

***SCHISTOSOMA HAEMATOBIIUM* INFECTION IN  
SCHOOL  
CHILDREN AND WOMEN OF REPRODUCTIVE AGE:  
THE EFFECT ON ANAEMIA, BLOOD PATHO-  
PHYSIOLOGICAL CHANGES IN SELECTED PARTS  
OF KWALE, KILIFI AND BIRTH WEIGHT  
OUTCOMES IN TANA RIVER, COUNTY,  
COAST PROVINCE, KENYA**

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***Schistosoma haematobium* Infection in School Children and Women  
of Reproductive Age: the Effect on Anaemia, Blood Patho-  
physiological Changes in Selected Parts of Kwale, Kilifi and Birth  
Weight Outcomes in Tana River, County, Coast Province, Kenya**

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**A thesis submitted in fulfillment for the degree of Doctor of  
Philosophy in Parasitology in the Jomo Kenyatta University of  
Agriculture and Technology**

**2013**

**DECLARATION**

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

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## **DEDICATION**

This long journey is dedicated to my family Kirote, Mdara, Nanzia and Iduri who walked this narrow path with me tirelessly – “the blessed will not struggle, he who owns will possess”, “*Mwenye rathi hasumbuki, mpewa hapokonyeki*” my late father once told me.

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## ACRONYMS

<b>AIDS</b>	Acquired Immune Deficiency Syndrome
<b>ANE</b>	Anaemia
<b>ANOVA</b>	Analysis of Variance
<b>BH</b>	Bohoni
<b>BCG</b>	Bacille Callimet Guerine
<b>BMI</b>	Body Mass Index
<b>BUN</b>	Blood Urea Nitrogen
<b>CDC</b>	Centre for Disease Control
<b>CN</b>	Chanani
<b>CW</b>	Chewani
<b>CI</b>	Confidence Interval
<b>CREAT</b>	Creatinine
<b>DALYs</b>	Disability Adjusted Life Years
<b>DNA</b>	Deoxyribonucleic Acid
<b>ECP</b>	Eosinphil Cationic Protein
<b>EDTA</b>	Ethylene Diamine Tetra-Acetic Acid
<b>ERY</b>	Erythrocytosis
<b>EOC</b>	Eosinophilia
<b>FUS</b>	Female Urogenital Schistosomiasis
<b>FGS</b>	Female Genital Schistosomiasis
<b>GPS</b>	Global Positioning System
<b>GLDH</b>	Glutamate Dehydrogenase

<b>GLMM</b>	General Linear Mixed –effect Model
<b>G/DL</b>	Grams per Deciliter
<b>HPCR</b>	Hypochromia
<b>HB</b>	Haemoglobin
<b>HIV</b>	Human Immunodeficiency Virus
<b>IDA</b>	Iron Deficiency Anemia
<b>INACG</b>	International Nutrition Anemia Consultative Group
<b>IL</b>	Interleukin
<b>ITN</b>	Insecticide Treated Net
<b>KEMRI</b>	Kenya Medical Research Institute
<b>LN</b>	Lenda
<b>LTS</b>	Lutsangani
<b>LBW</b>	Low Birth Weight
<b>LDCs</b>	Lesser Developed Countries
<b>LEC</b>	Leukocytosis
<b>LEKP</b>	Leukopenia
<b>MA</b>	Macrocytic Anemia
<b>MDA</b>	Mass Drug Administration
<b>MCR</b>	Microcytosis
<b>MJ</b>	Majengo
<b>MW</b>	Mwaluphamba
<b>MC</b>	Mwachinga
<b>MZ</b>	Mienzeni

<b>MDG</b>	Millenium Development Goal
<b>NNA</b>	Normocytic Normochromic Anemia
<b>NADH</b>	Nicotinamide Adenine Dinucleotide H
<b>NAD</b>	Nicotinamide Adenine Dinucleotide
<b>NTDs</b>	Neglected Tropical Diseases
<b>PI</b>	Principal Investigator
<b>QC</b>	Quality Control
<b>RBC</b>	Red Blood Cell
<b>RB</b>	Rabai
<b>STH</b>	Soil Transmitted Helminth
<b>SOPs</b>	Standard Operating Procedures
<b>SSC</b>	Scientific Steering Committee
<b>STD</b>	Sexually Transmitted Disease
<b>STI</b>	Sexually Transmitted Infection
<b>TDR</b>	Tropical Disease Research
<b>THRC</b>	Thrombocytosis
<b>THRP</b>	Thrombocytopenia
<b>TNF</b>	Tumor Necrosis Factor
<b>TBAs</b>	Traditional Birth Attendants
<b>UGS</b>	Urogenital Schistosomiasis
<b>UNAIDs</b>	United Nations and Aids
<b>WBC</b>	White Blood cell
<b>WHO</b>	World Health Organization

## ABSTRACT

In Kenya *Schistosoma haematobium* is predominantly prevalent in Coastal region. In the present study, comparing diagnosis of *Schistosoma haematobium* using blood in urine questionnaire and urine filtration (egg counts) revealed prevalence by both methods of between 0 and 91.3% in school children in Coastal region of Kenya. Using a threshold of 30% reported blood in urine to identify high (>50%) schools, yielded a sensitivity of 90.0% (95% CIs: 54.4–99.7%) and a specificity of 97.1% (95% CIs: 85.1–100%). The prevalence of *S. haematobium* in school children informed the present study on where to conduct further research of this infection in women of reproductive age. Two Counties (Tana River and Kwale) were selected to investigate schistosomiasis infection, anaemia, blood patho-physiological changes and birth weight outcomes in pregnant and Non-pregnant women. Pregnant women in Kwale County had a higher prevalence of infection with urinary schistosomiasis than non-pregnant 36.9% and 30.0%, they also had a higher intensity of infection 51.1 eggs/10mls and 40.9 eggs/10ml of urine respectively. Younger women aged between 16 and 26 years, had higher infection rates and intensity 37.6% and 56 e/10mls respectively. Out of two hundred and fifty (250) samples analysed for serum urea and creatinine as a proxy for kidney function; none had significant levels of pathology, either in pregnant or non-pregnant women. Fifty percent (50%) of the women had *S. haematobium* infection; among them 50% were suffering from anaemia (Hb less than 11g/dl and RBC count of less than  $4.2 \times 10^6 \mu\text{l}$ ) and other haematological conditions. The study has shown that there is elevated eosinophil counts in women who are infected with *S. haematobium* and equally high in pregnant women than non-pregnant women. This study reports decreased levels of thrombocytes (platelets) (thrombopenia) among pregnant women infected with *S. haematobium*. Using peripheral blood film examination and haematological indices analysis, 20.3% of the women, were diagnosed with Normocytic

Normochromic Anemia (NNA) while 50% were diagnosed with Macrocytic Anaemia (MA) (Macrocytosis). The possibility of women infected with *S. haematobium* delivering under-weight children was also investigated in 32 pregnant women in 5 villages in Hola, Tana River. One third of the pregnant women infected with *S. haematobium* delivered underweight children who were less than 2.5Kgs after full term (5/19=26.3%). In this study, preterm deliveries were not investigated since this was a quasi-longitudinal study. The study has established that, school children in parts of coastal region have high infection of *S. haematobium*. In conclusion, the study demonstrates high prevalence and intensity of *S. haematobium* infection in women of reproductive age in Kwale and Tana River County. The study also reports the different anemia conditions diagnosed in women of reproductive age in parts of Tana River County. It is thus, recommended that clinician be encouraged to investigate these specific anemia conditions, for proper management of anemia in pregnant women. Women in endemic areas spend a very large part of their reproductive lives involved in activities that expose them to infection and their treatment during gestation will not only improve on their health but alleviate their already poor nutritional status and birth weight outcomes.



## CHAPTER ONE

### 1.0 INTRODUCTION AND LITERATURE REVIEW

#### 1.1 Human schistosomiasis

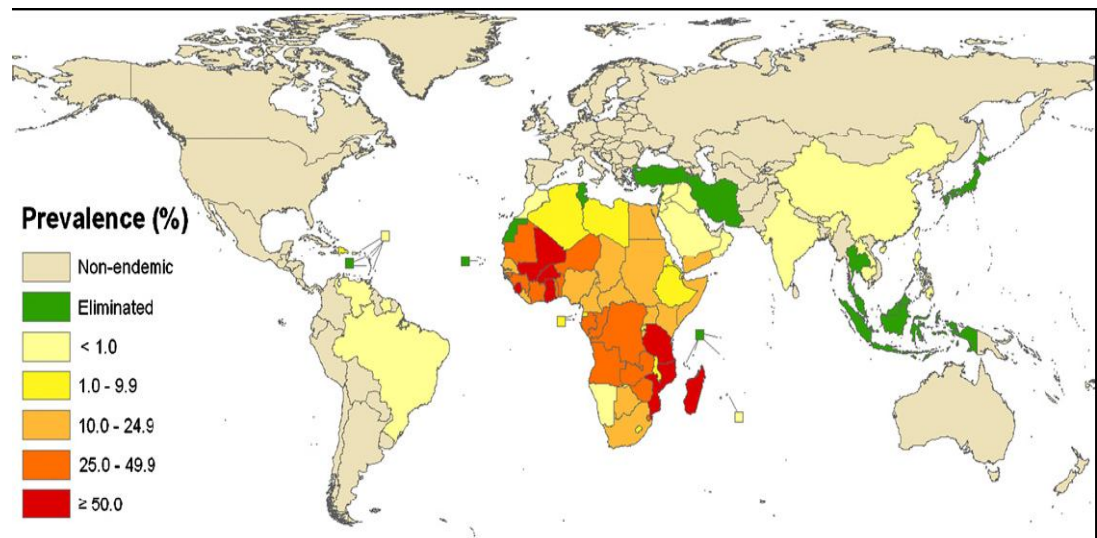
Schistosomiasis is a snail-transmitted disease caused by digenetic trematodes of the genus *Schistosoma* primarily infecting man. There are five main species that infect human being; *S. haematobium*, *S. mansoni*, *S. japonicum*, *S. intercalatum*, and *S. mekongi*. Other schistosome species are parasites of animals and birds some of which occasionally hybridize with human schistosomes. The different species exhibit distinct differences pertaining to size, the intermediate host, the final location in the definitive host and the number and morphological features of the eggs laid by the adult worms.

Adult males and females of *S. haematobium* reside in the venules of the pelvic plexus while the other species (*S. mansoni*, *S. japonicum*, *S. intercalatum*, and *S. mekongi*) are found in the mesenteric veins. Adult schistosomes are white-grey worms 1 to 1.5cm long and 0.2cm thick, with a cephalic and ventral sucker; the sexes are separated. The female worm is slender and cylindrical; the male is stouter with flattened lateral extensions which hold the female in a gynaecophoric canal. The female produces eggs with characteristic terminal or lateral spine depending on the species. Schistosomes have an average life span of 3 to 5 years, but may live for up to 30 years in the human body (Webbe, 1981).

#### 1.2 Epidemiology

Global statistics for mid-2003 suggest that almost 800 million individuals were at risk of schistosomiasis, 207 million were infected, 120 million suffered from clinical disease and 20 million exhibited severe morbidity (Chitsulo *et al.*, 2000; Steinmann *et al.*, 2006). An estimated 97% of all infected people are concentrated

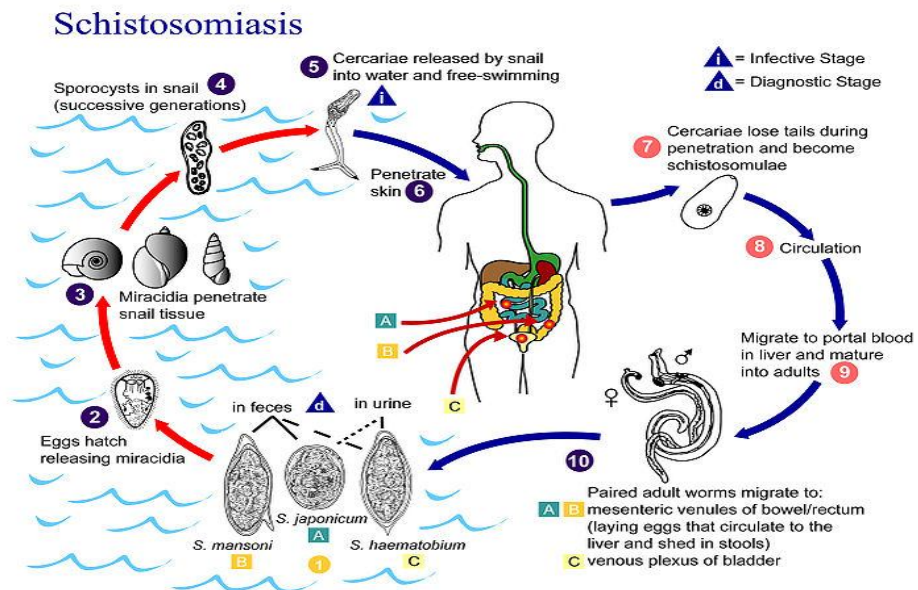
in Africa, which is partially explained by the social–ecological context detailed above, weak or non-existing health systems, poverty and general neglect (Bruun and Aagaard-Hansen, 2008; Hotez and Fenwick, 2009; Stothard *et al.*, 2009; Utzinger *et al.*, 2009). Fig. 1 shows a global map with estimated country-specific prevalence rates of schistosomiasis as of mid-2003.



**Figure 1.1: Current global distribution of Schistosomiasis stratified according to country-specific prevalence estimates. Source: Steinmann, *et al.*, (2006) and Utzinger, *et al.*, (2009).**

Geographical distribution of schistosomiasis is dependent on the distribution of the appropriate snail vectors. Transmission is linked to poor hygiene, human water contact patterns and the presence of the snail vector which can only breed in warm climatic conditions. *Schistosoma haematobium* primarily transmitted by aquatic pulmonate snails of the genus *Bulinus* is distributed in north and sub-sahara Africa, some islands of the Indian Ocean and Asia Minor). Similarly, *S. mansoni* (intestinal

schistosomiasis), is transmitted by aquatic snails of the genus *Biomphalaria*. Its distribution is wide spread in Africa south of the Sahara and some parts of Middle East. Schistosomiasis is typically an infection of the rural areas; however, urban schistosomiasis is an increasing problem in many countries especially in Africa (Steinmann, *et al.*, 2006). Population movements and hydraulic modifications of the environment have had a considerable impact on the extension of the disease in the last decades (Gryseels, 1991). While the distribution of infection has changed with 80 – 85% of current disease now found in sub-Saharan Africa. The number of people infected is not decreasing, furthermore, there is growing awareness that the impact of schistosomiasis, long under estimated, rivals that of Malaria and tuberculosis (Bergquist, 2002). In line with the scope of this thesis and publications thereof, these chapters will primarily be concerned with *S. haematobium* with examples of other species where applicable.



**Figure 1.2: Life cycle of Schistosomiasis. (Courtesy of CDC, 2010)**

The schistosome life cycle (Figure 1.2) undergoes an alteration of generations in two different hosts with the asexual, multiplying stage in the intermediate snail host and the sexual non-multiplying stage in the definitive host (human). Mature eggs hatch only in fresh water (with right temperature and light), releasing a ciliated free swimming larval stage known as miracidium, which remains infective for up to 48 hours and must find appropriate intermediate host to continue the life cycle. The miracidium penetrates the snail and transforms into primary sporocyst which intern develops into daughter sporocysts, migrate to the snail's digestive gland and mature and multiply (germ ball), eventually releasing many unisexual free swimming cercariae into water. When definitive host (man) is in contact with water, cercariae will penetrate the skin, shed its tail and transform into a schistosomulum, which then enters the blood stream. The schistosomulum is transported passively by the blood flow via the left side of the heart and lungs and to eventually reach the liver where the worms feed and attain maturity (male and female).

Depending on the species the paired worms either migrate to the mesenteric veins (*S. mansoni*, *S. japonicum*, *S. intercalutum*) or to the veins of the vesicle plexus (*S. haematobium*). It is in this location that the female will lay their eggs whose number varies depending on the species. Many eggs are trapped in the tissues as they are transported in the blood stream and are the cause of the observed morbidity. About half of the eggs penetrate through the bladder or intestinal wall and are excreted to the external environment via the urine or faeces to continue the life cycle.

### **1.3 Pathology and morbidity due to schistosomiasis**

Schistosomiasis is a chronic disease with infection occurring in early childhood, while due to continued water-contact activities, accumulation of worms may continue to early adulthood. Survival of the mature worms normally ranges from 2.5 – 12 years, but there have been reports of worms living for up to 30 years (Vermund, 1983). Schistosomiasis is a granulomatous disease which may affect several organs in the body including the urinary system. Smith and Christie, (1986) noted that hydroureter usually precedes hydronephrosis and that schistosomal hydro nephrosis passes from progressive renal pelvic dilatation to medullary atrophy and finally to nearly total effacement of the medulla before cortical atrophy occurs.

#### **1.3.1 Invasion stage**

Shortly after penetration of the cercariae (within a few hours), a pruritic papular rash can occur at the site of penetration. The skin manifestation mainly occurs in migrants and tourists coming into contact with schistosomes for the first time.

#### **1.3.2 Acute schistosomiasis**

Two to 10 weeks after the initial infection, the Katayama syndrome can develop mainly in non-immune visitors to endemic areas and more severe after *S. mansoni* infection (Zuidema., 1981; Visser et al., 1995). Presenting symptoms vary from general malaise, to severe illness. The most common clinical findings are, fever,

anorexia, headache, abdominal pains, myalgia, arthralgia, diarrhea, loss of weight, hepatomegaly, splenomegaly and urticaria (Stuiver, 1984, Doherty, *et al.*, 1996).

### **1.3.3 Early manifestation of *S. haematobium***

Pathology of *S. haematobium* infected individuals most frequently occurs in the bladder. In the active phase of infection, the pathological changes observed in the bladder wall including among others granulomas consisting of eggs and different types of inflammatory cell, sandy patches as a result of dead and calcified eggs and polypoid lesions, the extent of which depends on the intensity and duration of infection. This is more common in children and young adults (Garba, *et al.*, 2006). In highly endemic areas, bladder lesions can be detected in up to 89% of the population and major bladder lesions in 44% (Hatz *et al.*, 2001). The lesions produce the early symptoms (terminal) haematuria, dysuria, urinary frequency and supra pubic pain. It has been suggested that the loss of blood causes anaemia (Farid *et al.*, 1968).

Haematuria is the most common sign of urinary schistosomiasis and it has been estimated that infected individuals lose between 2.6 and 12.6 millilitres of blood per day, (Farid, *et al.*, 1968). In a well-designed, cross sectional survey conducted in Tanzania, *S. haematobium* infection was related to both decreased hemoglobin and decreased iron in adults, after adjusting for measures of socioeconomic status, malaria and hookworm (Tatala *et al.*, 1998). In school children (aged 5–14 years),

adolescents and adults (aged > 15 years) and the presence of *S. haematobium* infection was related to increased risk of anemia (Beasley et al., 1999).

#### **1.3.4 Late manifestation of *S. haematobium***

Eggs deposited in the tissues of the ureters can cause obstructive uropathy, thus hydronephrosis and hydronephrosis, which can be visualized by ultrasonography. One study showed that presence of lesions was related to the intensity of infection both on individual and population level (Hatz, 2001). Calcification of the bladder has been suggested as a risk factor towards the development of bladder cancer. Obstructive uropathy can give rise to gradual compression of the kidney parenchyma eventually causing non-functioning kidney. The glomerular and proximal tubular function of the kidney may remain intact for a long time (Browning *et al.*, 1984). Squamous cell bladder carcinoma may contribute to death attributed to *S. haematobium* infection.

In a highly infected population in Uganda (89% prevalence of infection) mortality was 2,600/100,000 of which at least 25% was due to *S. mansoni* infection resulting in 650/100,000 per year (Ongom and Brandley, 1972). Whereas the most typical symptoms of urinary schistosomiasis, namely, hematuria and proteinuria, are directly due to the emergence of eggs, symptoms of intestinal schistosomiasis are unspecific, including abdominal pain, diarrhea, and blood in stool. Eventually, a heavy and chronic *S. haematobium* infection can lead to bladder cancer and kidney failure, whereas chronic *S. mansoni* infections can lead to hematemesis, and heavy

*S. japonicum* infections to liver cirrhosis, which is a risk factor for colon and liver cancer (Utzinger et al, 2011).

#### **1.4 Burden of Schistosomiasis**

Current estimates of the global number of individuals with morbidity due to infection with schistosomes were based on assumption (not derived from data) that 10% of 200 million individuals infected have severe clinical disease and that of the remaining 180 million 50 – 60% also have symptoms (WHO, 1993). Most mortality caused by Schistosomiasis will be due to specific symptoms developed many years after the initial infection, making it difficult to ascribe them to the infection. Experts in Schistosomiasis morbidity believe that the calculations of the global burden of disease initiative significantly underestimate the burden of Schistosomiasis and needs revision (WHO, 2002). Infected persons who experience the low grade pathologies are high, however, the overall impact on Disability Adjusted Life Years (DALY's) lost due to these manifestations is greater than that of severe pathologies that are more directly life threatening (King *et al.*, 2005).

#### **1.5 Female Genital Schistosomiasis**

Female Genital Schistosomiasis (FGS) has been documented in various studies (Poggensee *et al.*, 1999; Kjetland *et al.*, 2005; Ajanga *et al.*, 2006). Up to 75% of women in areas endemic for urogenital Schistosomiasis may have female genital schistosomiasis (WHO 2009). The ova of *Schistosoma haematobium* are classically excreted in urine or trapped in the bladder wall. However, ova may become deposited in genital tissue causing chronic inflammation, fibrous tissue and



calcification. These so called ‘sandy patches’ areas of granulomatous lesions containing schistosome ova, are pathognomonic for *S. haematobium* infection and may occur both in urinary bladder and in the genital mucosa (Kjetland *et al.*, 2005). Vaginal washes from women with genital *S. haematobium* infection contain elevated levels of Eosinophil Cationic Protein (ECP) (Midzi *et al.*, 2003), suggesting that the lesions provide an enabling environment, perhaps even further increasing susceptibility to HIV 1 infection (Karanja *et al.*, 2002).

In general, these morbid effects are most pronounced in populations already at risk (nutritionally deprived, poor iron diets) or during times of increased metabolic and nutritional demand (during rapid growth after puberty and pregnancy) (Olds, 2003).

A research by Hinderaker (2002) showed that anaemia with Haemoglobin below 9g/dl was associated with nutritional factors such as Iron deficiency, foliate deficiency and vitamin A deficiency. Female Genital Schistosomiasis has been neglected during a period when considerable progress has been achieved in almost any other field of Schistosomiasis research (Olds, 2003).

### **1.5.1 Schistosomiasis in women of reproductive age**

Female Urogenital Schistosomiasis (FUS) is a lifetime disease acquired primarily in childhood by exposure to *S. haematobium*, one of the two main schistosomes transmitted in Africa. Female urogenital schistosomiasis is predominantly caused by *Schistosoma haematobium* and has been estimated by the World Health Organization to affect up to 45 million women living in sub-Saharan Africa (WHO, 2009). Adult *S. haematobium* worms inhabit blood vessels surrounding the urinary bladder and female genital tract and lay eggs that migrate through tissue of

proximate organs, causing chronic granulomatous inflammation most commonly in the urinary bladder, ureters, cervix, and vagina. Because the urinary and genital tracts are almost always both affected, the WHO has recently renamed this disease urogenital schistosomiasis, with detection of *S. haematobium* in the urine or genital tract being diagnostic (WHO, 2009).

In women, the clinical picture of urogenital Schistosomiasis may present with a range of signs and symptoms, including lesions of the cervix and vagina, vaginal bleeding, pain during sexual intercourse and nodules in the vulva. There may also be long-term irreversible consequences, including infertility. Previous policy has excluded pregnant and lactating women from the control of schistosomiasis using praziquantel treatment (WHO, 1998; 1999) but this policy was rescinded following a review in 2002 (WHO, 2002; Allen, 1998).

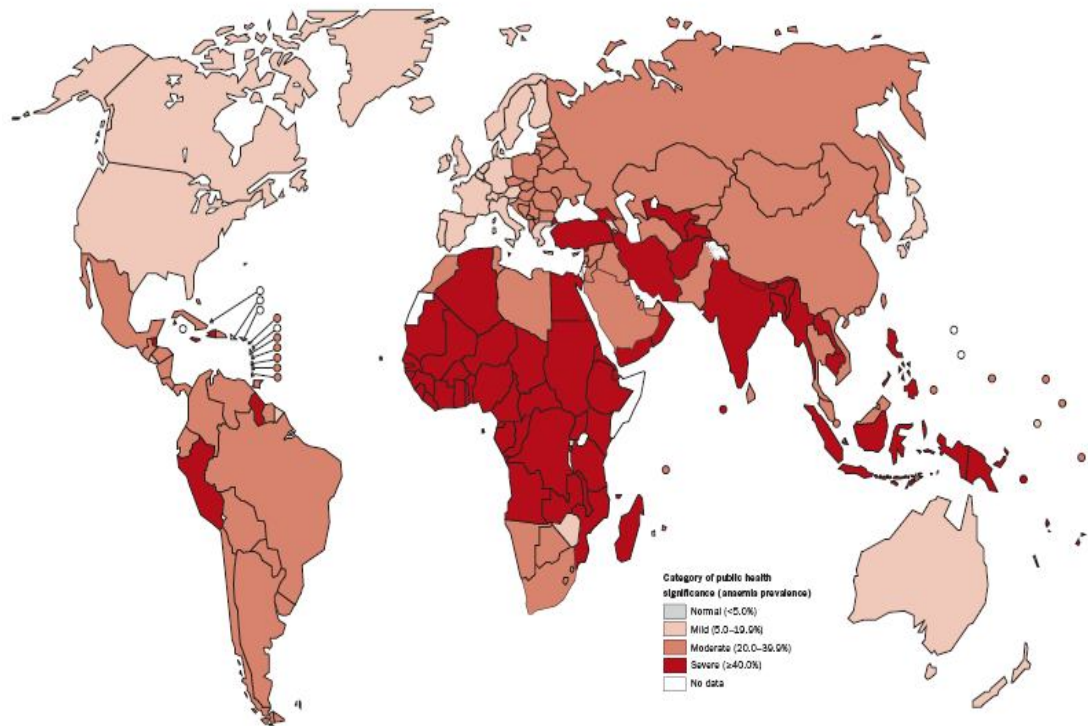
### **1.6 Anaemia during schistosomiasis infection**

Anaemia is the result of a wide variety of causes that can be isolated, but more often coexist. Globally, the most significant contributor to the onset of anaemia is iron deficiency (IDA) so that IDA and anaemia are often used synonymously, and the prevalence of anaemia has often been used as a proxy for IDA. It is generally assumed that 50% of the cases of anaemia are due to iron deficiency (WHO, 2001)), but the proportion may vary among population groups and in different areas according to the local conditions (Bothwell and Charlton, 1981). The main risk factors for IDA include a low intake of iron, poor absorption of iron from diets high in phytate or phenolic compounds, and period of life when iron requirements are

especially high ( thus growth and pregnancy). Among the other causes of anaemia, heavy blood loss as a result of menstruation, or parasite infections such as Hookworms, malaria, and schistosomiasis can lower blood haemoglobin (Hb) concentrations (Ayoya, *et al.*, 2006; Dreyfuss *et al.*, 2006).

Assessing the causes of anaemia is complex, especially where many different etiologies are involved simultaneously, as is the case in much of the developing world, (Sturrock *et al.*, 1996; Tatala *et al.*, 1998). Anaemia in the developing world is caused primarily by four processes (Weatherall, *et al.*, 1990), first, dietary insufficiencies of micronutrients including iron (exacerbated by pregnancy), B12 and folate. Second, iron deficiency caused by extra-corporeal blood loss: (i) blood loss in stools from Hookworm; (ii) in either stools or urine caused by schistosomiasis; and (iii) Trichuris, particularly with dysentery syndrome. Third, hemolysis (RBC destruction) caused by malaria and hemoglobinopathy (such as sickle-cell disease and thalassemias). Fourth, anaemia of inflammation and chronic diseases such as HIV, tuberculosis and, possibly, malaria and schistosomiasis (King *et al.*, 2005). .

Figure 3.1b Anaemia as a public health problem by country: Pregnant women



**Figure 1.3: Worldwide distribution map of anaemia in pregnant women (Courtesy of WHO Report, 2008).**

Globally, anaemia affects 1.62 billion people (95% CI: 1.50–1.74 billion), which corresponds to 24.8% of the population (95% CI: 22.9–26.7%) (Fig.3). The highest prevalence is in preschool-age children (47.4%, 95% CI: 45.7–49.1), and the lowest prevalence is in men (12.7%, 95% CI: 8.6–16.9%). World Health Organization regional estimates generated for preschool-age children and pregnant and non-pregnant women indicate that the highest proportion of individuals affected is in Africa (47.5–67.6%) (WHO, 2008).

World Health Organization definition of anaemia in pregnancy as haemoglobin (Hb) < 10.5 g/dl in women in the second trimester and severe anaemia as Hb < 7.0g/dl, which takes into account the expected plasma volume expansion during pregnancy (WHO, 1993). Heavy infection with *S. mansoni* has been shown to contribute to anaemia in pregnant women in an area in Tanzania (Ajanga *et al.*, 2006). Subsequently, they may end up with “subtle” morbidity including, stunting growth and reduced cognition and mortality (Nokes *et al.*, 1999). If egg-antigen-inflammatory processes contribute to poor pregnancy outcomes, then treatment during pregnancy could be too late. This, therefore, requires health initiatives to keep women of reproductive age free from infection (Friedman *et al.*, 2007).

In pregnant or lactating women in endemic areas, these conditions may be complicated further by malnutrition and low immunity resulting in more pathology related to liver and kidney function (King *et al.*, 2005). *Schistosoma mansoni* infection is associated with increased risk of anaemia (Ajanga *et al.*, 2006). A recent study in *S. japonicum* infected patients found anaemia across all infection levels while occult blood loss was observed only at the highest infection intensities, suggesting that blood loss alone does not explain the anaemia associated with Schistosomiasis, the authors suggested that anaemia of inflammation may be involved (Friedmann *et al.*, 2005). Interestingly, IL-10, which performs anti-inflammatory role both generally and during Schistosomiasis, has also been associated with anaemia of inflammation (Tilg *et al.*, 2002; Hoffmann *et al.*, 2000). In murine model of hepatosplenic Schistosomiasis, anaemia in some animals

worsened as the infection became chronic (Adewusi *et al.*, 1996). Chronic exposure to TNF- $\alpha$  has been associated with anaemia, ascites accumulation, thymic atrophy, and splenic enlargement (Hernandez-Caselles and Stutman, 1993). Most immunological studies in human Schistosomiasis focus on protective immune responses resulting in fibrosis and organomegaly (Birgitte, 2004; Dessein *et al.*, 2004).

Anaemia in pregnancy is an important contributor to maternal ill health and mortality especially around the time of delivery (Steketee, 2003). It is also associated with an increased risk of intrauterine growth retardation, premature delivery and low birth weight resulting in an increase in prenatal mortality (Brabin *et al.*, 2003). *Schistosoma haematobium* infections can lead to changes in both the male and female genital tracts that could promote transmission of sexually transmitted diseases. A study by Ahmed and Abdel Azia (1995) from Egypt, demonstrated that pregnant women with schistosomiasis had more impaired renal function than uninfected pregnant controls. Clinically apparent vulva, vaginal and cervical Schistosomiasis is a common gynaecological finding in areas where infection with *S. haematobium* prevails (Friedberg *et al.*, 1991).

Community based epidemiological studies on patho-physiological changes due to *S. haematobium* infections have not been elucidated exclusively in pregnant women. Qunhua *et al.*, (1997) demonstrated that significantly more women with *S. japonicum* infection showed chronic cervicitis and enlargement of the uterus. It is

not known if maternal Schistosomiasis affects the growth of the foetus in utero. However, there is evidence that women infected with *S. haematobium* deliver underweight children (Stegrist and Stegrist-Obimpeh, 1992). Control programmes aimed at reducing morbidity in *S. haematobium* infection can obviously not achieve their goals if one systematically ignores that infection of the genital tract is an important part of the disease spectrum in *S. haematobium* (Poggensee *et al.*, 1999).

### **1.7 Antenatal care of pregnant women in Kenya**

Antenatal care is the clinical assessment of mother and fetus during pregnancy, for the purpose of obtaining the best possible outcome for the mother and child. To achieve this objective, history and examination are complemented by screening and assessment using a combination of methods, including physical (height and weight), biochemical, haematological and parasitological. Antenatal care traditionally involves a number of 'routine' visits for assessment, on a regular basis throughout the pregnancy. In Kenya antenatal services can be offered in several public and private health facilities including dispensaries in the village. Despite this, antenatal care continues to be centered on clinical assessment, with emphasis on the regularity of visits, rather than a focus on what can be achieved at key visits during the antenatal period.

General advice regarding nutrition and lifestyle can be given at this time. Even a single antenatal nutritional education session during pregnancy has a significant effect on birth weight. Advice can be given regarding the avoidance of teratogens, including those linked with excesses such as vitamin A, cigarette smoking, while

ensuring an optimal dietary intake of folic acid which is given to the mother. Lack of education, traditional beliefs and customs, all or some of these reasons may discourage a pregnant woman from visiting a health facility to receive antenatal care and opt for Traditional Birth Attendants (TBAs).

### **1.7.1 Schedule of visits and examinations during pregnancy**

The pregnant woman is seen by a clinician/nurse as soon as possible following the first missed period. The ideal first 'antenatal' visit is at a pre-conception clinic where health education and risk assessment can be directed towards the planned pregnancy. In the local health facilities clinicians /nurses will deliver health education talks during hospital visits to encourage mothers to attend clinic days. At that time of visit, the patient's general health and wellbeing can be fully assessed and laboratory examinations carried out to screen for, hepatitis, anaemia, urinary tract infections, worm infections, malaria, HIV status, and appropriate action taken where indicated. It is during these visits that pregnant women can be diagnosed for schistosomiasis early and treatment instituted to reduce chances of poor birth outcomes due to high morbidity and infection complications. In Kenya as of now pregnant women are not treated with praziquantel during term because clinicians/nurses still believe pregnant women should not be treated for schistosomiasis; however, they can be treated after delivery. Incidentally, given our social status in the rural areas, women conceive again almost immediately without treatment for schistosomiasis. The disease builds up, transmission continues, rendering the women helpless at one point in their reproductive life. The



consequence of this disquieting re-infection may lead to hepatosplenomegaly, urinary tract lesions, cervical erosions, papillomatous lesions, risk of ectopic pregnancy, sterility and predisposing the women to various infections including HIV.

### **1.8 Malaria-Helminth Co-infection**

Malaria and helminth infections are the major parasitic diseases in developing countries and their epidemiologic coexistence is frequently observed, particularly in Africa (Helmbly, 1998). Across the continent, a number of helminth species share the same spatial extents as *P. falciparum*. The most ubiquitous of these are the Soil Transmitted Helminthes (STH) (*Ascaris lumbricoides*, *Trichuris trichiura*, and hookworms), which infect more than one third of the continent's population at any one time (Brooker, 2006), however, the schistosome (*S. haematobium*) have a more focal distribution than STH species (Brooker, 2007).

Although malaria and helminth infections are known etiological factors in tropical anemia (Menendez, 2000; Hotez, 2004; Friedman, 2005) the extent to which their combined presence might interact to further enhance the risk of anemia is poorly understood. However, the relevance of an integrated, non-disease-specific approach to controlling childhood anemia is an increasingly recognized strategy (Crawley, 2004), following a general move towards integrated disease control programs (Hotez, 2006; WHO, 2006).

### **1.8.1 Impact of co-infection on anaemia**

Anaemia is one of the most widespread and common health conditions afflicting individuals living in the tropics, and in Africa, it contributes to 23% of nutrition-related disability adjusted life years (WHO, 2002). The consequences of anaemia are particularly severe for children and pregnant women (Brabin, 2001; Crawley, 2004).

Chronic anaemia during childhood is associated with impairment in physical growth, cognition, and school performance, (Grantham-McGregor, 2001), whereas severe anemia accounts for up to one half of the deaths in children younger than 5 years of age (Korenromp, 2004). Although the etiology of anaemia is complex and multifactorial in origin, parasitic diseases, including *P. falciparum* and helminth infections, have long been recognized as major contributors to anemia in endemic countries (Hotez, 2004; Friedman, 2005; Ehrhardt, 2006). Malaria contributes to reduced hemoglobin concentrations through a number of mechanisms, principally by destruction and removal of parasitized red cells and the shortening of the life span of non-parasitized red cells, and decreasing the rate of erythrocyte production in the bone marrow (McDevitt, 2004).

Hookworm causes iron deficiency anaemia through intestinal blood loss (Hotez, 2004). *S. haematobium* also causes anemia by chronic blood loss as eggs penetrate the wall of the urinary tract (Friedman, 2005). Like malaria, anaemia caused by schistosomes can additionally arise from destruction of red blood cells and/or

dyserythropoiesis. *A. lumbricoides* and *T. trichuria* have little impact on iron status (Stephenson, 2000).

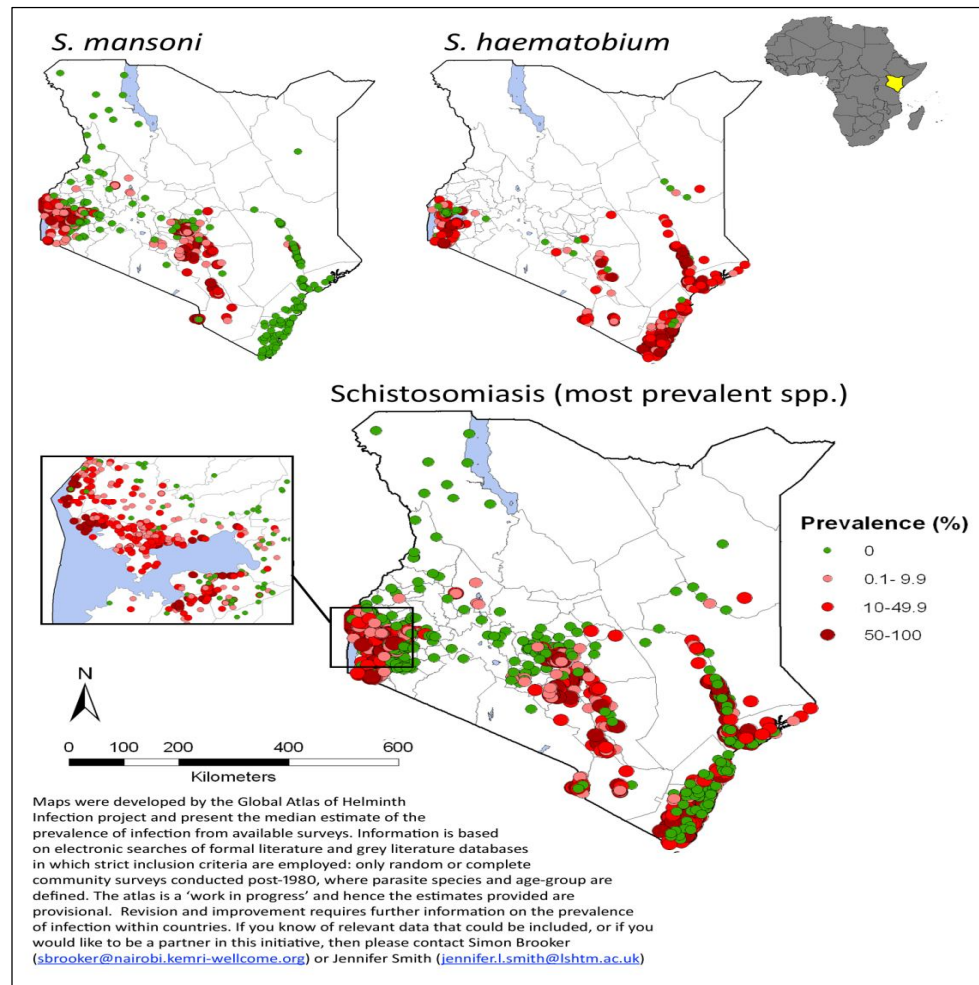
### **1.9 Control of schistosomiasis**

In the mid-1980s, morbidity control was adopted as the new global strategy for combating schistosomiasis. Before, the control or interruption of transmission was the declared objective, and hence snail control was a central feature of schistosomiasis control efforts. Morbidity control through large-scale, community – based chemotherapy has become in recent years the most widely advocated strategy, due to the development of safe, effective single dose drugs and adapted screening techniques, (Gryseels, 1990; Colley, 2001). Preventive chemotherapy is being recommended by WHO and partner organizations for the control of schistosomiasis in many endemic countries. It should be noted, however, that ‘preventive chemotherapy’ does not prevent an individual from an infection or re-infection (Utzing, 2011). Passive screening of the adult population in the community will complement the Mass Drug Administration (MDA) being administered in school age children in many countries. Repeated interventions will be necessary in view of the fact that a proportion of the population is always missed the first time and there are often migrations of infected people from other districts. However, wherever possible, control of vector snails can be initiated by covering irrigation channels, cleaning of canals or the use of concrete for irrigation canals can be useful, although this is a very expensive undertaking.

## 1.10 Schistosomiasis in Kenya

Current estimates show that more than three million Kenyans are infected with either one or both species of the schistosomiasis and approximately 10 million are at risk of infection (Muchiri *et al.*, 1996). In western Kenya, schistosomiasis is predominantly caused by *S. mansoni* (Brooker *et al.*, 2009b), and previous studies indicate that there is a direct relationship between the prevalence of *S. mansoni* and distance to Lake Victoria (Brooker *et al.*, 2001; Handzel *et al.*, 2003) such that schools within 5 km from the lakeshore can confidently be provided with mass treatment (Brooker *et al.*, 2001). On the coast of Kenya, schistosomiasis is exclusively caused by *S. haematobium*; whilst in other endemic regions of the country, both *S. mansoni* and *S. haematobium* occur (Fig. 1.4). In a pilot control programme initiated in 86 schools in Mwea District in central Kenya, a total of forty thousand (40,000) school age children were involved in a yearly treatment by trained school teachers for 4 consecutive years, Kihara *et al.*, 2007 demonstrated. The success of this pilot programme led to the implementation of a National school-based de-worming programme targeting more than 8 million school age children in endemic areas, out of whom 2 –3 million will be treated for schistosomiasis. While the effort is appreciated, control programmes do not include adult population, especially the most vulnerable group such as women of reproductive age. Also adults at high risk, such as farmers working in irrigation ditches, or freshwater fishermen, should also have access to praziquantel. The proposed study investigated the prevalence and intensity of *S. haematobium* infection in school children, women of child bearing age, anaemia (haemoglobin

levels), blood patho-physiological changes, classified anaemia, and birth weight outcomes in Kwale and Tana River counties.



**Figure 1.4 Prevalence of Schistosomiasis in Kenya (Courtesy of Simon Brooker, 2008)**

### 1.10.1 Statement of the problem

Lack of safe water supplies, inadequate sanitation, insufficient access to health care and prohibitive treatment costs all contribute to disease transmission and high morbidities, especially in infection with schistosomiasis. *S. haematobium* infection

that is predominant in Coastal region of Kenya is found to cluster in a subset of school age children with suggestions of synergistic effects on anemia, cognitive performance and stunting. In endemic areas, even adults are equally infected particularly the most vulnerable group such as pregnant women. Approximately, 10 million women in Africa have schistosomiasis in pregnancy. Female Genital Schistosomiasis (FGS) is predominantly caused by *S. haematobium*. During their reproductive years, women suffer severe morbidity and mortality because of FGS. Genital *S. haematobium* infection has been associated with human immunodeficiency virus (HIV) infection in one cross-sectional study and has been postulated to be a risk factor for HIV infection, (Kjetland, *et al.*, 2006).

In Kenya where treatment of pregnant women infected with schistosomiasis has not been embraced, women of reproductive age continue to be re-infected and harbour heavy loads of parasites, causing many untold suffering including anaemia, low birth weight and blood pathophysiological changes. These conditions are never addressed during pregnancy due to policies that do not allow pregnant women to be treated especially with praziquantel which is contraindicated.

### **1.10.2 Justification of the study**

In endemic areas women and young children are the most vulnerable groups to suffer from Schistosomiasis infections. Anaemia is the most easily measured of the “subtle” morbidities and is affected by presence as well as intensity of schistosome infection. It is also the most likely to be influenced by the host immune response

(King *et al.*, 2005). Fifty percent of women are infected with schistosomes in endemic areas, pre-disposing them to various complications before or during birth and contribute to maternal mortality; infant mortality and low birth weight.

Pregnant and lactating women are as susceptible to end organ damage as anyone else, resulting in major end organ morbidity. It has been hypothesized that infection with *S. haematobium* pre-disposes adult of active unprotected sexual behavior to infection with HIV and AIDS, thus, the interest in this vulnerable group. Avoiding treating pregnant women infected with Schistosomiasis adversely affects maternal iron stores and as a consequence adversely affects both their health and that of their unborn children (Friedman *et al.*, 2005).

The accuracy of iron deficiency assessment is improved by combining Hb estimation with independent measure of iron such as serum ferritin or erythrocytic protoporphirin (Rettmer *et al.*, 1999; McDade, 2002). Currently treatment of pregnant women infected with schistosomiasis is contraindicated in the country. Women in schistosomiasis endemic areas continue to be re-infected as most of them can not afford or get treatment in the health institutions almost throughout their parity life. The present study evaluated *S. haematobium* infection in women of reproductive age and pregnant women; the effect on anaemia, blood pathophysiological changes and birth weight outcomes, with a view to encourage treatment of pregnant women regularly in endemic areas. There are no known major side effects after pregnant women are treated with praziquantel, the treatment for schistosomiasis.

### **1.10.3 Hypotheses**

1. Primary school children in Kwale, Tana River and Kilifi Counties Coast Province do not have high prevalence of *S. haematobium* infection as determined by blood in urine questionnaire and parasitological urine examination.
2. Pregnant women infected with *S. haematobium* in parts of Kwale and Tana River Counties, Coast Province do not suffer from anaemia and other blood patho-physiological changes.
3. Pregnant women infected with *S. haematobium* do not have high levels of urea and creatinine in their blood than non- pregnant women in parts of Kwale County.
4. Schistosomiasis does not cause different types of anaemia in pregnant women and does not cause delivery of underweight children in parts of Tana River County.

### **1.10.4 General objective**

To determine the prevalence of *S. haematobium* infection in school children and women of reproductive age and their effect on anaemia, blood patho-physiological changes in selected parts of Tana River, Kilifi and Kwale Counties, Coast Province.

### **1.10.5 Specific objectives**

1. To determine the prevalence of *S. haematobium* infection in primary school children in parts Kwale, Kilifi and Tana River Counties, Coast Province



2. To determine the prevalence and intensity of *S. haematobium* infection in women of reproductive age in parts of Kwale County
3. To identify blood patho-physiological changes associated with *S. haematobium* infection in pregnant and non- pregnant women in parts of Kwale County
4. To determine the different types of anaemia and birth weight outcomes of pregnant and non-pregnant women infected with *S. haematobium* in parts of Tana River County
5. To determine the prevalence of malaria and hookworm infection in women of reproductive age in parts of Kwale and Tana River County

#### **1.10.1 Research questions**

1. What is the prevalence of *S. haematobium* infection in school children age between 5 and 18 years in Kwale, Tana River and Kilifi Counties of Kenya?
2. Do pregnant women infected with *S. haematobium* (female genital Schistosomiasis) have higher morbidity than uninfected pregnant women in endemic areas in parts of Kwale and Tana River Counties?
3. Do pregnant women infected with *S. haematobium* have low haemoglobin levels, low or high haematological indices and patho-physiological changes in women of reproductive age in parts of Kwale County, Coast province?
4. Do pregnant women infected with *S. haematobium* have high levels of

serum Urea and creatinine than uninfected women in parts of Kwale County?

5. Are pregnant women of reproductive age in Kwale and Tana River County infected with Malaria and Hookworms?



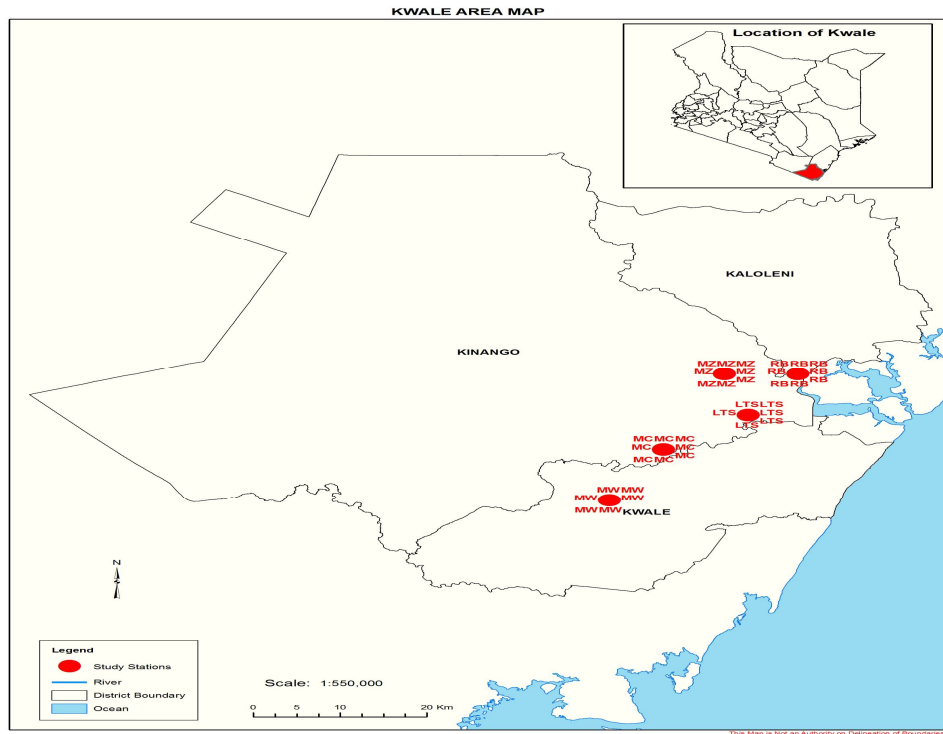
Tharaka and Isiolo District to the North, Lamu District to the South east, and Kilifi District to the south and the Indian Ocean to the Southwest, and Taita-Taveta to the south west. The district lies between latitude 00 (Equator) and 30 South, and longitude 380 300 East and 400 150 East. The major ethnic groups are the Pokomo, many of who are farmers, and the Orma and Wardey, who are predominantly nomadic pastoralists.

Tana River with an area of 38,446 km<sup>2</sup>, is one of the least populated districts in Kenya with a total population of about 250,000 (1999 national census) mostly concentrated along the river and the small urban centers. Over 90% of the total area in Tana River County is arid and semi-arid land. The main economic activities of the district are small-scale subsistence agriculture along Tana River, nomadic livestock activities in the rangeland and fishing along the river and coastal strip. There are two major irrigation schemes Bura and Hola. Despite the apparent adequate natural resources, the region remains marginalized from the rest of the country. Seventy two percent (72%) of the total population lives below the poverty line according to the 1997 poverty survey. The area is endemic for *S. haematobium*, where prevalence in school children ranges between 3 – 75% with an average of 44% across the District (Brooker *et al.*, 2009; Jimmy *et al.*, 2011). Women and children are the most affected.

### **2.1.2 Kwale County**

The study was also conducted in Kwale County, Coast province which is located in the South Eastern corner of Kenya, bordering Tanzania to the south and the Indian

Ocean to the east (latitudes 3.558° - 4.675°South and longitudes 38.452°- 39.663°East) (Fig. 2.2). Kwale County has an area of 8293 km<sup>2</sup> with a population of 583,330 persons. The annual growth rate is 2.6%. The mean household size is 6-8. Currently it is split into three (3) districts namely: - Kwale, Msambweni, Kinango



**Figure 2.2: Location of the study villages in Kwale County, Courtesy of Kenya National Bureau of Statistics 2011**

Key: MW= Mwaluphamba; MC=Mwachinga; LTS=Lutsangani; MZ=Mienzeni; RB=Rabai

Overall Kwale district has monsoon type of climate; hot and dry from January to April and cool between June and August. The district experiences a bimodal rainfall pattern with the short rains occurring between October to December and the long rains occurring between March and June/July. The average annual rainfall

ranges between 400mm and 1200mm. The three districts have four (4) livelihood zones that include livestock farming, mixed farming, fisheries and formal employment /tourism. Mixed farming (maize, cassava, beans, rice, coconuts, and sugar cane) is the main livelihood of the district although most of the district area is under livestock farming. The district meets 60% of its grains, vegetables and pulses requirements. The deficit is met from imports from other neighboring districts (mainly Taita Taveta) and upcountry. Currently malnutrition rates are at 3.2%.

### **2.1.3 Kilifi County**

Kilifi County, Coast Province lies at an altitude of between 0 metres and 120 meters above sea level and is usually hot and dry, with day temperatures of 35°C and 30°C at night. The average annual rainfall is 747mm (Data from Ministry of Agriculture, 2009). It covers an area of 730 Km<sup>2</sup> with a population of 152,000 and population density of 32.3 persons per Km<sup>2</sup>. The crops that are grown include coconuts, cassava, rice, vegetables, maize and beans. Coconuts are the main cash crops grown in various parts of the area. There are few seasonal rivers that transact the district from Eastern Province and several dams constructed for storing water during dry seasons.

### **2.2 Global Positioning System co-ordinate**

A hand held Global Positioning System (GPS) receiver (Etrex, 12 channels – Garmin) was used to collect co-ordinate readings in all the health facilities (villages) visited. These readings were then used to plot the exact position of the

study sites (health facilities in the villages) against digitalized ground maps and satellite images of Tana River, Kilifi and Kwale Counties.

### **2.3.0 Inclusion criteria**

School children who have not had treatment for schistosomiasis in the last one year and who consented or assented to be part of the study were included. All pregnant and non pregnant women (women of reproductive age), lactating and non-lactating, who consented to the research and had not been treated in the last 12 months were included in the study.

### **2.3.1 Exclusion criteria**

School children, who have had treatment in the last one year or did not consent or assent to be part of the study, were excluded. All adult female attending ante-natal clinics that did not consent to be part of the study and those that had been involved in Schistosomiasis control in the past 1 year were not included in the study. All women who appeared during the study but were not available at follow up, were not included in the analysis.

## **2.4. Sample size**

Assuming the prevalence of 50% and a level of significance of 5% the minimum number of samples required was calculated using Lemeshaw, (1990) formula;

$$n = \frac{Z_{\alpha/2}^2 (1 - a) P (1 - p)}{d^2}$$

n = Minimum sample size required

z = 1.96 (Standard error from the mean)

a = Absolute precision (at 5%)

p = 0.50 is the prevalence of *S. haematobium* in school children (Kihara *et al.*, 2011)

d = 0.05 (5%) required margin of error

$$\text{Hence } N = \frac{1.96^2 \times (1 - 0.05) \times 0.50 \times (1 - 0.5)}{0.1^2} \quad n = 173$$

Given the rate of compliance and pregnancies in different areas, a 25% sample was added, which then translated to a minimum sample size of 216.

## **2.5 Parasite Diagnosis**

### **2.5.1 Parasitological examinations**

Parasitological examinations of the eggs in urine were done using nuclear pore filtration technique as described by Kahama *et al.*, (1998). A fresh urine specimen was collected from each child between 10.00 and 14.00 h to coincide with the peak production of eggs by the blood fluke *S. haematobium*. A duplicate of 10 ml aliquot of urine was filtered through a 13-mm-diameter polycarbonate membrane with a 12µm pore size, examined under the microscope (40 x power) and the number of eggs of *S. haematobium* eggs were counted and the mean expressed as eggs/10 ml of urine.



### **2.5.2 Prevalence of *S. haematobium* infection in school children**

Before investigating the various research questions on women of reproductive age, prevalence of *S. haematobium* in Kwale, Kilifi and Tana River Counties was determined using a questionnaire of reported health problems developed by the Red Urine Study Group (1995) and the Partnership for Child Development (1999) to identify children experiencing blood in urine and parasitological egg counts.

### **2.5.3 Soil Transmitted Helminths in women of reproductive age**

The presence or absence of Soil Transmitted Helminths (STH) (Trichiuriasis, Ascariasis and hookworm infections) ova in stool was determined by Kato-Katz method (Katz *et al.*, 1972). A 41.7 mg stool smear was prepared using a sieve and a calibrated template. The smear was placed onto a glass slide and covered with glycerine-impregnated cellophane. This preparation was left to clear for a minimum of 45 minutes (Peters *et al.*, 1980). To identify hookworm eggs, the sample was examined within one hour of preparation.

### **2.5.4 Blood slides for malaria parasites**

Giemsa solution (10%) in buffered distilled or deionised water, pH 7.2, was prepared. The stain was gently poured onto the slide or by use of a pipette. The stain was allowed to stain for 10 minutes. Gently the stain was flushed off the slide by adding drops of clean water. The slides were placed film side downward in a slide rack to drain and dry, making sure the film does not touch the rack. The slides were examined using a compound microscope - 100x power and malaria parasite

identified (deep red chromatin and pale purplish blue cytoplasm). Malaria parasite densities were calculated by counting the number of asexual parasites per 200 leukocytes (or per 500 leukocytes, if the count is < 10 asexual parasites /200 leukocytes), assuming a leukocyte count of 8,000/ $\mu$ l. (WHO, 2007). A blood smear was considered negative when the examination of 100 high power fields did not reveal asexual parasites. Gametocytes were also determined from the thick blood smears. Thin smears were used to differentiate the different Plasmodium species. 10% of all the slides were then read again for quality control.

### **2.5.5 Preparation and Staining of Blood Films for haematology**

The thin films were made from a drop of blood spread evenly on a slide and stained.

### **2.6.0 Staining procedure**

Blood film slides were gently lowered into a coplin jar of acetic alcohol (3% acetic acid in 95% methanol), fixed for 1 minute, and then washed off the fixative with distilled water and drain. The slides were put on a rack and covered with 1ml of Leishman stain - 20 seconds. Then added 2ml of buffer pH6.8 and tipped the rack up and down to mix the solutions or blow gently, the slides were left to stain for 7 minutes. The slides were rinsed quickly in distilled water then treated with pH6.8 buffer for 2 minutes, rinsed again quickly in distilled water, shaken off the excess and dried on a warm (50°C) hot plate, or carefully blot dry with fibre-free blotting paper. The slides were then examined and blood cell morphology reported

appropriately.

## **2.7 Blood collection**

Carefully the left hand of the study subject was tied at the fore with a tourniquet (band), cleaned thoroughly with methylated spirit. Using a 5 ml syringe and needle, venous blood was gently aspirated. The tourniquet was then removed and a clean sterile ball of cotton wool placed on, the woman was asked to press gently for a few minutes and allowed to proceed for interview in the next room. Five (5) milliliters of blood drawn from the vein of the study subjects was divided as follows; 2ml was put in a special Ethylene Diamine Tetra-acetic Acid (EDTA) vial and rotated on the hand several times (10) to mix and 3mls of blood was put into a plain bottle (without anticoagulant). The EDTA blood was put in a cooler box with ice packs (temperature of about 8<sup>0</sup>C) until analysis in the laboratory within 2 hours after collection. The blood in the plain bottle was allowed to clot (one hour) and serum was collected by separating from the clot using a Pasteur pipette into a smaller vial. The samples were then stored at -80<sup>0</sup>C for biochemical analysis later in the laboratory.

## **2.8.0 Blood analysis using automated haematology analyzer**

### **2.8.1 Procedure for haematological indices**

Automated hematology analyzer was switched on 5 minutes before starting sample analysis. It was then let to prime for 5 minutes using isotonic saline while preparing the samples according to laboratory numbers (identification). Carefully, controls were taken from the refrigerator and brought to room temperature by rolling gently between the palms, then turning them upside down at least 30 times (thoroughly

mixing the plasma and the red blood cells). The nozzle of the machine was put right into the bottom of the control vials (supplied by the manufacturer). With the index finger the start count button of the machine was then pressed to allow aspiration of the blood into the different chambers of the machine. Within 30 seconds the machine counted all the cells and the different indices calculated; the results were then displayed onto the screen. The eighteen haematological control parameters were compared and standardized, ready for blood sample analysis. The auto analyzer was set with the study subject identifying numbers in the data base, whole blood was mixed gently. Any clotted samples were removed from the analysis. The analyzer nozzle was inserted (individual sample) to near bottom of the whole blood vial, start button was then pressed and whole blood was aspirated into the machine chambers. Within 30 seconds the results were displayed on the screen. The results were then printed by pressing a button. This data was then entered into Excel spread sheet in a computer for statistical analysis.

## **2.9 Kidney function test**

The machine used for the sample analysis was Clinical Chemistry Autoanalyzer Olympus 640 whilst Olympus 400 system. Olympus Diagnostica GmbH, Hamburg, Germany was used as a backup machine in case of any mechanical breakdown of the former. The Olympus is a discrete, random access clinical chemistry analyzer capable of performing a wide range of chemical tests in a single run.

### **2.9.1 Reagent preparation**

All reagents for the auto analyzer machine were commercially prepared to fit the required volumes and concentration. Reagents used in Olympus auto analyzer machine were in specific containers referred to as “reagent cartridges”. The reagent cartridges were bar-coded for the identification by the machine.

### **2.9.2 Calibration of the test**

To ensure that the values recovered from the patient sample assayed were both accurate and precise, the machine performed a calibration procedure for the parameters. The purpose of the calibration procedure was to determine the relationship between measured absorbance (or in case of ion selective electrodes, voltage potential) to known concentration of these same analytes contained in calibrator solutions (Olympus multi-calibrator for analyzed parameters). Calibration factors were installed once the relationship was achieved.

### **2.9.3. Quality control (QC) materials**

The assayed multiseria normal was used for the quality control of the analytical work during the study period. The QC multiseria were supplied in lyophilised form and were reconstituted as per the manufacturer’s preparation guide. For internal quality control assessment, the prepared QC multiseria was analysed daily or any other time samples for the study were being analysed.

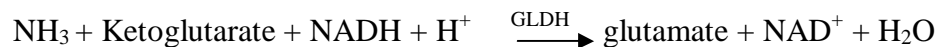
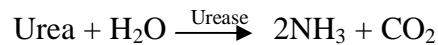
### **2.9.4 Olympus autoanalyzer**

Methods for specific analytes were programmed and stored in the microprocessor of the instruments. The autoanalyzer proportioned the required amount of reagent

and sample using the reagent and sample probes respectively. Reagent volumes were in the range of (50-300ul) and the sample volumes in the range of (2-20 µl). The various reactions occurred in the reaction compartment of the instrument at 37 degrees centigrades. After the reaction, results were obtained from the data manager either printed out or read from the screen.

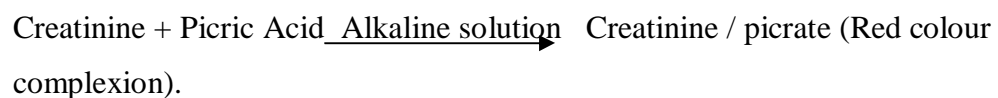
### **2.9.5 Blood Urea Nitrogen (B.U.N)**

BUN was analyzed using Glutamate Dehydrogenase (GLUDH). BUN reagent was used to measure the concentration of urea by an enzymatic rate method. In the reaction urea was hydrolyzed by urease to ammonia and carbon dioxide. Glutamate dehydrogenase (GLDH) catalysed the condensation of ammonia and –Ketoglutarate to glutamate with the concomitant oxidation of reduced B-nicotinamide adenine dinucleotide (NADH) to B-nicotinamide adenine dinucleotide (NAD).



### **2.9.6 Creatinine (CREAT)**

The creatinine reagent was used to measure the creatinine concentration by a modified rate Jaffe method. In the reaction, creatinine combined with picrate in an alkaline solution to form a creatinine – picrate complex. Thus, the principle is,



## **2.10 Sampling procedure**

Baseline data information on the prevalence of *S. haematobium* infection in Coast province was done using a pre-tested structured blood in urine questionnaire administered by school teachers and research officers from Kenya Medical Research Institute (KEMRI) to school children in several schools. Parasitological urine examination for eggs in the same children was also done to confirm the infection status. The questionnaire administrator was asking the children if they suffered from several ailments including passing urine with blood, (native language “kichocho”). This information was then used to identify the study sites where women of reproductive age were enrolled for the study in Kwale and Tana River County.

A multistage sampling procedure was adopted in this study. All villages were listed including their names, population size and the number of households, that formed the sampling frame. Pregnant women (1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> trimester) as well as women of reproductive age (Non-pregnant) were registered and enrolled in the study. Standard Operating Procedures (SOPs) were written for all study activities and were used throughout the study period in all the sites. The set ups were approved by KEMRI/Ministry of Health.

## 2.11 Neonate weight measurements

Immediately after delivery (1-12 hours), the weights of the neonates were measured by a trained community health worker, using a Baby weighing Scale - superior (salter type), either at the health facility or in the house (Fig. 7). If the delivery was at home, the trained community health worker visited the home a few hours later and weighed the neonate by placing him/her on the hanging trousers, then read the weight on the scale in grams, recorded the weight, the date, time and names of the mother. The mother was then advised to take the child for immunization as soon as possible according to the health guidelines. When the delivery was in a health facility, the same procedure was followed, the child immediately received immunization for polio and BCG (Bacilli Callimet Guerine).



**Figure 2.3: Showing the baby hanging weighing scale**



## **2.12 Field assistants**

Field assistants from the study area were recruited and trained on how to handle/administer research tools and were regularly monitored. Local administrators (chiefs and assistant chiefs) were incorporated into the study from the on-set through the implementation of the study and were orientated with a view to assisting in mobilizing and sensitizing the community including the men. It was envisaged that their involvement would increase participation of their members from the community, thus, building a sense of ownership and avoid any suspicion especially from the men. The core team members organized meetings with the help of the local administration and women leaders in the area to make sure they all understood the objectives of the study prior to implementation. The mothers were asked to visit the nearest health facility for sample collection and examination during specified dates and time.

## **2.13 Ethical consideration**

The study objective was explained to women in a Baraza (large group) by a trained study laboratory technician/nurse fluent in the local language. All women who accepted to be part of the study were asked to sign or place the finger marked with ink on the consent form. Permission to conduct the study was obtained from the District Medical Officers and clinicians/nurses stationed at participating dispensaries and health centers. Ethical approval was granted by the Kenya Medical Research Institute, Ethical Review Committee, Scientific Steering Committee

(SSC) No. 1319. Women diagnosed with schistosomiasis if not pregnant, received free treatment immediately. Women who were pregnant and diagnosed positive for *S. haematobium* were treated free of charge immediately post-term.

## **2.14 Data analysis and management**

Sensitivity of the school children questionnaire was calculated as the percentage of schools with prevalence of *S. haematobium*  $\geq 50\%$  and prevalence of reported blood in urine  $\geq 30\%$ , and specificity was calculated percentage of schools with prevalence of *S. haematobium*  $<50\%$  and prevalence of reported blood in urine  $<30\%$ . The Negative Predictive Value (NPV) and Positive Predictive Value (PPV) of the 30% threshold prevalence of reported blood in urine in identifying low and high prevalence schools for *S. haematobium* was calculated as follows: NPV = percentage of schools with reported blood in urine  $< 30\%$  which have a prevalence of *S. haematobium*  $< 50\%$ ; PPV = percentage of schools with a reported blood in urine  $\geq 30\%$  which have a prevalence of *S. haematobium*  $\geq 50\%$ . 95% exact binomial confidence intervals (CIs) were calculated (Guyatt, *et al.*, 1999; Ansell, *et al.*, 2001).

Generalized Linear Mixed-effect Models (GLMM) were used to explore the impact of pregnancy status on schistosomiasis prevalence and intensity. When examining the prevalence of infection, binomial regression analysis was used to account for the binomial character of the variable, thus, either pregnant or non-pregnant. When examining intensity of infection, a negative binomial regression analysis was used to control for the non-normal (non-gaussian) distribution of infection intensity

between individuals. Helminth infection intensities are typically highly over-dispersed (the minority of the population harbour heavy infection and the majority have light infections, or are uninfected) which can be reasonably be approximated by the negative binomial distribution.

Those aged 12 or younger (n=2) were excluded as thought likely not to be sexually active yet. One data point with a trimester of 8 was excluded as assumed to be a coding error. Hierarchical mixed effect models were used for the analysis. This allowed the schistosomiasis prevalence and intensity to vary between different areas at random. Allowing this to happen makes it easier to see if there is a general trend across all sites once the differences between sites have been taken away. In the analysis, egg count / prevalence was the dependent variable whilst Pregnancy status, Age and Area were all independent variables (Pregnancy status and Age are fixed effects whilst Area is included as a random effect).

Student's t-test was applied to test the difference in mean egg counts between age groups and/or sex of study subjects. Pearson's correlation co-efficiency was used to determine the correlation between haemoglobin levels, haematological indices (anaemia), egg counts (*S. haematobium*).

Regression analyses were carried out to explore the impact of pregnancy and schistosomiasis infection on haemoglobin (Hb) levels and resultant anaemia in Kwale and Tana districts, Kenya. Information on schistosomiasis infection and haemoglobin levels were available from 604 women (377 non-anaemic, and 227 anaemic). Schistosomiasis infection data were recorded from 614 women (410

uninfected, 204 infected). Pregnancy data were available on 621 women (191 pregnant and 430 non-pregnant). Regression analysis was used to explore the relationship between these factors and to control for other variables thought to be plausible contributors to anaemia: malaria, STH infection, age, weight, height, BMI (calculated as weight in kgs divided by the square of height in metres) in the two study areas either together or independently. Height, weight, BMI, STH infection and malaria were not found to be significantly associated with anaemia; they were dropped from the analysis. In this instance, normal linear regression was used where the outcome variable was found to be normally distributed, as in the case of Hb level, or binomial regression was used where the outcome variable was binary, or could be expressed as a proportion, as with presence/absence of Anaemia.

## CHAPTER THREE

### 3.0 PREPARING FOR NATIONAL SCHOOL-BASED DE-WORMING IN KENYA: THE VALIDATION AND LARGE-SCALE DISTRIBUTION OF SCHOOL QUESTIONNAIRES WITH URINARY SCHISTOSOMIASIS

#### 3.1 Introduction

In recent years, an increasing number of African countries have launched national school-based deworming programmes (Garba *et al.*, 2006; Kabatereine *et al.*, 2006; Tchuente-Tchuente´ and N’Goran, 2009). In Kenya, in 2008, the education and health sectors jointly established a national school-based deworming programme. In this, mass treatment, mebendazole was provided to all school children in districts identified as having a high prevalence of soil-transmitted helminth (STH) infection (Chunge *et al.*, 1985; Brooker *et al.*, 2009b). For schistosomiasis, the well-established focal distribution of infection necessitated that treatment with praziquantel is targeted only to schools with a high prevalence of infection.

In western Kenya, schistosomiasis is predominantly caused by *Schistosoma mansoni* (Brooker *et al.*, 2009b), and previous studies indicate that there is a direct relationship between the prevalence of *S. mansoni* and distance to Lake Victoria (Brooker *et al.*, 2001; Handzel *et al.*, 2003), such that schools within 5 km from the lakeshore can confidently be provided with mass treatment (Brooker *et al.*, 2001).

On the coast of Kenya, schistosomiasis is exclusively caused by *S. haematobium*; whilst in other endemic regions of the country, both *S. mansoni* and *S. haematobium* occur. To identify schools with a high prevalence of *S. haematobium*,

school-based questionnaires administered by teachers reliably identified high prevalence schools in a number of settings (Lengeler *et al.*, 2002; Brooker *et al.*, 2009a). Questionnaire surveys are now therefore recommended as a first step in implementing national schistosomiasis control (WHO, 1995; Christian *et al.*, 2002; Lengeler *et al.*, 2002) and have to date been implemented at national or sub-national scales in Cote d'Ivoire (N'Guessan *et al.*, 2007) and Tanzania (Clements *et al.*, 2008a,b). In implementing a questionnaire survey, it is essential that parasitological validation should precede any large-scale use of questionnaires (WHO, 1995; 2006). Results of the validation of school-based *Schistosoma haematobium* prevalence and blood in urine questionnaires in Kenya are presented here. These results then informed the researcher, on the possible areas to conduct a study on *S. haematobium* infection in women of reproductive age in Coastal region.

### **3.2 Literature review**

Helminth infections are parasitic worms found in the intestinal tract, urinary tract or blood of humans. The helminth species that cause the greatest human morbidity are the schistosomes, intestinal nematodes or commonly called Soil-Transmitted Helminths, (STH), and tissue nematodes, including human filariae that cause lymphatic filariasis and onchocerciasis (Muller, 2002). Schistosomiasis is a widespread parasitic disease of the tropics that places an enormous toll on the public health of affected regions. Of the 207 million people infected worldwide, 85% of the burden is concentrated in Africa south of the Sahara (WHO, 1999; Chitsulo *et al.*, 2000). An important feature of the disease is its focal distribution (Webb and Jordan, 1993). This results in a patchy distribution of risk, and

communities across a region or country. Praziquantel the drug of choice, is therefore not required everywhere and proper targeting is crucial, given the limited resources and the many other problems facing primary health care systems in sub-Saharan Africa (Brooker *et al.*, 2008).

Although helminth infections can infect all members of a population, it is clear that there are specific groups who are at greater risk of morbidity than others, and who are more vulnerable to the harmful effects of chronic infections. For schistosomes and STH, the most vulnerable groups are school aged children and women of child-bearing age, including adolescent girls (Hotez *et al.*, 2006; Brooker *et al.*, 2008).

The first step in targeting health interventions is to map the disease geographically and rank it according to the risk of infection and morbidity. In 1987, the first attempt to systematically map schistosomiasis on a global scale resulted in the Atlas of the global distribution of schistosomiasis (Doumenge *et al.*, 1987). A more recent effort using geographical information systems highlighted the scarcity of data for Africa (Brooker *et al.*, 2000), and underscored the need for a rapid and inexpensive epidemiological assessment tool that can be fully integrated within existing administrative systems.

The signs and symptoms of urinary schistosomiasis, such as blood in urine and pain when urinating, are clearly recognized by individuals, and it is not uncommon for school-aged children to report having blood in urine without having urinary schistosomiasis. Such specificity of this sign of *S. haematobium* infection in

endemic areas has allowed blood in urine to be incorporated into morbidity questionnaires which identify communities with a high prevalence of infection and warranting mass treatment (WHO, 1995). Such a tool, relying on simple school questionnaires, was developed more than a decade ago for *S. haematobium* and has since been validated in a variety of ecological, epidemiological, and socio cultural settings across sub-Saharan Africa, (Lengeler, 2002).

The use of reported blood in urine to identify high prevalence communities using a simple school based questionnaire was first developed and administered through the education system in Kilombero and Kilosa districts of Tanzania (Lengeler *et al.*, 1991a, b; Mafe *et al.*, 2000). A number of studies have subsequently demonstrated that morbidity questionnaires can rapidly and cost-effectively identify high-risk communities (Lengeler *et al.*, 2002). These studies confirm that morbidity questionnaires administered through the education system are accurate and allow for rapid and cost effective identification of high-risk communities of urinary schistosomiasis (Ansell and Guyatt, 2002).

Fortunately, much of the morbidity associated with infection can be reversed with the use of effective anthelmintic drug treatments (Keiser, 2008). The World Health Organization recommends mass drug administration with praziquantel (for schistosomes) in school age children (WHO, 2006).

### **3.3 Materials and methods**



A pilot study was conducted on 6182 children in 61 schools in Coast Province where school children were asked whether they pass blood in urine, and their answers compared with individual results of microscopy for *S. haematobium* eggs. This survey sought to evaluate whether blood in urine was a reliable indicator of infection in identifying both high prevalence (>50%) schools and infected individuals. Here, the same children were questioned about blood in urine and asked to provide a urine sample, which was parasitologically examined for eggs of *S. haematobium*, and the sensitivity of reported blood urine was evaluated at the school and the individual level. In the second phase, a blood in urine questionnaire was distributed by the Ministry of Education to all schools in Coast Province and administered by class teachers. Results from this survey were also compared with the results from the parasitological survey.

### **3.3.1 The Kenyan National Control Programme**

A number of pilot helminth control programmes have previously been undertaken in Kenya (King *et al.*, 1990; Magnussen *et al.*, 2001; Miguel & Kremer, 2004), but without an establishment of a national framework for school-based parasite control. To help lead the way for a national programme, in 2001, the Eastern and Southern Africa Centre of International Parasite Control (ESACIPAC) was established at Kenya Medical Research Institute (KEMRI), with support from the Hashimoto initiative, Japan. A pilot school health programme was initiated in 86 schools in Mwea District in central Kenya (Kihara *et al.*, 2007) and helped identify key components of a national school health programme.

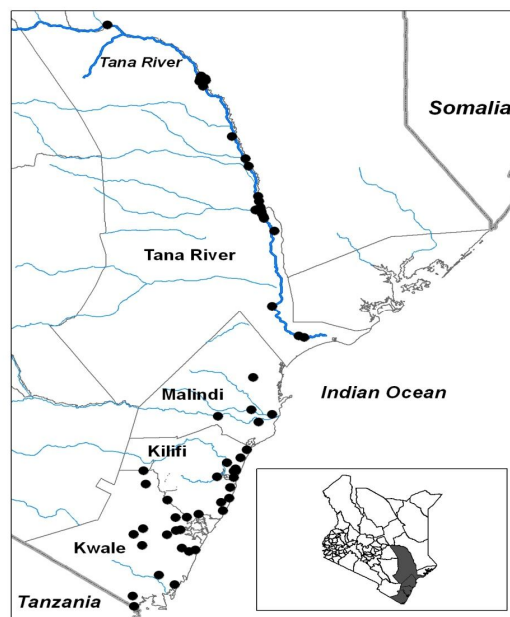
In 2005, the Kenya education sector constituency initiated the Kenya Education Sector Support Programme (KESSP), whose overarching goal is of enhancing access, equity and quality at all levels of education and training. To realize this goal, the programme established 23 investment programmes, one of which is the School Health, Nutrition and Feeding Investment Programme (Ministry of Education 2008). The aim of this programme is to provide school-based health, hygiene and nutrition skills and services to all school children. In 2008, implementation funds for school-based deworming were provided by the Ministry of Education, whilst technical and operational support and drugs were provided by a non-governmental organization (Deworm the World). In 2009, 3.4 million school children received mebendazole treatment for STH infection, and preparations were made for the targeted distribution of praziquantel in schools where schistosomiasis is prevalent.

### **3.3.2 Prevalence of *S. haematobium* infection in 61 schools**

The parasitological survey was conducted in 61 schools in eight of the 11 districts of Coast Province (Kilifi, Kinango, Kwale, Malindi, Kaloleni, Msambweni, Tana Delta and Tana River districts) (Figure 3.1). These nine districts were formally part of four larger districts (Kwale, Kilifi, Malindi and Tana River) in 1999 before district boundaries were revised throughout the country. A random selection of schools was based on the 1999 boundaries and population proportionate sampling based on the number of schools in each district. In each school, 10 boys and 10 girls (plus a reserve boy and reserve girl) were selected from each of classes 2–6

using random table numbers. A fresh urine specimen was collected from each child between 10.00 and 14.00 h to coincide with the peak production of eggs by the blood fluke *S. haematobium*. Up to 10 ml of urine was filtered through a 13-mm-diameter polycarbonate membrane with a 12 $\mu$ m pore size, and the number of eggs of *S. haematobium* eggs were counted and expressed as eggs/10 ml of urine.

A questionnaire of reported health problems was developed based on the questionnaires developed by the Red Urine Study Group (1995) and the Partnership for Child Development (1999). Each child was individually asked by a research team member whether he or she had experienced a number of health problems in the last 2 weeks, including headache, stomach ache, as well as whether he or she had passed blood in urine or had experienced kichocho (schistosomiasis) (appendix 4 and 5).



**Figure 3.1: Map of the 61 schools in Coast Province, included in the parasitological survey (Source Kihara, *et al.*, 2011)**

Ethical clearance for the parasitological survey was obtained from the Kenya Medical Research Institute- National Ethics Review Committee in Kenya. Meetings were held with education officials, the teachers and parents to explain the purpose of the study and to obtain approval for the study. Parents who did not want their children to participate in the study were free to refuse participation. Assent was also obtained from the children before samples were collected. This passive, opt-out method of parental permission is considered to be ethical and practical for informing participants in low-risk studies (Ellickson & Hawes, 1989), especially research used to directly guide government public health intervention. Analysis of diagnostic performance reported schistosomiasis (kichocho) was not readily understood by children and therefore not investigated here.

Associations at the school level were investigated by plotting the prevalence of reported blood in urine against the prevalence of *S. haematobium* infection. Previous analyses (Red Urine Study Group, 1995; Guyatt *et al.*, 1999; Ansell *et al.*, 2001) suggest that a prevalence of reported blood in urine  $\geq 30\%$  is equivalent to the prevalence of *S. haematobium*  $\geq 50\%$ . On this basis, sensitivity was calculated as the percentage of schools with the prevalence of *S. haematobium*  $\geq 50\%$  and reported blood in urine  $\geq 30\%$ , and specificity was calculated as percentage of schools with the prevalence of *S. haematobium*  $< 50\%$  and reported blood in urine  $< 30\%$ .

The negative predictive value (NPV) and positive predictive value (PPV) of the 30% threshold prevalence of reported blood in urine in identifying low and high prevalence schools for *S. haematobium* were calculated as follows: NPV = percentage of schools with a reported blood in urine <30%, which have a prevalence of *S. haematobium*  $\geq$ 50%; PPV = percentage of schools with a reported blood in urine  $\geq$ 30%, which have a prevalence of *S. haematobium* <50%, and 95% exact binomial confidence intervals (CIs) were calculated. Children in the parasitological survey were classified into four categories: true positives (self-reported blood in urine and eggs seen in urine); true negatives (blood in urine not reported and no eggs seen in urine); false positives (self-reported blood in urine but no eggs seen in urine); and false negatives (blood in urine not reported but eggs seen in urine).

The performance of reported blood in urine in identifying children infected with *S. haematobium* was assessed according to sex and age group (<10, 10–12 and 13+ years) in terms of sensitivity (the percentage of infected children who reported blood in urine) and specificity (the percentage of uninfected children who did not report blood in urine). Positive and negative predictive values were also calculated. All values and their 95% exact binomial CIs were calculated using the `digit` command in Stata version 10.0 (StataCorp, College Station, TX, USA), and non-overlapping CIs were indicative of a statistical difference between age and sex groupings. Significant differences between proportions were tested using logistic regression and correlations assessed with a Spearman rank test.

### 3.4 Questionnaire survey by school teachers

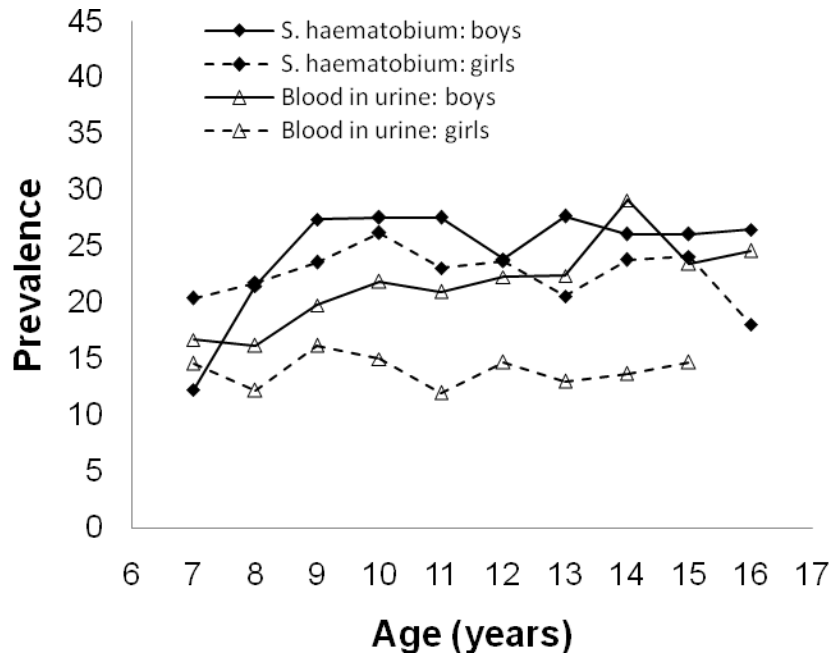
Prior to urine sample examination, questionnaires were delivered to the District Education Officers (DEO) in all districts of Coast Province. Subsequently, questionnaires were distributed to the head teachers of each school along with instructions on how class teachers should implement the questionnaire. The questionnaire was the same one used in the parasitological survey and included 15 health related problems. On the day of the survey, each child present was asked by the class teacher whether he/she had experienced any of the health problems in the last 2 weeks. Class totals were collated by the head teachers, and the questionnaires were returned to the DEO at the end of the month when the head teachers went to collect salaries.

The questionnaires for each district were then passed on to the national programme office in Nairobi. School results were linked to a national school database developed for the Ministry of Education (Ministry of Education 2008) and mapped using ArcGIS 9.3 (ESRI, Redlands, CA, USA). Of the 61 schools included in the parasitological survey, only 45 (74%) returned the questionnaire. For these schools, associations at the school level were investigated simply by plotting the prevalence of reported blood in urine against the prevalence of *S. haematobium* infection. The NPV and PPV of a reported threshold prevalence of blood in urine in identifying low and high prevalence schools for *S. haematobium* were calculated as follows: NPV = percentage of schools with a reported blood in urine <30%, which have a

prevalence of *S. haematobium* <50%; PPV = percentage of schools with a reported blood in urine  $\geq$ 30%, which have a prevalence of *S. haematobium*  $\geq$ 50%.

### **3.5 Results of parasitology examination and blood in urine in school children**

Sixty-one schools, which included 6182 children aged 5–20 years, were selected for the parasitological study. The number of children sampled in each school ranged from 79 to 113 children (median 105), with a similar number of boys (3077) and girls (3105) sampled. The overall prevalence of infection with *S. haematobium* was 24.5% [95% CIs: 23.4–25.6%], with prevalence ranging from 0 to 91.3% among schools. Prevalence of infection initially increased with age, then flattened out (Figure 3.2), and boys were significantly more infected than girls (26.0% vs. 23.0%,  $P = 0.006$ ). Overall, 18.2% (95% CIs: 17.2–19.2%) of children reported blood in urine, with more boys reporting than girls (22.5% vs. 13.9%,  $P < 0.001$ ). The prevalence of reported blood in urine increased with age among boys, but was constant with age among girls (Figure 3.2).

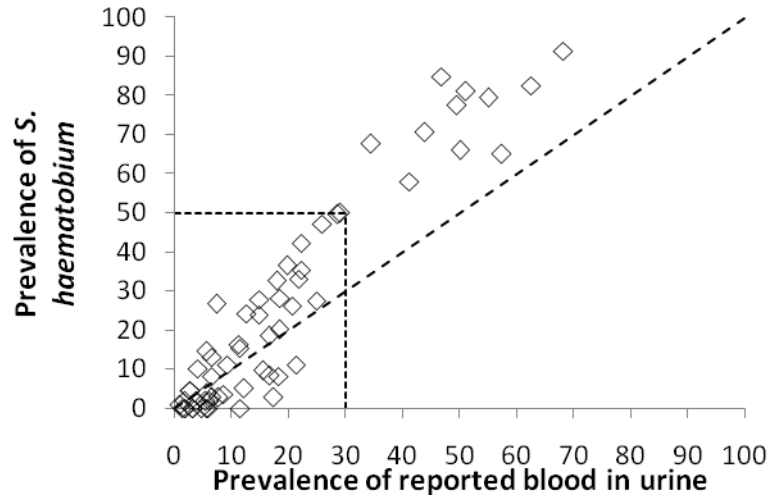


**Figure 3.2: Prevalence of *Schistosoma haematobium* and reported blood in urine by age group and sex.**

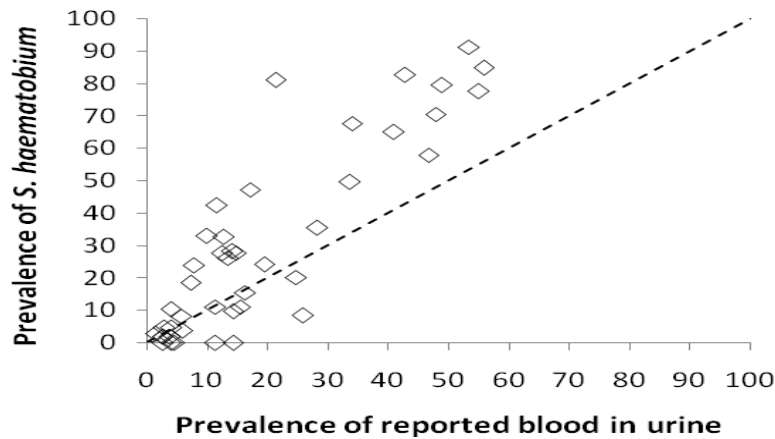
Figure 3a shows the relationship in the 61 schools between the prevalence of urinary schistosomiasis diagnosed by urine microscopy and the prevalence of reported blood in urine. There was a significant correlation between these prevalence ( $r = 0.94$ ,  $P < 0.001$ ), although the figure shows that reported prevalence of blood in urine underestimated the prevalence of infection by an approximately consistent amount, over the range of prevalence observed. The dotted line indicates equivalence of prevalence. Box indicates those schools with prevalence of reported blood in urine  $<30\%$  and prevalence of *S. haematobium*  $<50\%$ ; outside the box are



schools with prevalence of reported blood in urine  $\geq 30\%$  and prevalence of *S. haematobium*  $\geq 50\%$  (Figure 3.3 a & b).



**Figure 3.3(a): Relationship between the prevalence of infection with *S. haematobium*, diagnosed by microscopy and blood in urine in 61 schools**



**Figure 3.3(b): Relationship between the prevalence of *S. haematobium*, diagnosed by microscopy and reported blood in urine in 27 schools**

This relationship confirms that reported blood in urine is a reliable indicator of moderate and prevalence schools above 50%. For instance, using a threshold of 30% reported blood in urine to identify high (>50%) schools yields a sensitivity of 91.7% (95% CIs: 61.5–99.7%) and a specificity of 100% (95% CIs: 92.7– 100%) (Table 3.1). The ability of reported blood in urine to identify infected individuals is also reported. Table 3.1 indicates that questioning children about blood in urine has very poor sensitivity (48.9%) but high specificity (91.8%). Sensitivity was significantly better among boys (57.8%) than among girls (39.0%), and among boys, sensitivity marginally increased with increasing age, whilst among girls; sensitivity was highest among girls aged 10–12 years old.

**Table 3.1: The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of reported blood in urine**

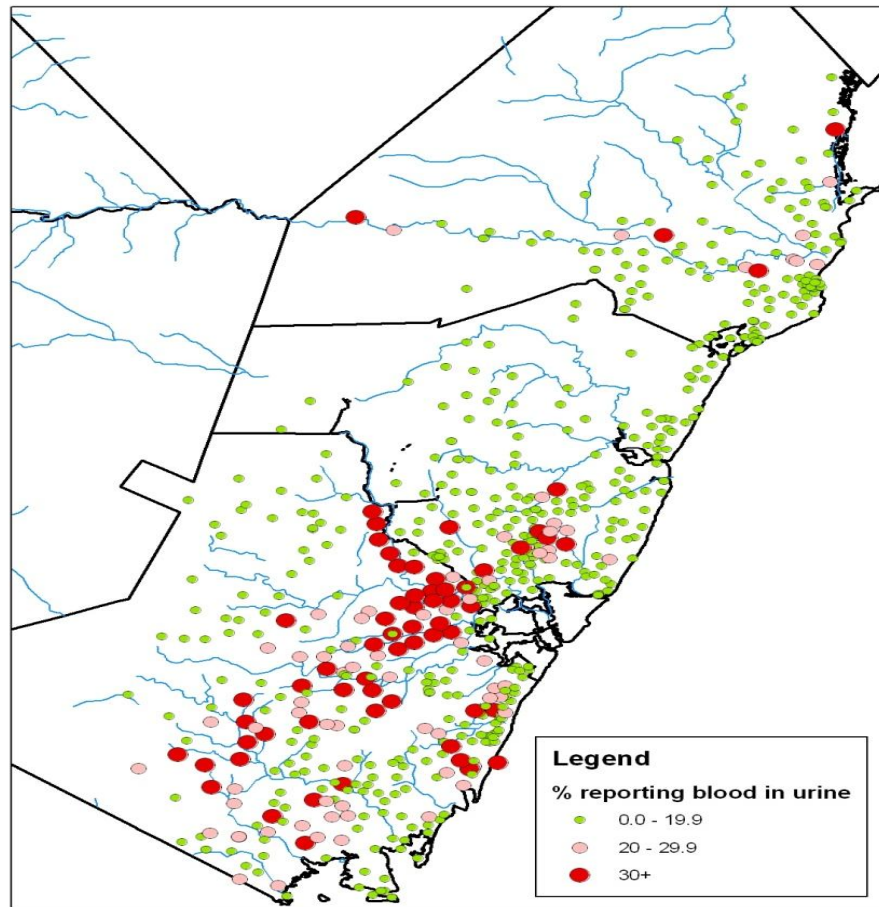
Overall			
Schools			
Sensitivity	91.7 (61.5-99.7)		
Specificity	100 (92.7-100)		
PPV	100 (71.5-100)		
NPV	98.0 (89.3-99.9)		
Children			
	All ages	Age <11 years	Age 11+ years
Boys			
Sensitivity	57.8 (54.2-61.2)	54.5 (47.8-61.2)	59.0 (54.9-63.0)
Specificity	90.0 (88.6-91.2)	91.7 (89.3-93.7)	89.3 (87.6-90.7)
PPV	67.0 (63.3-70.5)	69.1 (61.7-75.9)	66.2 (62.0-70.3)
NPV	85.8 (84.3-87.1)	85.5 (82.7-88.0)	85.9 (84.2-87.5)
Girls			
Sensitivity	39.0 (35.4-42.7)	35.7 (29.6-42.1)	40.7 (36.2-45.3)
Specificity	93.6 (92.5-94.6)	91.9 (89.8-93.8)	94.4 (93.1-95.5)
PPV	64.7 (59.9-69.2)	58.5 (50.1-66.6)	67.8 (62.1-73.3)
NPV	83.6 (82.3-85.0)	81.8 (79.0-84.3)	84.5 (82.8-86.2)

The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of reported blood in urine in (a) identifying school with prevalence >50% and (b) in diagnosing children infected with *S. haematobium* on the Kenyan coast, 2008–2009. Exact 95% binomial confidence intervals in parenthesis 23.4–

25.6%], with prevalence ranging from 0 to 91.3% among schools. Prevalence of infection initially increased with age, then flattened out (Figure 3.2), and boys were significantly more infected than girls (26.0% vs. 23.0%,  $P = 0.006$ ). Overall, 18.2% (95% CIs: 17.2–19.2%) of children reported blood in urine, with more boys reporting than girls (22.5% vs. 13.9%,  $P < 0.001$ ). The prevalence of reported blood in urine increased with age among boys, but was constant with age among girls (Figure 3.2). Figure 3.1a shows the relationship in the 61 schools between the prevalence of urinary schistosomiasis diagnosed by urine microscopy and the prevalence of reported blood in urine. There was a significant correlation between these prevalences ( $r = 0.94$ ,  $P < 0.001$ ), although the figure shows that reported prevalence of blood in urine underestimated the prevalence of infection over the range of prevalences observed microscopically.

As a part of the national control programmes, questionnaires were targeted for distribution to 1772 schools. Of the 61 schools with parasitological data, 45 (74%) schools returned the completed questionnaires. Figure 3.3b shows the relationship in these 45 schools between the prevalence of *S. haematobium* diagnosed by urine microscopy and the percentage of all children reporting blood in urine among those who responded as a part of the Ministry of Education questionnaire. There was a significant correlation between these prevalence ( $r = 0.91$ ,  $P < 0.001$ ). Using a threshold of 30% reported blood in urine to identify high (>50%) schools, yielded a sensitivity of 90.0% (95% CIs: 54.4–99.7%) and a specificity of 97.1% (95% CIs: 85.1–100%). Figure 3.4 presents the spatial distribution of reported blood in urine

based on the Ministry of Education questionnaire survey and highlights the marked focality of infection, with the highest prevalence occurring in Kwale District and in Mariakani of Kilifi District.



**Figure 3.4: The spatial distribution of reported blood in urine in 630 schools on the coast of Kenya (National school based deworming programme, Kihara *et al* 2011)**

### **3.6 Discussion**

Scaling-up of schistosomiasis control throughout sub-Saharan Africa serves to emphasize the role of operational research to ensure that programmes are implemented appropriately and cost-effectively. Both the focal distribution of schistosomiasis and the relatively high cost of praziquantel require that treatment is targeted to schools and communities at great risk of infection and disease in endemic areas. The data presented demonstrates that, within the context of a national deworming programme in Kenya, school questionnaires can reliably identify such high prevalence schools. The observed high sensitivity and specificity are consistent with research studies conducted elsewhere (Lengeler *et al.*, 2002; Nduka & Nwosu, 2008). The lower prevalence of reported blood in urine and the poor performance of reported blood in urine in identifying infected individuals, especially among older girls, corroborate with previous studies (Red Urine Study Group 1995; Lengeler *et al.*, 2002; Clements *et al.*, 2008a).

For instance, a survey among Tanzanian school children found that 28.2% of girls reported blood in urine compared with 48.6% of boys and that reported blood in urine had a sensitivity of 51.5% and a specificity of 79.2%, but sensitivity was significantly lower in girls than in boys (39.6% vs. 61.9%) (Guyatt *et al.*, 1999). Also in Tanzania, a 2004 national questionnaire survey was administered to over 2.5 million children in 12,399 schools across the country (Clements *et al.*, 2008b).

Analysis of differences of reported blood in urine by age and sex found that whilst the prevalence of *S. haematobium* increased with age among girls, the prevalence of reported blood in urine either flattened out or decreased, indicating the latter underestimated prevalence in older girls (Clements *et al.*, 2008a). It has been suggested that the onset of menses influences girls' willingness to report blood in urine. Five main limitations are identified in the present study. First, the use of a single urine filtration may have underestimated the prevalence of *S. haematobium* infection; however, the magnitude of the underestimation is unlikely to have led to a gross misclassification of schools.

Second, there is slight temporal disconnect between the time when the parasitological data were collected and when the questionnaire was distributed by the Ministry of Education, which may potentially reduce the validity of the validation of the government questionnaire survey. However, no mass treatment was undertaken between the parasitological survey and the government questionnaire survey, and although there may have been seasonal fluctuations in the two free-living aquatic stages, the miracidia and the cercaria, and of the infected snail hosts, it is unlikely that there would be significant fluctuations in the prevalence of human infection. This is because the life span of adult worms is substantially longer (3–6 years) than those of either infected snail hosts (weeks) or free-living stages (hours). A third limitation, and one related to the second limitation, is that individuals found to be infected in the parasitological surveys were treated and so may have contributed to an underestimation of reported blood in urine as a part of the government survey.

The fifth limitation is that, older girls who experience menstruation may report blood in urine yet it is not related to *S. haematobium* infection. However, these individuals represented only a small proportion of the total school population, and mass treatment was provided to high prevalence schools only after the questionnaire survey. Fourth, only 74% of schools returned the Ministry of Education questionnaire, and this may have introduced selection bias (to an unknown degree).

### **3.7 Conclusion**

The study is one of very few that effectively seeks to target praziquantel treatment at a national level. Linking the questionnaire survey results to the national schools database enables mapping of the spatial distribution of infection and estimation of the number of schools requiring mass treatment for urinary schistosomiasis.

Validation of the blood in urine questionnaire using school teachers and research officers has shown that the tool can successfully be administered by the teachers with equal results.

The results from this study have provided evidence that the blood in urine questionnaire can be used in a novel large- scale screening for defining the distribution and magnitude of *S. haematobium* infection in endemic areas.



For resource optimization, the blood in urine questionnaire is especially applicable in countries implementing large scale school based deworming programmes since teachers are readily available. However, the tool may not be as efficient in monitoring infections after treatment due to reduced pathology. It is difficult to predict how well the questionnaire will perform.

### **3.8 Recommendations**

A blood in urine questionnaire to screen for school children at highest risk of *S. haematobium* infection is well accepted and operationally feasible, and is faster and less expensive than standard parasitological diagnoses. It involves the active participation of teachers and schoolchildren, and can therefore be recommended especially for very remote schools in endemic areas.

Since primary schools are distributed in the remote areas, blood in urine questionnaire where urinary schistosomiasis is suspected, is thus recommended to cover large areas that would otherwise take long to investigate.

Teachers should be allowed to administer the blood in urine questionnaire in planned large scale school based deworming programmes.

## CHAPTER FOUR

### 4.0 Female Urogenital Schistosomiasis in women of reproductive age and pregnant mothers in Kwale County, Kenya

#### 4.1 Introduction

Urogenital schistosomiasis is a lifetime disease acquired primarily in childhood by exposure to *Schistosoma haematobium*, one of the two main schistosomes transmitted in Africa (WHO, 2009). Approximately 120 million people in Africa alone are estimated to be infected with *S. haematobium* which causes associated pathology in the urinary and genital tract. Female Urogenital Schistosomiasis (FUS) is predominantly caused by *S. haematobium* and has been estimated by the World Health Organization to affect up to 45 million women living in sub-Saharan Africa (WHO, 2009). Since the urinary and genital tracts are almost always both affected, the WHO has recently renamed this disease Urogenital Schistosomiasis (UG), with detection of *S. haematobium* in the urine or genital tract being diagnostic (WHO, 2009).

Chronic female genital-tract inflammation caused by *S. haematobium* has been associated with vaginal itching and discharge, (Kjetland *et al.*, 2008), post-coital bleeding, (Poggensee *et al.*, 2000) genitopelvic discomfort, (Leutscher *et al.*, 2008) marital discord, (Kjetland *et al.*, 1996), ectopic pregnancy (Laxman *et al.*, 2008) and infertility, (Poggensee *et al.*, 2001, Kjetland *et al.*, 2006). In a number

of clinical studies of women living in areas where *S. haematobium* is endemic, schistosoma eggs have often been observed to be associated with characteristic sandy patch lesions in the cervix and vagina (Kjetland *et al.*, 1996; Poggensee *et al.*, 2000; Poggensee, *et al.*, 2001; Kjetland, *et al.*, 2005; Swai *et al.*, 2006). Genital *S. haematobium* infection has been associated with human immunodeficiency virus (HIV) infection in one cross-sectional study (Kjetland *et al.*, 2006) and has been postulated to be a risk factor for HIV infection, (Feldemeir *et al.*, 1994, Hotez *et al.*, 2009).

Kidney function tests are a collective term for a variety of individual tests and procedures that can be performed to evaluate how well the kidneys are functioning. Eggs of *S. haematobium* are found in the blood circulation of infected individuals and are excreted through the same system into the bladder. Eggs of schistosomes have been found in many organs of the body of infected people. Some eggs are likely to find their way into the kidneys causing patho-physiological changes that can be diagnosed through certain tests in urine or blood.

Clinically apparent vulval, vaginal and cervical schistosomiasis is a common gynaecological finding in areas where infection with *S. haematobium* prevails (Friedberg, *et al.*, 1991). Female Urogenital Schistosomiasis affects up to 50% of women in highly endemic areas (Kjetland, *et al.*, 2006). Heavy infection with *S. mansoni* has been shown to contribute to anaemia in pregnant women in an area in Tanzania (Ajanga, *et al.*, 2006). If egg-antigen-inflammatory processes contribute to poor pregnancy outcomes, then treatment during pregnancy could be too late.

This, therefore, requires health initiatives to keep women of reproductive age free from infection (Friedman, *et al.*, 2007).

Female Urogenital schistosomiasis has been neglected for several years in endemic areas and especially in vulnerable groups such as women of reproductive age and children in the community. Community based epidemiological studies on pathophysiological changes due to *S. haematobium* infections have not been elucidated exclusively on women of reproductive age. In the Coastal region of Kenya, *Schistosoma haematobium* is exclusively prevalent, (between 2% and 75%) in school age children (Brooker *et al.*, 2009; Kihara *et al.*, 2011). The study was conducted on women of child bearing age in 5 villages in Kwale (*Lutsangani, Rabai, Mwachinga, Mienzeni and Mwaluphamba*). Treatment of the adult population has not been conducted in these villages at least in the past 4 years prior to enrollment into the study. The research objective number two was to determine the prevalence and intensity of *S. haematobium* infection in women of reproductive age, and whether the infection has any effect on kidney function in schistosomiasis endemic areas.

#### **4.2 Literature review**

Studies of *S. haematobium* epidemiology and clinical manifestations have focused predominantly on urinary tract infection. Genital schistosomiasis has received less attention, although histopathological studies have demonstrated that the female and male internal genitals are sites of egg-induced granulomatous inflammation (Gelfand, 1970; Gelfand, 1971; Wright, 1982). In a number of clinical studies of

women living in areas where *S. haematobium* is endemic, schistosoma eggs have often been observed to be associated with characteristic sandy patch lesions in the cervix and in the vagina (Kjetland, 1996; Poggensee, 2000; Poggensee, 2001; Kjetland, 2006). Eggs have also been detected in ejaculates from *S. haematobium*-infected men (Elem, 1987; Corachan, 1994; McKenna, 1997; Torresi, 1997; Leutscher, 2000;; Durand, 2004). In most areas of endemicity, infection prevalence and intensity normally peak during the second decade of life (Serieye, 1996; Kouriba, 2005).

On the community level, infected individuals may contribute to the burden of infection transmission, by transmission of *S. haematobium* to the community through passage of eggs in urine to the local water bodies (Feldermeir, 1994; Cohen, 1997; Chen, 2007). Chronic female genital-tract inflammation caused by *S. haematobium* has been associated with vaginal itching and discharge, (Poggensee *et al.*, 2006; Kjetland, 2008) post-coital bleeding, (Poggensee, 2000) genitopelvic discomfort, (Leutscher, 2008), marital discord, (Kjetland 1996) and infertility (Poggensee, 2001; Kjetland, 2006). Female urogenital schistosomiasis is a geographically clustered infection that disproportionately affects women younger than 30 years of age (Jennifer, 2011). These young women, who also have the highest risk for incident HIV infection and in whom genital lesions may be reversible if treated early, should be the focus of public health interventions aimed to reduce the prevalence of *S. haematobium* infection (Jennifer, 2011).

The health status of a woman before pregnancy is a crucial determinant of gestational morbidity and pregnancy outcomes. Poor nutritional status, deprived living environments and higher rates of infectious diseases contribute to maternal mortality, infant mortality and Low Birth Weight (LBW) (Kramer, 1987; Hasin *et al.*, 1996; Kramer and Victoria, 2001) in Lesser-Developed Countries (LDCs). Women who are underweight or short and those with anemia or infections are at increased risk of delivering LBW infant (Steketee, 2003; Kramer, 2003). Since, schistosomiasis causes both anemia (McGarvey, 1996; Friedman, 2005; Leenstra, 2006) and under nutrition (Stephenson, 1989; Friedman, 2005; Coutinho, 2005), maternal schistosomiasis could have deleterious consequences during pregnancy (Helling-Giese *et al.*, 1996). Treatment of this high risk group would be beneficial to both the mother and the unborn child.

#### **4.2.1 Praziquantel treatment and pregnancy**

Praziquantel (PZQ) was made available in 1979 and has been the mainstay of schistosomiasis control programs for decades (Davis *et al.*, 1981). Praziquantel has not been studied in pregnant or lactating women and was, therefore, designated as a ‘pregnancy class B’ drug. Federal Drug Administration (FDA: <http://www.fda.gov>) class B drugs are presumed to be safe based on animal studies but lack safety data from studies in pregnant women. In practice, this has led to withholding of treatment for pregnant and lactating women in most schistosomiasis-endemic countries. This is important in that women aged 18–25 years who live in schistosomiasis- endemic regions might spend almost 25% of their reproductive life pregnant and 60% of their reproductive time lactating. Delay

in treatment of more than one year results in significant morbidity among non pregnant women (Coutinho, 2006; Leenstra, 2006) and such morbidity could be further exacerbated in pregnant women, who have increased micro- and macro-nutrient requirements.

World Health Organization in 2002 in an informal conference held in Geneva, indicated that all Schistosome-infected pregnant and lactating women should be considered a high-risk group and Allen, 2002 suggested treatment be offered individually or during treatment campaigns. The WHO recommendation based the cost of withholding treatment on the expected morbidity that would be suffered by non pregnant women of reproductive age who might be left untreated during long periods of pregnancy and lactation. In the cost-benefit analysis, an emphasis was placed on examining the morbidity that women would be expected to experience, specifically schistosomiasis induced organ damage, which can progress over relatively short periods of time, anemia and pathologies that are largely reversible when treated early, such as hepatomegaly and urinary tract pathology. Despite this informal recommendation, the ministries of health of many endemic countries have not adopted this policy because of a lack of pregnancy safety data that had been rigorously collected (Friedman, 2007).

## 4.3 MATERIALS AND METHODS

### 4.3.1 Study site and subjects

This was a cross-sectional study conducted in Kwale County, Coast Province, Kenya. The district is located in the South Eastern corner of Kenya, bordering Tanzania to the south and the Indian Ocean to the east (latitude 3.558° - 4.675°South and longitude 38.452°- 39.663°East). The district covers an area of 8,295 km<sup>2</sup> and with an approximate population of 496,133. Kwale experiences continuous rains almost throughout the year in the higher parts of the district. Most inhabitants are subsistence farmers. The arid areas of the district have several dams and seasonal streams that are good breeding sites for snails, the intermediate host of schistosomiasis.

Women of reproductive age (here defined as between 16 – 45 years) were recruited into the study, and classified as pregnant or non-pregnant at the time of sample collection. Villages were selected primarily for their high *S. haematobium* prevalence (>40%) as determined by a study conducted by Kihara *et al.*, (2011). All participants were sensitized during a public Baraza (group gathering) where details of the study were explained by the research team members in their local language. Non consenting women or non consenting spouse were not included in the study.



#### **4.3.2 Parasitological examination for *S. haematobium* infection**

Parasitological examination of the eggs in urine was examined using nuclear pore filtration technique as described by Kahama *et al.*, (1998). The urine sample was collected between 10.00 am and 2.00 pm. A duplicate 10 ml aliquot of urine was filtered through 15-mm polycarbonate filters (GE polycarbonate, 12.0 Micron, 13mm diameter: Catalog No. K12CP01300; Water and process technologies). The filter was placed on a labeled slide and examined under a microscope within 6hrs. The mean counts of the two filters were recorded and expressed as eggs per 10ml urine.

#### **4.3.3 Blood collection**

Venous blood was collected in unsequesternated (plain) bottle simultaneously as urine samples were collected for parasitological examination. Five (5) milliliters of blood was drawn from the vein of the study subjects; 2ml was put in a sequestrenated vial and 5mls into a plain bottle (with no anticoagulant). The sequestrenated blood was put in a cooler box with ice packs (temperature of about 8<sup>0</sup>C) until analysis in the laboratory within 2 hours. The blood in the plain bottle was allowed to clot for 2 hours and serum collected by centrifuging for 5 minutes at 1500G and then separated for laboratory analysis.

#### **4.3.4 Kidney function tests**

The machine used for sample analysis was Clinical Chemistry Autoanalyzer Olympus 640(Olympus Diagnostica GmbH, Hamburg, German). This is a discrete, random access clinical chemistry analyzer capable of performing a wide range of

chemical tests in a single run, including urea and creatinine.

#### **4.3.5 Olympus auto-analyzer**

Methods for specific analytes were programmed and stored in the microprocessor of the instruments. The auto-analyzer proportioned the required amount of reagent and sample using the reagent and sample probes respectively. Reagent volumes were in the range of (50-300ul) and the sample volumes in the range of (2-20  $\mu$ l). The various reactions occur in the reaction compartment of the instrument at 37 degrees centigrade. After the reaction, results were obtained from the data manager either printed out or read from the screen.

#### **4.3.6 Blood Urea Nitrogen (BUN)**

Blood urea nitrogen was analyzed using Glutamate Dehydrogenase (GLUDH).

BUN reagent was used to measure the concentration of urea by an enzymatic rate method. In the reaction urea was hydrolyzed by urease to ammonia and carbon dioxide. Glutamate dehydrogenase (GLDH) catalysed the condensation of ammonia and  $\alpha$ -Ketoglutarate to glutamate with the concomitant oxidation of reduced B-nicotinamide adenine dinucleotide (NADH) to B-nicotinamide adenine dinucleotide (NAD).

#### **4.3.8 Creatinine (CREAT)**

The creatinine reagent was used to measure the creatinine concentration by a modified rate Jaffe method. In the reaction, creatinine combined with picrate in an alkaline solution to form a creatinine – picrate complexone.

#### **4.4 Ethical consideration**

The study objective was explained to women in a Baraza (large group) by a trained study laboratory technician/nurse fluent in the local language. All women who accepted to be part of the study were asked to provide written informed consent or place their mark on the consent form. Permission to conduct the study was obtained from the District Medical Officers and clinicians/nurses stationed at participating dispensaries and health centers. Ethical approval was granted by the Kenya Medical Research Institute, Ethical Review Committee, SSC No 1319 (appendix 8). Women diagnosed with urogenital schistosomiasis if not pregnant, received free treatment immediately. Women who were pregnant and diagnosed positive for *S. haematobium* were treated free of charge immediately after delivery.

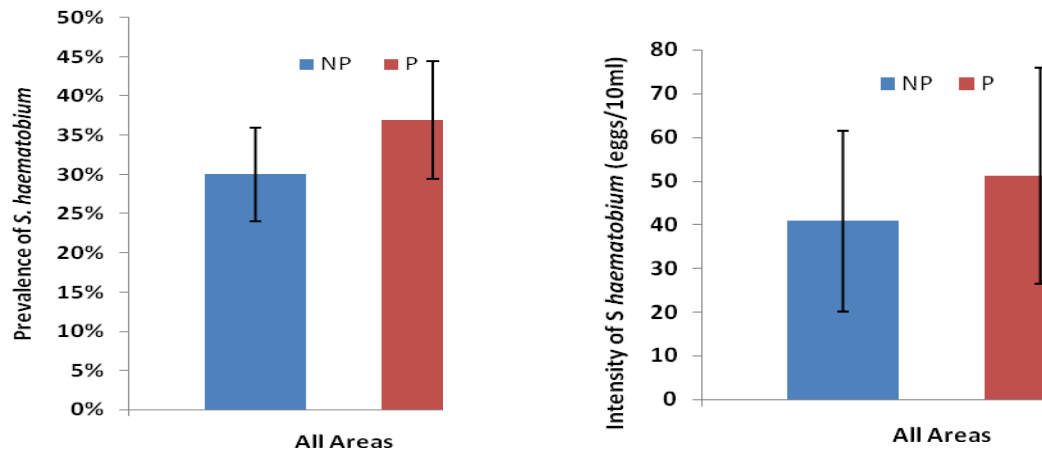
#### **4.5 Statistical analysis**

Analysis was based on prevalence and intensity of the infections in relation to age group, gestation period using Chi-square test ( $X^2$ ) and regression analysis. Student's t-test was applied to test the difference in mean egg counts between age groups and/or sex of study subjects (Kirkwood and Sterne, 2003). When examining the prevalence of infection, binomial regression analysis was used to account for the binomial character of the variable; thus, either pregnant or non-pregnant. When examining intensity of infection, a negative binomial regression analysis was used to control for the non-normal (non-gaussian) distribution of infection intensity between individuals. The Generalized Linear Mixed-effect Models (GLMM) allowed the schistosomiasis prevalence and intensity to vary between different

areas (the random effect) but allowing any trend between pregnant and non-pregnant women (the fixed effect) to be identified.

#### **4.6 Results of the prevalence of *S. haematobium* in women of reproductive age in Kwale and the effect on kidney function**

The relationship between pregnancy, non pregnancy and infection with urinary schistosomiasis was investigated in women of reproductive age. The villages/locations investigated were as follows: MZ: Mienzeni, LT: Lutsangani, MW: Mwaluphamba, RB: Rabai, MC: Mwachinga. Samples were taken from a total of 411 women, between the ages of 16 and 37 years, split between pregnant (158) and non-pregnant women (253). Mwaluphamba village had higher mean egg counts for both non-pregnant (87) and pregnant (59) women, followed by Mienzeni with mean egg count of 43 and 39 respectively. The village with lowest mean egg count was Lutsangani 21 for non-pregnant and 12 for pregnant women.

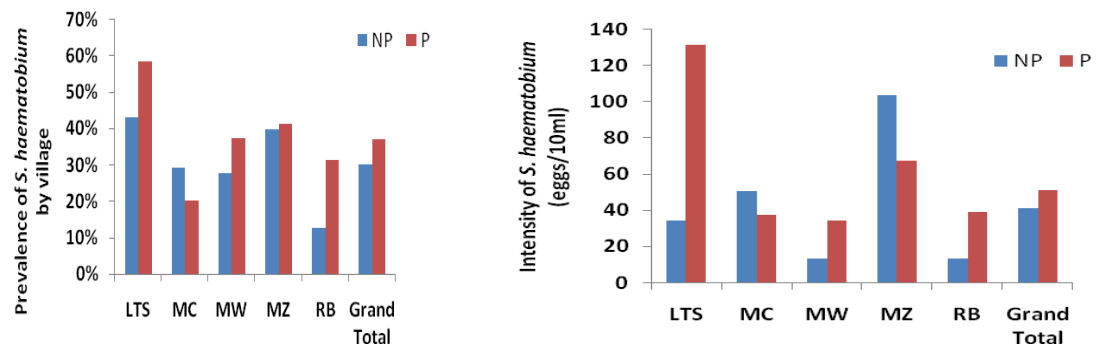


**Figure 4.1: The prevalence and intensity of *S. haematobium* in women of reproductive age in 5 villages of Kwale County**

P= Pregnant, NP = Not pregnant

Pregnant women had a higher prevalence of infection with urinary schistosomiasis than non-pregnant women (Pregnant: 36.9% (95%CI: 29.4-44.5%), Non-pregnant: 30.0% (95%CI: 24.1-25.9%),  $Z= 1.43$ ,  $P= 0.153$ ). They also had a higher intensity of infection (Pregnant: 51.1 eggs/10ml (CI: 26.5-71.9 eggs/10ml), Non-pregnant: 40.9 eggs/10ml (CI: 16.2 – 61.5 eggs/10ml),  $Z=0.629$ ,  $P=0.529$ ) (Fig. 4.1).

There was significant variation in infection intensity and prevalence between villages, as shown in the figures 4.2. Infection prevalence in pregnant women ranged from 20.0% in MC to 58.3% in LTS. Correspondingly, infection intensity in pregnant women ranged from 34.3 eggs/10ml in MW to 131.3eggs/10ml in LTS. Bearing in mind that 50eggs/10ml is the criterion for ‘High’ infection intensity as set out by the WHO, 1999 and 2006. For non-pregnant women prevalence ranged from 12.5% in RB to 42.9% in LTS, and intensity from 13.1 eggs/10ml in RB to 103.5 eggs/10ml in MZ.

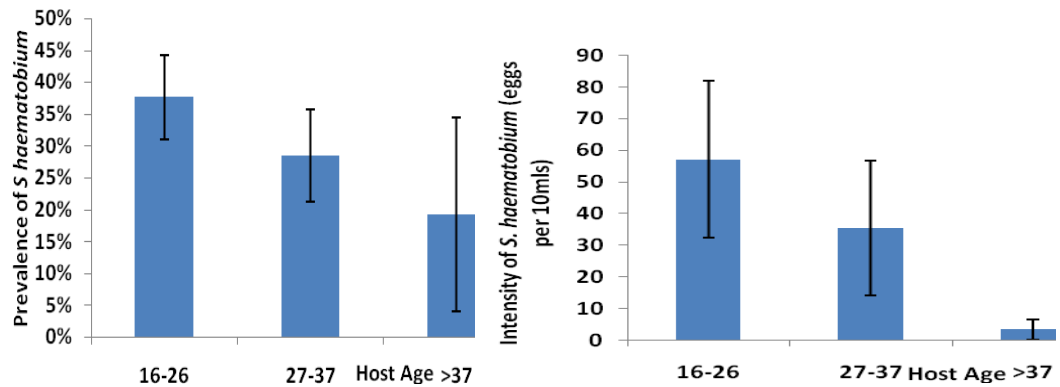


**Figure 4.2: Prevalence and intensity of infection from the different villages**

P = Pregnant, NP = Not pregnant

#### 4.6.1 Prevalence and intensity of *S. haematobium* by age group

Prevalence and intensity of infection with *S. Haematobium* in women of reproductive age compared by age group (there is no known standardization of the adult female age groups), the age groups were stratified in a ten years difference for convenience of showing the changes in the following age categories: 16-26, 27-37 and >37 (Fig.4.3).

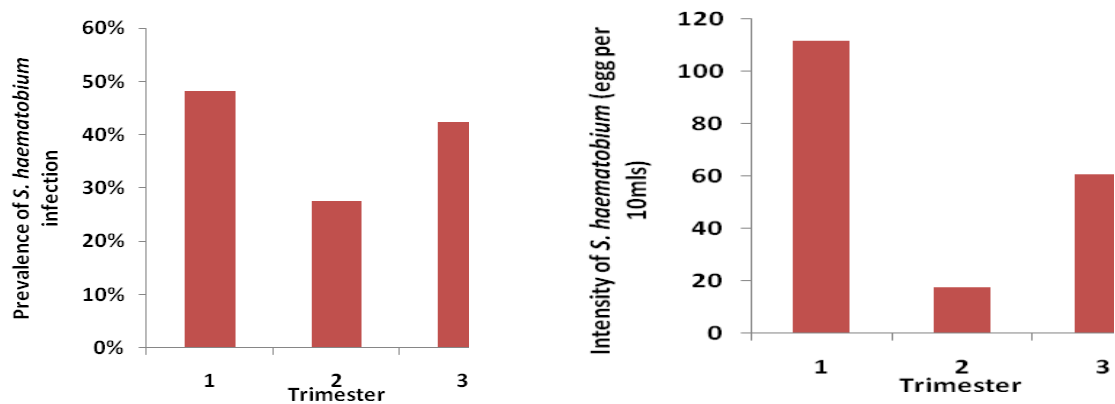


**Figure 4.3: Comparison of prevalence and intensity of urinary schistosomiasis by age groups in women of reproductive age in the study cohort**

As has been found with other studies previously, both the prevalence and intensity of infection with urinary schistosomiasis peak at late-adolescence to young adulthood (in our case, 16-26, although there were no younger women in this cohort to check whether they would be higher) and then declines in the 27-37 age-group, and even further in the >37 age-group (Fig. 4.3). There was a significant and substantial reduction in prevalence; reducing from 37.6% to 28.5% to 19.2% (Chi-squared test for trend,  $\chi^2=5.65$ ,  $P=0.017$ ). Intensity falls from 57.16 eggs/10ml to 35.46 e/10ml to 3.31 eggs/10ml (One way analysis of variance, ANOVA = 1.789,  $P=0.168$ ) although this reduction is quite substantial, it does not reach statistical significance, probably due to the sample size obtained in this study.

#### 4.6.2 Comparison of *S. haematobium* infection with gestation period

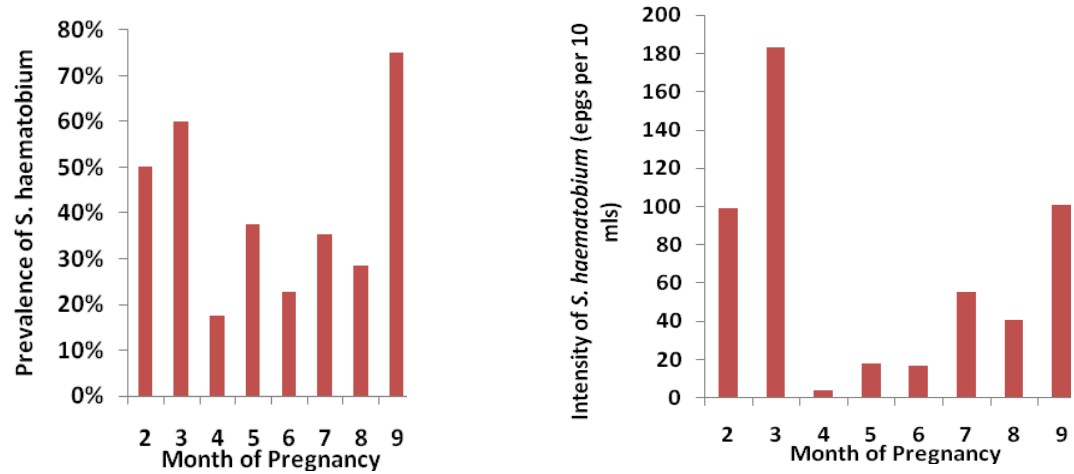
In this Part of the study the prevalence and intensity of infection with the gestation period, expressed as either the month of pregnancy, or the trimester (3-monthly intervals) of pregnancy were compared.



**Figure 4.4: The comparison of infection with trimester of pregnancy**

As can be seen in Figure 4.4, the level of *S. haematobium* infection prevalence and intensity was highest in the first trimester of pregnancy, then dropped in the second, and rose again in the third trimester, probably because of the lack of treatment during pregnancy. In figure 4.5 below, there is no clear pattern as to the relationship, however, intensity does seem to be highest in the first trimester (second and third month), then fall away dramatically in the second trimester (4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup> month) before building again for the remainder of the pregnancy (7<sup>th</sup> 8<sup>th</sup> and 9<sup>th</sup> months). Again the reason for this observation can only be hypothesized as the one in the trimester analysis.





**Figure 4.5: Prevalence and intensity of *S. haematobium* infection against the gestational age in months**

#### **4.6.3 Significance of infection in pregnant and non-pregnant women**

The model indicates that on average, across all villages the prevalence or intensity of *S. haematobium* infection is the same in pregnant and non-pregnant women.

Adjusting for age the prevalence of infection was 32.76% in pregnant women and 32.73% in non-pregnant women. Similarly the mean intensity was 41.91egg/10mls in pregnant women and 38.22 egg/10ml in non-pregnant women.

#### **4.7 Results of kidney function test**

Of the 411 women who participated in the study, 250 serum samples were collected and analysed for Urea (BUN) and creatinine to determine the kidney function. Out of all samples analysed, none of the women showed higher than normal serum creatinine,  $>115\mu\text{l}$  (normal is between 55 and  $115\mu\text{l}$ ) or Urea  $> 8.3\text{mmols}$  (normal is between 1.7 and  $8.3\text{mmols/l}$ ) in their blood.

#### **4.8 Discussion**

The results of this cross-sectional study demonstrates that *S. haematobium* infection is highly prevalent in women of reproductive age in Kwale County, Coast Province and that higher infection rates are observed in pregnant women than in non-pregnant women. However, in the areas where the study was conducted, some women did not participate due to various reasons including, availability, cultural attitudes and fear of reaction from their spouse (husband).

There was no significant difference between women with light infection (39.63%) 1-49 eggs/10ml and heavy infections (48.63%)  $>49$  eggs/10ml, according to WHO (1999), classification of schistosomiasis infection intensity. Although the analysis did not reach statistical significance, this could probably be related to the sample size obtained in this study population. The intensity of infection follows the same trend as for prevalence whereby the younger ages of 16 – 35 years consistently harbour heavier parasitic burdens, as measured by excreted egg counts. This association between age and schistosomiasis infection has also been demonstrated by other researchers who reported FUS with younger

age of 18 -29 years consistent with the natural history of the disease and which has important public health ramifications (Jennifer, *et al.*, 2011). These reductions in infection markers with age are thought to be related to changing patterns of exposure and/or behaviour, thus, less time-contact with infested water sources, and less frequent visits to suitable snail habitats in the area. They could also be related to the development of acquired concomitant immunity or a combination of these factors.

In normal circumstances women between these ages are not a focus of school-based anti-schistosomal treatment campaigns in sub-Saharan Africa, but the substantial burden of FUS in this age group argues strongly for targeted FUS treatment. Comparing the prevalence and intensity of *S. haematobium* infection in the study cohort shows higher levels among pregnant women, (36.9%) than in non pregnant women (30%). Correspondingly, the intensity was also slightly higher in the pregnant (mean 51.1 eggs/10ml) than in the non pregnant women (40.9 eggs/10ml).

However, even in this selection, the prevalence and intensity of *S. haematobium* varied among villages where Lutsangani and Mwaluphamba had higher prevalence and intensity than other areas, typical of the focal nature of schistosomiasis. Studies by other researchers in Nigeria and Kenya, have also reported on seasonality of schistosomiasis transmission (Ozumba *et al.*, 1989; Sturrock *et al.*, 2001). Facts which are strongly supported by Lwambo and colleagues (Lwambo *et al.*, 1999) who observed that transmission of *S. haematobium* is generally more wide spread than *S. mansoni* and that it is more seasonal with highest transmission occurring

after the rainy season when snails are no longer estivating. Our analysis has shown high infection with *S. haematobium* in some villages (Lutsangani) probably due to the continuous exposure to infested sites and consistent lack of intervention such as mass de-worming in this area.

Previously, de-worming has been avoided during pregnancy and lactation because of safety concerns; however, in areas where women are pregnant or lactating for over half of their reproductive lives, this may result in treatment delays and high morbidity (WHO, 1994). Incidentally, this may be the case in the present study cohort since treatment of pregnant women with praziquantel has not been embraced in the health care delivery systems in Kenya. In 2002, WHO recommended the use of praziquantel during pregnancy (after the 1<sup>st</sup> Trimester) in areas where schistosomiasis is endemic, in addition to evaluation of birth outcomes.

Women aged 18–25 years who live in schistosomiasis-endemic regions might spend almost 25% of their reproductive life pregnant and 60% of their reproductive time lactating (Jennifer, 2011). The effects of delays to treatment could be exacerbated in pregnant women who have increased micro- and macro-nutrient requirements (Coutinho *et al.*, 2006; Leenstra *et al.*, 2006). This research has revealed prevalence and intensity of *S. haematobium* infection of 37.6% and 56e/10mls respectively in young women aged between 16 and 26 years. This result is in agreement with studies by Jennifer (2011) who demonstrated that those individuals in younger age groups (18-29 years) were significantly associated with FUS and is consistent with natural history of schistosomiasis. This is also the age at

highest risk for HIV transmission UNAIDS, (2008). Also, Woolhouse (1998) showed peak levels of schistosomiasis infection are higher and occur at a younger age in populations subject to high levels of transmission but are lower and occur at an older age in populations subject to lower levels of transmission.

The intensity of infection in the present study decreases with increase in age (age group). This could be attributed to concomitant immunity acquired over time due to continuous re-infection and the fact that women of reproductive age may not be treated for a long time especially in the rural communities where praziquantel tablets are not readily available, even in district hospitals. Alternatively, it may be explained by the highly non-linear relationship between infection prevalence and intensity and exposure, typically observed with schistosome infections.

For the first time in Kenya, this study has reported the prevalence and intensity of *S. haematobium* infection in pregnant women by the varying stages of pregnancy (trimester) and gestational age (in months) of the women. In this study, women in their early stages of pregnancy (1<sup>st</sup> trimester), had higher infection with *S. haematobium*, then declined in those in their second trimester and increased slightly in those that are in the third trimester. No particular reason could be attributed to this observed decline except to hypothesize that; it may be due to the seasonality of schistosomiasis transmission in this area and the period of the year when the samples were collected (September/October). It could also be that infection was acquired in early childhood with no treatment over a long period, plus water contact

behaviour of the women especially in the rural set-up and/or transmission dynamics. Thirdly, the high infection intensity could be associated with possible lowered immunity of the mothers during progression of the pregnancy or other infections not investigated in this study.

Interestingly, pregnant women showed low intensity but moderate prevalence of infection during their second trimester. No one specific reason can be hypothesized from these results but to speculate on a multiple of aforesaid factors mentioned previously. The same trend was also observed when infection intensity by month of gestation was compared. However, it is worth noting here that, the small sample sizes that result when a cohort is split into 9 categories in this study may have produced some confounding results which will be best to investigate further with a much larger sample size.

The serum creatinine and urea levels in the women were normal in all the subjects analysed. This is in conformity with a study by Mohamberry, *et al* (1987) who also reported normal creatinine, urea and urates in school children infected with *S. haematobium*. However, Lehman *et al*, (1970), working in Egypt found evidence of functional renal impairment in a high proportion of their patients who showed bilateral obstructive uropathy on pyelography or showed bacterial infection or both. In the present study, these conditions were not investigated; however, more detailed studies on renal and liver function of infected subjects in endemic areas could assist in understanding other patho-physiological changes that may be manifested in patients infected with *S. haematobium*.

Praziquantel treatment of human schistosomiasis during pregnancy and lactation was avoided from the time it became available in 1979, until an informal consultation by the World Health Organization in 2002 (WHO, 1998; 2003) which recommended treatment of pregnant women after the first trimester. A study by Tweyongyere *et al.*, (2009), suggests that praziquantel treatment against *S. mansoni* during pregnancy has similar efficacy as praziquantel treatment in non-pregnant women, despite the presence of lower levels of anti-schistosomal antibodies and tendency to reduced boosting of both antibody and cytokine responses observed during pregnancy. The WHO recommendation based the cost of withholding treatment on the expected morbidity that would be suffered by non-pregnant women of reproductive age who might be left untreated during long periods of pregnancy and lactation.

In the cost–benefit analysis, emphasis was placed on examining the morbidity that women would be expected to experience, specifically schistosomiasis induced organ damage, which can progress over relatively short periods of time, anemia and pathologies that are largely reversible when treated early, such as hepatomegaly and urinary tract pathology (Friedman *et al.*, 2007). It is estimated that 16 million women will acquire the genital manifestation of *S. haematobium* infection and that, if cured, 120,000 new cases of HIV could be averted through regular praziquantel treatment in the next decade (Hotez, *et al.*, 2009).

#### **4.9 Conclusion**

The present study has demonstrated the prevalence of 50% and intensity of 103.5eggs/10mls of *S. haematobium* in women of reproductive age in Kwale County. In this particular study, infected women were not treated before delivery because the clinicians still adhere to the old teachings that pregnant women infected with schistosomiasis should not be treated during term but after delivery.

The study has shown that pregnant women are equally or more infected with schistosomiasis than non pregnant women and their treatment will not only relieve them of the burden of disease during gestation, but also probably improve on their birth outcomes. Infection with *S. haematobium* did not affect kidney function in women of reproductive age in Kwale County.

#### **4.10 Recommendations**

The following recommendations were made from this study;

From this study it is suggested that the inclusion and expansion of the age group (16 – 37 years) of the reproductive women be treated as high risk groups during any MDA.

Notwithstanding some challenges due to effects of Praziquantel on pregnancy, treatment of women of reproductive age after the 1<sup>st</sup> trimester is recommended in schistosomiasis endemic areas.



It is recommended that all clinicians, nurses and doctors be advised and/or retrained on management/treatment of pregnant women infected with schistosomiasis after the first trimester as recommended by World Health Organization (WHO, 2002).

## CHAPTER FIVE

### 5.0 INVESTIGATION OF HAEMATOLOGICAL CONDITIONS AND BLOOD PATH-PHYSIOLOGICAL CHANGES OF WOMEN SUFFERING FROM FEMALE GENITAL SCHISTOSOMIASIS IN KWALE COUNTY, KENYA

#### 5.1 Introduction

Schistosomiasis (bilharzia) is a neglected tropical disease caused by trematode parasitic worms of the genus *Schistosoma*. Approximately 207 million persons are infected with schistosomiasis worldwide (Steinman *et al.*, 2006) which leads to the loss of approximately 1.53 million Disability Adjusted Life Years (DALYs) (Gryseels *et al.*, 2006; King and Dangerfield-Cha, 2008). The greatest burden of the disease is found in school age children in sub-Saharan Africa (Van de Warf *et al.*, 2003; Elagba *et al.*, 2006)). Overt pathology results from granulomatous inflammation and fibrosis in response to eggs that are trapped in the hepatic sinusoids, which leads to hepatosplenic disease. *S. haematobium* resides in the venous plexus, which surrounds the urinary bladder. Pathology is caused by chronic inflammation and scarring of the bladder and urogenital tract because eggs are passed through the bladder wall and out in the urine. The contribution of schistosomiasis to morbidity and mortality has recently been reassessed using reported symptoms and available and predicted prevalence-of-infection data. Such data estimate global deaths to be as high as 200,000 per year, in comparison with earlier estimates of 11,000–15,000 (WHO, 2005).

Female Genital Schistosomiasis (FGS) is characterized by the presence of schistosome eggs/worms in the upper or lower genital tract. Such a disease manifestation was reported for the first time in 1889 when Madden observed a tumorous growth that consisted of numerous egg granulomas in the vagina of a young woman from Egypt. Clinically apparent vulval, vaginal and/or cervical schistosomiasis has been reported from both endemic and non endemic areas. This has led to the Gender Task Force of the World Health Organization Tropical Research Programme (WHO-TDR) to include in the list of scientific areas that deserve high research priority (Van de Warf, 2003). Several studies have shown that FGS is a common manifestation in *S. haematobium* infection, with prevalence rate ranging from 30% to 75% in some communities (Poggensee *et al.*, 1999). Other studies have indicated that schistosome ova deposition may be the cause of genital papillomatous tumours, leukoplakia, polyps, and ulcers similar to sexually transmitted diseases (STD), (Leutscher *et al.*, 1997; Poggensee *et al.*, 2000).

Pregnant or lactating women infected with schistosomiasis in endemic areas, may be complicated further by malnutrition and low immunity resulting in more pathology related to liver and kidney function (King *et al.*, 2005). Currently, there are inadequate data that address schistosomiasis during pregnancy and possible patho-physiological changes that they suffer. Available information indicates that 10 million women in Africa have schistosomiasis during pregnancy (WHO, 2002). Schistosomiasis causes debilitating nutritional, hematologic and cognitive deficits, with substantial morbidity and mortality in non-pregnant populations [Continho, 2006; Leenstra, 2006; Ezeamama, 2005). Infection with *S. mansoni* has been shown

to contribute to anaemia in pregnant women in an area in Tanzania (Ajanga *et al.*, 2006; Liljestrand *et al.*, 1986). Assessing the causes of anaemia is complex, especially where many different etiologies are at play simultaneously, as is the case in much of the developing world (Sturrock *et al.*, 1996; Tatala *et al.*, 1998).

Despite the potentially enormous at-risk population, little is known about the schistosome-specific morbidities that are experienced by pregnant and lactating mothers and their newborns (Friedman, 2007). Thrombopoietin is constitutively produced by the liver and kidneys, but its actual activity with precursor cells is regulated by the number of circulating platelets because of the expression of the corresponding receptor on the surface, which tunes the activity (Harker *et al.*, 2000).

It is estimated that over 9.1 million (~ 25%) of Kenyans are infected with schistosomiasis (WHO, 2010). In endemic areas, 50% of women of reproductive age are infected with one or both species of schistosomes (Poggensee *et al.*, 1999). The objective of this cross-sectional study was therefore, to determine anaemia status, haematological conditions and blood pathophysiological changes in adult women suffering from female genital schistosomiasis Kwale County, Kenya. The effects of these changes on pregnant women were also investigated with a view to improving on treatment strategies of these- at risk groups in Kwale and any other areas where *S. haematobium* is endemic. Apparently, these patho-physiological changes have not been fully investigated in women of reproductive age residing in

schistosomiasis endemic areas, in the context of treatment algorithms for administration of praziquantel in pregnant women. The study aimed to inform and prelude discussions of urogenital schistosomiasis as a neglected yet urgent public health challenge to women of reproductive age in areas where transmission occurs. Hookworm and malaria infections were also investigated since these infections cause anaemia of significant level. All women found infected with hookworm or malaria parasites were not included in the data analysis.

## **5.2 Literature review**

Anaemia is a global public health problem affecting both developing and developed countries with major consequences for human health as well as social and economic development. It occurs at all stages of the life cycle, but is more prevalent in pregnant women and young children. In 2002, Iron Deficiency Anaemia (IDA) was considered to be among the most important contributing factors to the global burden of disease (WHO, 2002). It is generally assumed that 50% of the cases of anaemia are due to iron deficiency (WHO, 2001), but the proportion may vary among population groups and in different areas according to the local conditions. Infectious diseases, particularly parasitic diseases, also lead to both extracorporeal iron loss and anemia of inflammation, which decreases bioavailability of iron to host tissues (Julia and Jenniffer, 2011). The main risk factors for IDA include a low intake of iron, poor absorption of iron from diets high in phytate or phenolic compounds, and period of life when iron requirements are especially high, thus, growth and pregnancy. Among the other causes of anaemia, heavy blood loss as a result of menstruation, or parasite infections such as hookworms, ascaris, and schistosomiasis can lower blood haemoglobin (Hb) concentrations (Dreyfuss *et al.*, 2000; Friedman *et al.*, 2005).

The health status of a woman before pregnancy is a crucial determinant of gestational morbidity and pregnancy outcomes. Poor nutritional status, deprived living environments and higher rates of infectious diseases contribute to maternal mortality, infant mortality and Low Birth Weight (LBW) (Kremer, 2003) in Lesser Developed Countries (LDCs). Women who are underweight or short (according to body mass index) and those with anemia or infections are at increased risk of delivering LBW infants (Steketee, 2003). Since schistosomiasis causes both anemia (Leenstra, 2006; Friedman, 2005) and undernutrition (McGavey, 1996), maternal schistosomiasis could have deleterious consequences during pregnancy.

In communities in where the prevalence of Schistosomiasis is at least moderate in children (50–90%) women continue to have urogenital schistosomiasis throughout their teens and twenties (Clements *et al.*, 2006; Jennifer, *et al.*, 2011). This is also the age at highest risk for HIV transmission (UNAIDS, 2008). Women between the ages of 18 and 29 years are not a focus of school-based anti-schistosomal treatment campaigns in sub-Saharan Africa, but the substantial burden of FUS in this age group argues strongly for targeted FUS treatment. The study by Jennifer, *et al.*, (2011) demonstrated that, although *S. mansoni* may be geographically isolated near the Lake shores, *S. haematobium* may be more widespread in inland villages throughout the region, placing more women at risk for urogenital schistosomiasis and potentially, increased HIV transmission.

In a cross-sectional survey of 972 pregnant women conducted in northwest Tanzania, showed that heavy infection with *S. mansoni* was associated with

increased risk of anemia (adjusted odds ratio = 1.87, 95% confidence interval = 1.07–3.27, P = 0.026) (Ajanga *et al.*, 2006). These effects persisted after controlling for potential confounders. Increased intensity of infection was associated with both reduced hemoglobin levels and increased prevalence of anemia. Control for potential confounders and the robust study methodology provides evidence of a relationship between *S. mansoni* and anemia during pregnancy. This is important because anemia is a risk factor for maternal mortality and LBW (Abouzhar, 1998; Murray, 1998).

## **5.3 Materials and Methods**

### **5.3.1 Study area**

This was a cross-sectional study conducted in Kwale County (Figure 2.2), which is located in the South Eastern corner of Kenya, bordering Tanzania to the south and the Indian Ocean to the east. Kwale County has an area of 8293 km<sup>2</sup> with a population of 583,330 persons (Kenya national census, 2009). The annual growth rate is 2.625%. The mean household size is 6-8. Currently the district is split into three (3) districts namely: - Kwale, Msambweni and Kinango.

### **5.4 Parasitological examinations of urine samples**

Parasitological examination of the eggs in urine was done using nuclear pore filtration technique previously described by Kahama (1998). The urine samples were collected between 10.00 am and 2.00 pm. A duplicate 10 ml aliquot of urine was filtered through 13-mm polycarbonate filters (Nuclear pore R; Costar Europe

Ltd., Badhoevedorp, the Netherlands), Then placed on a labeled slide and examined under a microscope within 6hrs. The mean counts of the two filters were recorded and expressed as eggs per 10ml urine. The presence or absence of Soil Transmitted Helminths (STH) (Trichiuriasis, Ascariasis and hookworm infections) ova in stool was determined by Kato-Katz method (Katz *et al.*, 1972). A 41.7 mg stool smear was prepared using a sieve and a calibrated template to contain 41.7 mg. The smear was placed onto a glass slide and covered with glycerine-impregnated cellophane. This preparation was left to clear for a minimum of 45 minutes (Peters *et al.*, 1980). To identify hookworm eggs, the sample was examined within one hour of preparation. These results were important for the study to rule out presence of hookworm parasites which also causes anaemia

#### **5.4.1 Blood slides for malaria parasites**

A 10% Giemsa solution was prepared in buffered distilled or deionised water, pH 7.2. The solution was gently poured onto the slide using a pipette. The slide was then left to stain for 5-10 minutes. Gently the stain was flushed off from the slide by adding drops of clean water until no stain drips. The slide was then place on a drying rack, making sure the film does not touch the rack. On the thick film, the background will be clean and free from debris; leukocyte nuclei should be a deep, rich purple, and the malaria parasites should have deep red chromatin and pale purplish blue cytoplasm. At the periphery of the thick film, erythrocytes are not lysed and schuffner's stippling may be apparent in *P.vivax* and *P.ovale* infections. The slides were reported as positive or negative for malaria parasites in both the



thick and thin film. The results were important to rule out malaria infection which also causes anaemia.

## **5.5 Haematological analysis**

Fresh sequestriated blood was analysed for 24 different haematological parameters using Nihon-Kohden celltac automated haematology analyzer model MEK-6410, NIHON KOHDEN CORPORATION – 1-31-4 NISHIOCHIAI – Shinjuku – KU- Tokyo – 161-856 Japan. The parameters included, white blood cell count, red blood cell count, haematocrit, mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration, platelet crit, lymphocytes, monocytes, granulocytes, macrocytosis, eosinophils, red cell distribution width, packed cell volume, mean platelet volume, platelet distribution width, erythrocytosis, anaemia, microcytosis, hypochromia, eosinophilia, thrombocytosis, thrombopenia, leukocytosis, leucopenia, anisocytosis.

### **5.5.2 Procedure for coulter counter haematological analysis**

Women of reproductive age in each village were assembled in the local health facility between 9.00 Am and Noon. The fore arm was cleaned with methylated spirit and allowed to dry. Using a sterile needle and syringe, five milliliters of venous blood was collected aseptically. Two Milliliters of freshly collected whole blood was put into a sample container containing anticoagulant (EDTA). The sample was then gently shaken up and down for 30 minutes and put in a cooler box with ice packs (cool enough to keep temperature at ~ 20<sup>0</sup>C) ready for transportation to the laboratory. The remaining three Milliliters of blood was put in a plain bottle

and allowed to clot for approximately 1 hour. Enough serum transferred into clean appendoff tube. Analysis was done in a central laboratory in Kwale District Hospital.

In the laboratory, the automated analyzer was switched on 5 minutes before analysis; the machine was primed for 5 minutes. The haematological controls were then removed from the refrigerator and rolled on the hands to bring to warm temperature. The low, normal and high standard controls (supplied by the manufacturer) were then run through the nozzle by putting it into the solution and pressing the button on the machine. The count button switch was pressed to allow the machine to automatically aspirate the required volume. Within 15 seconds the machine then printed the results from the attached printer. The details of the sample (patient) were entered into the analyzer. Whole blood was aspirated automatically after inserting the nozzle, and then pressed the count button. After 15 seconds results of the blood analysis were printed from the computer attached to the machine.

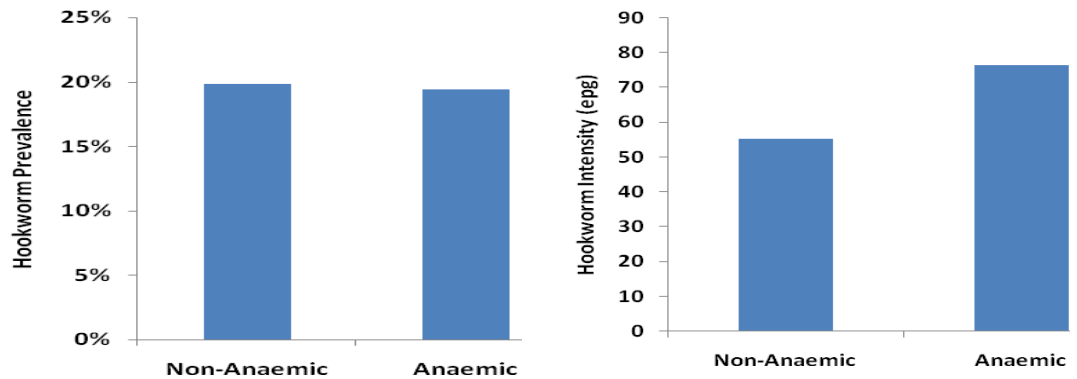
## **5.6 Statistical analysis**

For data entry, Excel spread sheet was used, where individual raw data was transferred from the original forms (hard copy). Pearson's correlation co-efficiency was used to determine the correlation between haemoglobin levels, haematological indices, egg counts (*S. haematobium*). The association between infection and the different haematological biomarkers were examined by multiple regression analysis. Normal linear regression was used where the outcome variable was found

to be normally distributed, as in the case of Hb level, or binomial regression was used where the outcome variable was binary, or could be expressed as a proportion, as with presence/absence of Anaemia. The model was fitted to the data and checked whether adding Infection status information significantly improves the fit of the model.

### **5.7 Results of hookworm and anaemia in women of reproductive age in Kwale County**

Hookworm is also a known risk factor for anaemia. In the present study out of 512 women investigated for hookworm infection, 102 (20%) were prevalent with the infection. However, there is no difference between hookworm prevalence in those with anaemia (19.4%) and those without anaemia (19.9%), although there is a slight (non-significant) increase in hookworm intensity in those with anaemia (76.4 epg) compared with those women without anaemia (55.4 epg). However, both of these are classified as 'light' infections according to WHO (1999) classification of intensity of soil transmitted helminths. These results are shown in figure 5.1.



**Figure 5.1 Prevalence and intensity of hookworm in women of reproductive age in Kwale County**

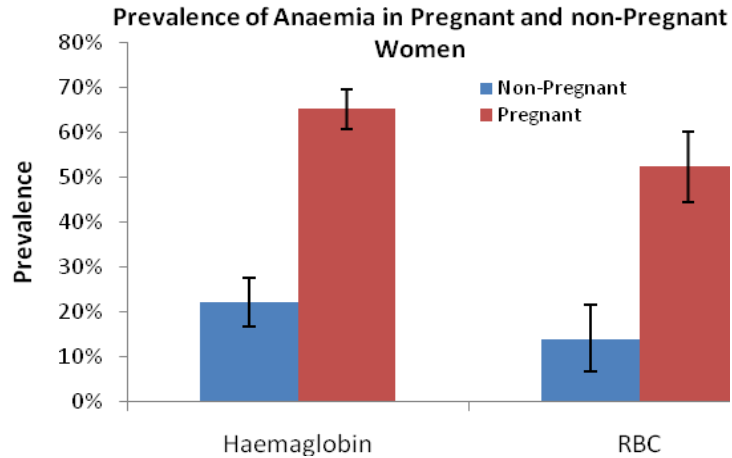
## **5.8 Results of the haematological analysis**

### **5.8.1 Anaemia in pregnant and non pregnant women**

This part of the study, the presence or absence of anaemia was measured in two ways, either through a Haemoglobin (Hb) count, where a reading below 11g/dl of blood was considered as anaemic or using Red Blood Cell (RBC) count, where a result below  $4.2 \times 10^6 \mu\text{l}$  was classified as anaemic. Red blood cells count gives more quantitatively the amount of cells circulating in the body thus, allows for more information to be generated on the different haematological conditions that may prevail. The results of both of these parameters are shown Figure 5.2.

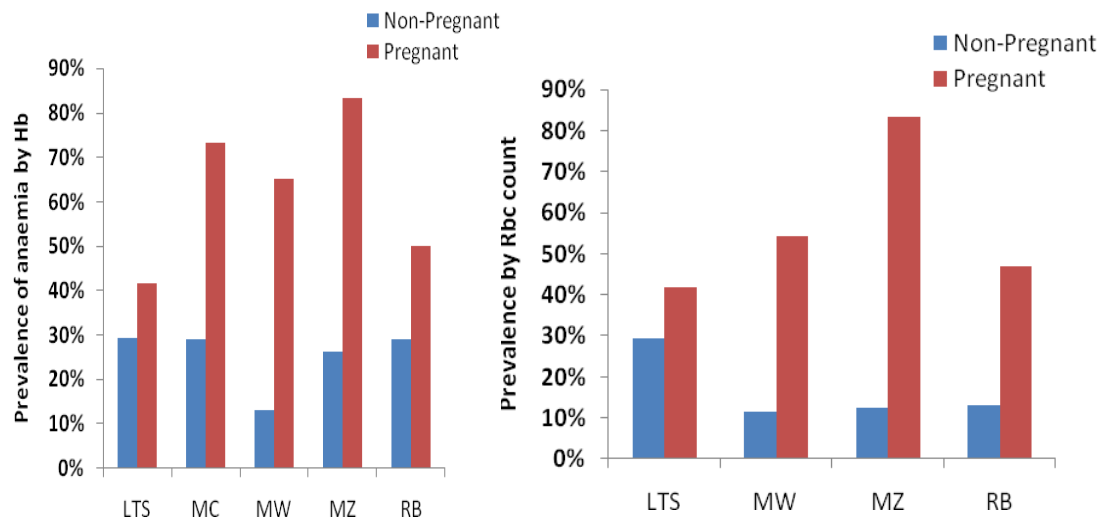
When measuring for Hb alone the presence of anaemia was 65.2% in pregnant women and 22.2% in non-pregnant women ( $Z=8.46$ ,  $P<0.001$ ). When measuring for

RBC count, the prevalence of anaemia was 52.3% in pregnant women and 14.1% in non-pregnant women ( $Z=6.39$ ,  $P<0.001$ ). Results are shown in Figure 5.2



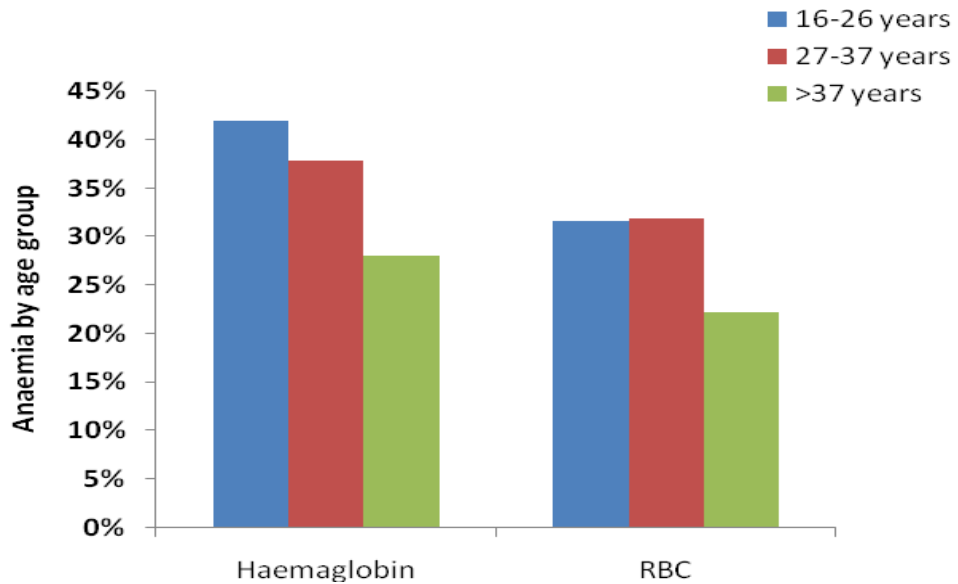
**Figure 5.2: Comparison of anaemia in pregnant and non pregnant women as determined by Hb concentration and RBC count**

The pattern of anaemia across the villages that took part in the study appears to be the same when measured by Hb method and by RBC method. However, there is a difference in the prevalence of anaemia in pregnant and non pregnant women across the villages. This is shown on figure 5.3. Note, blood from Mwachinga was not analysed for RBC due to the time it took to process the samples after refrigeration for more than 8 hours.



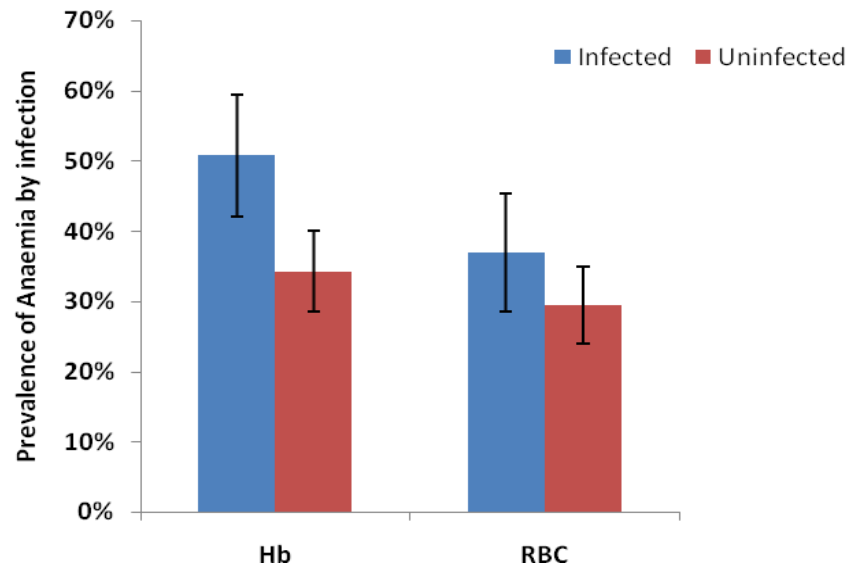
**Figure 5.3: Anaemia (Hb and RBC counts) in pregnant and non-pregnant women by village**

With regard to the relationship between anaemia and age, the graph below (fig. 5.4) shows a general downward trend. That is, with an increase in host age, there is a general reduction in the prevalence of anaemia. This is more pronounced when measuring with Hb, although is still evident with RBC.



**Figure 5.4: Anaemia (Hb and RBC count) by age group of the women**

The graph below (Fig. 5.5) shows the relationship between infection status (with schistosomiasis) and the prevalence of anaemia. It can be seen that the prevalence of anaemia is higher in infected women when compared to uninfected. This holds when using either the Hb approach (Pregnant: 50.8%, non-pregnant: 34.2%,  $Z=3.13$ ,  $P= 0.001$ ) or when using the RBC approach (Pregnant: 37.0%, non-pregnant: 29.4%,  $Z=1.49$ ,  $P=0.135$ ), although the difference is only significant when using the Hb approach.

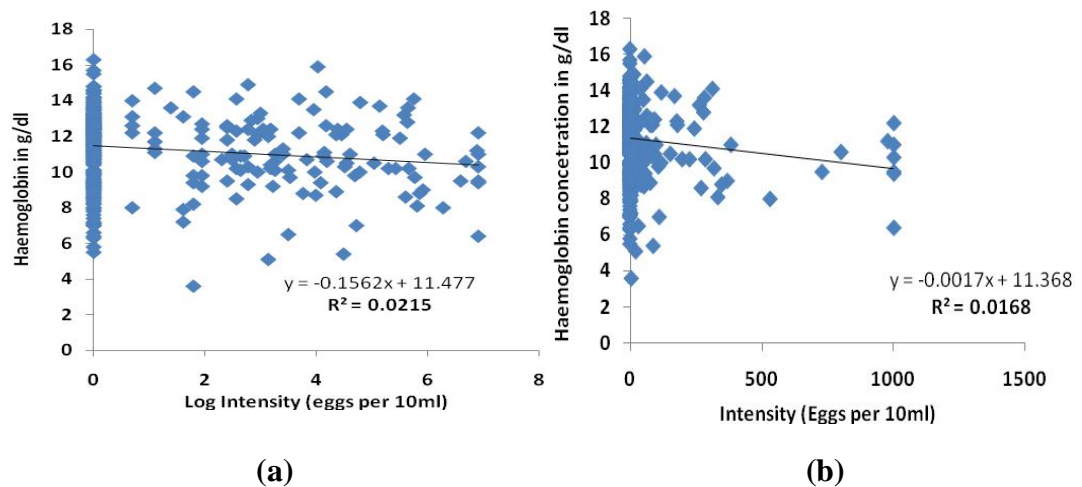


**Figure 5.5: Relationship between *S. haematobium* infected women and anaemia status (Hb and RBC count)**

### **5.8.2 Correlation between *S. haematobium* infected women and anaemia**

The figure below (5.6a &b) showing the correlation between prevalence and intensity *S. haematobium* infected women and haemoglobin concentration. The picture is a little less clear when looking at the correlation of intensity of infection with *S. haematobium* and Hb levels in both pregnant and non pregnant women in Kwale County.

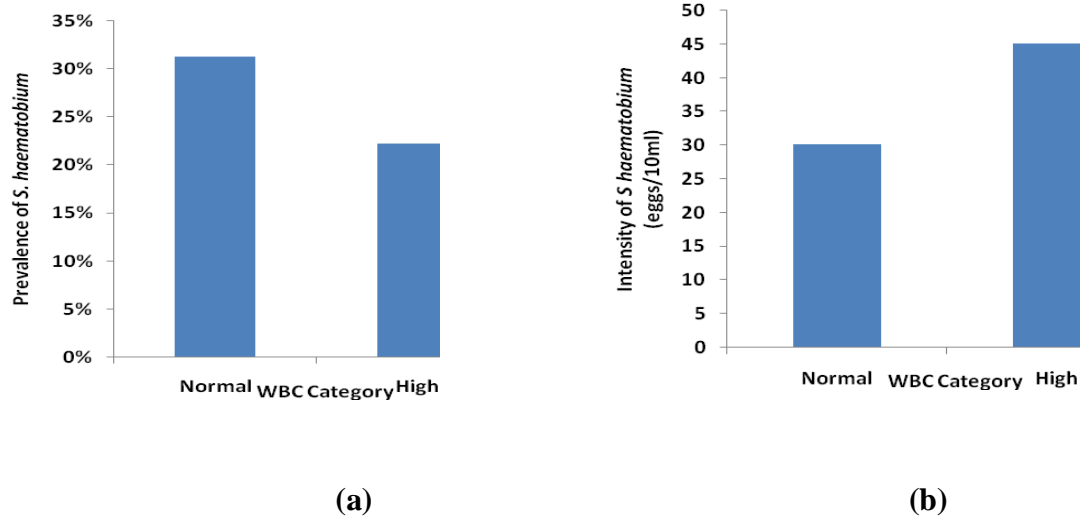




**Figures 5.6 (a) and (b): Correlation of egg per 10mls and log intensity with haemoglobin concentration in blood.**

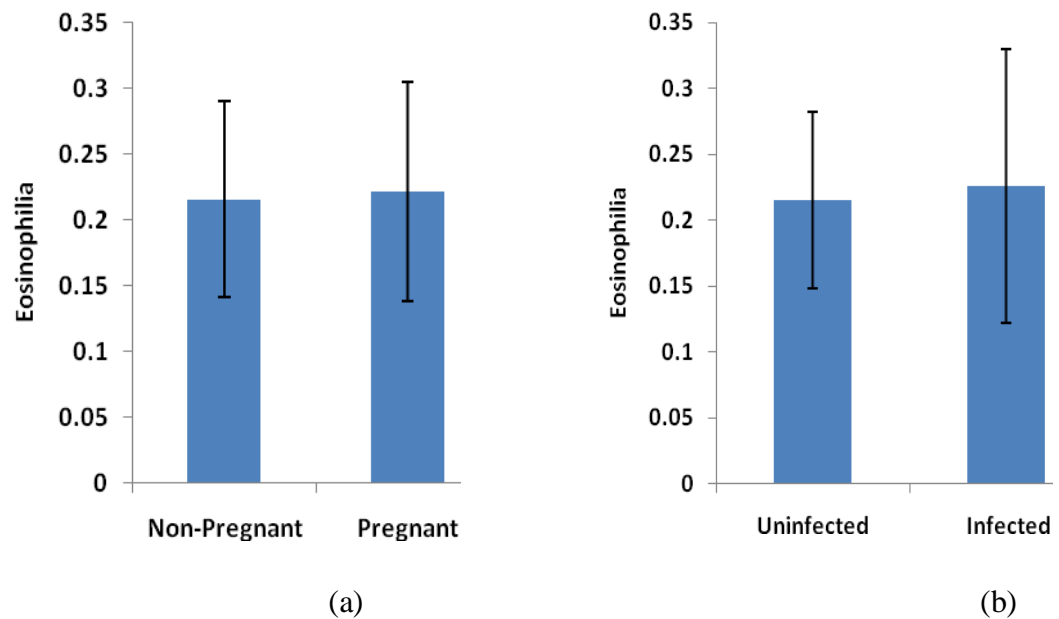
### 5.8.3 Comparing pregnant and non-pregnant women blood granulocyte counts

As part of the blood tests carried out on this cohort of women, White Blood Cell (granulocytes) counts were measured using automated haematology analyzer. Below they are compared by infection status. Figure 5.7 a and b showing the relationship of *S. haematobium* infection with granulocyte level, granulocytes counts of between 42-85% was classified as 'Normal' whilst less than 42% was classified as 'Low'. It was seen that, there was high prevalence and intensity of infection in those women who had 'Normal' granulocyte levels (Prevalence: 31.4% in Normal, 25.9% in Low,  $Z = 0.60$ ,  $P = 0.55$ ; Intensity: 32.6 eggs/10ml in 'Normal', 17.6 eggs/10ml in 'Low',  $Z = 1.10$ ,  $P = 0.27$ ).



**Figure 5.7 (a) and (b): Showing the levels of total granulocytes counts in blood of women infected with *S. haematobium*.**

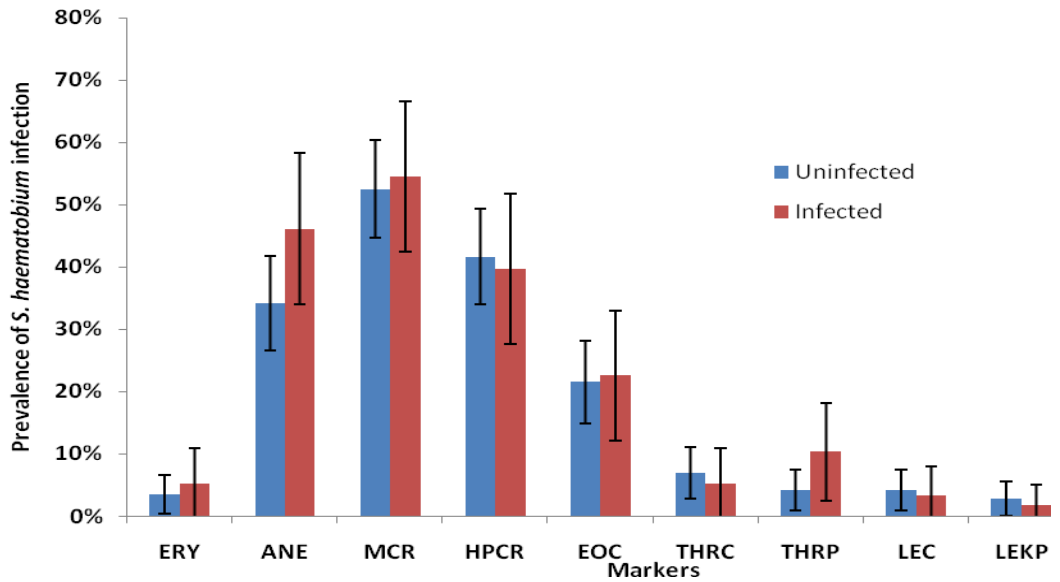
Presence of Eosinophilia was classified as Eosinophil count of more than 500 Eosinophils per microliter of blood. Levels of Eosinophilia were slightly higher in infected pregnant women than infected non-pregnant women (22.1% *c.f.* 21.6%). Similarly the presence of Eosinophilia was slightly higher in those women infected with *S. haematobium* (22.6% *c.f.* 21.5%) as shown in figure 5.8a&b



**Figure 5.8: Eosinophilia in pregnant and Non-pregnant women infected with *Schistosoma haematobium***

#### **5.8.4 Haematological conditions assessed in infected pregnant women and non-infected pregnant women**

A range of haematological conditions were also investigated in the blood of the cohort of pregnant and non-pregnant women in the five study villages (Figure 5.9). The following blood conditions were tested (legend on page 108). With regard to haematological markers and infection with *S. haematobium*, there were no clear-cut significant differences in the various parameters tested. However, the tests for anaemia (46.2% *c.f.* 34.2%,  $Z=1.65$ ,  $P=0.10$ ) and for Thrombopenia (10.3% *c.f.* 0.4%,  $Z=1.68$ ,  $P=0.09$ ) showed values that were higher for infected individuals and were marginally significant ( $P<0.1$ ).



**Figure 5.9 The relationship between haematological markers and infection with *S. haematobium* in women of reproductive age in Kwale County**

**Legend:**

**ERY** = Erythrocytosis, **ANE** = Anaemia, **MCR** = Microcytosis,  
**HPCR** = Hypochromia, **EOC** = Eosinophilia, **THRC** = Thrombocytosis  
**THRP** = Thrombocytopenia, **LEC** = Leukocytosis, **LEKP** = Leukopenia

**5.8.5 Discussion**

Hookworm is a major contributor to anaemia and even light hookworm loads are associated with low haemoglobin levels (Bates, *et al.*, 2007). Women of reproductive age in the study group in Kwale County had significant levels of hookworm in both pregnant and non-pregnant women, although interestingly both the prevalence and intensity were higher in pregnant women than non-pregnant women and both of these reached statistical significance, (P=0.041). In both infected and non-infected women, anaemia was reported at almost 20%.

In the present study, women of reproductive age in a rural community in Kwale County were examined for anaemia (both Hb and RBC count) and pathophysiological changes in relation to infection with *S. haematobium*. Fifty percent (50%) of the women had *S. haematobium* infection among them 50% were suffering from anaemia (Hb less than 11g/dl and RBC count of less than  $4.2 \times 10^6$   $\mu$ l) and other haematological conditions. The figures given conform to World Health Organization standard to allow for comparison with other studies (WHO, 2008). Yet, as Sarin (1995) had argued, the standard threshold for anaemia (Hb 11g/dl) may be considered too high in the context of many developing countries. In view of this, here RBC count is added to compare the anaemia in terms of the amount of blood in circulation for both infected and uninfected women in schistosomiasis endemic area in lower Kenyan Coast.

Anaemia is one of the most evident morbidities associated with schistosome infections, for both *S. mansoni* and *S. haematobium* and is the result of blood loss caused by schistosome eggs penetrating the walls of the blood vessels. Anaemia is an important issue in pregnant women particularly in developing countries. Anaemia is a multi-factorial morbidity, in so much as there are many causes, such as malaria, hookworm, schistosomiasis infection, nutrition. In this cross sectional study, not all anaemia would have been caused by schistosome infection but, whatever the cause; it is an important result and one that requires further research in terms of specific haematological conditions and other co-founding factors. In order to rule out the possibility of hookworm or malaria being the cause of anaemia in the

study women, all women were examined for the parasites either in urine for STH or blood for malaria. All samples that were positive for hookworm or any STH were not included in the final analysis of the results. In Kenya pregnant and lactating mothers and children under 5 years, are provided with long lasting insecticide treated bed nets free of charge. All the women examined, were negative for malaria parasites during the study period.

In the present study, pregnant women had substantially and significantly higher levels of anaemia than non-pregnant women and this was the case whether anaemia was measured by Hb or by RBC count. This pattern is replicated across all of the village locations that took part in the study. There is a smaller discrepancy between anaemia rates in Lutsangani village, but still a higher prevalence in pregnant women compared to non-pregnant women.

Other studies have found the prevalence of anaemia in pregnancy varies considerably because of differences in socioeconomic conditions, lifestyles and health-seeking behavior across different cultures. Pregnant women are at special risk of anaemia due to increased iron requirement during pregnancy, short birth intervals (blood loss) and prolonged lactation (iron loss), especially when combined with parasitic and helminthic infection may lead to iron and/or folate deficiency (Kagu *et al.*, 2007). Although this study did not investigate the factors associated with the high prevalence of anaemia in pregnant women in Kwale County, it is most

likely the same co-founding factors contribute to the low haemoglobin levels in the study group.

There is a general increase of anaemia with increase in infection and increase of age, however, there is decline in anaemia status in women above 37 years of age. This is more pronounced when measuring by Hb, although it is still evident with RBC counts. The reasons for this could be that older women are less likely to be pregnant and have lower intensity of schistosome parasites due to concomitant immunity. Incidentally, the same age groups (16 – 37 years) have higher infection rates with *S. haematobium* as observed in another cohort study in Kwale County. However, a study by Knopp, (2010) showed that, people infected with one or several helminth species concurrently were not at a higher risk of anemia compared with non-infected individuals, unlike in the present study. We overruled malaria as the cause of anaemia since in all the blood slide examined, non was positive for malaria parasites at the time of conducting the study. Findings of Jennifer (2011) showed that, in communities in which the prevalence of Schistosomiasis is at least moderate in children (50–90%) (Clements *et al.*, 2006) women continue to have urogenital schistosomiasis throughout their teens and twenties. This is also the age at highest risk for HIV transmission (UNAIDS, 2008). Women between the ages of 18 and 29 years are not a focus of school-based anti-schistosomal treatment campaigns in sub-Saharan Africa, but the substantial burden of FUS in this age group argues strongly for targeted FUS treatment.

Studies have shown that a strong relationship between anaemia and schistosomiasis exists even after controlling for other co-infections and dietary factors among pregnant women and children, (King, *et al.*, 2005). The underlying mechanisms proposed range from social determinants to complex immune interactions (Ajanga *et al.*, 2006; Ayoya *et al.*, 2006; King and Dangerfield, 2008). This indicates that there are many underlying factors that may compound the anaemia (low Hb) in pregnant women in Schistosomiasis endemic areas. The  $R^2$  value for correlation of egg intensity and haemoglobin (Fig.5.6a and 5.6b) concentration indicates that only approximately 1.5% of the reduction in Hb is explained by the increase in intensity. Because counts of helminth infection are non-normally distributed (they are often over-dispersed or skewed in any given population) an attempt was made to normalize the data by log-transforming it (6b). This also showed a down ward trend but again the correlation was weak, suggestive of many co-founding factors which were not investigated in this study.

In some other studies, research has shown that, where transmission in communities is high, NTDs are a major cause of mild and moderate anaemia (Hb 7–11.9 g/dl). For example, 66% of pregnant Tanzanian women (Ajanga *et al.*, 2006) and 50%–62% of Tanzanian children (Stoltzfus *et al.*, 1997; Beasley *et al.*, 1999) living in high-risk areas for helminth and/or schistosomiasis infections have anaemia. *S. mansoni*, *S. japonicum*, and *S. haematobium* cause intestinal or urinary blood loss. Studies from Kenya, Niger, Tanzania, and elsewhere have shown that, despite the presence of confounding factors, haemoglobin levels of children and pregnant



women correlate negatively with egg count in both *S. mansoni* and *S. haematobium* (Gilgen *et al.*, 2001, Beasley *et al.*, 1999; Ajanga *et al.*, 2006).

Women infected with *S. haematobium* had 'Normal' granulocyte levels (31.4% in Normal, 25.9% in Low). Intensity: 32.6eggs/10ml in 'Normal', 17.6eggs/10ml in 'Low'), suggesting that the immune response to the *Schistosoma* infection may have raised the granulocyte levels in the normal group. This scenario may be as a result of decrease in Eosinophils which in conjunction with other immune systems such as Th 2 cytokine milieu induced by helminthes may be thought to drive the antibody response to *S. haematobium* infection in the women. This has been suggested in other studies on malaria infection where the cytokine milieu could also favour either pro- or anti- inflammatory reactions during malaria infection (Diallo *et al.*, 2004).

In areas where parasitic diseases are common, as is the situation in large parts of sub-Saharan Africa, these are often the cause of eosinophilia in endemic areas thus, schistosomiasis is no exception. Peripheral blood eosinophil counts were conducted in this study as a more sensitive marker of parasitic infection than microscopy blood film examination. Eosinophil activation generally occurs in inflamed tissues (Weller, 1994) but circulating eosinophils from helminth- infected individuals exhibit an activated phenotype, suggesting that parasite infection may up regulate eosinophil function (Thorne and Mazza, 1991; Mawhorter *et al.*, 1996).

Several studies have demonstrated an association between eosinophilia and host protection against schistosomes (Sturrock *et al.*, 1983; Butterworth *et al.*, 1985; Hagan *et al.*, 1987). Eosinophils make up a high proportion of the leukocyte infiltrate of the egg granuloma in *Schistosoma* sp. infections. (Moore, 1997). In *S. haematobium* infections in humans, this is reflected by the cellular composition in the urine where eosinophils constitute a significant proportion of the leukocytes present (Bhatt, 1984; Eltoun, 1989; Reinemert *et al.*, 1993). This study has shown there is slight elevated eosinophils in circulating blood of women who are infected with *S. haematobium* and equally high in pregnant women than non-pregnant women.

Other studies, Mohamed, 2006 showed significant rise in the level of total leukocyte counts that could be attributed to the rise in eosinophils, but likewise a significant rise was encountered in monocytes, lymphocytes and neutrophils. This indicated a general immunological response. A number of systems employing antibodies and/or eosinophils, neutrophils, monocytes were produced to kill schistosomes. A significant relationship was observed between the degree of eosinophilia and the intensity of infection (Davis, 1981). Many studies on Eosinophils have been conducted using urine Eosinophiluria as a marker for morbidity in school children (Reinemert *et al.*, 1993). This study did not compare the blood eosinophils with eosinophiluria because it would require more time and a different design; however, it will be interesting to measure the same in other studies, especially in pregnant women infected with schistosomiasis.

The significance of haematological conditions associated with *S. haematobium* infection was investigated. The various haematological markers studied in infected and uninfected women of reproductive age showed increased levels in women infected with *S. haematobium*, except macrocytosis, granulocytosis and anisocytosis which did not reveal any significant change, thus they were not included in figure 5.9. This has also been documented by Mohammed (2006) who showed that *S. haematobium* infection can influence the normal levels of some haematological and biochemical constituents of blood. Until recently, there has been a lack of valid biomarkers to distinguish the causes of anemia. Hemoglobin and ferritin have proven to be poor diagnostic tools (Chaparro, 2008), largely due to the fact that ferritin is decreased in the context of iron deficiency, but increased in the context of anemia of inflammation as iron is sequestered in storage forms.

Relatively few studies have investigated the interactions between schistosomes and elements of host haemostatic defense mechanisms, of which platelets are a significant component (Nurden, 1980). Reduction in circulating platelet numbers occurs about 2 days after percutaneous infection of mice with *S. mansoni* cercariae. This is approximately the time at which some schistosomulae would be expected to be entering the blood system of the host (Wilson and Lawson 1980). In another study, worm burdens were significantly increased in mice that were thrombocytopenic at the time of infection, when compared with normal untreated mice (Stanley, 2003). In this present study, decreased levels of thrombocytes (platelets) (thrombopenia) among women infected with *S. haematobium*, than non-pregnant women are reported. It is then our hypothesis that as has been shown by

other researchers, these decreased platelets may be associated with *S. haematobium* infection, despite lack of a larger comparative data in the study. Further research may reveal the pertinent innate immune activity of platelet in the defense mechanism of schistosomiasis infection.

Schistosomiasis has been a neglected tropical disease and, as a result, little attention has been paid to women with FGS. Given the safety, efficacy, and low cost of PZQ, programs are continuing large-scale periodic treatment of at-risk populations without diagnosis. Special attention must be given to pregnant and lactating women to decrease the disease burden and improve pregnancy and fetal outcomes. Observational results by Ouma, (2005) from matched cohorts in Kwale suggest that the benefits of childhood or adolescent treatment for schistosomiasis can persist into adulthood, particularly if the therapy is given into adolescence.

Prevention of this disease must occur during adolescence because transmission is common due to children swimming in contaminated waters. If programs can target this population, the likelihood of improving women's reproductive life and well-being will be directly affected (Nawal, 2010).

## **5.9 Conclusion**

From the results of the present study, the following conclusions were made;

Female genital schistosomiasis causes anemia among pregnant women.

Anemia was more severe among older women suffering from FGS compared to the younger women.

Other blood patho-physiological conditions occur during infection with schistosomiasis and they could be associated with disease progression and worsening of the already serious multiple conditions in pregnant women.

### **5.10 Recommendations**

Women of reproductive health in Kwale County do not only suffer from anemia but also some other blood pathological disorders. They should therefore be investigated especially during ante- natal visits to identify and manage the disorders effectively.

Women of reproductive age in Kwale County spend a very large part of their reproductive lives involved in activities that expose them to schistosomiasis infection and their treatment during MDA will not only improve their health but alleviate the already poor nutritional status. It is thus, recommended that schistosomiasis infected women of reproductive age in endemic areas should be treated during gestation period (after 1<sup>st</sup> Trimester) to avoid development of serious deleterious conditions later in their parity life.

## CHAPTER SIX

### **6.0 CLASSIFICATION OF ANAEMIA IN WOMEN OF REPRODUCTIVE AGE INFECTED WITH *S. HAEMATOBIIUM* AND THEIR LOW BIRTH WEIGHT OUTCOMES IN TANA RIVER COUNTY, COAST PROVINCE**

#### **6.1 Introduction**

Women in developing countries account for 95% of anaemic pregnancies in the world. The pallor of anaemia was associated with weakness and tiredness long before its cause was known. Now it is recognized that even without anaemia, mild to moderate iron deficiency has adverse functional consequence (Scrimshaw, 1990). The contribution anaemia makes to maternal morbidity and mortality and its association with low birth weight in sub-Saharan Africa is well documented (Shulman, 1999; Guyatt & Snow, 2001). The prevalence of anaemia in pregnancy varies considerably because of differences in socio-economic conditions, lifestyles and health-seeking behaviors across different cultures.

Pregnant women are at special risk of anaemia due to increased iron requirement during pregnancy, short birth intervals (blood loss) and prolonged lactation (iron loss), especially when combined with parasitic and helminthic infection may lead to iron and/ or folate deficiency (Kagu *et al.*, 2007). Other risk factors include socio-demographic (age, level of formal education and areas of residence), obstetric factors (gravidity, parity, plurality of pregnancy-multiple or singleton, etc.) and behavioural factors (utilization of prenatal care, smoking and alcohol usage), as well as medical associated conditions (Kagu *et al.*, 2007).

High prevalence of anaemia observed among illiterate mothers is an indication that ignorance may have contributed to the anaemia, as such women do not have the economic power and knowledge on the recommended nourishing food in pregnancy (Kagu *et al.*, 2007). Iron deficiency anaemia during pregnancy increases prenatal risks for mothers and neonates and increases overall infant mortality. Moreover, iron-deficient animals and humans have impaired gastrointestinal functions and altered patterns of hormone production and metabolism. The latter include those for neurotransmitters and thyroidal hormones which are associated with neurological, muscular, and temperature-regulatory alterations that limit the capacity of individuals exposed to the cold to maintain their body temperature. In addition, DNA replication and repair involve iron-dependent enzymes (WHO, 2001).

One hypothesis to explain why nutritional supplementation appears to be necessary but not sufficient to eliminate growth shortfalls is that chronic infection and colonization of the gut by fecal bacteria impedes nutrient absorption and creates low-level immune system stimulation, a condition called tropical enteropathy (Lunn, 2000). The same observation detected in children with environmental enteropathy (an inflammatory disorder of the intestine), could probably be more pronounced in pregnant women continually infected with parasitic infections that are known to have adverse effects even to the unborn child.

Studies have demonstrated that pregnant women infected with schistosomiasis develop severe anemia, have low birth weight infants, and an increased infant and maternal mortality rate, (Friedman *et al.*, 2007; helling-giese *et al.*, 1996; Siegrist and Siegrist Obimpeh, 1992). Schistosomiasis has been detected in the placenta and newborns have been diagnosed with the disease, thus confirming congenital infection. Data suggest that infected women have a higher rate of spontaneous abortions and a higher risk for ectopic pregnancies (Bahrami *et al.*, 2006; Laxman *et al.*, 2008). The causal relationship between anaemia and schistosomiasis exists even after controlling for other co-infections and dietary factors among pregnant women and children. The underlying mechanisms proposed range from social determinants, nutrition to complex immune interactions (Ayoya *et al.*, 2006).

Because the Neglected Tropical Diseases (NTDs) are the most common infectious among the world's poorest people (Hotez *et al.*, 2007), including girls and women, there is now a strong case to be made for controlling the NTDs as a means of directly addressing MDG 3 and MDG 5. Schistosomiasis is one of the most important NTDs especially in Sub-Saharan Africa. Studies conducted over the last two decades provide an evidence base that the NTDs are important factors that (i) impair reproductive health in developing countries; (ii) increase the transmission of sexually transmitted infections (STIs); and (iii) promote stigma and gender inequality. For these reasons, interventions focused on NTD control and elimination could offer an opportunity for improving the health and rights of girls and women in the poorest countries of Africa, Asia, and Latin America and the Caribbean (Hotez, 2009).



The main objective for assessing anaemia is to inform decision-makers on the type of measures to be taken to prevent and control anaemia. Morphology of peripheral erythrocytes as a proxy to identify iron deficiency (presence of hypochromic and microcytic cells) and vitamin B12 and folate deficiency (presence of macrocytic cells) will contribute to understanding the type of anemia affecting women of reproductive age in schistosomiasis endemic areas. This implies that in addition to the measurement of Hb concentration, the causes of anaemia need to be identified considering that they may vary according to the population. The objective of this study was to determine the different types of anaemia and birth weight outcomes of pregnant and non pregnant women infected with *S. haematobium* parts of Tana River County. Identification and classification of the specific anaemia other than iron deficiency in a given locality may increase reliability of intervention and management of the condition especially in the high risk groups such as pregnant women in schistosomiasis endemic areas.

## **6.2 Literature review**

Adaptation to pregnancy involves major changes in maternal metabolism in order to satisfy growing demands of the pregnancy outcome. The continuous physiologic adjustments affect the metabolism of all nutrients (Victoria *et al.*, 2001). When the body is infected with pathogens, the normal outcome of pregnancy is greatly compromised, leading to various conditions including haematological, immunological and physiological. An important external risk factor for pregnant women and newborn health is unbalanced nutrition of pregnant women (Darwal *et*

*al.*, 1993; Hasin *et al.*, 1996; Mathews *et al.*, 1999; Pondo *et al.*, 1997), besides many other factors such as mother age, her height and weight, smoke, alcoholic beverages, social factors, hard work, stress, etc. Otherwise, the woman who is in good health and who has maintained good nutrition prior to conception as well as during pregnancy has the best chance for a pregnancy without complications, a healthy baby and the ability to nurse (Robinson and weigley, 1991).

Anaemia is an indicator of both poor nutrition and poor health. The most dramatic health effects of anaemia, such as increased risk of maternal and child mortality due to severe anaemia, have been well documented (Bothwell, 1981; Scholl, 1994). The amount of iron in the human body is approximately 2,500 mg. More than half of this amount, approximately 1,700mg is present in the hemoglobin of red blood cells (INACG, 1989). A certain amount of iron is lost daily due to basal iron losses with epithelial cells from internal and external surfaces. About one-third is lost from the gastrointestinal tract. A tiny fraction of iron is lost in urine or sweat. Significant iron loss occurs in women during menstruation. Median monthly loss of blood during menstruation has been estimated at 35 ml (the upper limit of normal is about 80 ml), which is equivalent to more than 12 mg of iron (Lee, 1999).

Studies from developing countries suggest that 120mg elemental iron and 1mg folic acid are the optimum daily dosages needed to prevent anaemia in pregnant women. But WHO recommends, for all pregnant women, if there are no high risk factors a combination tablet containing 60mg of elemental iron and 250µg of folate to be taken twice a day. Where this is not available, tablets such as ferrous sulphate,

containing 60mg of elemental iron, should be given twice a day together with one folic acid tablet (1 mg) (Van den Broek *et al.*, 2000).

Normal Hb distributions vary with age, sex, and physiological status, especially during pregnancy (Koller, 1982). WHO haemoglobin (Hb) thresholds were used to classify individuals living at sea level as anaemic (WHO, 2001). Hb concentration is the most reliable indicator of anaemia at the population level, as opposed to clinical measures which are subjective and therefore have more room for error. Measuring Hb concentration is relatively easy and inexpensive, and this measurement is frequently used as a proxy indicator of iron deficiency. However, anaemia can be caused by factors other than iron deficiency. In addition, in populations where the prevalence of inherited haemoglobinopathies such as sickle cell anaemia or thalassemia is high, the mean level of Hb concentration may be lowered. This underlines that the etiology of anaemia should be interpreted with caution if the only indicator used is Hb concentration.

Normocytic normochromic anaemia is a morphologic diagnosis made on a peripheral blood film and complete blood count values. This can be due to a fall in the number of circulating red cells and is often due to blood loss (haemorrhage) or red cell breakdown (haemolysis). The first steps in managing a patient with suspected anemia are to confirm the diagnosis and to determine the morphologic type. The normocytic normochromic type has many causes, two of the most common being chronic disease and drug therapy. The anemia of chronic disease is often mistaken for iron-deficiency anemia and treated with iron supplements.

Symptoms of normochromic normocytic anemia develop slowly and often go undetected. These may include constipation, loss of appetite, headaches, irritability, noises in the ears, difficulty in concentration and memory. Moreover, people feel fatigue, weakness, depression, dizziness, paleness, brittle nails, pale lips soreness of the mouth, and a cessation in menstruation.

Macrocytic anemia is blood with an insufficient concentration of hemoglobin in which the erythrocytes are larger than their normal volume. Especially common causes of macrocytic anemias are megaloblastic anemias, in which cells are larger because they cannot produce DNA quickly enough to divide at the right time as they grow, and thus grow too large before division. Causes for the DNA synthetic problem range from lack of certain vitamins needed to produce DNA (notably folate and vitamin B12). Macrocytosis is a relatively common finding in the era of automated blood cell counters, with prevalence estimates ranging from 1.7% to 3.6% (Davidson *et al.*, 1978; Breedveld *et al.*, 1981).

One in four people is affected by anaemia; the poorest women, particularly when pregnant and children < 5 years are the most anaemic and have least access to services and interventions to mitigate anaemia. The WHO regions of Africa and South-East Asia have the highest risk, where about two thirds of preschool-age children and half of all women are affected (WHO, 2008).

In order to effectively combat it, however, the contributing factors must be identified and addressed appropriately and in time. In settings where iron deficiency is the most frequent cause, additional iron intake is usually provided

through iron supplements to vulnerable groups; in particular pregnant women and young children. Unlike Vitamin A which can be delivered as a massive dose every six months, iron supplements need to be consumed daily by the most vulnerable groups, making it more difficult to provide a constant supply. Food based approaches to increase iron intake through food fortification and dietary diversification are important, sustainable strategies for preventing IDA in the general population. In settings where iron deficiency is not the only cause of anaemia, approaches that combine iron interventions with other measures are needed (WHO, 2008).

Rodent models provide strong evidence that schistosomiasis infection leads to deleterious pregnancy outcomes. In some studies 60 female mice were 'moderately' infected with 40 *S. mansoni* cercariae (the infective larval stage), and seven weeks later were mated with uninfected males. A control group of uninfected female mice was bred in parallel. There were 146 pregnancies and 50 surviving infants (34.2%) in the infected group, in contrast to 121 pregnancies and 93 surviving infants (76.8%) among controls. The pregnancy outcomes were 13% versus 2% abortion, 10.9% versus 0% maternal death and 41.7% versus 21% infant death in the experimental group versus the controls, respectively. Moreover, the weight of the offspring from infected pregnancies at two and four weeks of age was significantly less than that of controls (mean weight of babies was 1.37 g and 1.01 g at two and four weeks, respectively ( $P < 0.01$ ), (Nurden, 1993).

These mice were followed for three or four subsequent pregnancies, with their infections maintained, and similar trends were observed. This indicates that a pattern of adverse outcomes is maintained even during chronic infection, and represents a more appropriate model for human populations in which schistosomiasis are endemic and chronic. The study also showed that the longevity of moderately infected pregnant mice was significantly lower than that of both infected mice that did not become pregnant and non-infected mice that had repeated pregnancies. This indicates that there is likely to be an interaction between schistosomiasis infection and pregnancy such that the combination is potentially more pathogenic than either condition alone.

Eosinophilia refers to a health condition resulting due to presence of excessive eosinophils (type of white blood cell) in blood or body tissues. Eosinophils are produced in the bone marrow existing normally in the bloodstream and gut lining, which helps the body fight infection from parasitic organisms. Most common cause for eosinophilia are parasitic infections (such as hookworm, schistosomiasis), allergic conditions and certain types of drug reactions. Eosinophils participate in the immune response against helminthic parasites by discharging their cytotoxic granular contents onto the parasites, which kills them. (Capron, 1991; Davidson, 1985).

Lymphocytosis is a condition characterized by elevated amounts of lymphocytes, which are a type of white blood cell, in the body. There are three main types of

lymphocytes: natural killer cells, T cells, and B cells. Each is important when it comes to defending the body from illness and disease (Davidson, 1985).

### **6. 3 Study design and methodology**

#### **6.3.1 Study area**

Tana River with an area of 38,446 km<sup>2</sup>, is one of the least populated districts in Kenya with a total population of about 250,000 (1999 national census) mostly concentrated along the river and the small urban centers. Over 90% of the total area in Tana River County is arid and semi-arid land. The main economic activities of the district are small-scale subsistence agriculture along Tana River, nomadic livestock activities in the rangeland and fishing along the river and coastal strip. There are two major irrigation schemes such as Bura and Hola. Despite the apparent adequate natural resources, the region remains marginalized from the rest of the country. Seventy two percent (72%) of the total population lives below the poverty line according to the 1997 poverty survey. The area is endemic for *S. haematobium*, where prevalence in school children ranges between 3 – 75% with an average of 44% across the District (Brooker *et al.*, 2009; Jimmy *et al.*, 2011).

#### **6.3.2 Study population**

The study was conducted on pregnant and non-pregnant women in 5 villages in Tana River County

### **6.3.3 Design**

This was a quasi-longitudinal study focused on women of reproductive age (between 16 and 48 years) and more so those infected with *S. haematobium* and their newborns. Blood samples used in this analysis were obtained from the women recruited in a central place (local health facility) after prior sensitization and consenting in writing and through their spouses. As a follow-up to blood pathological changes, birth weight outcomes of the newborns were measured only once immediately after delivery.

### **6.4 Neonate weight measurements**

Immediately after delivery (less than 12 hours), the weights of the neonates was measured by a trained community health worker, using a Baby weighing Scale - superior (salter type), either at the health facility or in the house. If the delivery was at home, the trained community health worker visited the home a few hours later and weighed the neonate by placing him/her on the hanging trousers, then read the weight on the scale in grams, recorded weight, the date, time and names of the mother. The mother was then advised to take the child for immunization as soon as possible according to the health guidelines. When the delivery was in a health facility, the same procedure was followed, the mother and the child received immunization immediately.

### **6.5 Preparation and Staining of Blood Films for haematology examination**

The films were made from a drop of blood spread evenly on a slide and stained. A small drop of well-mixed blood was placed in the centre line of a slide about 1 cm



from one end, then placed a spreader in front of the drop at an angle of about 30° to the slide; moved it backwards to make contact with the drop. The drop of blood was spread along the slide. After the slide was made, it was left to dry in the air. The identifying number of the sample was written at the edge with a pencil before staining.

## **6.6. Leishman's stain for blood smears**

### **6.6.1 Staining procedure**

Blood film slide was gently lowered into a coplin jar of acetic alcohol (3% acetic acid in 95% methanol), fixed for 1 minute, and then washed off the fixative with distilled water and drain. The slides were put on a rack and covered with 1ml of Leishman stain - 20 seconds. Then added 2ml of pH6.8 buffer and tipped the rack up and down to mix the solutions or blow gently, the slides were left to stain for 7 minutes. The slides were rinsed quickly in distilled water then treated with pH 6.8 buffer for 2 minutes, rinsed again quickly in distilled water, shaken off the excess and dried on a warm (50°C) hotplate, or carefully blot dry with fibre-free blotting paper. The slides were then examined and blood cells morphology reported appropriately.

## **6.7. Methods of statistical analysis**

Regression analyses were carried out to explore the impact of schistosomiasis infection on subsequent birth weight in Tana River County, Kenya. Information on both infection and birth weight were available on a cohort of 32 women and their

babies (18 infected, 14 uninfected). Regression analysis was used to control for the impacts of all collected data that was considered to be a biologically plausible contributor to the child's birth weight. In this analysis, these variables included: schistosomiasis infection, mother's age, mother's height, mother's weight.

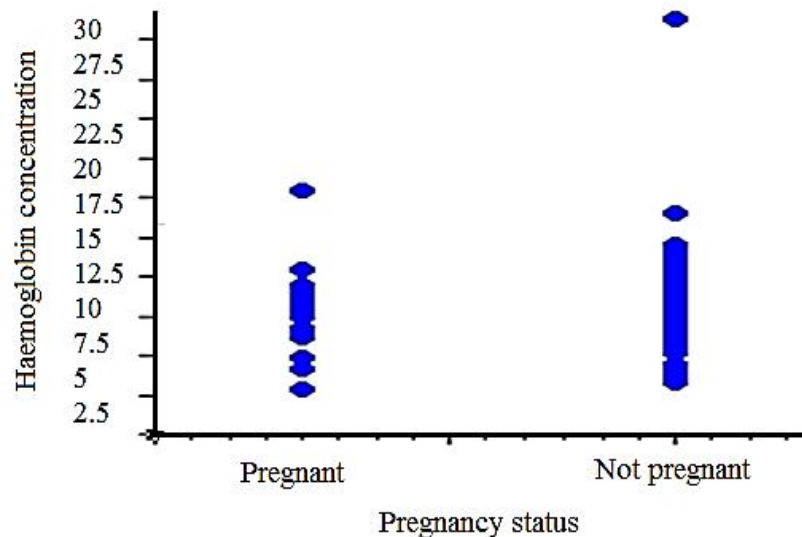
Negative binomial regression analysis was used to control for the non-normal (non-gaussian) distribution of infection intensity between individuals. That is, the distribution of infection is heavily over dispersed (the minorities of the population harbour heavy infection and the majority have light infection, or are uninfected) and this distribution can reasonably be approximated by the negative binomial distribution. There was found to be no impact of area on the results such as no clustering of the data at area level, so this was removed from the analysis. In the analysis Birth Weight was the dependent variable whilst infection status, mother's age, mother's height and weight are all independent variables. The models was then fit to the data and then see if adding infection status information significantly improves the fit of the model. Analysis of Variance (ANOVA) was used to compare mean haemaoglobin concentration in pregnant and non pregnant women in Tana River County.

### **6.8 Results of classification of anaemia and birth weight outcomes in women of reproductive age in Tana River**

The different types of anaemia and white blood cell counts were investigated in pregnant and non pregnant women from parts of Tana River County, Coast Province. The research of different types of anaemia was conducted and data analysis done while controlling for other plausible variables including hookworm

infections and malaria as possible causes of anaemia. In this research all women of reproductive age diagnosed with any soil transmitted helminthes was excluded from the final data analysis.

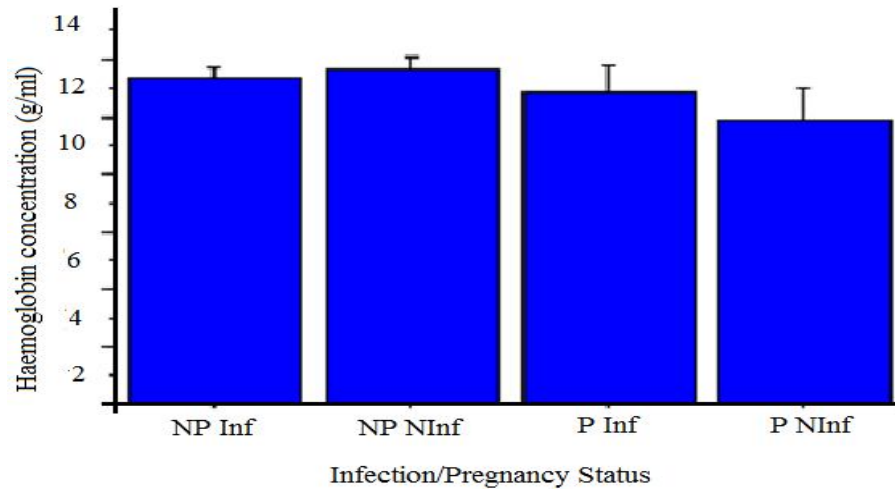
There was an inverse relationship between infection with schistosomiasis and anaemia as measured by haemaoglobin concentration. However, due to other cofounding factors including intensity may alter the relationship. In this study the correlation of haemoglobin concetration in pregnant women and non pregnant women was calculated (figure 6.1) and there was significance ( $P < 0.001$ ).



**Figure 6.1 showing haemoglobin concentration in pregnant and non pregnant women in parts of Tana River County**

Using ANOVA, pregnancy status of the women was compared against infection with *S. haematobiun* and anaemia as measured by haemoglobin concetration in Tana River County. There was significant difference where women who are pregnant and infected had lower haemoglobin than non pregnant infected ( $P < 0.05$ ) (Figure 6.1). Like wise, women who were not pregnant and not infected had

significant levels compared to women who were pregnant and not infected ( $P < 0.05$ ) (Figure 6.2).



**Figure 6.2 showing haemoglobin concentration, infection with *S. haematobium* and pregnancy status**

Key: **NP Inf** = Not pregnant infected, **NP ninf** = Not pregnant not infected

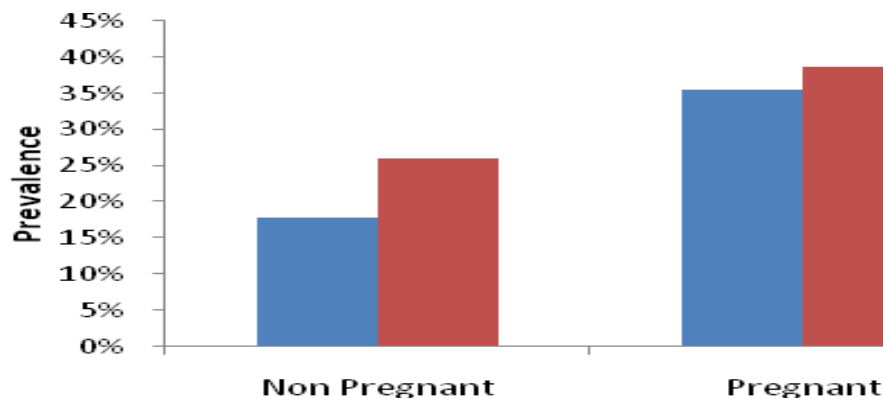
**P inf** = Pregnant infected, **P Ninf** = Pregnant not infected

Specifically, two types of anemia were recorded as Normocytic Normochromic Anaemia (NNA) and Macrocytic anaemia (MA) (macrocytosis). In addition, two markers of white blood cell counts (WBC) were also reported as Eosinophilia and Lymphocytosis, (Table 6.1).

**Table 6.1: Different types of anemia and white blood cells investigated**

Parameters	No. of subjects	Negative	Positive	% positive
Normocytic Normochromic Anaemia	212	169	43	20.3
Macrocytic anaemia (Macrocytosis) (MA)	212	89	123	50.0
Eosinophilia	212	119	93	43.9
Lymphocytosis	212	204	8	3.8

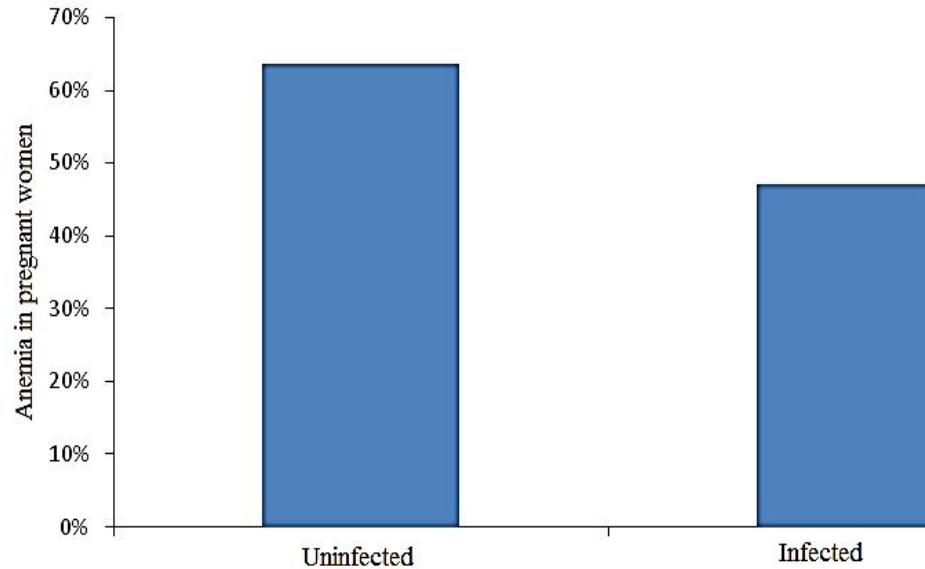
Impact of pregnancy status on normocytic normochromic anaemia (blue bars) and macrocytosis (red bars) were investigated (Figure 6.3). There was evidence for pregnant women having higher levels of significant Normocytic Normochromic Anaemia ( $P \leq 0.05$ ). Non-pregnant women had significant Macrocytic anaemia than pregnant women ( $P \leq 0.10$ ) in parts of Tana River County.



**Figure 6.3 Showing the impact of pregnancy status on Normocytic Normochromic Anaemia (blue bars) and Macrocytosis (red bars)**

Blood markers, Normocytic Normochromic Anaemia and Macrocytosis were correlated with pregnancy and *S. haematobium* infection status. The impact was

non-significant largely due to the small sample size obtained in this study Figure 6.3.



**Figure 6.4 showing the impact normocytic normochromic and macrocytic anaemia on pregnant women infected with *S. haematobium***

### **6.9. Correlation of three anaemia conditions (NNA, MA) with haemoglobin levels and red blood cell counts.**

Both Normocytic Normochromic Anaemia (NNA) and Macrocytic Anaemia (MA) were significantly negatively associated with haemoglobin (Hb) levels after controlling for age (the higher the haemoglobin, the lower the NNA/MA) (Table 6.2).

**Table 6.2: Correlation of Anaemia condition with Hb levels**

Variable	Comparison Group	Effect Size	P-Value
Intercept		11.967055	<0.0001
NNA	No NNA	-2.525417	<0.0001
MA	No MA	-2.468893	<0.0001
Age	Continuous	0.014204	0.0778

Model outputs from exploring the impact of types of anaemia (NNA and MA) on haemoglobin levels, indicate significant levels on both types, however, there is no significance when compared with age of the women. All two anaemia measures (NNA and MA) were significantly negatively associated with levels of red blood cells (RBCs); the higher the prevalence of each of these measures then the lower the corresponding level of RBCs (Table 6.3).

**Table 6.3: Correlation of Red blood cell with the different anaemia conditions**

(P-Values are highlighted green where they reach statistical significant ( $P \leq 0.05$ ) or yellow where they approach significance ( $P \leq 0.10$ )).

Variable	Comparison Group	Effect Size	P-Value
Intercept		4.247152	<0.0001
NNA	No NNA	-0.43096	<0.0001
MA	No MA	-0.49911	<0.0001
Age	Continuous	0.000123	0.955

Model outputs from exploring the impact of types of anaemia (NNA, MA) on Red Blood Cell levels shows significance, but no significance when compared with age of the women. P-Values are highlighted light green where they reach statistical significant ( $P \leq 0.05$ ).

With regard to White Blood cell Counts, there was evidence of higher cases of eosinophilia in individuals infected with *S. haematobium* and lower cases in pregnant individuals (Table 6.4)

**Table 6.4: Eosinophilia in relation to *S. haematobium* infection**

Variable	Comparison Group	Effect Size	P-Value
Intercept		-0.71202	0.10118
Schistosomiasis Infection	Uninfected	1.105165	0.0010
Pregnancy Status	Not Pregnant	-1.11558	0.0157
Age	Continuous	0.007746	0.46931

Model outputs from exploring the impact of *S. haematobium* infection and pregnancy on eosinophilia indicate statistical significant ( $P \leq 0.05$ ), there is no statistical significance when compared with age of the women.

### **6.9.1 Prevalence of Soil Transmitted Helminths and Malaria parasites**

All women of reproductive age enrolled in the study were examined for malaria parasites and any soil transmitted helminthes. None of the samples had malaria parasites and those that had any STH parasites (Hookworm, *Ascaris lumbricoides* and *Trichiuris trichiura*) were excluded from the study. However, those that were found positive, they were all treated with Albendazole at the nearest health facility.

### **6.9.2 Birth weight outcomes**

To investigate the effect of *S. haematobium* infection on birth outcomes in parts of



Tana River County, 32 women were followed almost immediately after live singleton birth (1 – 12 hours after delivery) either in the village or in the nearest Government health facility. Weights of babies were recorded in 5 villages in Hola, Tana River. Out of 32 live births recorded, eight (8) (25%) were recorded as having low birth weights (< 2500g) and 24 (75%) had normal (by WHO standards) live birth weights ( $\geq 2500$ g). Of those mothers diagnosed with *S. haematobium* infection, 5/19 (26.3%) of their neonate children were underweight, and the mothers who were negative for *S. haematobium* at the time of the study, 3/13 (23.1%) were underweight.

## **6.10 Discussion**

Anaemia is the second most common cause of disability in the world (Murray and Lopez), and is reflected in several of the global Millennium Development Goals (United Nations, 2007). The association between anaemia and NTD burden and control is well established for helminthiasis and schistosomiasis, and there are examples of these programmes using population changes in haemoglobin levels as a monitoring tool (Bates *et al.*, 2007). Schistosomiasis causes both anemia and under nutrition, thus, maternal schistosomiasis could have deleterious consequences during pregnancy (Leenstra *et al.*, 2006). The health status of a woman before pregnancy is a crucial determinant of gestational morbidity and pregnancy outcomes.

Anaemia in pregnancy is defined as a haemoglobin concentration < 11.0 g/dl or <10.5 g/dl in the second half of pregnancy. The additional demands placed on

maternal iron stores by the growing foetus, placenta and the increased maternal red cell mass though partially offset by cessation of menstruation and increased absorption of iron during pregnancy leads to an increased demand of iron (Liljestrand *et al.*, 1986). Requirement during first trimester is low, 0.8mg per day, but this rises considerably during the second and third trimester to a high of 6.3mg/day. The total iron requirement over the whole pregnancy is about 1000mg (Liljestrand *et al.*, 1986).

Anaemia in Tana River is prevalent in women of reproductive age according to this study in five villages. Pregnant women are inimical in that, they are more likely to suffer from anaemia than non pregnant women. The condition is even worse when pregnant women are infected with parasites such as schistosomes. Results from the study indicated that pregnant women infected with *S. haematobium* have low haemoglobin than non pregnant infected women. This has also been demonstrated by Qunhua *et al.*, (2000) that pregnant women infected with schistosomiasis develop severe anemia.

In the present study a total of 212 women of reproductive age in parts of Tana River County were investigated for the different types of anaemia, white cell count, birth weight outcomes and *S. haematobium* infection after controlling for malaria and hookworm infections. Using peripheral blood film examination (blood counts) and coulter counter haematological indices analysis, 20.3% of the women, were diagnosed with Normocytic Normochromic Anaemia while 50% were diagnosed with Macrocytic Anaemia (Macrocytosis). There are several causes that can lead to

these conditions; however, certain parasitic infections are known to highly contribute to the spontaneous blood loss especially in pregnant women. Several studies (Ajanga *et al.*, 2006; Jennifer *et al.*, 2011; Kagu *et al.*, 2007) have shown that infection with parasites can lead to anaemia (blood loss). Macrocytosis tends to be underestimated, since about 60% of patients present without associated anemia (Colon-otero *et al.*, 1992), unless there are other accompanying abnormalities noted. No complications arise from macrocytosis itself as an isolated finding; however, its identification can provide important information regarding the presence of an underlying disease state (Colon-Otero *et al.*, 1992). Pregnant women showed significantly higher levels of NNA and ANE ( $P \leq 0.05$ ) than non-pregnant women in the study, same as macrocytic anaemia ( $P \leq 0.10$ ).

Milder forms of  $\alpha$ -thalassemia and sickle cell anaemia which can now be detected by DNA methods are associated with hypochromic and normocytic anaemia (Bowden *et al.*, 1985; William *et al.*, 1996) were not investigated in the study, however, there are no reported cases of these haemoglobinopathies in the cohort study subjects. Correlation of the NNA and MA with haemoglobin as assessed by coulter counter analysis revealed a negative association meaning the higher the prevalence of either of the anaemia, the lower the corresponding red blood cell count. Treatment of normochromic normocytic anemia varies and depends on the cause of anemia. The most important strategy being to diagnose the cause and manage the subsequent progression, anemia will then subside automatically.

It is generally agreed that individuals infected with schistosomiasis, have elevated levels of Eosinophils either by the blood smear examination and/or WBC counts as determined by coulter counter cell analysis. The present study sort to find out whether there is Eosinophilia in pregnant women infected with *S. haematobium*. Our results indicate that there is evidence of higher cases of eosinophilia (as determined by both methods) in individuals infected with *S. haematobium* and lower in pregnant women. We did not investigate other immunological reactions in this cohort, however, this observation could be due to the fact that, pregnant women tend to have low immunity during pregnancy and can easily develop various other conditions that are associated with progression of the disease and/or infection. Several other studies have demonstrated an association between eosinophilia and host protection against schistosomes (Butterworth, 1985; Sturrock, 1983).

The possibility of women infected with *S. haematobium* delivering under-weight children was investigated in 32 pregnant women in 5 villages in Hola Tana River County. Data from this study, though not adequate at the time, indicate that pregnant women infected with *S. haematobium* delivered underweight children after full term (5/19=26.3%). This is in agreement with another study by Siegrist 1992, which showed preterm (37 weeks) deliveries were 34.8% in the study group vs. 23.8% in the control group. The births weights in term deliveries (37 weeks) were not significantly different (3012 g vs. 3103 g). In the preterm deliveries, the birth weight was significantly lower in the infected group (1768 g vs. 2457 g).

In this study, preterm deliveries were not investigated since this was a quasi-longitudinal study, however, even mothers who were not infected with *S. haematobium* (3/13=23.1%) delivered under weight children.

More than 50% anaemia in women of reproductive age in parts of Tana River County has been reported. Investigation of a larger sample population of pregnant women in schistosomiasis endemic areas of Kenya may reveal much more information and preferably longitudinal study is warranted.

### **6.11 Conclusion**

Women in *S. haematobium* endemic areas in parts of Tana River County are susceptible to high infection rates, leading to various conditions including different types of anaemia being experienced. The study has reported the effect of *S. haematobium* infection on birth weight outcomes of pregnant women in Tana River County. The inherent limitation of this study include among others, the modest number of pregnant women recruited in Tana River County and the few number of pregnant women who were followed for the birth weight outcome after a period of between 2 and 9 months.

### **6.12 Recommendations**

Pregnant mothers can also be supplemented with multiple micronutrients which contain vitamins and minerals to alleviate their iron stores and/or blood levels.

Combating anaemia may be enhanced by consuming foods that are a good source of iron, like meat, green vegetables, legumes, fish and fortified grains.

Mothers attending ante-natal clinic in schistosomiasis endemic areas and presenting with symptoms of anaemia should be screened for the different types of anemia conditions among other investigations.

Clinicians should be advised to investigate for specific anaemia conditions to properly diagnose the etiological factors and proper management of the patients.

## CHAPTER SEVEN

### 7.0 GENERAL CONCLUSIONS AND RECOMMENDATIONS

#### 7.1 General conclusions

##### 7.1.1 Prevalence of *S. haematobium* infection in primary school children in parts Kwale, Kilifi and Tana River Counties, Coast Province

The objective of was to determine the prevalence of *S. haematobium* in school children in Coastal region of Kenya. The study found that in *S. haematobium* endemic areas, blood in urine questionnaire administered by school teachers after thorough instructions compared primarily well with those administered by research officials in identifying schools with high prevalence as well as children with the infection. At an operational level, the questionnaire correlated favourably with urine microscopic egg counts and was a useful predictor of schools to be targeted for mass drug administration.

The blood in urine questionnaire when used in a National schistosomiasis control programme is attractive in terms of the low cost, its simplicity, logistical relevance, quicker response in terms of delivery of results and effective approach as a community level diagnostic tool (Mafe, *et al.*, 2000; Ansell and Guyatt, 2002; Brooker, *et al.*, 2009). However, low prevalence and intensities may be missed out, thus, it is important to include or supplement the surveys with other rapid diagnostic procedures such as dipstick, protein in urine to improve on the accuracy of the data. As observed by other researchers, questionnaire which ask the child to

recall the presence of blood in urine are less sensitive in girls particularly older girls. In view of the low prevalence of *S. haematobium* after treatment with praziquantel, blood in urine questionnaire may perform poorly after implementation.

### **7.1.2 Prevalence and intensity of *S. haematobium* infection in women of reproductive age in parts of Kwale County**

*Schistosoma haematobium* infection is highly prevalent in Sub-Saharan Africa. Circumstantial, biological and epidemiological evidence strongly suggest there is continuous re-infection with *S. haematobium* on women of reproductive age in endemic areas. There is considerable presumptive association of *S. haematobium* infection with HIV as suggested by some authors. The plausible association where co-endemicity of these infections occur, a salient effect on the health of especially pregnant women is more prominent and causes high morbidity and mortality and it is underestimated by the different programmes addressing maternal health in the community.

This is only a preliminary study of the Female Genital Schistosomiasis in women of reproductive age in Kenya. It may have some limitations as compared with other prospective studies in the communities involving school children (school deworming programmes). Many factors are associated with Female Genital Schistosomiasis such as, parity, personal hygiene, environmental sanitation, educational levels, living conditions and traditional practices. Based on available



information, women showing symptoms of FGS should be diagnosed appropriately and advised with proper information on the causes, consequences and protection against contracting schistosomiasis. Despite these results showing that FGS does not cause any conditions in the kidney, it is empirical to carry out further studies on other body organs where the systemic circulation of the parasite may cause organ damage.

Recently WHO indicated that all schistosome-infected pregnant and lactating women should be considered high-risk group and should be offered treatment individually or during treatment campaigns. However, in Kenya this has not been the case and women in schistosomiasis endemic areas have had to contend with the infection for almost the entire parity period due to the policy of non treatment of pregnant women during term. Despite lack of pregnancy safety treatment data in endemic areas, policies of the Government (Ministry of Health) should be reviewed to include pregnant and lactating women in MDA campaigns for schistosomiasis control.

### **7.1.3 Blood patho-physiological changes associated with *S. haematobium* infection in pregnant and non- pregnant women in parts of Kwale County**

Although haemoglobin estimation is the most common indicator for anemia world wide, it does not reflect the actual iron stores in the body and has low sensitivity especially in Africa where malnutrition is also rampant. Addition of some other anemia indicators such as red blood cell count may complement the diagnosis of anemia, notwithstanding tests such as serum ferritin and soluble transferrin

receptor. Of note in this study is the almost equal comparable information on anemia as estimated by Hb estimation and RBC count in women of reproductive age. Anemia was higher in women infected with *S. haematobium* and even higher in pregnant women across all the villages. It is highly unlikely that the anemia in these women was biased by unmeasured confounding factors such as socio-economic status of the study participant since the general area is the same in terms of nutrition and diseases epidemiology.

It is well documented that circulating eosinophil from helminth-infected individuals may up regulate eosinophil function, which may lead to increased production as observed in women infected with *S. haematobium*. The same infected women also had thrombocytopenia, however, this was not completely associated with the infection but is indicative of patho-physiological changes that occur and may subsequently lead to progression of morbidity due to schistosomiasis infection.

The ecological pattern of urinary schistosomiasis in Kenya and anemia associated with the infection identifies the very high-risk group (women of reproductive age). Their treatment and any other additional control measures are needed to consolidate the progress made on preventive chemotherapy through school deworming programmes.

#### **7.1.4 The different types of anaemia and birth weight outcomes of pregnant and non-pregnant women infected with *S. haematobium* in parts of Tana River County**

Infectious diseases particularly parasitic diseases, lead to both extracorporal iron loss and anemia of inflammation which decreases bioavailability of iron to host tissues. Despite enormous global burden of disease due to anemia and the multiple causes of anaemia in LDCs, diagnostic tests to distinguish primary etiology are limited in rural set up health institutions. Once anemia is defined, many algorithms then classify it as microcytic, normocytic normochromic or macrocytic according to the mean cell volume and the morphological features presented in a blood smear. In Tana River County, women infected with *S. haematobium* showed low haemoglobin concentration in their blood. This confirms the fact that schistosomiasis can cause anaemia in endemic areas.

In the present study two types of anemia were diagnosed in women of reproductive age infected with *S. haematobium* in Tana River County. Treatment of the infection is important and investigation of the underlying causes is equally beneficial. In *S. haematobium* endemic areas, non-nutritional causes of anemia if investigated and controlled, will reduce the pathogenesis of anemia in pregnancy and improve on child growth in utero. More information and data on different types of anaemia due to schistosomiasis infection are needed to address the efficacy of treatment during pregnancy and child birth outcomes in endemic areas. Information on specific micro-nutrient supplementation of pregnant women infected with schistosomes is

lacking. Such information and /or longitudinal study will improve on the knowledge of patho-physiological changes that are observed in women of reproductive age especially in Africa.

#### **7.1.5 Study limitations**

The inherent limitations of this quasi-longitudinal study on health and infections in pregnant women are that previous history, basic life style, community engagements and subsequent events are unknown. Women of reproductive age in Kwale and Tana River County may also have been suffering from other infections. Interractions of malaria and hookworm as well as nutritional status in the mechanisms of anaemia may have been a limiting factor in estimating and measuring the actual haemoglobin levels in women of reproductive age.

Other reasons for the high prevalence of anaemia in these women are that, the survey participants most likely would belong to the poorest population in the Country with significant low socio-economic status. Further more, there was limitation in the sample size that was enrolled in the study, especially the pregnant women who delivered after full term. Overral, despite the aforementioned potential limitations in this study, it still represents one of a few that has looked at schistosomiasis infection in pregnancy and the associated pathophysiological changes thereof.

#### **7.2 GENERAL RECOMMENDATIONS**

From this study the following recommendations are made;

Because schistosomiasis is focal in transmission, questionnaires are now readily available for rapidly screening for urinary schistosomiasis in endemic areas. These processes and features lay the foundation for future control of *S. haematobium* in Kenya, thus it is recommended school teachers be involved in the quick diagnosis of blood in urine in their respective schools.

It is envisaged that, in areas of lower prevalence, questionnaire-based screening tools have proven valid. They have been used both to identify communities with high prevalence, and to identify individual cases that would then be further screened and treated, or treated presumptively as it has been recommended for the Kenya National school-based deworming programme.

In the present study, *S. haematobium* prevalence, intensity and morbidity data obtained from pregnant women in Coastal region in particular may have potential policy implications, as current WHO guidelines and national policies focus on mass deworming school-aged children. Available information on treatment of schistosomiasis infected pregnant women in Kenya indicates that this high risk group is almost always avoided by control programmes for various reasons. It is against the back ground of this research that treatment of pregnant women is strongly advocated especially following the WHO guide lines that recommend treatment of pregnant women after the 1<sup>st</sup> trimester. In view of this, it is recommended that, medical doctors, clinicians, nurses and health workers, should be informed of the WHO recommendation on treating pregnant women infected with schistosomiasis after 1<sup>st</sup> trimester during ante-natal visits and whenever there

is mass drug administration. Findings emerging from this investigation merits further research on implication of the infection and proper management of pregnant women infected with schistosomiasis.

It is recommended that anemia estimates be encouraged during surveys which assess the prevalence of factors that contribute to anaemia – not only iron deficiency, but also infectious diseases and other micronutrient deficiencies in pregnant and lactating women. The understanding of how these factors vary by geography, level of development, and other social and economic factors will make it easier to design interventions that are more effective and integrative in addressing multiple contributing factors at the same time.

Deworming is important to prevent the direct pathological effects of worms and as hypothesized, maternal helminth infection may have long-term effects on the development of the fetal immune system and both risks and benefits for disease susceptibility in later life. Thus, from this study it is recommended that women of reproductive age in particular, where community-based approaches would offer unique benefits in complementing school-based mass deworming, be screened for schistosomiasis infection regularly either in surveys or at the Ante-natal clinic.

Access to treatment, particularly for pregnant women in schistosomiasis endemic areas, seems to be a more important determinant of outcome of child birth that is why it is recommended for mothers to be treated early enough in pregnancy to

avoid serial complications during delivery. It is empirical to summarise these general conclusions and recommendations and further research from this study in this last chapter.

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## **9.0 APPENDICES**

### **9.1 Appendix 1: Research information sheet**

**Title: *Schistosoma haematobium* infection in school children and women of reproductive age: the effect on anemia, blood patho-physiological changes in selected parts of Kwale, Kilifi and birth weight outcomes in Tana River, County, Coast Province, Kenya**

#### **Introduction**

We the staff of Kenya Medical Research Institute and Ministry of Health are conducting research in the communities on the presence of Schistosomiasis (Bilharzia), malaria and worms in order to establish the seriousness of the disease in the area. Information on the presence of the types of bilharzia parasites and the seriousness of the disease in the community especially pregnant women will assist in providing information on the treatment strategies of infected individuals. This will also assist in establishing the extent of anaemia in women who are infected with one or more of the parasites.

#### **What I will do**

We will visit selected villages in your District after public awareness meeting with community members in chiefs' barazas. In the villages, we intend to liaise with the teachers and community leaders to collect urine, blood and stool samples to check for worms. We will also further examine those adults found to be infected with either parasite for their physical condition and/or any abnormality in the area around the abdomen (ultrasonography). Collection of stool and urine samples and physical examination is non-invasive and there will be no injury onto you at any

moment. However, collection of blood samples from a finger prick may inflict some little pain that will disappear with a few seconds and does not cause loss of blood in any way. After all the examinations, the women in the community will be treated with the appropriate drugs for worms after delivery to avoid any complications.

Why I have come to you and what I need you to do

As woman/pregnant woman, we need your permission and cooperation with us to facilitate the examination in or around a health facility. Your neonate (newly born baby) will also be examined for weight and anaemia (Hb concentration.) immediately after delivery and thereafter every 6 months to monitor growth improvement. You will also be followed over the same period to check for any changes in your haemoglobin levels and nutritional status after delivery and treatment.

### **Risks and benefits**

Collection and examination of stool/urine samples has no injury on to you or your baby in the womb and after delivery. Physically external examination and ultrasonography of body organs (liver, spleen, bladder) to measure the extent of the disease, has no adverse effects at all except minor discomfort. The drugs (Praziquantel and Albendazole) that will be used for treatment are known to be safe and are commonly used in Kenya. Examination of the neonate(s) will not affect him/her in any way whatsoever. Information generated, will be very useful in making decisions on how treatment should be carried out on pregnant women in areas where Schistosomiasis infections occur.

## **Confidentiality**

All information which will be obtained from this work will not be disclosed to anybody except you and will only be used by the investigators for purposes of report writing. No personal information will be included in the report without your written consent and authority.

## **Condition for participation**

You and/or your child have been selected for the study, but the final decision to participate is yours. You are free to accept or reject the inclusion of your child or you're self in the study. After accepting to take part in the study, you can withdraw any time and that will not affect the benefits that you would get during the process of the study.

## **If you have any questions**

The team from Kenya Medical Research Institute and Ministry of Health will be readily available to answer any questions. You can contact Mr. Jimmy H. Kihara, or Dr. Charles Mwandawiro at Kenya Medical Research Institute, ESACIPAC, P. O. Box 54840 00200, Nairobi, Tel. 254-020-2722541. You can also contact The Secretary, KEMRI/National Ethical Review committee, P. O. Box 54840 00200 Nairobi, E-mail [Kemri-hq@nairobi.mimcom.net](mailto:Kemri-hq@nairobi.mimcom.net).

## 9.2 Appendix 2: Informed consent to participate in the study

### DECLARATION OF THE PREGNANT WOMAN

I being over 18 years and having full capacity to consent for myself, have been informed about the study entitled:

*Schistosoma haematobium* infection in school children and women of reproductive age: the effect on anemia, blood patho-physiological changes in selected parts of kwale, kilifi and birth weight outcomes in tana river, counties, coast province, kenya

Under the direction of the study investigator, Mr. Jimmy H. Kihara

The purpose, nature, duration, inconveniences, hazards and procedures of the study have been explained to me thoroughly by the responsible officer(s).

I have understood the information about the study and what will happen. I have been given

the opportunity to ask any questions concerning the study and these (if any) have been answered to my satisfaction.

I understand that I am free to withdraw my child or myself from the study any time without any loss or penalty. My refusal to participate will involve no penalty or loss of benefits to which my family is otherwise entitled.

Mark one box with **X**

I hereby agree to allow my child/myself to take part in this study

I do not wish my child/myself to participate in this study

Parent Name		Identity number		
Village				
		Date of birth	Sex	Age

Child Name		Identity number	
Village			
	Date of birth	Sex	Age

Witness: I hereby confirm that the study has been explained to the parent/school child. All questions (if any) have also been answered to his/her satisfaction, and he/she has of his/her own free will consented to take part in the study.

Name of witness:		
Signature of witness:		Date:

Name of person explaining the study.....

### **9.3 Appendix 3: Reference questionnaire form, guideline for interviewing the pupils about their health**

The Government of Kenya acknowledges that major impediments to national development and poverty reduction are ill-health and disease. The ill-health is partly due to water, sanitation and hygiene related factors. Other factors are parasitic infections, infectious diseases, macro and micronutrient deficiencies, sexually transmitted infections, HIV and AIDS, tobacco and drug abuse. For this reasons, the government is committed to promote quality health care to all school going children through the recently launched comprehensive school health programme by the Ministers of Education & Public Health and Sanitation. Since school children are considered to be agents of change in the society; they will disseminate positive health messages in the community. It is hoped that this will

improve effective learning, realization of their productive potential and educational goals in their many years of schooling.

The questionnaire provided should be completed by each class teacher and returned to the Head Teacher, who will then deliver it to the education office in the area.

The class teacher required to fill the name of the school, date, class, stream, total number of boys and girl. In addition, the teacher will fill in his or her name in the space provided on the form. You will notice that the top page of the form has pupils from number 1 to 20. Each number represents one pupil. Therefore the boxes provided below number 1, will all be filled for all the named conditions (example headache, cough scabies etc.) for child number 1. The same applies to all the other numbers on page 1 and the following page. If there are more than 40 pupils in your class, please continue on a sheet without a number, and insert numbers appropriately.

NB: If the child suffers from the kind of diseases/infection stated; indicate with a tick (√). If not indicate with X

Example

**10.0 Appendix 4: School health programme; class questionnaire form form for teacher to interview the pupils about their health**

Pupils	n.k.	1	2	3	4	5	6	7	8	9	10	11
Admission Number	2345	434	356	447								
Age (years)	12	10	12	10								
Sex (M or F)	M	F	F	M								
Headache	<input type="checkbox"/>	√	√	x								











## 11.0 Appendix 6: Definitions of haematological indices

**Erythrocytosis (ERY):** An increase in the total red cell count, often a consequence of a range of non-hematogenic systemic disorders.

**Microcytosis (MCR):** A hematologic condition characterized by smaller than normal erythrocytes

**Macrocytosis (MA):** The presence of abundant RBCs with a mean cell volume (MCV) of >100 femolitres (fL) and a diameter greater or equal to 8.5µm.

**Hypochromia (HPCR):** The lack of complete saturation of the erythrocyte stroma with haemoglobin, which leads to anaemia

**Eosinophilia (EOC):** An abnormally high number of Eosinophils in the blood. This is often caused by parasitic diseases where they are present

**Thrombocytosis (THRC):** is a blood disorder in which the body produces a surplus of platelets (thrombocytes).

**Thrombopenia (THRP):** is a blood disease or disorder characterized by an abnormally small number of platelets in the blood circulation


**Leukocytosis (LEC):** Is a condition characterized by an elevated number of white cells in the blood, it may affect all types of White Blood Cells (Monocytes, Lymphocytes, Basophils, Neutrophils, Eosinophils)

**Leukopenia (LEKP):** Refers to a reduced number of white blood cells in general

**Granulocytopenia:** refers to a decreased number of all of the granulocytes-type blood cells (Neutrophils, Eosinophils, and Basophils).

**Anisocytosis:** The excessive inequality in the size of Red Blood Cells circulating in the blood.

## 12.0 Appendix 7: Ethical clearance certificate



**KENYA MEDICAL RESEARCH INSTITUTE**

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**KEMRI/RES/7/3/1** **APRIL 09, 2008**

**FROM:** SECRETARY, KEMRI/National Ethical Review Committee

**THRO':** Dr. C Mwandawiro,  
CENTER DIRECTOR, ESACIPAC *forwarded*  
NAIROBI *K. Mwangi*

**TO:** Mr. Hussein Jimmy Kihara (ESACIPAC)

**RE:** **SSC No. 1319 (Rev):** Schistosomiasis infection in pregnant women: The effect on hemoglobin concentration (Anaemia), nutritional status and birth weight

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Dear Sir,

Make reference to your letter dated 19 March 2008.

The Committee is satisfied that the issues raised by the Committee have been adequately addressed. Due consideration has been given to ethical issues and the study is granted approval from today the 9<sup>th</sup> APRIL 2008 to APRIL 8<sup>th</sup> 2009.

Please note that any changes to the research study must be reported to the Scientific Steering Committee and to the Ethical Review Committee prior to implementation. This includes changes to research design, equipment, personnel, funding or procedures that could introduce new or more than minimum risk to research participants.

Respectfully,

*R. C. Kithinji*  
**R. C. Kithinji,**  
**For: Secretary,**  
**KEMRI/NATIONAL ETHICAL REVIEW COMMITTEE**

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*In Search of Better Health*

## **Appendix 8: Leishman's stain for blood smears**

### **Reagents**

0.15% Leishman powder in 100% methanol. Use after 24hrs.

Phosphate buffer (Sorensen)

Stock A: 0.2M sodium di-hydrogen orthophosphate (mw 156).

To prepare dissolve 3.12g in 100ml distilled water.

Stock B: 0.2M di-sodium hydrogen orthophosphate (mw 142).

To prepare: dissolve 2.83g in 100ml distilled water.

## **Appendix 9: Reagents and equipment**

1. Hemolynac – 3N Nihon Kohden – Firenze Sri; MEK – 6410 shinjuku – KU-Tokyo Japan
2. Claenac – CE – Nihon- Kohden – Firenze S.R.I
3. Isotonac – 3 – EC- Nihon – Kohden – Firenze SRI
4. Normal, high and low counts controls
5. Printer – Epson – Lax 300+II