CLEARANCE OF SOLUBLE EGG ANTIGEN IN *SCHISTOSOMA HAEMATOBIUM* INFECTION IN CHILDREN AFTER PRAZIQUANTEL TREATMENT.

BY

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DECLARATION

I declare that this thesis is my own original work and has not been presented for a degree in any other university or institution.

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DEDICATION

This thesis is dedicated to my wife, Ruth Kirote, my children Mdara, Nanzia, Iduri and Mrisha and to my late father and mother whose words, *"mwenye radhi hasumbuki, mpewa hapokonyeki"* inspired me.

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ABSTRACT

Schistosomiasis, a disease caused by trematode worms of the genus *Schistosoma,* afflicts both humans and animals. *Schistosoma mansoni* and *Schistosoma haematobium* are the two most important human species found in Africa. The schistosome life cycle involves an alteration of generations in two different hosts, with the asexual multiplying stage in the intermediate snail host (*Biomphalaria* or *Bulinus*) species and a sexual non-multiplying stage in the definitive host (human). Mature adult female worms deposit eggs into the mesenteric veins of the small intestine or blood vessels around the plexus of the bladder. Diagnosis of schistosomiasis is based on microscopic detection of eggs in urine/ stool or abdomen scanning using ultrasound machine or reaction of reagent strips dipped in urine. These methods are cumbersome, time consuming, inaccurate and expensive. Recently, an enzyme linked immuno-sorbent assay for detection of *S. haematobium* soluble egg antigen (SEA) has been developed. Detection of the antigen derived from the eggs in urine and those trapped in the tissues, is a non-invasive method that is simple, specific, reliable and gives an indication of an active infection. The objective of the study was to establish whether or not there is an age and sex-related difference in the clearance of SEA and whether SEA can serve as a marker of pathology. This study was conducted in Jimba location, Kilifi District, Coast Province of Kenya, where *S. haematobium* is endemic. Urine from school children aged between 4 – 18 years were examined for eggs of *S. haematobium* using urine samples filtration technique. ELISA was performed on urine samples from children examined for egg counts. The overall prevalence of *S. haematobium* was 92.8% with

higher infection rates in males (95.45%) than in females (89.50%). All schistosome egg positive children (a total of 372) were treated with a single oral dose 40-mg of praziquantel per kg body weight. A cohort of one hundred and fifty eight pupils consistently participated in the study. Follow up was conducted over a period of 33 days. Both the clearance of SEA and egg counts showed a gradual significant decrease after praziquatel treatment. There was a significant difference in the prevalence of *S. haematobium* between male and female children ($t = 2.164$, $P < 0.05$). There was no statistical difference in the clearance of SEA and egg counts in urine between male and female children. There was also a significant difference between the egg count clearance in age groups ≤ 5 years and 9 – 11 years $P = 0.012$), and 12 – 14 years ($P = 0.019$ respectively. 9 –11 years. Comparison of SEA clearance among the various age groups indicated a significant difference between age groups ≤ 5 years and 6 – 8 years ($P = 0.012$), 9 - 11 years ($P = 0.003$) and 12 – 14 years ($P = 0.006$) respectively. The results showed that there was a correlation between clearance of *S. haematobium* soluble egg antigen and egg counts in urine of infected children (pearson's correlation coefficient) ($r = 0.981$, $P < 0.010$). Also *S. haematobium* SEA would be detected in urine even when there were no eggs being excreted any more. The results would be helpful in the development of a SEA based assay for field diagnosis of morbidity in endemic areas. These results suggest that SEA assay can be used in *S. haematobium* endemic areas where prevalence is high or low and is highly sensitive irrespective of age or sex of the children.

CHAPTER ONE

1. 0. INTRODUCTION

1.1. Schistosomiasis.

Schistosomiasis (bilharziasis) a disease caused by digenetic trematodes in the class Trematoda and in the genus *Schistosoma* afflicts both humans and domestic animals. Six species of *Schistosoma* primarily infect humans; *S. haematobium* (Bilharz, 1852), S. *mansoni*, *S. japonicum* (Katsurada, 1904), *S. intercalatum* (Fisher, 1934), S. *mekongi* (Voge et al., 1978), and *S. malaysiensis* (Greer *et al.,* 1980,). It is currently rated second only to malaria in socio-economic and public health importance in tropical and sub-tropical regions of the world (Iarotski and Davis, 1981; WHO, 1985; Doumenge *et al.,* 1987). Figure 1 shows the global distribution of schistosomiasis.

Over 200 million people in 76 countries of the world are infected and approximately 600 million persons are at risk in Africa, Asia, South America and the Caribbean Islands. Approximately, 90 million persons in 53 countries are infected with *S. haematobium* (WHO, 1993). The figure could, however, be higher in view of the continued establishment of man-made dams and irrigation schemes all over Africa, which results in expanding the breeding habitats of the bulinid intermediate hosts (McMullen *et al.,* 1962). Eight hundred thousand deaths per year are associated with the disease in Africa alone, accounting for 85% of the infected people (Newport and Colley, 1993). The two most important species are *S. haematobium* and *S. mansoni,* both of which are endemic in Africa.

In Kenya, schistosomiasis has progressively increased over the past three decades. Current estimates show that more than three million Kenyans are infected with either one or both species of the parasites and approximately10 million are at risk of infection (Muchiri *et al.*, 1996). *S. haematobium* is endemic throughout the Coastal, Eastern, Western and Northeastern regions (Kinoti, 1971 (a and b); Warren *et al.,* 1978). *Schistosoma mansoni* is mainly found in Machakos, Kitui, parts of Kirinyaga, Kiambu and along the shores of lake Victoria (Highton, 1974: Arap Siongok, *et al.,* 1976).

The distribution of the disease follows the distribution of the intermediate hosts (*Bulinus and Biomphalaria*) and is found at altitudes ranging between 10m and 1500m above sea level. The main intermediate hosts for *S. haematobium* along the coast are *Bulinus globosus* and *B. nasutus* members of the *B. africanus* group (Brown *et al.,* 1981; Otieno, 1999). Many countries in sub-Saharan Africa including Kenya, have several freshwater snails, which transmit trematodes that afflict human health (most notably *S. mansoni* and *S. haematobium*) and livestock health e.g. *S. bovis*, and S. *matheii* (Diesfeld and Hecklan,1978; Brown, 1981;1994).

S. haematobium like other trematode worms, have a complex life cycle and develop by a succession of stages, which involve alternating parasitic and free living forms. In the mammalian host, the adult worms produce a large number of eggs. A single female *S. haematobium* produces 300 eggs per day (WHO, 1985).

These eggs are passed out in urine or become trapped in the host tissues. The eggs, which are passed out through urine, hatch in fresh water to release free-swimming

forms (miracidia), which invade aquatic fresh water snails of the genus *Bulinus* (Jordan and Webbe, 1982).

In the snail, the miracidia transform into sporocysts, which undergo asexual multiplication and are released as cercariae into the water in large numbers. This stage infects man after skin penetration and transforms into a schistosomulum. It migrates via the lymphatics and blood vessels through the heart and lungs to the hepatic portal system. Maturation to adulthood, pairing and mating takes place in the liver where the paired worms migrate to the pelvic area, and in about 4-7 weeks after infection, egg production starts (Warren and Peters, 1967).

The embryo protected by the eggshell develops into a ciliated organism (miracidia) within six days (Sturrock, 1993) and begins to secrete proteolytic enzymes, which are released through ultramicroscopic pores in the eggshell. The proteolytic enzymes digest the surrounding host tissues, enabling eggs to pass out of the venules into the bladder. The egg reach water in urine and soon hatch releasing a fully developed free-swimming miracidia to continue the life cycle (Warren, 1973). A terminal spine characterizes *S. haematobium* eggs.

1. 2. Chemothrapy

Several effective and safe drugs have been developed for treatment of schistosomiasis such as praziquantel, metrifonate and oxamnoquine. Praziquantel is currently the drug of choice for the treatment of schistosomiasis. There are three recognized types of drug regimes: mass chemotherapy which is aimed at the whole population without prior diagnosis; selective population chemotherapy which is used especially for treatment of children since they are the most heavily infected and

likely to develop severe disease and targeted chemotherapy which is aimed at a selected section of the population with heavy infection who contribute highly to the contamination of the environment with schistosome eggs. Though chemotherapy reduces the prevalence and intensity of infection, re-infection often occurs after treatment (WHO, 1999; WHO, 1985).

1. 3. Diagnosis of *S. haematobium*

The various techniques used to diagnose *S. haematobium* are microscopy, which is expensive and inaccurate especially for light infections; ultrasonography which is also expensive and requires trained manpower to operate; and the use of antigens which tests the existence of antibodies but not necessarily active infections (Feldmeier and Poggensee, 1993) Serological techniques are used to improve the diagnostic record in endemic areas characterized by low level of transmission (Kremsner *et al.*,1994). In clinical settings, they are developed for diagnostic purposes either to demonstrate the appearance of antibody or to detect parasitic antigens (Lisette *et al.*, 1997).

At various stages of the parasite life cycle including cerceriae, adult worms and eggs, antigens are secreted into the circulation of the host (Abdel-Hafez *et al.*, 1983; Hayunga *et al.*, 1986). Egg antigens are mainly present in the hatching fluid of viable eggs (Hassan *et al.*, 1992; Ripert *et al.*, 1988; Bosompen *et al.*, 1996). Hassan *et al,:* (1998) used a monoclonal antibody 128C3 to detect antigens in serum of *S. haematobium* infected school children and showed a sensitivity of 100% and a specificity of 99% with no cross reactivity with other parasites. Unlike the detection of adult worm antigens that provide information on the worm burden, the detection

of soluble egg antigen (SEA) in urine could provide more information on the egg burden and might be a better parameter for the assessment of morbidity in *S. haematobium* infection (Kahama *et al.*, 1998).

1. 4. Justification

Kenya has a population of 28 million people (Government of Kenya population census, 1999) and it is estimated that three million or just fewer than 10% of the population are infected with either urinary or intestinal schistosomiasis, whereas a further 10 million inhabitants are at risk of infection. Majority of patients suffering from schistosomiasis come from nine endemic districts in which prevalence exceeds 25% (MOH annual medical report, 1980).

Schistosomiasis is the fourth most frequently noted infectious disease in the Coast Province and the fifth in Eastern and Nyanza Provinces. It causes economic losses due to work and school absenteeism, thereby reducing productivity and increase school dropout rate in endemic areas.

Rapid inexpensive and reliable diagnostic methods are necessary in the treatment, morbidity assessment, monitoring, evaluation and control strategies (Uga *et al.*, 1989). Parasitological methods, which rely on the microscopic detection of ova of *S. haematobium* in urine, are cumbersome, have low sensitivity and are time consuming (WHO, 1985). *Schistosoma haematobium* egg counts is the standard method of diagnosis; however, variation in the parasite egg excretion may lead to incorrect diagnosis and estimates in prevalence and intensity of infection (Feldmeier and Poggensee, 1993).

Detection of circulating egg antigens provides a simple measure of the degree of infection with *S. haematobium* (Zwingenberger *et al.,* 1989). Since soluble egg antigen is secreted by live miracidia developing within the eggs around the bladder tissues, its detection is indicative of active *S. haematobium* infection and can be developed as a simple tool for the assessment of morbidity in *S. haematobium* infection (Kahama *et al.,* 1998). During clearance of soluble egg antigen (SEA) from the body, it is not well understood whether there is any difference in the clearance rate between sex, age or intensity of infection (egg counts). In addition to being a diagnostic tool, SEA in urine can also be used in quantifying the true effect of chemotherapy and for monitoring drug resistance (Kahama *et al.,* 1998).

1. 5. Hypothesis

After treatment with praziquantel, the *S. haematobium* soluble egg antigen (SEA) continues to be detected in urine over a period of time and there is no difference in the clearance rate of SEA in relation to age, sex and egg load (morbidity).

1. 6. 0. OBJECTIVES OF THE STUDY

1. 6.1. General objective

To assess the clearance rate of *S. haematobium* soluble egg antigen (SEA) from infected school children after treatment with praziquantel.

1. 6. 2. Specific objectives

1. To determine the rate of the day-to-day clearance of *S. haematobium* soluble egg antigen from the human body after treatment with a single dose of 40mg praziquantel /kg-body weight.
2. To determine age and sex related differences in the clearance of soluble egg antigen (SEA) in urine following treatment with praziquantel.
3. To determine the difference in the mean egg counts between age groups and sex of the infected school children.
4. To correlate the levels of *S. haematobium* soluble egg antigen (SEA) with egg counts in urine before praziquatel treatment.

CHAPTER TWO

2. 0. LITERATURE REVIEW

2. 1. Geographical distribution of schistosomiasis

Schistosomiasis is endemic in 76 countries of the world and 600 million people are exposed to infection due to poverty, ignorance, poor housing, substandard hygienic practices and the availability of few, if any, sanitary facilities. Figure 1 shows the global distribution of schistosomiasis by species.

S. chistosoma mansoni, which causes intestinal schistosomiasis, is endemic in most African countries, parts of Arabia, in northern and eastern parts of South America and in some Caribbean Islands. *Schistosoma japonicum*, which also causes intestinal schistosomiasis, occurs in the Philippines, Japan, China, and some parts of Thailand and Indonesia. Also, *S. mekongi* occurs in the Far East. *S. chistosoma malaysiensis* occurs in Malaysia. *Schistosoma intercalatum* also causes intestinal schistosomiasis and occurs in the forest areas of West and Central Africa and the islands of Sao Tome. *S. chistosoma haematobium* causes urinary schistosomiasis and occurs in most African and some Middle Eastern countries (WHO, 1985).

This geographical distribution of the disease is dependent on the distribution of the snails (Kinoti, 1971a,b). In areas where the two species of snails coexist, also, both *S. haematobium* and *S. mansoni* infections occur. Schistosome hybrids also exist. Natural hybrids between *S. haematobium* and *S. intercalatum* have been found in, Cameroon. In South Africa, hybrids between *S. matheei* and *S. haematobium* and /or *S. mansoni* have been found (Kruger, *et al* 1986a).

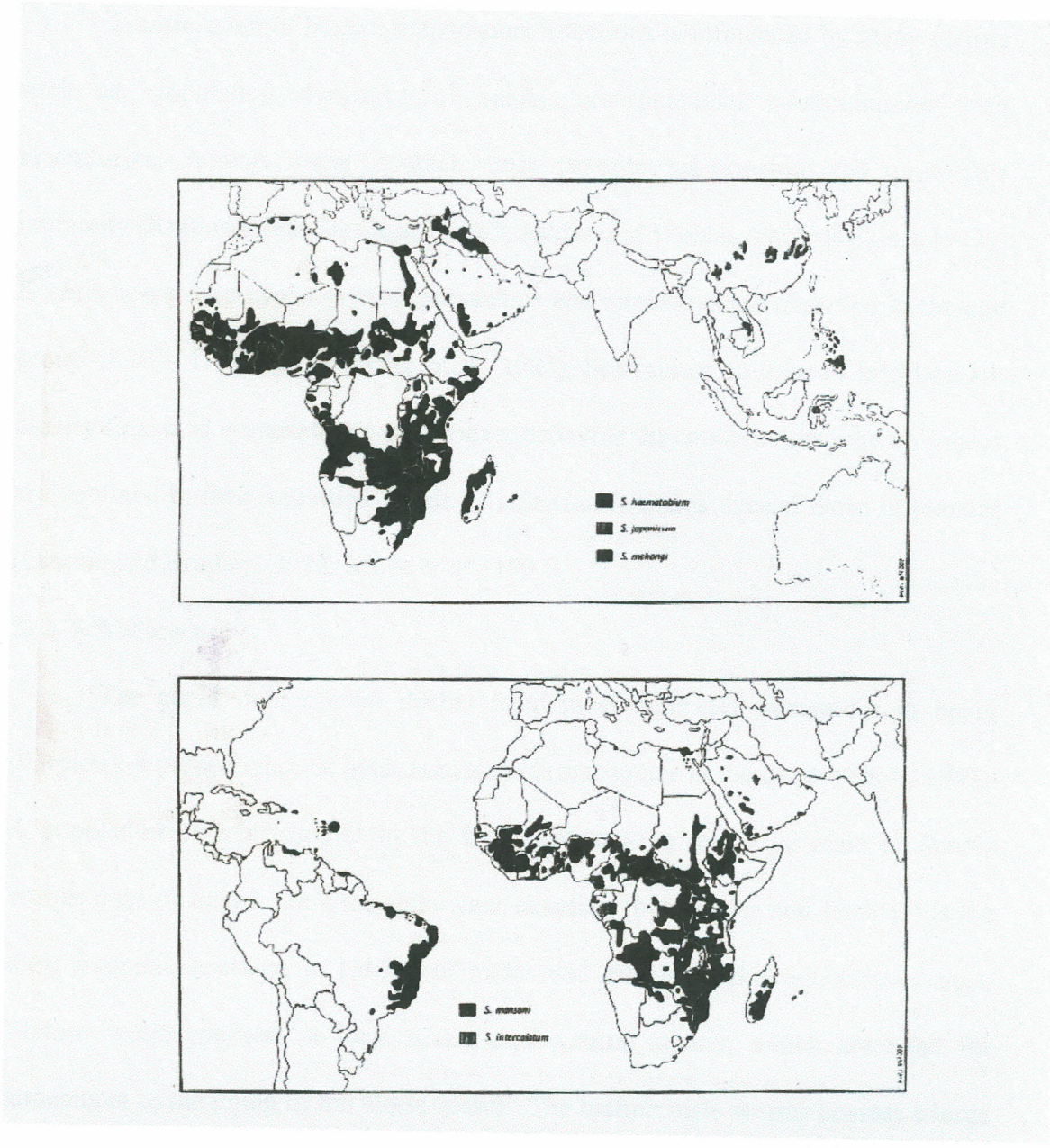


Fig. 1. Global distribution of schistosomiasis (Courtesy WHO, 1987).

Top: *Schistosoma haematobium*, *S. japonicum* and *S. mekongi*.

Bottom: *S. mansoni* and *S. intercalatum*.

Transmission of human schistosome infections is influenced by many factors such as, population dynamics of snails, environmental contamination with faeces/urine, human water contact, host parasite relationship and protective immunity (Rollinson and Southgate, 1987; Jordan and Webbe, 1982; Wilkins, 1977). *S. chistosoma haematobium* peak prevalence and intensities are observed in the age group of 10 – 15 years (Doehring *et al.*, 1983). Sex-related patterns of infection are closely linked to sex-related water contact behavior. In communities where females are confined in their activities, levels of infection in males exceed those in females (Ongom and Bradley, 1972; Kloos *et al.*, 1997).

2. 2. Schistosomes

The genus *Schistosoma* differs from most digenetic trematodes in being dioecious, a consequence of heteromorphic chromosomes in the ovum (Short, 1983). A population of schistosomes in the final host may be unisexual male or female worms only or mixed. In nature, the later situation (both males and females) is the most frequent, resulting in pairing of males and females and production of eggs. Mature worms possess an oral sucker and ventral sucker, which are used for attachment to the lining of the blood vessel. The mature male worms possess a large ventral groove, the gynaecophoric canal, in which the female is retained during pairing. The external surface is characterized by an abundance of papillae in some species.

2. 3. The life cycle of schistosomes

Schistosomiasis infection involves two different hosts in its life cycle; the asexual multiplication takes place in the molluscan intermediate host. The

intermediate hosts for *S. mansoni* and *S. haematobium* belong to the genus *Biomphalaria* and *Bulinus,* respectively. The non-multiplication stage occurs in the definitive vertebrate hosts (man, cattle, goats, rodents, monkeys etc).

Excreted eggs hatch when in contact with freshwater and the escaping ciliated larvae (miracidia) live freely in the water for up to 72 hours. The miracidia has to penetrate an appropriate intermediate snail host for further development (Sturrock, 1993). In the suitable snail vector, the miracidium is transformed into mother sporocytes (primary), which in turn produces many daughter sporocytes (secondary). The daughter sporocytes migrate to the gonads and digestive glands where they grow and multiply, eventually releasing many unisexual cercariae through the snail tissues into the water in a process that is dependent on temperature and light. The free living cercariae have a short life span and most of them die within 48 hours; thus, they must penetrate a human definitive host within this short span of time for the life cycle to continue (Sturrock, 1993) (Fig. 2).

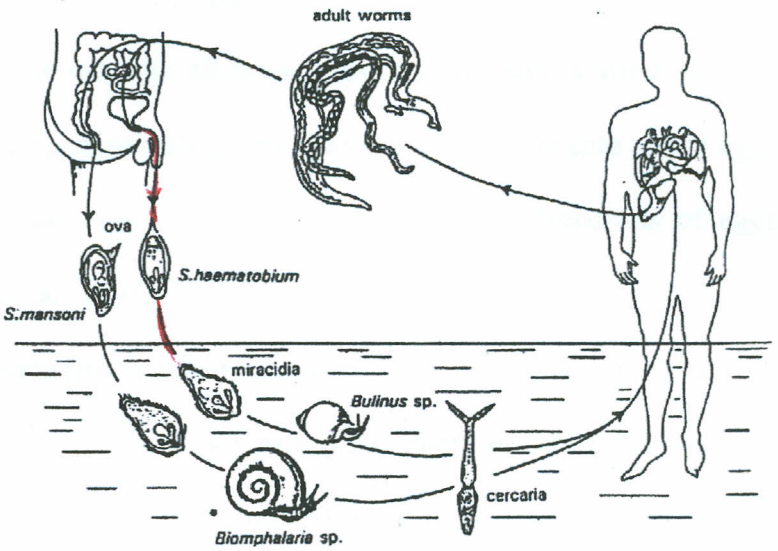
Humans become infected when the skin is exposed to water containing cercariae. On penetrating the human skin, the cercariae lose their tail and become adapted to the greater osmotic environment of the body. They subsequently transform into schistosomula. Under the skin, a schistosomulum stays for about 48 hours, elongates and is then transported passively by the blood flow via the left side of the heart and the lungs to eventually reach the liver where the worms pair and attain maturity. The length of the pre-patent period may vary (Hsu and Hsu, 1958; Loker, 1983; Basch, 1991). In *S. mansoni,* it may be as short as 35 days while in *S.*

haematobium the sexual maturity is reached by day 31and the pre-patent period is about 70 days (Webbe and James, 1971; Clegg *et al.*, 1976).

Depending on the schistosome species, the paired worms either migrate to the mesenteric veins (*S. mansoni,*) or to the veins of the vesicle plexus especially of the bladder (*S. haematobium*). The female worm may vacate the gynaecophorical canal to be able to migrate further into the venule, where she develops and lay her eggs (Sturrock, 1993). Subsequently, the female retreats into a larger venule thereby allowing the smaller venule to contract around the egg. These eggs retain their position after the body of the parent worm has been withdrawn. The embryo protected by the eggshell develops into a ciliated organism (miracidia) within 6 days (Sturrock, 1993). The embryo within the egg matures rapidly and secretes a proteolytic enzyme, which passes through the porous eggshell and causes necrosis of the vessel wall and adjacent tissue.

Passage of the egg through the vein wall and tissues to the lumen or bladder is aided by the release of proteolytic enzymes secreted by the miracidium. This migration takes several days to weeks and more than 50% of all eggs deposited remain trapped within the body tissues of the host, with many ending up in the liver. In the tissue around the plexus of the bladder, the miracidia secretes soluble egg antigens, which are released through ultra microscopic pores on the eggshell into the circulation. The antigens then pass through the kidney into the urine in the bladder (Hayunga *et al.*, 1987; Kahama *et al.*, 1998). The eggs that may reach water either in urine or faeces soon hatch releasing fully developed free-swimming miracidia to continue the life cycle (Warren, 1973). The process of egg excretion including the

proportion of the eggs trapped in the tissues and that excreted through the bladder wall is not clearly understood and estimates rely on studies from animal models (Rollinson and Simpson, 1987).



Life cycle of *Schistosoma mansoni* and *Schistosoma haematobium* (Courtesy of Dr. A.M. Deelder)

Fig. 2. Life cycle of schistosomes

2. 4. Pathology

The three distinct syndromes caused by schistosomiasis include cercariae dermatitis or "swimmers itch", acute schistosomiasis or Katayama fever and chronic schistosomiasis (Bruijuing, 1982). The *Schistosoma* species infecting man affect the urinary and intestinal system and involvement of the ovaries in schistosomiasis has also been reported (Tiboldi, 1978). There have also been reports of renal myloidosis

and nephritic syndromes in patients with schistosomiasis (Barsoum *et al.*, 1979; Strausbaugh *et al.*, 1978).

There is also evidence that urinary schistosomiasis can predispose to bladder carcinoma (Mostafa *et al.*, 1995). There are other ectopic sites of schistosomiasis involvement including the central nervous system and the lungs (Scrimgeour and Ciadusek, 1985). The pathogenesis and clinical manifestation of schistosomiasis are essentially the same for all the species of schistosomes infecting man but differences arise with respect to differences in location of the parasite and in egg-laying capacity of the worms. Severe disease begins with the deposition of eggs in the tissues (Mostafa *et al.*, 1995).

2. 5. 0. Morbidity in schistosomiasis

The egg is the major pathogenic agent in *Schistosoma* infection, while adult worms induce only limited pathological damage to the host (Warren, 1973). The pathological consequence of *Schistosoma* infection varies from sub-clinical and mild, to very severe, depending on the intensity of infection as reflected in the number of eggs, which are deposited in the body (Cook *et al.*, 1974). In mild infections, the granulomatous reactions and subsequent fibrotic changes are limited, whereas in heavier *S. haematobium* infection, extensive fibrosis of the urinary bladder and ureter may occur. This may lead to upper and lower urinary tract lesions like ureteric stenosis, hydroureter, hydronephrosis and bladder carcinoma (Bruijuing, 1982).

Initial symptoms of chronic schistosomiasis may include diarrhea, dysentery, abdominal pain, loss of weight, proteinuria and haematuria. Severe infection may

result in portal hypertension, hepatomegaly, splenomegaly, ascites, esophageal and gastric varices for intestinal schistosomiasis or obstruction of the urinary tract for urinary schistosomiasis (Anderson, 1985). Migrating, *S. haematobium* worms may be lodged in the veins of the female genital organs where egg deposition in the vagina and cervix results in lesions leading to female genital schistosomiasis (FGS) (Feldmeier *et al.*, 1994). Up to 75% of the women in *schistosomiasis haematobia* endemic areas may suffer lesions of the uterine, cervix, vagina or vulva. Female genital schistosomiasis could be a predisposing factor to the spread of sexually transmitted diseases including HIV (Feldmeier *et al.*, 1994; 1995).

Bleeding varices are the major causes of death. Five to ten percent of infected individuals develop serious disease. This proportion shows regional and ethnic differences (Gryseels, 1989). It is not yet known whether the differences result from variations in transmission dynamics, genetic/immunological or parasite related factors or the interaction with other infections such as malaria. There is evidence of an association between egg counts and morbidity. This is probably due to a time lag between heavy infection and development of severe chronic pathology (Gryseels, 1989).

2. 5. 1. Schistosome dermatitis or swimmers itch

Cercarial penetration of the skin can provoke a rash consisting of round erythematous papules caused by an acute inflammatory reaction to cercariae. This is known as cercarial dermatitis or swimmers itch (Boros, 1989). The symptoms are usually mild. Severe dermatitis in human may occur in infections with bird or

mammal schistosome cercariae; however, they do not mature in man but may migrate to central nervous system and cause pathology.

2. 5. 2. Katayama fever

Acute schistosomiasis or Katayama fever is often seen on primary infection in non-immune individuals visiting endemic areas (Boros, 1989; Cheever and Yap, 1997). It is an acute toxemic syndrome associated with the maturation of schistosomula and the onset of egg production and occurs 3 to 9 weeks after infection (Boros, 1989). Patients suffering from Katayama fever experience daily afternoon fevers, malaise, chills, perspiration, headache, slight cough and diarrhea. Extensive urticaria occurs in huge patches on various parts of the body, and generalized lymphodenopathy. The syndrome is self- limiting with a few fatal cases reported. The already existing and cross-reacting antibodies produced in response to the presence of the developing worms could lead to allergic bronchitis (Warren, 1973).

2. 5. 3. Chronic schistosomiasis

The main impact of schistosomiasis on public health is due to the chronic nature of the disease. Half the number of eggs are not excreted, but are trapped in the tissue or swept into the peri-portal circulation and get trapped in the presinusoidal capillary venules of the liver. A chronic granulomatous inflammatory response is induced by antigens secreted by miracidium through microscopic pores within the egg-shell (Mitchell, 1990).

Miracidia in eggs secrete soluble egg antigen (SEA), the preparation that contains the moieties that can induce and elicit granuloma formation and its

regulation (Boros and Warren, 1970). Granuloma formation has been studied in murine models of schistosomiasis and shows a co-coordinated influx of lymphocytes, eosinophils, macrophages, neutrophils, mast cells, and fibroblast around eggs trapped in tissue (Mitchell, 1990).

Induction of granulomas requires an intact T-cell response, specific orchestration of T-cell and macrophage derived cytokines and induction of chemo-tactic factors. Six to eight weeks after infection, the balance shifts from inflammation promotion to suppressing cytokines with commencement of fibrosis (Wahl *et al.*, 1997). The active acute granulomatous response gradually becomes down modulated into the chronic phase in 12-18 weeks (Cheever and Yap, 1997; Wahl *et al.*, 1997).

2. 6. 0. Control of Schistosomiasis

The global strategy to control schistosomiasis is aimed at reducing morbidity through effective chemotherapy (WHO, 1985). In some cases targeted therapy has a significant impact on *S. haematobium* transmission (Muchiri *et al.*, 1996). However, rapid, inexpensive and reliable diagnostic methods are necessary in the treatment, morbidity assessment, evaluation and control strategies (Uga *et al.*, 1989).

2. 6. 1. Integrated control measures

Non-specific disease controls are aimed at the interruption of the life cycle by reduction or elimination of snail population by mollusciciding, biological control or environmental manipulation. Also, included is health education, provision of safe water, and sanitation. However, these control measures are expensive and difficult to initiate.The best results for the control of schistosomiasis are achieved in population-

based chemotherapy combined with focal molluscicide and health education (Muchiri, *et al* 1996).

2. 6. 2. Praziquatel treatment

Praziquantel, 2-Cylohexylcarbonyl-1, 2, 3, 6, 7, 11 b-hexahydro pyrazino [2, 1-alisoquino-4-one, is a synthetic heterocyclic antihelminthic agent (Anon, 1982; Pearson and Guerrant, 1983; Andrews, 1981). Praziquantel is used for the treatment of schistosomiasis caused by all *Schistosoma* species pathogenic to human beings and is now the drug of choice. It is able to kill mature schistosomal worms, while developing parasites of between two and five weeks of age (pre-patent infection) are less susceptible (Sabah *et al.,* 1986). Praziquantel is lethal to mature *S. mansoni* eggs, while embryonic eggs suffer little drug-related toxicity (Richards, 1989).

Studies in animals with schistosomal infections caused by *S. mansoni* indicate that all worms are dead in 7 days following treatment with the drug. The number of worms affected and the degree of injury increases with time post-treatment (Andrews, 1981).

The exact mechanism of antischistosomal activity of praziquantel has not been fully elucidated (Pearson and Guerrant, 1983; Andrews, 1981; McMahon and Kolstrup, 1979). It appears to kill susceptible adult schistosomes in vivo directly. Male worms appear to be more susceptible than females. ((McMahon and Kolstrup, 1979; Webbe *et al.*, 1981; Shaw and Erasmus, 1983).

Studies in animals with experimentally induced schistosomal infections indicate that praziquantel is active against all developmental stages of schistosomes including the miracidia and cercariae (the free-swimming larvae that emerge from

the intermediate snail host (Andrews, 1981). In addition to killing, the drug causes dead or dying worms to be dislodged from their sites of residence in the mesenteric or pelvic veins to the liver where they are retained and subsequently elicit host tissue reactions e.g. phagocytosis (Pearson and Guerrant, 1983; Webbe *et al.*, 1981; Andrews, 1981; McMahon and Kolstrup, 1979; Mehlhorn *et al.*, 1981).

The dislodgement of the worms appears to result principally from contraction and paralysis of their musculature and subsequent immobilization of their suckers, which causes them to detach from the blood vessel wall, thereby allowing passive dislodgement by normal blood flow. The drug-induced contraction and subsequent paralysis in the contracted state appear to result from an increased permeability of the cells membrane of susceptible worms to calcium and consequent influx of calcium ions.

Evidence from studies in animals with schistosomal infections, indicates that praziquantel is readily taken up by schistosomes and that the drug-induced dislodgement of schistosomes to the liver is rapid, occurring within 1 hour after administration of a single oral dose (Pearson and Guerrant 1983; Andrews, 1981; Mehlhorn *et al.*, 1981); contraction and paralysis of the worms occur almost immediately (Andrews, 1981; Mehlhorn *et a.l*, 1981; Wegner, 1984) but may be reversible at sub therapeutic plasma concentrations of praziquantel (less than 960 pmol/l) (Andrews, 1981).

Following administration of praziquantel, intense focal vacuolization and subsequent disintegration occur at distinct sites of the schistosomal integument. Vacuolization and disintegration of the tegument results in the formation of a defect

in the surface of the worms and cause most schistosomes to lose their normal copulatory position thus subsequently reducing oviposition (laying of eggs) (Wegner, 1984).

Evidence from animal studies indicate that praziquantel-induced vacuolization of schistosomal integument begins within 15 minutes following administration of the drug and that after 1 day the tegument is largely destroyed where vacuolization had occurred; eosinophilic granulocytes from the host attach to the vacuolized regions and subsequently infiltrate the interior of the schistosome resulting in a progressive internal lyses (Leopold *et al.,* 1978; Buhring *et al.*, 1978).

Approximately, 80% of an oral dose of the drug is absorbed from the gastrointestinal tract. However, because of rapid rate of metabolism in the liver, only a small proportion reaches the systemic circulation unchanged Ref. Peak serum concentrations of praziquantel occur approximately 1-3 h after oral administration of the usual dose of the drug. Following oral administration of a single dose of 40 mg/kg body weight in healthy adults, peak serum drug concentrations of 3.2 nmol/l occurred at 1-2h. In studies in rats, concentrations of free (unbound) praziquantel in CSF is reported to be 14-20% of the concurrent total (free plus protein-bound) plasma concentration. (Patzschke *et al.*, 1979).

Praziquantel is rapidly and extensively metabolized principally in the liver via hydroxylation to monohydroxylate and polyhydroxylated metabolites. Following a single oral dose of the drug, approximately 70 – 80% of the dose is excreted in urine within 24 hours, principally as metabolites but less than 0.1% of an oral dose is

excreted in urine unchanged. (Pearson and Guerrant, 1983; Leopold *et al.*, 1978; Patzschke *et al.*, 1979).

Praziquantel also penetrates the blood-brain barrier and is, therefore, used in treating central nervous system schistosomiasis to minimize the toxicity due to eggs present in and around the nervous tissue. Tolerance to Praziquantel treatment of *S. mansoni* infected community in Senegal has recently been reported (Stelma *et al.*, 1997; WHO, 1999). Thiong'o *et al.*, (2002) also reported evidence of resistance/tolerance of praziquantel to *S. mansoni*-infected school children in Machakos area of Kenya. Increasing the dose and duration of praziquantel therapy is recommended to obtain maximum effect (Richards *et al.*, 1989).

2. 7. 0. Diagnosis of schistosomiasis

Diagnosis is not only essential for the medical care of the individual patient but also for screening a population for either mass treatment or epidemiological studies. Furthermore, reliable diagnostic results are indispensable in the evaluation of the effects of chemotherapy or other large-scale interventions. (Uga *et al.*, 1989) The current diagnostic tools for schistosomiasis fall into three categories: direct parasitological and immunological methods.

2. 7. 1. Direct parasitological methods

The diagnosis of schistosomiasis is usually based on the microscopic detection of eggs in urine or stool. The eggs of the human *Schistosoma* species are characteristic and easy to identify. They are large (70-180 μm) and have a typical terminal (*S. haematobium, S. intercalatum*) or lateral spine (other species) (Rollinson and Southgate, 1987). The distribution of *S. haematobium* ova in urine is not

proportional even with thorough mixing of the sample (Doehring *et al.*, 1983). Sensitivity of any particular method depends not only on intensity of infection but also on the sample size and the intrinsic loss rate of the eggs from the tissue (Feldmeier *et al.*, 1993).

Therefore, parasitological examinations need to be repeated several times to get an accurate impression of the individual intensity of infection, otherwise light infections are easily missed by a single routine examination (De Vlas and Gryseels 1992). Thus, the sensitivity of any method will decrease with decreasing intensity of infection irrespective of the method used (Feldmeier *et al.*, 1979).

Development of filtration techniques (Peters *et al.*, 1976; Mott and Cline, 1980) and Kato-Katz fecal thick smear method (Kato and Mura, 1954; Katz *et al.*, 1972) has been very useful in developing reasonably sensitive techniques that are simple and applicable under field conditions for diagnosis of schistosomiasis. In these techniques microscopic examination is used to detect the characteristic eggs (WHO, 1993).

2. 7. 2. Indirect methods

2. 7. 2. 1. Physical/clinical examination

In the past years, a number of indirect indicators of schistosomiasis-related pathology for both intestinal and urinary schistosomiasis have been applied in community diagnosis and control operations (Gryseels, 1989; Savioli *et al.*, 1990). Ultrasonography can detect hepatomegaly, splenomegaly and periportal hepatic fibrosis. However, ultrasonographical detectable fibrotic lesions show important regional differences (Hagan and Gryseels, 1994). Observation for gross haematuria

is a simple and reliable indication of heavy urinary schistosomiasis in endemic areas. (Murare and Taylor, 1987).

2. 7. 2. 2. Biochemical tests

Presence of micro-haematuria, proteinuria and leukocyturia are reported to be useful indicators for urinary schistosomiasis and can be determined with semi-quantitative reagent strip methods (Feldmeier *et al.,* 1982; Mott *et al.,* 1985; Murare and Taylor, 1987). These reagents (which react with blood in urine to produce blue color) show a high sensitivity, but a low specificity (Murare and Taylor, 1987)). In addition, notable variations, which depend on geographical area, endemicity, cultural practices, age, sex and even time of sample collection, limit the application of haematuria as a diagnostic tool (Feldmeier and Poggensee, 1993).

Although these techniques may offer a feasible diagnosis in endemic areas, they can by no means be used to make a definitive diagnosis. Rather, hepato-splenomegaly and blood in urine or stool, point to a long list of differential diagnoses of which schistosomiasis forms one of the major components (Gryseels and Polderman, 1991). Evaluation of pathology relies on ultrasonography and various studies have used this approach in an epidemiological setting (Hatz *et al.*, 1992). This, however, requires trained physicians and may be difficult to use routinely. To achieve better *S. haematobium* morbidity control, a simple tool that can be used in the field is needed (Kahama *et al.,* 1999).

2. 8. 0. Immunological methods

2. 8. 1. Antibody detection

A major breakthrough in the control of schistosomiasis has been the development of a rapid, inexpensive and reliable sero-diagnostic methods such as the enzyme linked immuno-sorbent assay (ELISA) to supplement parasitological tests involving egg counts (Hirata *et al.,* 1986). The *S. haematobium* soluble egg antigen attaches to coated monoclonal antibody Mcab 290-2- E 6- A, then reacts with a conjugate anti SEA after continuous shaking at 37° C for one hour. For colour development para-nitrophenyl phosphate (pNPP) in diethanolamine (DEA) is added and the optical density is read at 405 nm after 30 minutes and overnight incubation. The optical density measured is equivalent to the amount of SEA in the urine. Wide ranges of tests are described for the demonstration of specific antibodies, including the use of crude purified or recombinant antigens and the detection of specific antibody isotypes (Doenhoff *et al.,* 1993).

Monoclonal antibodies against genus specific antigens permit the quantification of circulating antigens resulting from schistosome infections that commonly occur in Africa (Feldmeier *et al.,* 1986). However, such techniques do not necessarily detect active infection, making their utility in intervention studies difficult.

2. 8. 2. Antigen detection

Newly formed schistosome eggs are composed of 30-40 vitelline cells and one fertilized ovum, which are encapsulated in an eggshell. The eggs are still immature when laid and it takes approximately 6 days for the embryo to reach the

miracidial stage. Eggs take over 6-10 days to reach the lumen of the gut or bladder (Jordan *et al.,* 1993).

The lifespan of the eggs retained in tissue is not clear: but it is the estimated to range from 14-32 days for *S. mansoni* (Moore *et al.,* 1977; Maldonado, 1959). The synthesis and release of proteins and glycoproteins during miracidial development has been demonstrated in *S. japonicum* eggs in *vitro*. The source of excreted/secreted products from immature eggs is likely to be the collapsing vitelline cells and the products from mature eggs consisting of miracidial secretions (Kawanaka and Carter, 1992).

The soluble egg antigen released by the egg, which includes proteins, polysaccharide and glycoproteins are responsible for the egg-induced pathology (Xu *et al.,* 1990). Egg antigens may vary considerably in concentration among schistosome eggs of different geographic origin (Hamberger *et al.,* 1982).

The quantitative diagnosis of schistosome infections through detection and quantification of circulating antigens such as, the circulating anodic antigen (CAA) and the circulating cathodic antigen (CCA), both of which are adult worm derived antigens have been used in *S. haematobium, S. mansoni, S. japonicum* and *S. intercalatum* (De Jouge *et al.,* 1990; Kremsner, *et al.*, 1994; Van Etten *et al.*, 1994).

Schistosome circulating antigens are of interest for both immuno-diagnosis and immuno-pathology of the disease. Circulating antigens may play a role in the pathology of schistosomiasis (induction of granuloma by egg antigens) and the evasion by the parasite of the effector mechanism of the host's immune system. Circulating antigens may also play a role in the induction of tolerance in children

born to infected mothers and in chronically infected adults (De Jouge *et al.,* 1990; Deelder, *et al.,* 1994).

The development of monoclonal antibody (Mab) based sandwich ELISA has made it possible to detect CAA and CCA in a sensitive and highly specific manner (De Jouge *et al.*, 1990; Deelder *et al.*, 1989). Since the cut off value for these assays are low, this in turn means that they can be applied even in cases of light infections. Significant correlations were found between the antigen levels in both serum and urine as compared to egg output levels, and pathology as determined by reagent strip index (RSI) (Kremsner *et al.,* 1994). Generally, antigen detection is based on monoclonal antibodies that have a high affinity on the antigen molecule giving them nearly 100% specificity. The assay can be applied at community level where there can be cross-infection reactions with other helminthic infections (Kahama *et al.*, 1998).

All developmental stages of schistosomes secrete antigens. A number of antigens are present in the hatching fluid, which surrounds the miracidia in the eggshell. Preliminary characterization of this antigen showed that it is was a hydrophobic polypeptide of approximately 41 kd (Hayunga *et al.,* 1987). Miracidia enclosed in the mature eggs of *S. haematobium* secrete soluble egg antigen (SEA) that reach the surrounding tissues through the microscopic pores in the egg- shell. Analysis of SEA on polyacrylamide gel electropheresis has shown the presence of about 20 major proteins, 14-16 of which appears to be immunogenic (Kahama *et al.*, 1998).

The antigen detected in urine is derived from the eggs in urine and those trapped in the bladder tissue and plexus. The detection of soluble egg antigen (SEA) provides more information on the egg burden and might be a good parameter for assessment of pathology and drug tolerance in an infected person (Kahama *et al.*, 1998). Nourel *et al.* (1994) reported the use of monoclonal antibodies based sandwich enzyme linked immunosorbent assay (ELISA), for the quantitative determination of SEA in both serum and urine of *S.mansoni* infected individuals. Amanor *et al.* (1996) characterized murine antibodies against *S. haematobium* antigens and described some species-specific antibodies that could be utilized in immuno-diagnosis of infected individuals.

Immunological techniques that rely on detecting parasite antigens or antibodies in blood or urine offer simplicity and reliability for mass screening. A study carried out in Kwale, Coast Province, Kenya, indicated that urine ELISA detects *S. haematobium* egg-positive persons with a specificity of 91% and a sensitivity of 97% making it a useful test for diagnosing urinary schistosomiasis (Uga *et al.*, 1989). Soluble egg antigen (SEA) is derived from the activity of the mature miracidium enclosed inside the eggshell. Kahama *et al.* (1998) evaluated the SEA assay and showed significant correlation between the levels of SEA with egg counts, haematuria and ultrasound detectable pathology.

The sensitivity of the SEA-ELISA within different egg count categories compared well with micro-haematuria with both techniques detecting around 100% of individuals with egg counts above 50 per 10ml (i.e. the heavily infected category). As noted by Kahama *et al.* (1998), SEA-assay could be applied in the assessment of

vaccine trials especially to evaluate the effect of anti-fecundity vaccines. Another area where use of SEA detection is expected to have an input is in the diagnosis of women infected with genital schistosomiasis. Kahama demonstrated the presence of SEA in vaginal lavage collected from women suspected to have female genital schistosomiasis (FGS) (Kahama *et al* 2001; *personal communication, unpublished results*).

CHAPTER THREE

3. 0. MATERIALS AND METHODS

3. 1. Study area

The present study was undertaken in Jimba Sub-location in Ruruma location, Kaloleni Division in Kilifi District, Coast Province, Kenya (Fig. 3). It borders Mwatsunga and Mleji sub-locations to the East and North respectively, Rabai location to the South, Mariakani location to the Southwest and Kayafungo to the Northwest. Kilifi District has a population of 544,303. The location (Ruruma) has a population of 3670 which is 0.67% of the Kilifi District population scattered over 15.5 square kilometers. Ruruma location has 1648 males and 2022 females and a population density of 237 persons per square kilometer (Kenya population census, 1999).

3. 2. Topography

Jimba sub-location consists of plain land with gradual gradients and a few seasonal rivers. To the Southern and Eastern region there are rivers and streams that flow eastwards. These streams and rivers and in some cases dams empty their waters into river Kombeni that finally drains into Mto Jomvu and Tudor Creek of the Indian Ocean, further east. Jimba River, which is 10 meters below Jimba Primary School drains into Kombeni River. After the rains, the river forms large pools of water, which are the breeding sites for the snail *Bulinus nasutus,* an intermediate host and transmitter of *S. haematobium*. The piped water supply system in this area is rarely operational and as such most people rely on river water for their supply.

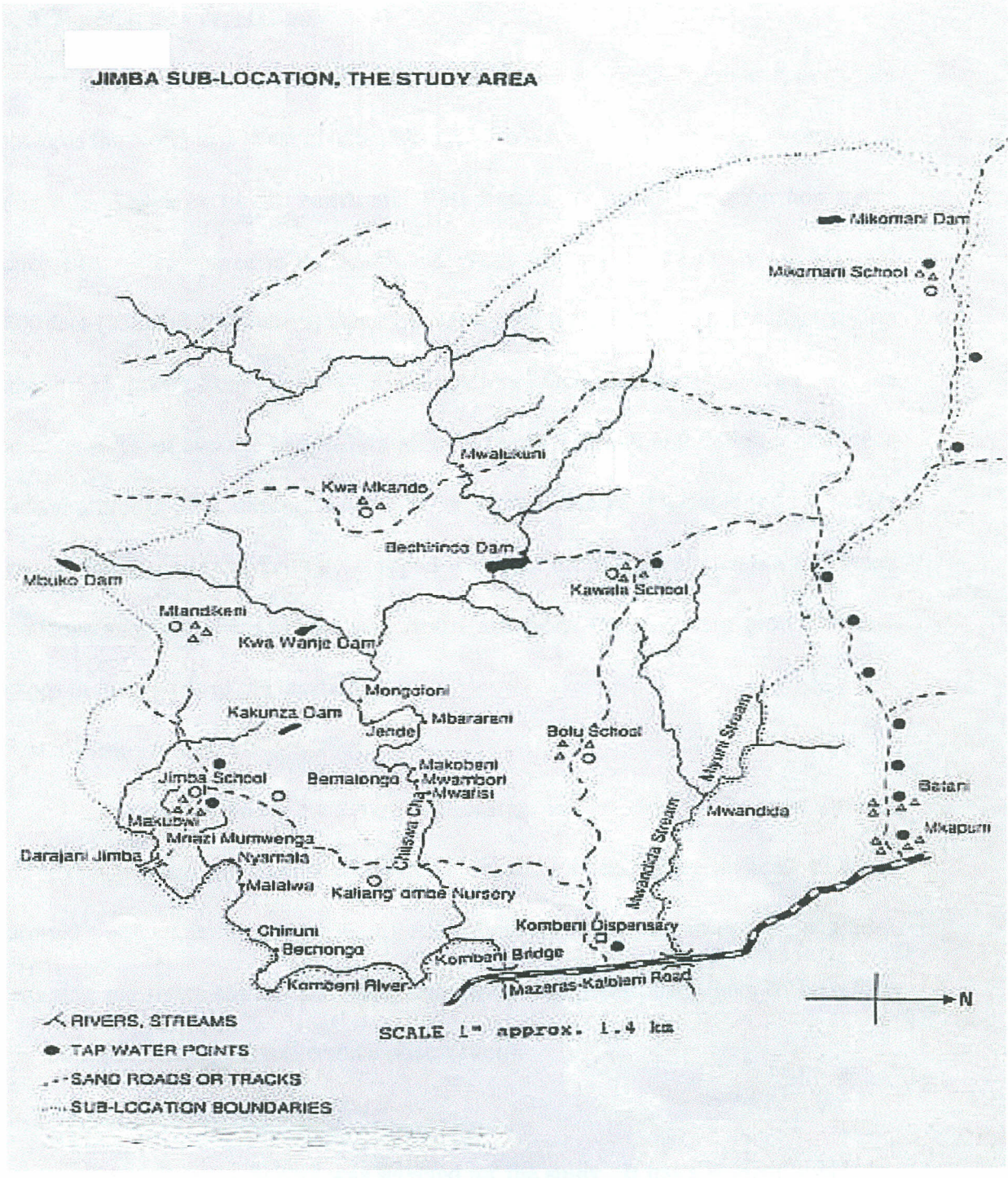


Fig.3. A map showing Jimba location and the school.

- Jimba school

3. 3. Rainfall and vegetation

Rainfall in this sub-location determines the type of vegetation as it decreases towards the North and West in Kilifi District as a whole and Jimba is no exception to this rule. The area to the North and East has more dense vegetation and many coconut trees compared to the South and West. At an altitude of between 500 and 800 feet (152 and 250 meters) above sea level, and lying in the coastal range (region above 135 meters from sea level), it is a medium potential zone for agriculture. The area receives an average annual rainfall of between 735 mm and 980 mm and mean temperatures of 22°C minimum and 30°C maximum. Maize as a staple food is widely grown seasonally and often intercropped with root crops such as cassava and sweet potatoes and other cereals like rice, beans and peas. Coconuts are grown as cash crops in most parts of the location.

3. 4. Communication

Communication within Jimba sub-location is hampered by the total absence of telephones and by unmaintained roads, making it extremely difficult to travel around the location except on foot. All children trek on foot to and from school, crossing the rivers sometimes twice, a situation that predisposes them to infections including schistosomiasis (Personal observation).

3. 5. Study population

Jimba Primary School was selected for the study. It has a student population of 401 with ages ranging from 4-18 years (median 11 years). Before commencement of the study, permission was obtained from the pupils, parents, the education office, the Medical Officer of Health and local administration after explaining the purpose

and benefits of the study. The Director of Medical Services, Ministry of Health, granted permission to carry out the research in the area. For ethical reasons, all children found positive were treated with one dose of praziquantel (40- mgs/kg body weight).

The minimum sample size (n) was 96 as calculated by the formula of Lwanga and Lemeshow (1991) where $N=Z^2\ 1 - ap(1-p)/d^2$, Z = 1.96 (Std errors from the mean), a = absolute precision at 5%, p = prevalence rate and d = 0.05. However, a total of 401 children were incorporated into the study, from which 158 were selected and urine samples were collected over a period of 33 days.

3. 6. Exclusion/ inclusion criteria

Those students with no egg count in urine and those that did not agree to be part of the study were excluded. All students positive with eggs in their urine who agreed to be part of the study and were consistent through out the study period were included. Some students negative for *S. haematobium* eggs in urine were selected as negative control. Urines samples were also collected from a non-endemic area, Sengani in Kangundo. This area is known to be free of *S. haematobium* transmission. Kangundo is approximately 450 km from the study area and 70 km from Nairobi.

3. 7. Benefit to study

All students who agreed to be examined for *S. haematobium* infection and were found positive at the beginning and the end of the study were treated with 40-mgs/kg body weight praziquantel. Both the examination and the subsequent treatment were given free to the pupils who participated in the study. At the end of

the study, all the pupils who were clinically found to have fungal infections, other worms and malaria were also treated with the appropriate drugs.

3. 8. 0. Serological Analysis

3. 8. 1. Preparation of monoclonal antibodies

For preparation of anti-egg monoclonal antibody (Mabs), Balb/c mice were immunized with 100 µg of hatching fluid antigen (HF schistosome antigen). Booster immunization was given after 2 weeks and thereafter every fourth week with 50 µg of HF antigen. Final booster of 50 µg was given three days prior to fusion. The spleen cells were fused with SP2/0 mouse myloma cells three (fusion 290) or five (fusion 291) months post primary immunization. Hybridoma cells were selected and cloned by limiting dilutions. The monoclonal antibodies were then produced by induction of asciatic fluid in mice. Isotypes were determined in the HF- ELISA using anti-mouse isotype-specific peroxidase conjugate. Centrifuging the supernatant for 10 minutes at 2000 g purified the antibodies. The pellet was then discarded. Ammonium sulphate (689 µl) solution was added to the supernatant at 25°C and stirred for 2.5 h. The solution was then centrifuged for 15 minutes at 9000 g and the supernatant discarded. The pellet was then dissolved in buffered potassium phosphate saline (pH 7.8), dialyzed against distilled water twice and then lyophilized (De Jouge *et al.*, 1990). The monoclonal antibodies with isotype IgM were labeled with Biotin-xx-hydrazide and IgG monoclonal antibodies were labeled with sulfosucciminidyl-6-(Biotinamido) hexanoate, (immunopore NHS-LC-Biotin Rock IL) according to the manufacturer's instructions.

3. 8. 2. Preparation of antigens

Adult schistosome worms were collected by perfusion of hepatic portal system of golden hamster at seven weeks (*S. mansoni*) or at 16 weeks (*S. haematobium*) after infection. Eggs were isolated from infected hamster gut (*S heamatobium*) by means of homogenization and trypsinization. Adult worm antigen (AWA), soluble egg antigen (SEA) and the trichloroacetic acid soluble (7.5% w/v) fraction of SEA (SEA-TCA) were prepared according to the method by Deelder *et al.* (1976). *S. mansoni* hatching fluid was prepared according to Amador and Wright, (1982). Briefly, 400,000 eggs were incubated in distilled water for 2 h at 28°C under a light source. After removal of eggs and miracidia by centrifugation and filtration, the hatching fluid was freeze-dried.

3. 8. 3. Isolation and preparation of *S. haematobium* egg antigen by homoginization (TCA)

Preparation of *S. haematobium* antigen was done by homogenizing 1.5 – 2 gm eggs in a small homogenizer in phosphate buffer saline at 0^0 C, then transferred the homogenate to a glass tube and sonificated for 3 minutes at level 7 (Branson Sonic Power Company, sonifier B-12 power supply and converter) at 0^0 C. It was centrifuged for 20 minutes at 25,000 g and 4^0 C. Supernatant was collected and re-homoginized the pellet in PBS at 0^0 C then centrifuged for 20 minutes at 25,000 g and 4^0 C. Supernatant was collected and added to the first supernatant. The mixture was then centrifuged 25,000 g for 15 minutes at 4^0 C, collected the supernatant and re-suspended the pellet in PBS. Trichloroacetic acid solution was added to the antigen solution, to make the final concentration of 7.5% (w/v), and then stirred for

20 minutes at room temperature. It was then centrifuged for 15 minutes at 25,000 g at 4^0 C. The supernatants were combined and dialyzed against distilled water at 4^0 C and changed twice, then lyophilized and stored at 4^0 C.

3. 8. 4. Preparation of reagents

3. 8. 4. 1. Antibody solution

The antibody solution was made by diluting the purified 290-2-E6-A (1 gm) in 0.1 M Carbonate bicarbonate buffer, pH 9.6 to a final concentration of 5 μg/ ml.

3. 8. 4. 2. Blocking solution: 0.1% (w/v) Bovine serum albumin (BSA)

This was made by adding and dissolving 0.1 mg of BSA in 10 ml of Carbonate bicarbonate buffer.

3. 8. 4. 3. Assay buffer

This was prepared by adding 100 ml of phosphate buffer saline (PBS) into 1 ml of 30% between 20 and 100 mg BSA and then adjusting the pH to between 7.2 and 7.8.

3. 8. 4. 4. Biotin solution (290-2-E6-A BDACH)

The biotin solution was diluted in the ratio: 1000 in the assay buffer. This was then added into100 ml of 2% fetal calf serum (FGS).

3. 8. 4. 5. Streptavidin conjugate (1:3000)

This solution was prepared by diluting streptavidin alkaline phosphate in the ratio 1:3000 in the assay buffer.

3. 8. 4. 6. Paranitrophenyl phosphate (PNPP) substrate solution

This solution was made by adding10 mg of PNPP in 10 ml Diethylamine buffer.

3. 8. 4. 7. Carbonate buffer pH 9.6

This solution was prepared by adding and dissolving 5.98 gm $NaHCO_3$ and 3.03 gm Na_2CO_3 in 1000 ml of distilled water.

3. 8. 4. 8. 20% Trichloroacetic acid solution (w/v)

This solution was prepared by dissolving 200 g TCA (MW=163.39) in one liter distilled water and then stored at 4^0 C until use.

3. 8. 4. 9. 0.1 M DEA buffer pH 9.6

This solution was prepared by dissolving 0.95 gm of $MgCl_2$ in 10 ml distilled water. Diethanolamine buffer is prepared by adding 4.75 ml of DEA and 250 µl $MgCl_2$ solution into 450 ml of distilled water. The pH is adjusted to 9.6 and the volume made to 500 ml with distilled water.

3. 9. 0. Collection of urine

Urine samples were collected between 10.00 am and 2.00 pm before treatment and follow-ups on the study group on alternate days for 14 times in total. The school children drank one glass of orange juice before samples were collected each time to enhance the chances of obtaining enough urine. Information on the study subjects which included identification number, name, sex, age, class of each child, parasitological examination and SEA profile were recorded on a data sheet. Urine samples from a cohort of 158 school children infected with *Schistosoma haematobium* were collected from each child before treatment with Praziquatel (Day 0) and on alternate days from day one through to day 33 post-treatment. These urine samples were examined for *S. haematobium* eggs and soluble egg antigen (SEA).

3. 9. 1. Collection of urine for soluble egg antigen assay

A 10 ml fresh urine sample for the soluble egg antigen (SEA) assay was collected in a nunc polysorb tube (Nunc, Roskilde, Denmark) each time urine was collected for parasitological examination. The samples were immediately placed in a cooler box with ice bags. They were then frozen within six hours of collection and stored at -20^{0}C until further analysis.

3. 9. 2. Parasitological examination of urine

To determine presence or absence of *S. haematobium* ova in the urine, filtration method using nucleopore filters was used (Peters *et al.,* 1976). Briefly, two aliquots of 10 ml urine samples were filtered and the filters placed on a single slide labeled with the identification number of the child and date. The slides were transported to a central laboratory and examined within a period of 6 hrs. The eggs were counted on both filters using a microscope and expressed as the mean number of eggs per 10 ml of urine. An average of egg counts from the two filters was then calculated and used to determine the pre-treatment prevalence and intensity of infection. Clogging of filters often presented logistic problems but when it occurred the urine sample was diluted 100 times with normal saline and filtration repeated. The results obtained after examination were then expressed as eggs counted per 10 ml urine.

3. 10. 0. Quantification of soluble egg antigen (SEA) in urine

All steps (except the addition of substrate) were performed on a heat shaking incubator system set at 37° C (Mushens and Scott, 1990). Between all steps, washing was performed with 200µl wash buffer (20x diluted phosphate buffer saline) per well

(four times). Microtitre plates were coated with 50 µl per well of anti-SEA McAb 290- 2-E6-A (5 µg/ml) and incubated overnight at room temperature. One hundred microlitre per well of 0.1% Bovine serum albumin (BSA) was then added to block the non-binding sites and incubated at 37^0C for one hour. Seventy milliliters of urine was heated at 70^0C for 30 minutes and left to return to room temperature. Twenty-five microlitres per well of heat-treated urine was then added in duplicate. Seventy-three microlitres of 3160 ng/ml of the standard SEA solution was added to G1 and H1 well on the plate and 50 µl assay-buffer added in well G 2 till G12, and H2 till H12. Twenty-three microlitres was pippetted from well G1/H1 and transferred to G2/H2, mixed well and transferred again 23 µl to G 3/H 3 through to G10/H10. Pooled reference standards (300, 60, 20, 500, 50, normal urine, and blank) were added into wells 11 and 12 row A to H, respectively, and then incubated at 37^0C for one hour. Fifty microlitres per well of Biotin conjugate of anti-SEA McAb plus 2% Fetal Calf Serum (FCS) were added and incubated at 37^0C for one hour. Fifty microlitres per well of alkaline phosphatase conjugate of streptavidin (Sigma Chemical Company St.louis Missouri USA) were added and incubated for 30 minutes at 37^0C. One hundred microlitres per well of substrate p-nitrophenyl phosphate (PNPP) in DEA were added and incubated at room temperature in the dark for 30 minutes. The absorbance was measured at 405nm and 620nm after 30 minutes incubation at room temperature (to check for the high positives). Samples were then put in the dark again overnight at 4^0C (to check for the low positives). The values were read against a standard curve prepared from known (SEA) standard concentrations.

3. 11. 0. Data management and analysis

Chi-square test was used to compare the prevalence between male and female children infected with *S. haematobium*. Since neither the egg counts nor the SEA concentration data were normally distributed, a logarithm $\log_{10}(x+1)$ transformation was performed on the data. ANOVA was used for comparison of log mean eggs and SEA excretion between and within different age groups of children before praziquantel treatment. Post ANOVA tests were used for multiple comparison of the mean differences between various age groups. The tests were conducted at 95% confidence interval and significance level of 5% ($P < 0.05$) was considered significant. The Student's *t*-test was then applied to test for the differences in mean SEA or egg counts between age groups or sex of the study children. Pearson's correlation coefficient was computed to check the association between SEA concentration and egg counts in urine during the study period.

CHAPTER FOUR

4. 0. RESULTS

4.1. Prevalence and intensity of urinary schistosomiasis in Jimba Primary School.

Out of the 401 urine samples examined for schistosome eggs at baseline, 92.8% (372) were positive for eggs of *S. haematobium* (Table1). Of the 372 children positive for *S. haematobium* eggs, 210 were males (95.5%) and 162 were females (89.5%). This showed a significantly higher prevalence in males than in females ($\chi^2 = 5.23$; $P < 0.05$). Of 158 children who consistently presented themselves for urine examination throughout the study period, 74 were males and 84 were females. These children had an age range of 4 – 18 years with a median of 11 years.

Table 1: Prevalence of *Schistosoma haematobium* infection in children attending Jimba primary school.

	Number examined	Number positive	Prevalence (%)
Males	220	210	95.5
Females	181	162	89.5
Total	401	372	92.8

4. 2. Secretion of SEA and excretion of eggs in urine

Of the 158 school children enrolled in the study, 16 (10.1%) continued excreting eggs and soluble egg antigen (SEA) in urine throughout the 33 days after treatment with praziquatel; 4 (2.5%) who were originally not excreting eggs and secreting SEA started excreting eggs and secreting SEA on day 8 and continued doing so up to day 33; 10 (6.3%) continued secreting SEA up to day 33 but stopped excreting eggs on day 15 and 17; 9 (5.7%) who were originally not secreting SEA started secreting SEA but not eggs on day 31, 32 and 33; 26 (16.5%) who were originally secreting SEA and excreting eggs in urine stopped secreting SEA and excreting eggs on days, 15, 17, 22, 24 and 26 after treatment.

These same children started excreting eggs and secreting SEA from day 29 up to day 33; 48 (30.4%) who were originally secreting both eggs and SEA in urine stopped on days 15 and/or 17 after treatment. However, these same children started secreting SEA on days 29, 30, 31, 32 and 33; 29 (18.4%) who were originally excreting both SEA and eggs stopped on day 15 up to the last day; and 16 (10.1%) of the children who were not secreting SEA and excreting eggs continued like that way up to day 33.

Fig. 4. Daily median egg counts in urine before and after praziquantel Treatment.

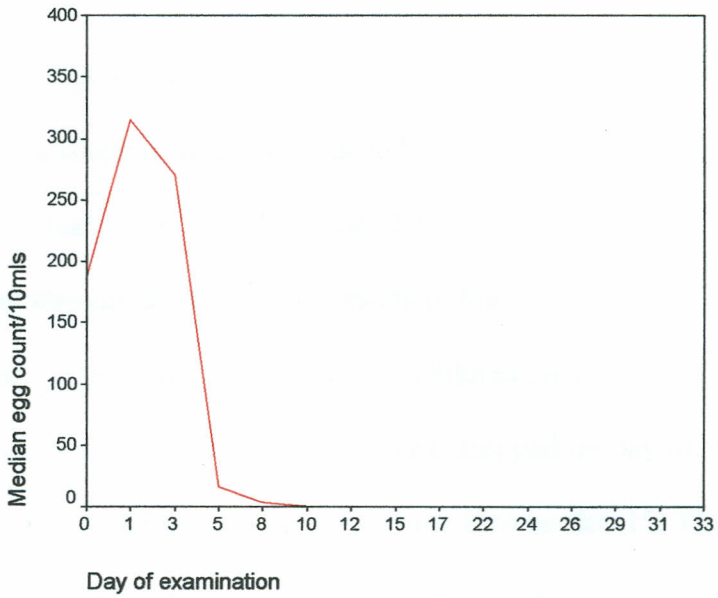
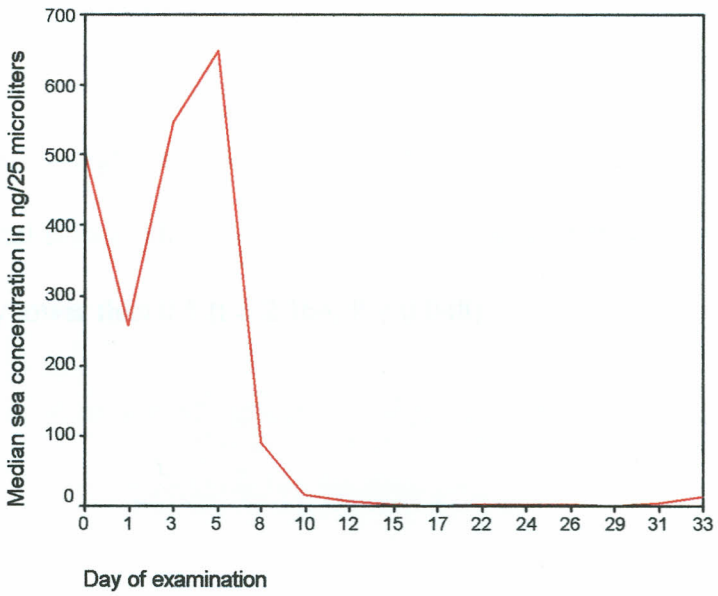


Fig. 5. Daily median SEA levels in urine before and after praziquantel treatment.



4. 3. Day to day comparison of median *S. haematobium* egg counts and SEA levels in urine of school children examined.

The results in figure 4 show that there was an increase in median egg output after treatment with a single dose of praziquantel in the first two days and thereafter started reducing to reach the zero level on day nine. The level of egg output remained at zero all through to day 33. The results in Figure 5 show that immediately after treatment of *S. haematobium* infected children with a single dose of praziquantel (40 mgs/ kg body weight), SEA levels dropped on day one and started rising to a peak on day 5 and then decreased to near zero on day 15. This continued at that level until day 31 when SEA levels started rising again.

4. 4. Daily secretion and excretion of SEA and egg counts in urine by sex.

Figure 6 shows the mean daily egg counts/10 ml of urine excreted by the male and female children over a period of 33 days post treatment with praziquantel. The highest geometric mean was recorded in both sexes on day seven; but male children had significantly higher egg counts than female children (male 127.8 versus female 63.6 eggs/10 ml urine). The lowest geometric means for females was 0.3 and for male significantly lower than 0.5 ($t = 2.164$, $P = 0.048$).

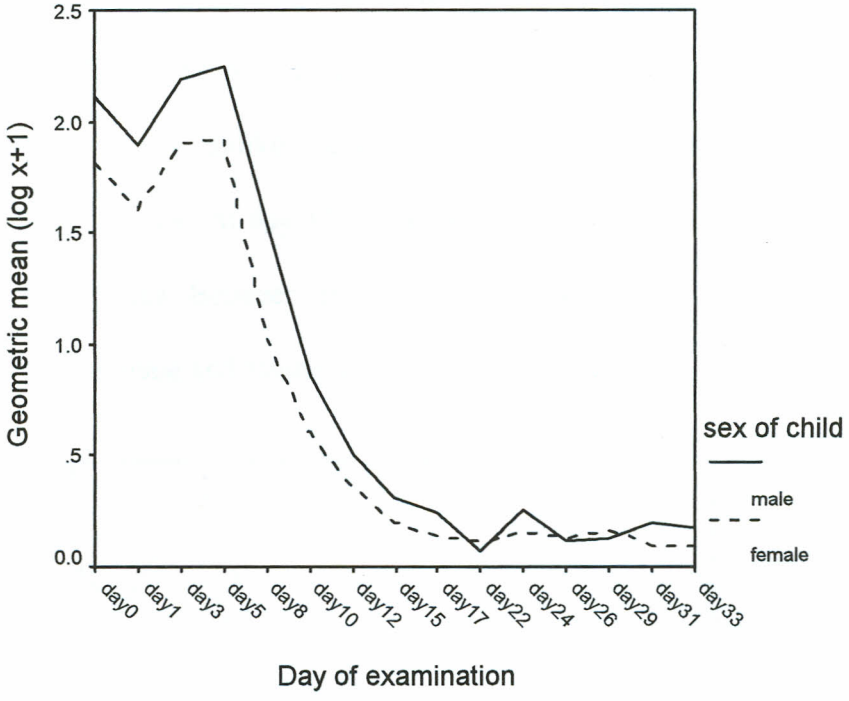


Fig. 6. Daily excretion of eggs per 10 ml of urine in male and female children before and after praziquantel treatment.

After praziquantel treatment, there was an initial slight drop of mean egg output on day 2 (post - treatment), then an increase on day 4, reaching the peak egg count excretion on day 5. There after, a sharp reduction over the next two weeks occurred for both sexes. However, on day 24, there was a slight increase in the male children, which dropped on day 26 and remained stable at that level and rose again on day 31. The pattern of egg excretion was the same for both male and female children throughout the study period but egg excretion in the male children remained higher than that of the female children for the first 20 days. This trend remained lower up to day 33. However, on day 24 and 31 to 33, there was a slight increase in egg excretion in the male than the female children.

Figure 7 shows the clearance of SEA in urine from the body during the 33 days post praziquantel treatment for both male and female children. A similar trend

as that observed for the egg excretion in urine was observed in male children. The curve for SEA excretion for male children remained higher than that of the female children. However, unlike the egg excretion, the SEA clearance did not decline below the 0.5 mark. At day 15, some SEA could still be detected in urine though in smaller quantities. Between days 29 and 33, there was an increase in the SEA in urine for both male and female children.

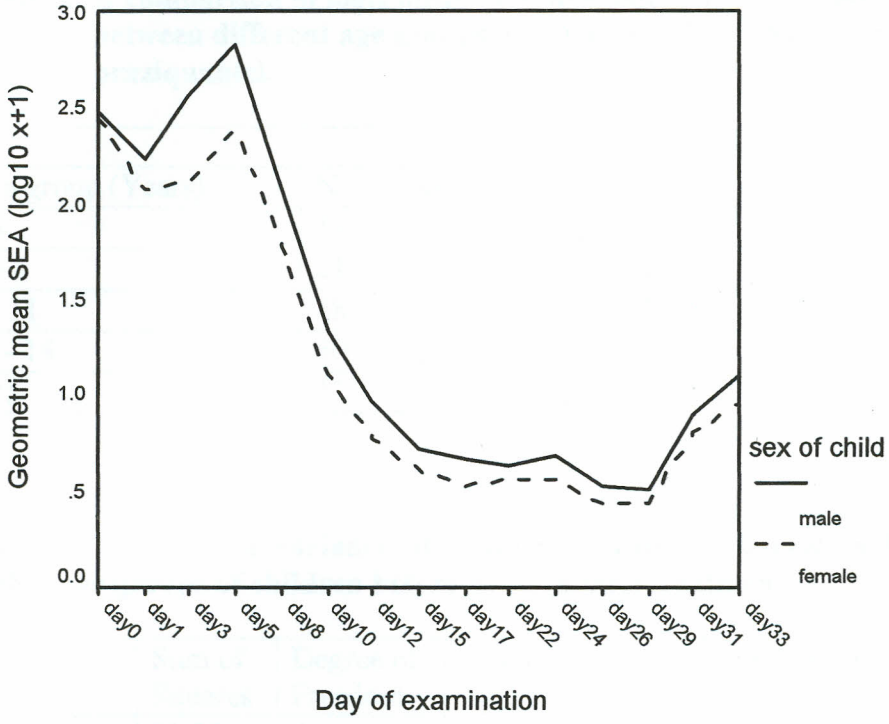


Fig. 7. Daily variation of SEA concentration in urine of male and female children before and after praziquantel treatment.

4. 5. Day to day variations of *S. haematobium* egg counts in urine by age groups.

The variations of *S. haematobium* egg excretion patterns in urine in age groups ≤ 5 years, 6 – 8 years, 9 – 11 years, 12 – 14 years, and ≥ 15 years were investigated to assess whether there were differences between and within the different age groups before praziquantel treatment (Table 2). The analysis of

variance on the data in Table 2 indicated that there were significant differences between and within age groups ($F = 6.51$, $P < 0.001$) (Table 3). Post ANOVA tests performed on the data on table 2 indicated that there were significant differences in egg excretion between age group ≤ 5 years and 9-11 years ($P = 0.012$) and 12-14 years ($P = 0.019$) (Table 4).

Table. 2: A comparison of log_{10} mean of *S. haematobium* egg count between different age groups of children before treatment with praziquantel.

			95% confidence interval	
Age group (Years)	N	Mean ± SEM	Lower limit	Upper limit
≤ 5	14	0.87 ± 0.32	0.19	1.56
6 – 8	21	1.92 ± 0.20	1.51	2.33
9 - 11	48	2.19 ± 0.14	1.92	2.46
12 - 14	60	2.11 ± 0.11	1.89	2.33
≥ 15	11	1.46 ± 0.35	0.70	2.24

Table. 3: Analysis of variance of *S. haematobium* egg excretion between and within age groups of children before praziquantel treatment.

	Sum of Squares	Degree of Freedom	Mean square	F	Significance
Between groups	23.29	4	5.82	6.51	0.000
Within groups	133.29	149	0.90		
Total	156.58	153			

Table. 4: Post anova test for the mean difference (MD) in *S. haematobium* egg excretion between various age groups of children before praziquantel treatment

				95% confidence Interval	
Age group (Years)	Age groups (Years)	MD ± SEM	Significance	Lower Limit	Upper Limit
≤ 5 Vs	6 - 8	-1.05 ± 0.33	0.092	-2.21	0.10
	9 – 11	-1.32 ± 0.29	0.012	-2.41	-0.22
	12 – 14	-1.24 ± 0.28	0.019	-2.32	-0.15
	≥ 15	-0.59 ± 0.38	0.926	-2.06	0.89
6 – 8 Vs	9 – 11	-0.26 ± 0.25	0.960	-0.97	0.44
	12 – 14	-0.19 ± 0.24	0.995	-0.86	0.49
	≥ 15	0.47 ± 0.35	0.953	-0.84	1.77
9 – 11 Vs	12 – 14	0.08 ± 0.18	1.000	-0.42	0.58
	≥ 15	0.73 ± 0.32	0.538	-0.53	2.00
12 – 14 Vs	≥ 15	0.65 ± 0.31	0.660	-0.61	1.92

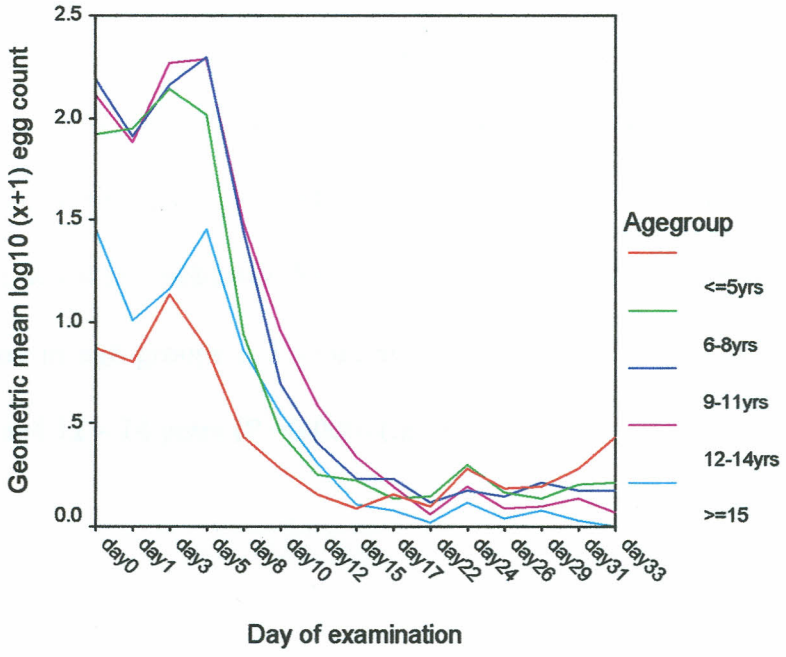


Fig. 8. Comparison of the geometric mean of *S. haematobium* eggs count /10 ml urine by age group before and after treatment.

In all age groups, the eggs decreased on day 1, then markedly increased between day 4 and 8 after which egg counts dropped to below 0.5 geometric mean. The geometric mean for the lowest age group (≤5 years) and the highest age group (≥15 years) were slightly lower than for the middle age groups (6-8, 9-11, 12-14 years). The counts picked again on day 24 for all the age groups, then decreased slightly and continued to be low throughout. However, in the age group ≤ 5 years, the egg count increased from day 29 onwards. In the ≥ 15 years age group the lowest egg counts were observed on days 31, 32 and 33 after praziquantel treatment (fig. 8).

4. 6. Mean of *S. haematobium* SEA clearance in children of different age groups before praziquantel treatment.

Table 5 shows a comparison of the clearance of soluble egg antigen (SEA) before treatment with a single dose of praziquantel between and within the various age groups. The anova result for the data on Table 5 indicates that there were significant differences between and within the various age groups of children before praziquantel treatment ($F = 7.54$, $P < 0.001$) (Table 6). Post anova tests performed on the data in Table 5 indicates that there were significant differences in the mean SEA excretion in age groups ≤ 5 years and 6 –8 years ($P = 0.012$), 9 – 11 years ($P = 0.003$) and 12 – 14 years ($P = 0.006$) (table 7).

Table. 5: Comparison of log_{10} mean of *S. haematobium* SEA between different age groups of children before praziquantel treatment.

			95% confidence interval	
Age group (Years)	N	Mean ± SEM	Lower limit	Upper limit
≤ 5	14	0.97 ± 0.37	0.17	1.76
6 – 8	21	2.63 ± 0.27	2.06	3.19
9 - 11	50	2.76 ± 0.18	2.40	3.12
12 - 14	62	2.62 ± 0.13	2.35	2.90
≥ 15	11	1.76 ± 0.40	0.87	2.65

Table. 6: Analysis of variance of *S. haematobium* SEA secretion between and Within age groups of children before praziquantel treatment.

	Sum of Squares	Degree of Freedom	Mean square	F	Significance
Between groups	43.46	4	10.86	7.54	0.000
Within groups	220.35	153	1.44		
Total	263.81	157			

Table. 7: Post anova test for the mean difference (MD) in *S. haematobium* SEA secretion between various age groups of children before praziquantel treatment

				95% confidence Interval	
Age group (Years)	Age groups (Years	MD ± SEM	Significance	Lower Limit	Upper Limit
≤ 5 Vs	6 - 8	-1.66 ± 0.41	0.012	-3.06	-0.25
	9 – 11	-1.80 ± 0.36	0.003	-3.09	-0.50
	12 – 14	-1.66 ± 0.36	0.006	-2.92	-0.39
	≥ 15	-0.79 ± 0.48	0.822	-2.49	0.90
6 – 8 Vs	9 – 11	-0.14 ± 0.31	1.000	-1.10	0.83
	12 – 14	-0.003 ± 0.30	1.000	-0.91	0.92
	≥ 15	0.87 ± 0.45	0.607	-0.66	2.39
9 – 11 Vs	12 – 14	0.14 ± 0.23	1.000	-0.50	0.78
	≥ 15	1.00 ± 0.40	0.322	-0.45	2.45
12 – 14 Vs	≥ 15	0.86 ± 0.39	0.478	-0.57	2.30

Significance at P < 0.05 level, Vs = versus

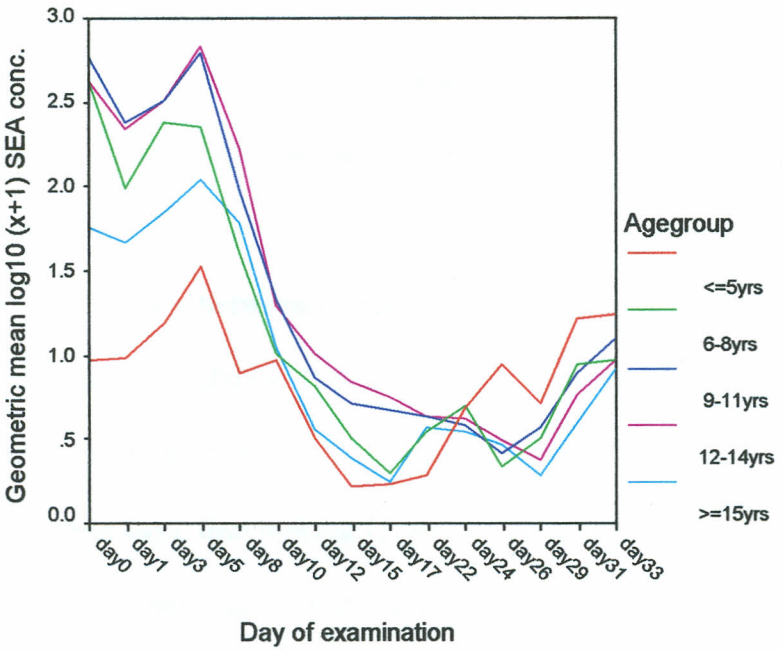


Fig.9. Comparison of the geometric mean *S. haematobium* SEA concentration by age group before and after Praziquantel treatment.

Figure 9 shows the clearance of *S. haematobium* SEA from the body before and after treatment with praziquantel as determined in urine by ELISA. There were variations in SEA clearance in the different age groups. Age group ≤ 5 years had the lowest SEA levels in urine, while age groups 9-11 years and 12-14 years had the highest. The lowest age group ≤ 5years and ≥15years had no significant drop on day one. Soluble egg antigen increased slightly on day 2 for all the age groups. However, there was a sharp decrease in urine SEA in age groups ≤ 5years, 6- 8years and 12-14years on day six all through to day 16 and/or 17. Soluble egg antigen increased slightly in days 18 to 21 for age groups 6-8 years, and ≥ 15years. However, there was increased SEA in children aged ≤ 5years between day 22 and 26 but decreased slightly in the subsequent 3 days. Between day 29 and 33, urine SEA increased in all the age groups. Thus, after treatment with praziquantel, soluble egg antigen (SEA)

was not completely cleared from the body of infected children in all the age groups. The urine samples collected from non-schistosomiasis transmission area (Kangundo) were run concurrently. No Soluble egg antigen (SEA) was detected in those urine samples.

4. 7. Correlation between mean egg counts and mean soluble egg antigen

Figure10 shows the correlation between eggs and SEA clearance as determined in urine during the study period (33 days). The samples were collected on alternate days, thus making 15 time points of urine collection.

The mean eggs counts and mean soluble egg antigen (SEA) levels were significantly positively correlated ($r = 0.981$, $P < 0.01$).

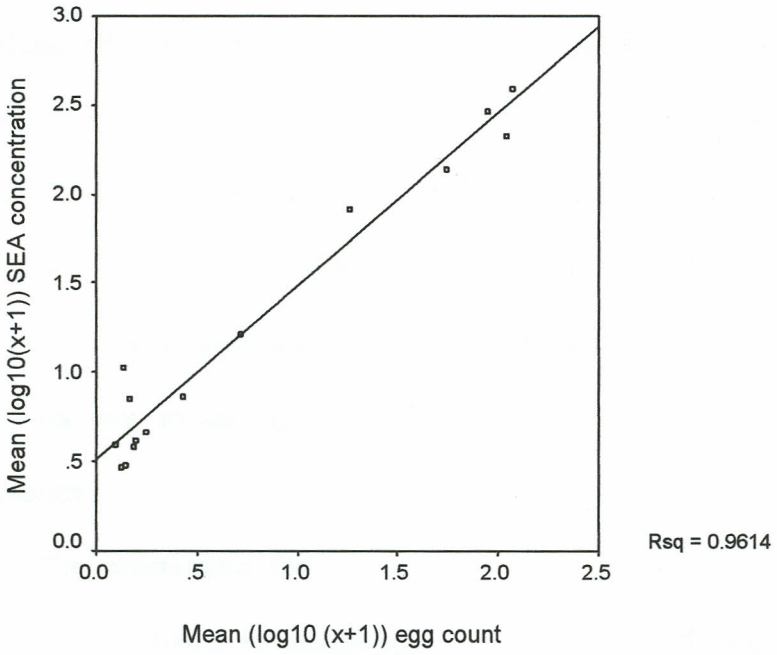


Fig.10. Correlation between mean egg counts and mean SEA concentration in urine.

CHAPTER FIVE

5. 0. Discussion

The present study has examined the dynamics of circulating antigens (SEA) levels in urine (pre and post-treatment), over a period of 33 days after administering a single dose 40-mg of praziquantel / kg body weight. The study also compared the relationship between clearance of SEA and egg counts against age and sex of the school children.

The prevalence of *S. haematobium* infection in school children was 92.8%. Male children had higher infection rate (95.5%) than the females (89.5%). This is in agreement with a study carried out by Kahama *et al.* (1998) who reported the prevalence of *S. haematobium* in Mariakani, Coast Province of 84.8% with 53.4% being males and 46.4% female. The high prevalence observed in males than in females could be attributed to the difference in water contact patterns where males contact water more often than females (Kloos *et al.*, 1997). The male children in this area tend to spend many more hours in water than female children as they play and swim, thereby increasing their chances of acquiring the infection (personal observation).

The observation that 16 cases (10.1%) of the 158 children treated with praziquantel continued to excrete eggs and SEA in urine throughout the study period (33 days) could be associated with low cure rates of praziquantel when a single dose of 40-mg/kg body weight is used. Stelma *et al.* (1997) observed cure rates of between 70 – 100% when a single dose was used. Improved cure rates were observed if praziquantel is administered in split doses given up to 6 hours (El-Masry

et al., 1988). Giboda and Smith (1994) recommended that two doses of praziquantel administered at a 9 – 10 days interval to eliminate all eggs in tissue.

That 4 (2.5%) of the 158 children examined who were originally negative for eggs and SEA in urine turned positive 8 days after praziquantel treatment indicated that there was continuous development of worms in the body of the host. That 10 (6.3%) of the 158 children examined continued excreting SEA in urine after treatment throughout the study period and were negative for egg counts at day 15 and/or 17 indicated that SEA is rapidly reduced but not completely cleared from the body even after treatment. This supports the gradual pattern of decrease of both egg counts and SEA after treatment. This observation is in agreement with that of other researchers who observed that circulating antigens are cleared within days to weeks following successful treatment (De Jonge *et al.*, 1989; Van Lieshout *et al.*, 1992). Other schistosome circulating antigens have also been reported to decrease gradually following chemotherapy including both adult worms and egg antigens (Madwar *et al.*, 1988; Hassan *et al.*, 1992).

That nine (5.7%) of the 158 children who were not excreting SEA originally became positive (excreted SEA) at day 31 and 33 while negative for egg counts at the same time could have been caused by the miracidia in the eggs, which secrete SEA into the circulation, before the eggs have been excreted. This agrees with studies by Kahama *et al.* (1999) who observed that SEA could be demonstrated in urine even when egg output was very low, or had stopped all together.

That twenty-six (16.5%) of the 158 children were excreting SEA and eggs initially, then converted to negative (no eggs or SEA in urine) at days 15, 17, 22, 24

and 26 after treatment with a single dose and again started excreting eggs and SEA on day 29 through to day 33 could be explained by a re-infection and/or maturity of young worms which were lodged in the tissues and which were not affected by the drug. This therefore agrees with the observation that young children and teenagers tend to re-acquire infection more rapidly than do the adults because of their greater frequency of contact with infected water (Mahmoud *et al.,* 1983; Butterworth *et al.*, 1984). That twenty nine (18.4%) of the praziquantel treated children stopped excreting SEA or schistosome eggs in urine 15 days after treatment, indicated that the drug had cleared the infection in that group of children.

Forty eight (30.4%) of the children who were originally secreting SEA and excreting eggs in urine stopped on day 15 and/or 17 after treatment, then became positive for both on day 29 through to day 33 is indicative of worms that continued to develop or a re-infection. Sixteen (10.1%) of the children who were not secreting SEA or excreting eggs in urine continued like that through out the study period indicating they did not acquire infection.

The increase in egg counts and SEA in urine on day 2, 3, 4, and 5 may be attributed to the release of more eggs and SEA from the tissue into the circulation after the parasite and miracidia had died. The role of protective immunity has also been cited as a possible explanation of the age related pre-disposition to re-infection (Wilkins *et al.*, 1987; Butterworth *et al.,* 1985,1988). The slight drop in egg excretion and SEA in urine after treatment between day zero and day one may be due to the effect of the drug on the parasite. After treatment with praziquantel, the worms die and dislodge from their sites of residence in the mesenteric or pelvic veins then

get transported to the liver. Here they are retained and subsequently illicit host tissue reactions (Pearson and Guerrant, 1983; Webbe *et al.*, 1981), making them unable to continue laying eggs.

The rapid increase of SEA and egg counts on day 6 may be attributed to the release of the same from the tissues due to the drug activity in the body. However, that SEA was slightly increased at day 31 onwards could imply a re-infection had occurred and/or further development of young worms that were not affected by the treatment. The soluble egg antigen assay detected the infection earlier than parasitological examination, indicating a higher sensitivity for SEA.

A significant difference was also observed in the clearance of eggs and SEA from the body between male and female children ($P < 0.05$) due to intense water contact by the boys while swimming in stagnant infected waters in the area (Personal observation). These results also agree with those of Kahama *et al.* (1998; 1999) who observed that SEA levels were significantly higher in males than in females. The high egg counts and SEA levels observed in age groups 6 – 8 years, 9 – 11 years and 12 – 14 years, before treatment may be attributed to more worms in the circulation due to the intense water contact activities in these age groups. This observation also agrees with that of Kloos *et al.* (1997).

The observed significant difference in egg excretion and SEA concentration between the age group ≤ 5 years and ≥ 15 years could be attributed to the low worm burden in the lower age group and less exposure and concomitant immunity in the older age group, which is acquired by individuals in endemic areas (Butterworth *et al.*, 1985). The observed significant difference in SEA concentration in urine

between age group ≤ 5 years, 9 – 11 years and 12 – 14 years could be attributed to the lower worm burden in the lower age group and the high worm burden in the higher age (9 – 14 years) with less concomitant immunity.

That there was direct correlation between soluble egg antigen clearance and egg counts in urine of infected school children suggests that the eggs (miracidia) were the source of SEA ($r = 0.981$, $P<0.01$). This was further confirmed by their similar pattern of secretion and excretion. These findings agree with those observed by Nibbelling *et al.* (1998) and Kahama *et al.* (1999) who demonstrated that SEA could quantitatively be detected in urine of *S. haematobium* infected children and that SEA levels correlated well with egg counts and pathology as determined by haematuria and ultrasonography. Undoubtedly, the detection of antigens especially (SEA) in urine would be preferable as it involves a non-invasive method of sample collection. The circulating antigens are a reflection of an on going infection and since the SEA is secreted by live miracidia around tissues, the detection could correlate well with pathology.

Simplification of SEA assay into a dipstick like technique that would be easier to apply in the field will be a step forward in achieving rapid diagnosis in morbidity control programme of *S. haematobium* infection and thereby reducing transmission. Comparison of the clearance of SEA and egg counts between sexes and age groups has contributed more information on the possibility of using SEA as a diagnostic tool as well as a morbidity marker in schistosomiasis infection. A SEA dip stick-like assay when developed could be a better diagnostic tool to be used in *S. haematobium* endemic areas where there is high and low prevalence.

In conclusion, comparison of the clearance of *S. haematobium* soluble egg antigen and egg counts pre and post treatment in both male and female children indicated similar patterns throughout the study period; statistical significant differences occurred in the clearance of SEA and egg counts in some age groups of treated school children and none in others; soluble egg antigen (SEA) assay is able to diagnose *S. haematobium* infection even when eggs are not being excreted in urine and soluble egg antigen(SEA) assay can be used to monitor drug tolerance in infected individuals after treatment since SEA excretion correlates with egg output.

CHAPTER SIX

6. 0. Recommendations

From the outcome of this study, it is recommended that:

- two doses of praziquantel (40 mg/kg body weight) be administered at an interval of 10 -15 days to kill the young worms that will not have been initially affected by the drug. Praziquantel does not act on immature worms and undeveloped eggs and therefore allows them to proceed to maturity and continue excreting eggs into the circulation.
- SEA assay should be standardized and improved into a dipstick- like technique that can be used in *S. haematobium* endemic areas. In this study, significant correlation exists between SEA and egg counts, thus, indicating the level of pathology or infection intensity.
- use of soluble egg antigen (SEA) in mixed *S. haematobium* and *S. mansoni* infections should be evaluated. The clearance of SEA in adults should also be investigated. Soluble egg antigen (SEA) can be compared with Eosinophil cationic protein to assess pathology in relation to ultrasonography and egg counts in children and adults in *S. haematobium* transmission areas.
- a combined assay of SEA and other circulating antigens (CCA and CAA) should be investigated to provide more details on both worm and egg loads and thus add information on worm fecundity. These antigens have direct relation with worm burden.

- soluble egg antigen (SEA) in urine of *S. haematobium* infected individuals should be investigated further as a potential marker of resistance/tolerance to anti-schistosomal drugs.

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APPENDICES

Appendix 1. Median SEA levels and egg counts

MEDEGC	MEDSEA	DAY
187.30	501.60	0
315.50	258.00	1
270.30	545.90	3
15.50	649.00	5
3.00	90.30	8
.50	16.30	10
.00	6.60	12
.00	2.20	15
.00	.45	17
.00	2.10	22
.00	2.40	24
.00	1.30	26
.00	.43	29
.00	3.90	31
.00	14.00	33

Appendix 2. Aggregate log egg count by sex

Sex		Log egc	Gm eggc	day
Male	1	2.11	127.8	0
Female	2	1.81	63.6	0
Male	1	1.89	76.6	1
Female	2	1.61	39.7	1
Male	1	2.2	157.5	2
Female	2	1.91	8.3	2
Male	1	2.24	172.8	3
Female	2	1.92	82.2	3
Male	1	1.54	33.7	4
Female	2	1.02	9.5	4
Male	1	0.86	6.2	5
Female	2	0.6	3.0	5
Male	1	0.5	2.2	6
Female	2	0.36	1.3	6
Male	1	0.31	1.04	7
Female	2	0.19	0.54	7
Male	1	0.24	0.7	8
Female	2	0.14	0.4	8
Male	1	0.07	0.2	9
Female	2	0.11	0.3	9
Male	1	0.25	0.8	10
Female	2	0.15	0.4	10
Male	1	0.11	0.3	11
Female	2	0.13	0.3	11
Male	1	0.13	0.3	12
Female	2	0.16	0.4	12
Male	1	0.2	0.6	13
Female	2	0.1	0.3	13
Male	1	0.17	0.5	14
Female	2	0.1	0.3	14
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Appendix 3. Aggregate log SEA by sex

Sex		Log seac	gmseac	day
Male	1	2.48	300.9	0
Female	2	2.45	280.8	0
Male	1	2.23	168.8	1
Female	2	2.06	113.8	1
Male	1	2.57	370.5	2
Female	2	2.11	127.8	2
Male	1	2.82	659.7	3
Female	2	2.38	238.9	3
Male	1	2.07	116.5	4
Female	2	1.78	59.3	4
Male	1	1.34	20.9	5
Female	2	1.11	11.9	5
Male	1	0.96	8.1	6
Female	2	0.76	4.6	6
Male	1	0.73	4.4	7
Female	2	0.61	3.1	7
Male	1	0.66	3.6	8
Female	2	0.53	2.4	8
Male	1	0.63	3.3	9
Female	2	0.56	2.6	9
Male	1	0.68	3.8	10
Female	2	0.56	2.6	10
Male	1	0.52	2.3	11
Female	2	0.43	1.7	11
Male	1	0.51	2.2	12
Female	2	0.45	1.8	12
Male	1	0.9	6.9	13
Female	2	0.8	5.3	13
Male	1	1.11	11.9	14
Female	2	0.96	8.1	14

Appendix 4. Aggregate egg count by age group

Agegrp		Logegc	gmeggc	day
≤ 5yrs	1	0.87	6.4	0
6-8yrs	2	1.92	82.2	0
9-11yrs	3	2.19	153.9	0
12-14yrs	4	2.11	127.8	0
≥15yrs	5	1.46	27.8	0
≤ 5yrs	1	0.80	5.3	1
6-8yrs	2	1.95	88.1	1
9-11yrs	3	1.91	80.2	1
12-14yrs	4	1.88	74.9	1
≥15yrs	5	1.01	9.2	1
≤ 5yrs	1	1.14	12.8	2
6-8yrs	2	2.14	137	2
9-11yrs	3	2.16	143.5	2
12-14yrs	4	2.27	185.2	2
≥15yrs	5	1.16	13.5	2
≤ 5yrs	1	0.87	6.4	3
6-8yrs	2	2.01	101.3	3
9-11yrs	3	2.30	198.5	3
12-14yrs	4	2.29	194	3
≥15yrs	5	1.46	27.8	3
≤ 5yrs	1	0.43	1.7	4
6-8yrs	2	0.94	7.7	4
9-11yrs	3	1.44	26.5	4
12-14yrs	4	1.48	29.2	4
≥ 15yrs	5	0.86	6.2	4
≤ 5yrs	1	0.28	0.9	5
6-8yrs	2	0.46	1.9	5
9-11yrs	3	0.69	3.9	5
12-14yrs	4	0.96	8.1	5
≥15yrs	5	0.55	2.5	5
≤ 5yrs		0.15	0.4	6
6-8yrs	2	0.25	0.8	6
9-11yrs	3	0.41	1.6	6
12-14yrs	4	0.59	2.9	6
≥15yrs	5	0.31	1	6
≤ 5yrs	1	0.08	0.2	7
6-8yrs	2	0.23	0.7	7
9-11yrs	3	0.23	0.7	7
12-14yrs	4	0.34	1.2	7
≥15yrs	5	0.10	0.3	7
≤ 5yrs	1	0.15	0.4	8
6-8yrs	2	0.14	0.4	8
9-11yrs	3	0.23	0.7	8
12-14yrs	4	0.20	0.6	8

≥15yrs	5	0.08	0.2	8
≤ 5yrs	1	0.09	0.2	9
6-8yrs	2	0.14	0.4	9
9-11yrs	3	0.11	0.3	9
12-14yrs	4	0.06	0.1	9
≥15yrs	5	0.02	0.04	9
≤ 5yrs	1	0.28	0.9	10
6-8yrs	2	0.30	0.9	10
9-11yrs	3	0.18	0.5	10
12-14yrs	4	0.19	0.5	10
≥15yrs	5	0.12	0.3	10
≤ 5yrs	1	0.18	0.5	11
6-8yrs	2	0.17	0.5	11
9-11yrs	3	0.15	0.4	11
12-14yrs	4	0.09	0.2	11
≥15yrs	5	0.04	0.1	11
≤ 5yrs	1	0.20	0.6	12
6-8yrs	2	0.13	0.3	12
9-11yrs	3	0.22	0.7	12
12-14yrs	4	0.09	0.2	12
≥15yrs	5	0.08	0.2	12
≤ 5yrs	1	0.28	0.9	13
6-8yrs	2	0.20	0.6	13
9-11yrs	3	0.18	0.5	13
12-14yrs	4	0.14	0.4	13
≥15yrs	5	0.03	0.07	13
≤ 5yrs	1	0.43	0.7	14
6-8yrs	2	0.22	0.7	14
9-11yrs	3	0.17	0.5	14
12-14yrs	4	0.06	0.1	14
≥15yrs	5	0.01	0.1	14

Appendix 5. Aggregate SEA by age group

Agegrp		Logseac	gmseac	day
≤ 5yrs	1	0.97	8.3	0
6-8yrs	2	2.63	425.6	0
9-11yrs	3	2.76	574.4	0
12-14yrs	4	2.62	415.9	0
≥15yrs	5	1.76	56.5	0
≤ 5yrs	1	0.98	8.5	1
6-8yrs	2	1.99	96.7	1
9-11yrs	3	2.38	238.9	1
12-14yrs	4	2.34	217.8	1
≥15yrs	5	1.66	44.7	1
≤ 5yrs	1	1.18	14.1	2
6-8yrs	2	2.38	238.9	2
9-11yrs	3	2.50	315.2	2
12-14yrs	4	2.51	322.6	2
≥15yrs	5	1.85	69.8	2
≤ 5yrs	1	1.53	32.9	3
6-8yrs	2	2.36	228.1	3
9-11yrs	3	2.80	629.9	3
12-14yrs	4	2.83	675.1	3
≥15yrs	5	2.04	108.6	3
≤ 5yrs	1	0.89	6.8	4
6-8yrs	2	1.60	38.8	4
9-11yrs	3	1.98	94.5	4
12-14yrs	4	2.22	164.9	4
≥15yrs	5	1.78	59.3	4
≤ 5yrs	1	0.98	8.5	5
6-8yrs	2	1.00	9	5
9-11yrs	3	1.33	20.4	5
12-14yrs	4	1.29	18.5	5
≥15yrs	5	1.04	9.9	5
≤ 5yrs	1	0.51	2.2	6
6-8yrs	2	0.81	5.5	6
9-11yrs	3	0.87	6.4	6
12-14yrs	4	1.00	9	6
≥15yrs	5	0.56	2.6	6
≤ 5yrs	1	0.22	0.7	7
6-8yrs	2	0.51	2.2	7
9-11yrs	3	0.71	4.1	7
12-14yrs	4	0.84	5.9	7
≥15yrs	5	0.39	1.5	7
≤ 5yrs	1	0.23	0.7	8
6-8yrs	2	0.29	0.9	8
9-11yrs	3	0.67	3.7	8
12-14yrs	4	0.76	4.8	8

≥15yrs	5	0.25	0.8	8
≤ 5yrs	1	0.28	0.9	9
6-8yrs	2	0.54	2.7	9
9-11yrs	3	0.64	3.4	9
12-14yrs	4	0.63	3.3	9
≥15yrs	5	0.57	2.7	9
≤ 5yrs	1	0.68	3.8	10
6-8yrs	2	0.69	3.9	10
9-11yrs	3	0.58	2.8	10
12-14yrs	4	0.63	3.3	10
≥15yrs	5	0.55	2.5	10
≤ 5yrs	1	0.94	7.7	11
6-8yrs	2	0.33	1.1	11
9-11yrs	3	0.41	1.6	11
12-14yrs	4	0.49	2.1	11
≥15yrs	5	0.47	1.9	11
≤ 5yrs	1	0.71	4.1	12
6-8yrs	2	0.50	2.2	12
9-11yrs	3	0.57	2.7	12
12-14yrs	4	0.38	1.4	12
≥15yrs	5	0.29	0.9	12
≤ 5yrs	1	1.22	15.6	13
6-8yrs	2	0.94	7.7	13
9-11yrs	3	0.89	6.8	13
12-14yrs	4	0.76	4.8	13
≥15yrs		0.60	2.9	13
≤ 5yrs	1	1.24	16.4	14
6-8yrs	2	0.98	8.5	14
9-11yrs	3	1.10	11.6	14
12-14yrs	4	0.97	8.3	14
≥15yrs		0.92	7.3	14

Appendix 6. Aggregate correlation data

Logegc	Logsea
1.95	2.46
1.74	2.14
2.04	2.33
2.07	2.59
1.26	1.91
0.72	1.22
0.43	0.86
0.25	0.67
0.19	0.59
0.09	0.59
0.2	0.61
0.12	0.47
0.15	0.48
0.16	0.85
0.13	1.03