine against schistosomiasis mansoni. *American Journal of Tropical Medicine and Hygiene*, **66**: 348–358.

Todt, J.C., Whitfield, J.R., Ivard, S.R. and Boros D.L. (2000). Down regulation of interleukin-12, interleukin-12R expression/activity mediates the switch from Th-1 to Th-2 granuloma response during murine schistosomiasis mansoni. *Scandinavian Journal of Immunology*, **52**: 385-392.

Tsang, V.C. and Wilkins, P.P. (1997). Immunodiagnosis of schistosomiasis. *Immunological Investigations*, **26**:175-188.

Van Dam, G.J., Wichers, J.H., Ferreira, T.M.F., Ghati, D., Van Amerongen, A. and Deelder, A.M. (2004). Diagnosis of schistosomiasis by reagent strip test for detection of circulating cathodic antigen. *Journal of Clinical Microbiology*, **42**: 5458–5461.

Van Lieshout, L., Polderman, A.M., Visser, L.G., Verwey, J.J. and Deelder, A.M. (1997). Detection of the circulating antigens CAA and CCA in a group of Dutch travellers with acute schistosomiasis. *Tropical Medicine &International Health*, **2**:551–557.

Verity, C.K., McManus, D.P. and brindley, P. J. (2001). Vaccine efficacy of recombinant cathepsin D spartic protease from *Schistosoma japonicum*. *Parasite Immunology*, **23**: 153-162

Viana, I. R., Corre^a-Oliveira, R.., Carvalho, O. S., Massara, C. L., Colosimo, E., Colley, D. G. and Gazzinelli, G. (1995). Comparison of antibody isotype responses to

Schistosoma mansoni antigens by infected and putative resistant individuals living in an endemic area. *Parasite Immunology*, **17**: 297–304.

von Lichtenberg, F., Sadun, E. H., Cheever, A. W., Erickson, D. G., Johnson, A. J. and Boyce, H. W. (1971). Experimental infection with Schistosoma japonicum in chimpanzees. *American Journal of Tropical Medicine and Hygiene*, **20**: 850–893.

Warren, K.S. and Peters, P.A. (1967). Comparison of penetration and maturation of Schistosoma mansoni in the hamster, mouse, guinea pig, rabbit, and the rat. *American Journal of Tropical Medicine and Hygiene*, **16 (6)**: 718–722.

Wang, X., Li, S. and Zhou, Z. (1999). A rapid one-step method of EIA for detection of circulating antigen of Schistosoma japonicum. *Chinese Medical Journal (English Edition)*, **112**:124-128.

Warren, K. S. 1973. The pathology of schistosome infections. *Helminthological Abstracts Series A*, **42:** 590-633.

Weinzettl, M. and Jurberg, **P.** (1990). Biological control of *Biomphalaria tenagophila* (Mollusca, Planorbidae), a schistosomiasis vector, using the fish *Geophagus brasiliensis* (Pisces, cichlidea) in the laboratory or in a seminatural environment. *Memorias do Instituto Oswaldo Cruz*, **85**: 35–38.

WHO, (2002). Prevention and control of Schistosomiasis and Soil-transmitted Helminthiasis, Report of a WHO Expert Committee, World Health Organization Technical Report Series, volume 912.

Woolhouse, M.E. and Hagan, P. (1999). Seeking the ghost of worms past. *Nature Medicine*, **5**: 1225–1227.

Woolhouse, M.E. (1989). The effect of schistosome infection on the mortality rates of *Bulinus globosus* and *Biomphalaria pfeifferi*. *Annals of Tropical Medicine and Parasitology*, **83**: 137–141.

Wynn, T.A. (1996). Development of an antipathology vaccine for schistosomiasis. *Annals of the New York Academy of Sciences*, **797**: 191–195.

Yole, S.D., Pemberton, R., Reid, D.D.F., and Wilson, R.A. (1996). Protective immunity to *Schistosoma mansoni* induced in the olive baboon *Papio anubis* by the irradiated cercariae vaccine. *Parasitology*, **112**: 37-46.

Zhang, W. and Wong, C.M. (2003). Evaluation of the 1992–1999 world bank schistosomiasis control project in China. *Acta Tropica*, **85**: 303–313.

APPENDIX I

HARRIS' HEAMATOXYLIN STAIN

Hamatoxylin crystals	5.0 gm
Alcohol, 100%	50 ml
Ammonium or potassium alum	100 gm
Distilled H_2O	1000 ml
Mercuric Oxide (red)	2.5 gm
Glacial acetic acid	30 ml

EOSIN STAIN

Eosin Stock Solution

Eosin Y	1 gm
Distilled H_2O	20 ml
95% ETOH	80 ml

Eosin Working Solution (used to stain slides)

Eosin Stock Solution	250 ml
80% ETOH	750 ml
Glacial Acetic acid	5.0 ml