

**MORBIDITY PATTERNS, SPATIAL DISTRIBUTION AND  
TREATMENT OF *SCHISTOSOMA HAEMATOBIMUM* AND SOIL  
TRANSMITTED HELMINTHES IN PRIMARY SCHOOL CHILDREN IN  
THE TANA DELTA OF KENYA**

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**PhD Reg. No W80/80039/2008**

**A thesis submitted in partial fulfillment of the degree of Doctor of  
Philosophy (PhD) of the University of Nairobi**

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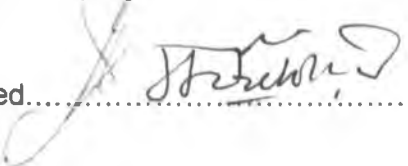
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## **DEDICATION**

This thesis is dedicated to my wife L. Njeri, my daughters C. Wanja and S. Nyawira, for their endurance during my long absence from home while undertaking this study, and to all children who, due to the socio-economic status of their families, unjustifiably suffer neglected diseases.

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## ACRONYMS AND ABBREVIATIONS

**BMI:** Body Mass Index

**CONTRAST:** A Multidisciplinary Alliance to Optimise Schistosomiasis Control and Transmission Surveillance in sub-Saharan Africa

**DALYs:** Disability-Adjusted Life Years

**DANIDA:** Danish International Development Agency

**DBL-CHRD:** Danish Bilharziasis Laboratory – Centre for Health Research and Development

**DVB & NTD:** Division of Vector-Borne & Neglected Tropical Diseases

**ECP:** Eosinophil Cationic Protein

**EPX:** Eosinophil Protein X

**GIS:** Geographical Information Systems

**GMI:** Geometric Mean Intensity

**IFN- $\gamma$ :** Interferon-gamma

**IL-10:** Interleukin-10

**IL-6:** Interleukin-6

**KEMRI:** Kenya Medical Research Institute

**MPO:** Macrophage-derived myeloperoxidase

**STH:** Soil-Transmitted Helminths (*Trichuris trichiura*, *hookworms* and *Ascaris lumbricoides*)

**TNF- $\alpha$ :** Tumour Necrosis Factor-beta

**UNITID:** University of Nairobi Institute of Tropical and Infectious Diseases

**WHO:** World Health Organisation

## DEFINITION OF OPERATIONAL TERMS

**Anaemia:** Haemoglobin concentration below 11.5 g/dl of blood

**Eosinophilia:** Presence of eosinophils above 7% of all white blood cells in peripheral blood

**Faecal ECP:** ECP levels in stool specimens of the sturdy participants

**Faecal EPX:** EPX levels in stool specimens of the sturdy participants

**Haematuria:** Presence of blood in urine

**Low Body Mass Index:** Body mass index below 18.5 kg/m<sup>2</sup>

**Macrohaematuria:** Presence of blood in urine as observed with the naked eye

**Microhaematuria:** Presence of blood in urine as detected by dipstick test

**Morbidity:** Abnormality of observed in the study participants as a result of *S. haematobium* or STH infections

**Pathology:** Deviation from a healthy or normal condition

**Reported haematuria:** Presence of blood in urine as reported by the study participants

***S. haematobium*-infected:** Children whose urine samples were positive for *S. haematobium* eggs by microscopy

**Serum cytokines:** Cytokine levels in serum specimens of the sturdy participants

**STH-infected:** Children whose stool samples were positive for STH eggs by microscopy

**Study participants:** Children who participated in the study

**Urinary cytokines:** Cytokine levels in urine specimens of the sturdy participants

**Urinary ECP:** ECP levels in urine specimens of the sturdy participants

## ABSTRACT

**Background:** *Schistosoma haematobium* and soil-transmitted helminthic (STH) infections are important public health problems in Kenya but their prevalence, intensities and the resultant morbidity vary widely from one endemic focus to another in the country. There is, therefore, an urgent need for investigations on the extent of disease burden, risk factors associated with the infections and effects of treatment from different endemic settings as a background for designing and implementing programs for successful control of these infections.

**Objective:** To assess morbidity patterns, response to treatment and spatial distribution of *S. haematobium* and STH infections in school-going children of the Tana Delta District, coastal Kenya.

**Methods:** At baseline, urine samples were collected from primary school children and examined for *S. haematobium* eggs, haematuria, and eosinophil cationic protein (ECP) and selected cytokines [interleukin (IL)-6, interferon (IFN)- $\gamma$ , tumour necrosis factor (TNF)- $\alpha$  and IL-10] levels. Stool samples were also collected and examined for soil-transmitted helminth eggs, ECP and eosinophil protein X (EPX) levels. One sample of venous blood was taken from each child and tested for haemoglobin level, serum IL-6, TNF- $\alpha$ , IFN- $\gamma$  and IL-10 levels. Height and weight of each child were taken and each child was subjected to ultrasound examination of the urinary tract for *S. haematobium* infection-related morbidity. The children were interviewed on their behaviour in relation to infection with *S. haematobium* and STH, related symptoms. At the end of the baseline survey each child was treated with praziquantel (40 mg/ kg body weight) and albendazole (400 mg).

During the follow-up survey, 3 months after treatment, stool and urine samples were examined for *S. haematobium* and STH eggs and haematuria as in the baseline. The weight of each child was also recorded. A household survey was conducted during which parents were interviewed to elucidate the socio-economic conditions which would predispose to infections. Geographical co-ordinates of the main houses in the households and the local water contact points were also recorded. *p*-values less than 0.05 were considered significant in all statistical tests.

### Results:

#### Prevalence and intensity of *S. haematobium* and STH infections

A total of 262 children, 67 from Kau with a mean age of 9.6 years and 195 from Ozi, with a mean age of 9.7 years, were recruited into the study. The prevalence of *S. haematobium* infections was 98.5% and 92.3% in Kau and Ozi, respectively. The prevalence of *T. trichiura* infections was 85.1% and 89.2% in Kau and Ozi, respectively. The prevalence of



hookworm infections was 68.7% and 85.1% in Kau and Ozi, respectively whereas the prevalence of *A. lumbricoides* infections was 32.8% and 49.7% in Kau and Ozi, respectively.

Similarly, high geometric mean intensities (GMI) of *S. haematobium* infections were observed (63.1 eggs/ 10ml of urine and 54.3 eggs/ 10ml of urine in Kau and Ozi, respectively). In contrast, low GMI of STH infections were observed among the children according to the grading by WHO. For *T. trichiura* infections the GMI were 44.4 eggs/ g of stool and 110.7 eggs/ g of stool in Kau and Ozi, respectively. For hookworm infections, the GMI were 20.2 eggs/ g of stool and 117.2 eggs/ g of stool in Kau and Ozi, respectively. For *A. lumbricoides* infections, the GMI were 2.8 eggs/ g of stool and 12.8 eggs/ g of stool in Kau and Ozi, respectively.

To elucidate the effects of STH on *S. haematobium* and vice versa, during co-infections, the GMI of STH infections were compared between children with heavy and light *S. haematobium* infections. Children with heavy *S. haematobium* infections had significantly lower GMI of *T. trichiura* infections than children with light *S. haematobium* infections ( $p=0.028$ ) whereas children with heavy *S. haematobium* infections had significantly higher GMI of hookworm infections than those with light *S. haematobium* infections ( $p=0.043$ ). There was no significant difference in GMI of *A. lumbricoides* infections between children with heavy and light *S. haematobium* infections ( $p=0.87$ ).

#### **Relationship between *S. haematobium* and STH infections and morbidity**

To shed light on the relationship between *S. haematobium* and STH infections and morbidity, proportions of children with different morbidities were compared between different levels of infections. Significantly higher proportions of children with heavy *S. haematobium* infections had microhaematuria, ultrasound-detectable urinary tract pathology and anaemia than those with light *S. haematobium* infections ( $p<0.001$  and  $p=0.007$ ,  $p=0.06$ , respectively). There was no significant difference in the proportions of children with low body mass index (BMI) between children with heavy and light *S. haematobium* infections ( $p=0.68$ ). When compared between different groups of STH infection intensities, there was a significant positive relationship between hookworm infection intensity and anaemia ( $p<0.001$ ) but *T. trichiura* or *A. lumbricoides* infections were not related to anaemia. On the other hand, *T. trichiura* or *A. lumbricoides* infection intensities were significantly positively related to low BMI ( $p=0.006$  and  $p=0.027$ , respectively) but hookworm infections were not related to BMI.

#### **Relationship between *S. haematobium* and STH infections and inflammatory markers**

Analyses were carried out to elucidate how inflammatory markers (ECP and EPX) were related to infection intensities and morbidity. There was a significant positive correlation

between *S. haematobium* egg counts and urinary ECP levels ( $r=0.46$ ,  $p<0.001$ ,  $n=211$ ). Children with microhaematuria and those with ultrasound-detectable urinary tract pathology had significantly higher levels of urinary ECP than those without these conditions ( $p<0.001$ ,  $n=241$  and  $p=0.037$ ,  $n=200$ ; respectively). The relationship between *S. haematobium* egg counts and urinary ECP was not significantly influenced by infections with STH. Faecal ECP and EPX were analysed for 186 children. There was a significant positive correlation between faecal ECP and faecal EPX levels ( $r=0.32$ ,  $p<0.001$ ). There was no significant relationship between faecal ECP levels and *T. trichiura*, hookworm or *A. lumbricoides* egg counts ( $p=0.31$ ,  $p=0.50$  and,  $p=0.21$ , respectively). Although faecal EPX levels were significantly positively related to hookworm egg counts ( $r=0.24$ ,  $p=0.001$ ), they were not significantly correlated to *T. trichiura* or *A. lumbricoides* egg counts ( $r=0.14$ ,  $p=0.05$  and;  $r=0.08$ ,  $p=0.27$ , respectively).

#### **Relationship between cytokine levels, *S. haematobium* infection and urinary ECP**

Serum and urine samples from 158 children were analysed for IL-6, TNF- $\alpha$ , IFN- $\gamma$  and IL-10 levels in order to assess the relationships between the cytokines, *S. haematobium* infection and related morbidity. There was no significant linear relationship between serum and urinary IL-6 ( $r=0.01$ ,  $p=0.94$ ), IFN- $\gamma$  ( $r=-0.09$ ,  $p=0.24$ ), TNF- $\alpha$  ( $r=-0.07$ ,  $p=0.36$ ) and IL-10 ( $r=0.06$ ,  $p=0.43$ ). There was no significant difference in levels of serum IL-6, TNF- $\alpha$ , IFN- $\gamma$  or IL-10 between children with light and heavy *S. haematobium* infections ( $p=0.83$ ,  $p=0.86$ ,  $p=0.96$ , and  $p=0.79$ , respectively). Similarly, there was no significant difference in levels of urinary TNF- $\alpha$  or IFN- $\gamma$  between children with light or heavy *S. haematobium* infections ( $p=0.21$  and  $p=0.22$ , respectively). However, children with heavy *S. haematobium* infections had significantly higher levels of urinary IL-6 ( $p<0.001$ ) and significantly lower levels of urinary IL-10 ( $p=0.002$ ) than children with light *S. haematobium* infections. In addition, children with ultrasound-detectable urinary tract pathology and microhaematuria had significantly higher levels of urinary IL-6 ( $p=0.009$ ,  $p<0.001$ , respectively) than children without urinary tract pathology and microhaematuria. On the contrary, children with ultrasound-detectable urinary tract pathology and microhaematuria had significantly lower levels of urinary IL-10 ( $p=0.034$ ,  $p=0.005$ , respectively) than children without urinary tract pathology and microhaematuria. When analysed in relation to co-infection with STH, the relationships between these cytokines and *S. haematobium* infections were not significantly influenced by any of the STH species considered. Moreover, urinary IL-6 levels were significantly positively related to urinary ECP levels ( $r=0.54$ ,  $p<0.001$ ) whereas urinary IL-10 levels were significantly related to urinary ECP levels ( $r=-0.21$ ,  $p=0.012$ ).

### **Effects of combined praziquantel-albendazole treatment on *S. haematobium* and STH**

Baseline and follow-up urine and stool samples from 171 children were analysed for the effects of combined praziquantel-albendazole treatment on *S. haematobium* and STH egg counts. The treatment resulted in highly significant reductions ( $p < 0.001$ ) in the prevalences of *S. haematobium* (77.9%), *T. trichiura* (15.1%), hookworm (69.0%) and *A. lumbricoides* (91.5%). Similarly, there were highly significant reductions ( $p < 0.001$ ) in the GMI of *S. haematobium* (99.0%), *T. trichiura* (62.9%), hookworm (98.0%) and *A. lumbricoides* (97.3%). Analysis of the relationship between *S. haematobium* infection intensity and effects of treatment on prevalence of STH infections revealed a two-fold higher reduction of *T. trichiura* infection prevalence in children with heavy *S. haematobium* than in those with light infections but the reductions were approximately the same for hookworm and *A. lumbricoides* in children with heavy and light *S. haematobium* infections.

### **Relationship between behavioural, socio-economic and environmental risk factors, and *S. haematobium* and STH infections**

The relationships between *S. haematobium* or STH infections and selected behavioural, socio-economic and environmental risk factors among the children were also assessed. Out of the 262 children recruited at baseline, 211 were interviewed with regard to their behavioural exposure (swimming, walking in flood water, use of latrines and eating fruits in the fields). Among these factors, only use of latrines was significantly negatively related to the prevalences of *T. trichiura* and *A. lumbricoides* ( $p = 0.029$  and  $p = 0.041$ , respectively) but not *S. haematobium* or hookworm infections. Similarly, only use of latrines was significantly negatively related to GMI of *A. lumbricoides* infections ( $p < 0.001$ ) but not any of the other three helminth species studied.

One hundred and ninety-six households were visited for studies on socio-economic risk factors (structure of housing, source of water for domestic use, social class, presence of latrines, parents' economic activity, and parents' education level) and geospatial distribution of the infections. Only 10 out of 192 households had pit latrines. There was a significant negative relationship between social class and the prevalence and GMI of hookworm infections ( $p = 0.003$  and  $p = 0.043$ , respectively). There was also a significant relationship between the GMI of *S. haematobium* infections and sources of water for domestic use ( $p = 0.009$ ) with children from households using swamps as the main source having higher GMI than those using river or wells as their main sources. No spatial clustering of *S. haematobium* and STH infections was observed in the two villages.

**Conclusion:** The findings of this study indicate that the two study villages were highly endemic for both *S. haematobium* and STH with the infections resulting in important morbidity in primary school-age children. Anaemia and low BMI were highly prevalent

among the children with *S. haematobium* and hookworm as the main parasitic causes of anaemia and *T. trichiura* and *A. lumbricoides* as the main parasitic causes of low BMI. During co-infections, there were some antagonistic interactions between *S. haematobium* and *T. trichiura* which resulted in lower intensities of *T. trichiura* infection in children with heavy *S. haematobium* infections and vice versa. On the contrary there were positive interactions between *S. haematobium* and hookworm which resulted in high intensities of hookworm infections in children with heavy *S. haematobium* infections and vice versa. The infections with *S. haematobium* resulted in urinary tract pathology that was clearly reflected by urinary ECP, IL-6 and IL-10 levels. Faecal ECP and EPX were poor markers of intestinal pathology due to STH, except hookworm, infections in the children. Combined praziquantel-albendazole treatment was highly effective against *S. haematobium* and STH during co-infections. The results suggested that *S. haematobium* may lead to an *S. haematobium* intensity-dependent enhancement of the effects of combined praziquantel-albendazole treatment against *T. trichiura* in co-infected children. Lack of toilets and prevailing unhygienic conditions in the study villages led to widespread environmental contamination with *S. haematobium* and STH eggs thus obviating the role of other socio-economic and environmental factors in transmission of these infections. On the basis of the findings from the present study, it is recommended the Kenyan Ministry of Public Health and Sanitation facilitates sustained control efforts in the area.

# **CHAPTER ONE: INTRODUCTION AND LITERATURE REVIEW**

## **1.1 Introduction**

### **1.1.1 Global Importance of Human schistosomiasis**

Human schistosomiasis or bilharziasis is a chronic disease caused by infection with one or more of the six species of digenetic trematodes, belonging to the genus *Schistosoma* and which are known to naturally infect humans (Chitsulo *et al.*, 2000; van der Werf *et al.*, 2003; Gryseels *et al.*, 2006; Webster *et al.*, 2006). It is estimated that schistosomes infect about 207 million individuals worldwide among an estimated at-risk population of 779 million people (Steinmann *et al.*, 2006; Molyneux, 2004; Hotez & Kamath, 2009). About 120 million of the infected individuals are asymptomatic whereas 20 million suffer severe disease, resulting in between 1.7 and 4.5 million disability-adjusted life-years (DALYs) (Chitsulo *et al.*, 2000; van der Werf *et al.*, 2003; Steinmann *et al.*, 2006). The disease is mainly confined to the resource-poor communities in tropical and sub-tropical regions. Sub-Saharan Africa bears about 93% of the global schistosomiasis burden with more than 192 million individuals or 25% of the total regional population already infected with schistosomes (Hotez & Kamath, 2009). School-age children account for the largest proportion of the infected individuals and those suffering severe morbidity in the region (Chitsulo *et al.*, 2000; van der Werf *et al.*, 2003; King *et al.*, 2005; Hotez & Kamath, 2009). It is important to note that the above figures are basically inferential, based on the findings of scattered studies over vast regions with unaccounted for inherent heterogeneities.

### **1.1.2 Global Importance of Soil Transmitted Helminthiasis**

Soil transmitted helminthiasis are caused by infections with helminth species whose eggs or larvae are found in soil. Such helminths include *Trichuris trichiura* and *Ascaris lumbricoides* which are transmitted through ingestion of infective eggs from contaminated soil, as well as hookworm (*Ancylostoma duodenale* and *Necator americanus*) and *Strongyloides stercoralis* transmitted through active skin penetration by infective larvae in the soil (Gilles, 1996). Three of these helminths namely; *T. trichiura*, *A. lumbricoides* and hookworm are the focus of the present study.

Human trichuriasis is caused by the nematode *Trichuris trichiura*, commonly known as whipworm, which usually resides in the caecum. The parasite has a cosmopolitan distribution and is estimated to infect over 1 billion individuals worldwide with 162 million of these residing in sub-Saharan Africa (Hotez & Kamath, 2009). It has been estimated to account for a loss of at least 6.4 million DALYs worldwide, 1.7 million of them in sub-Saharan Africa, (Supali *et al.*, 2010; Hotez & Kamath, 2009; Molyneux *et al.*, 2005). In total, *T. trichiura* is estimated to cause morbidity in over 46 million individuals, most of them children in sub-Saharan Africa, and cause over 10,000 deaths annually (WHO, 1998).

Hookworm disease in humans is mainly caused by two species of nematodes namely *Ancylostoma duodenale* and *Necator americanus* both of which have a worldwide distribution. Both species are prevalent in Kenya but *N. americanus* has been reported to be more prevalent in coastal East Africa (Ashford *et al.*, 1993; Stoltzfus *et al.*, 1997). It is estimated that about 1.3 billion individuals are currently infected with hookworm resulting in about 65,000 million deaths annually (WHO, 2002; Supali *et al.*, 2010). In sub-Saharan Africa, it is estimated that about 1.98 billion individuals, including 40 – 50 million school-age children, are infected with hookworm which results in a loss of about 7.5 million DALYs in the region against a global estimate of 22.1 DALYs (Hotez & Kamath, 2009).

Ascariasis in humans is caused by the largest human intestinal nematode, *Ascaris lumbricoides*. The parasite has a cosmopolitan distribution but the largest burden of disease due to it is borne by Asia, Africa and Latin America (O'Lorcain & Holland, 2000). It is estimated that there are over 1 billion people infected with the parasite, about 15% of whom suffer related morbidity, which results in a loss of over 10 million DALYs worldwide (O'Lorcain & Holland, 2000; Bethony *et al.*, 2006; Hotez & Kamath, 2009). In sub-Saharan Africa, the parasite infects over 173 million people, mainly school-age children, resulting in a loss of about 2.2 million DALYs lost to the infections (Bethony *et al.*, 2006). In total, *A. lumbricoides* infections are estimated to result in over 60,000 deaths (WHO, 1998).

## **1.2 Literature Review**

### **1.2.1 Human Schistosomiasis**

#### **1.2.1.1 Distribution of Human Schistosomes**

Two important schistosome species, in terms of public health, are *S. haematobium* and *S. mansoni*. The former mainly causes urogenital schistosomiasis and is widespread in many parts of Africa and the Middle East whereas the latter mainly causes intestinal form of schistosomiasis in Africa, the Middle East, the Caribbean, Brazil, Venezuela and Suriname (Gryseels *et al.*, 2006). Others include *S. intercalatum* and the related *S. guineensis*, which are endemic in the rain forest areas of Central Africa where they cause intestinal schistosomiasis (Webster *et al.*, 2006). *S. japonicum* and *S. mekongi* are endemic in Asia where they cause intestinal schistosomiasis mainly in China, Indonesia and the Philippines. Whereas the focus of *S. japonicum* in Asia is wide, *S. mekongi* is mainly restricted to the Lao People's Democratic Republic and some districts of Cambodia (Gryseels *et al.*, 2006).

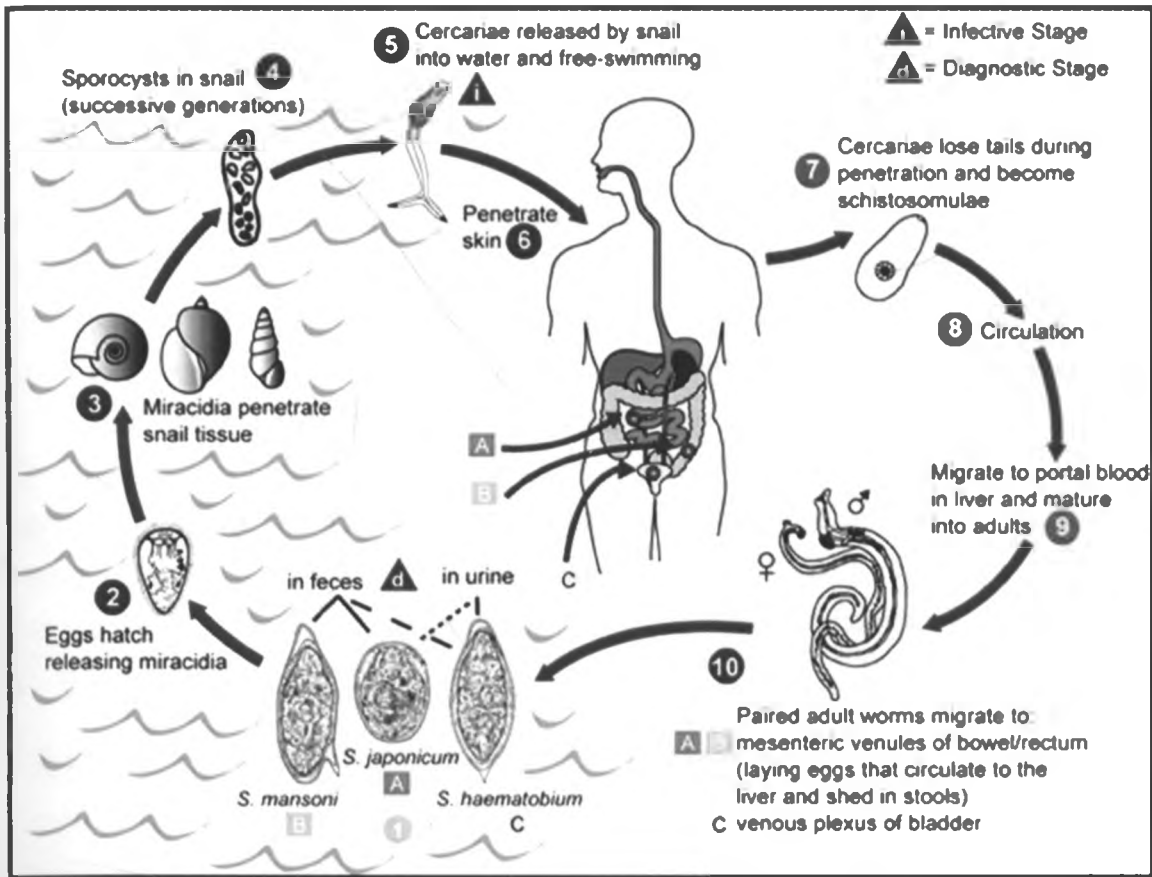
#### **1.2.1.2 Life Cycle of Human Schistosomes**

Figure 1.1 briefly describes the general life cycle of human schistosomes. The cycle has also been reviewed by Sturrock (1993) and Gryseels *et al.* (2009). In the human host, the adult male and female worms live in permanent copula, with the long slender female held

within the gynaecophoric groove of the short stout male. *S. haematobium* worm pairs reside in the venous plexus of the bladder, ureters and kidneys. Those of intestinal schistosomiasis-causing worms reside in the mesenteric vessels. The adult female worm, which measures between 7 and 20 mm in length, releases hundreds of eggs daily. It is estimated that one female *S. haematobium* worm can lay about 200 eggs daily and that of *S. mansoni* can lay 300 eggs daily whereas *S. japonicum* can lay 3,000 egg daily (Gryseels *et al.*, 2006). About 50% of these eggs are carried by blood and penetrate through the walls of the blood capillaries and the urinary tract or intestines where they are subsequently voided in urine (*S. haematobium*) or stool (*S. mansoni*, *S. japonicum*, *S. mekongi*, *S. intercalatum*). The rest of the eggs are transported by blood and lodged in various tissues in the body.

When the eggs reach fresh water, they hatch to release free-swimming miracidia that infect fresh water snails. If these miracidia infect the right snail species, *Bulinus* sp. for *S. haematobium* and *S. intercalatum*, *Biomphalaria* sp. for *S. mansoni* or *Oncomelania* sp. for *S. japonicum*, each miracidium develops into a sac-like stage called sporocyst with numerous germinal cells. Each sporocyst forms numerous daughter sporocysts each of which forms numerous cercariae, the stage infective to humans. This asexual reproduction results in enormous magnification of the number of parasites emanating from infection of the snail host with just one or just a few miracidia. About five weeks after snail infection with miracidia, cercariae escape from the host snail through the soft body parts into the water where they percutaneously infect the vertebrate host including humans.

Following penetration of human skin by cercariae, the parasites transform to schistosomulae within the dermal tissues and enter the blood circulation. The young worms migrate in blood through the lungs and eventually to the liver where they mature in 4 to 6 weeks. Male and female worms pair up, mate and migrate through blood to the perivesicular region or mesenteric region for *S. haematobium* and other schistosomes, respectively. The female worm starts producing eggs and may continue doing so for 3 to 5 years in untreated individuals leading to chronic schistosomiasis and even death (Gryseels *et al.*, 2006).



**Figure 1.1:** Life cycle of *Schistosoma* spp.  
 (Adopted from CDC; <http://www.dpd.cdc.gov/dpdx/html/schistosomiasis.htm>; accessed on 25th June 2011)

### 1.2.1.3 Human Schistosomiasis in Kenya

The distribution of *S. haematobium* and *S. mansoni* infections in Kenya is shown in Figure 1.2. In Kenya two species, *S. haematobium* and *S. mansoni*, are found. Infections with these parasites have been reported in all Counties, with the Tana River County having the highest prevalence of *S. haematobium* infections (WHO, 1987; Brooker *et al.*, 2009). Transmission of *S. mansoni* does not occur in the coastal zone except in a small focus in Taveta District. Several studies on *S. haematobium* infections have been carried out at the coast spanning from Msambweni District in the south to Malindi District in the north. This has resulted in a wealth of information regarding, among other aspects, transmission patterns (Clennon *et al.*, 2004; Clennon *et al.*, 2006), morbidity (Kahama *et al.*, 1999a; King *et al.*, 2004; Wamachi *et al.*, 2004) and the effects of treatment (King, 2006) among schoolchildren. On the other hand, information on *S. haematobium* infections in the three districts to the north of Malindi namely; Tana Delta, Lamu West and Ijara is restricted to prevalence figures obtained mainly from brief reports of the prevalences by the then Division of Vector Borne Diseases (DVB & NTD), Ministry of Health, (WHO, 1987; Brooker *et al.*, 2009).



#### **1.2.1.4 Schistosoma haematobium**

About 112 million individuals, mostly school-age children, in sub-Saharan Africa alone are estimated to be infected with *S. haematobium* and a considerable number of them are suffering significant morbidity (Gryseels *et al.*, 2006; Hotez & Kamath, 2009; Rollinson, 2009). Moreover, of the 300,000 annual deaths attributable to schistosomiasis in sub-Saharan Africa, 150,000 are due to infections with *S. haematobium* thus underlining the importance of the parasite in the region (Rollinson, 2009). However, compared to *S. mansoni* infections, little is understood about epidemiology, immunology and pathology of *S. haematobium* infections (Rollinson, 2009). For example, the parasite is widely distributed along the entire Kenyan coast but most of the information about it emanates from studies in the southern coast as very little is documented about the infections in the northern parts of the coast (WHO, 1987; Brooker *et al.*, 2009). This is important because the two regions are not only environmentally different but the communities living in them are also culturally different.

#### **1.2.1.5 Risk Factors for Infection with S. haematobium**

The risk factors for infections with schistosomes are such as climatic, biological, political, demographic, economic, social, and cultural factors (Bruun & Aagard-Hansen, 2008). For example, being a water-borne disease, schistosomiasis is endemic among resource-poor communities characterised by low socio-economic and hygiene standards compounded by intense water contact during agricultural or recreational activities (Steinmann *et al.*, 2006). The effect of demography on schistosomiasis has been highlighted by various studies that have shown that in many endemic communities age, sex and gender are strongly related to infective behavior and therefore have a significant effect on infection prevalence (Bruun & Aagard-Hansen, 2008). Among children in most endemic communities, swimming is one of the most important factors predisposing to infections with schistosomes (Handzel *et al.*, 2003; Clennon *et al.*, 2004). This may result in boys, who have a higher tendency to play and swim in local vector snail-infested water bodies compared to girls, of the same age group in an area.

#### **1.2.1.6 Pathology Associated with S. haematobium Infections**

Early stages of infections with *S. haematobium* are associated with non-specific signs and symptoms like dermatitis, fever, fatigue, myalgia, malaise, non-productive cough, and eosinophilia (Gryseels *et al.*, 2006; Meltzer *et al.*, 2008). Adult female worms of *S. haematobium* release hundreds of eggs in the venous plexus of the bladder, ureters and kidneys every day. About half of the eggs manage to penetrate the capillaries and urinary tract walls resulting in bleeding lesions that manifest as haematuria during heavy *S. haematobium* infections. The rest of the eggs are deposited in tissues mainly in the

bladder wall, ureter and kidneys where they provoke granulomatous inflammation, ulceration, and pseudopolyposis (Chen & Mott, 1989; Smith & Christie, 1986). The resultant morbidity leads to dysuria, frequent micturation, proteinuria, eosinophiluria and haematuria (Gryseels *et al.*, 2006; Reimert *et al.*, 2000). In advanced stages, urinary schistosomiasis is strongly associated with renal failure, bladder carcinomas and even death (Rollinson *et al.*, 2009; Gryseels *et al.*, 2006). Chronic urinary schistosomiasis may also cause other non-specific health problems like anaemia, weight loss, growth retardation and cognitive impairment (Gryseels *et al.*, 2006). However, for reasons that are not well understood, only a few infected individuals have heavy infection intensities and suffer overt morbidity in any endemic setting (van der Werf *et al.*, 2003). It is hypothesized that concurrent infections with other helminths can at least partially account for this variation (Wamachi *et al.*, 2004; King *et al.*, 2001).

Infections with helminths are characterised by profound antibody and cytokine immune responses which play a key role in pathology development (Moreau & Chauvin, 2010). However, it is cytokine responses that play a major role in resistance as well as pathology of helminth infections (MacDonald *et al.*, 2002; Maizels *et al.*, 2004; Moreau & Chauvin, 2010). Helminth-induced cytokines are broadly categorized into: 1) the pro-inflammatory Th<sub>1</sub> cytokine responses characterised by elevated production of interleukin (IL)-2, tumour necrosis factor (TNF)- $\alpha$ , and interferon (IFN)- $\gamma$ , and 2) anti-inflammatory Th<sub>2</sub> responses characterised by significant production of IL-4, IL-5 and IL-10 (Maizels *et al.*, 2004). In *S. mansoni* infections, there is pronounced pro-inflammatory Th<sub>1</sub> cytokine responses during the acute phase of infections (de Jesus *et al.*, 2002). This shifts to anti-inflammatory Th<sub>2</sub>-type responses during the chronic phase at the onset of egg deposition in tissues (Araujo *et al.*, 1996; Corrêa-Oliveira *et al.*, 1998). A cytokine profile that is similar to that observed in *S. mansoni* infections has been suggested in infections with *S. haematobium* in a Zimbabwean population (Mduluzza *et al.*, 2003). However, this Th<sub>1</sub>/Th<sub>2</sub> dichotomy is not always obvious among *S. haematobium* or *S. mansoni* endemic populations as a mixture of both Th<sub>1</sub> and Th<sub>2</sub> cytokine responses has been observed in some individuals and both are thought to play an important role in pathological development as well as mediating resistance to infection (Abath *et al.*, 2006; Corrêa-Oliveira *et al.*, 1998). The reasons for this lack of clear-cut dichotomy among endemic populations are not clear.

#### **1.2.1.7 Urinary Schistosomiasis Morbidity Assessment**

In heavy infections, haematuria resulting from haemorrhage due to damage on the urinary tract mucosa is an indirect indicator of *S. haematobium* infection (Gryseels *et al.*, 2006). Haematuria is particularly useful in highly endemic areas where large scale intervention activities involving chemotherapy are being undertaken (Savioli *et al.*, 1990). Specific laboratory diagnosis of urinary schistosomiasis is based on microscopic

examination of urine for *S. haematobium* eggs either through centrifugation or filtration using polycarbonate filters (Cheesbrough, 1998; WHO, 1998). In field studies and control programmes, urine filtration technique has been the method of choice for many years owing to the fact that it is quantitative whereas centrifugation is preferred for routine medical laboratory diagnosis (Cheesbrough, 1998; WHO, 1998). However, this method has the disadvantage in that it is less sensitive when the infection intensities are low (Bergquist *et al.*, 2009).

Different methods are used to assess different forms of morbidity due to infections with *S. haematobium*. It is believed that almost half of schistosome eggs are lodged in host tissues where they invoke inflammation and thus morbidity (Chen & Mott, 1989; Smith & Christie, 1986). Schistosome egg counts have therefore been used as indirect indicator of the level of morbidity due to *S. haematobium* infection (Leutscher *et al.*, 2008). Tissue-lodged *S. haematobium* eggs also invoke inflammation and production of cytotoxic compounds like the eosinophil cationic protein (ECP) high levels of which are also found in the urine of infected individuals (Reimert *et al.*, 2000; Leutscher *et al.*, 2000). Urinary ECP is therefore considered a useful tool to study morbidity due to *S. haematobium* infection, especially during early pathology (Reimert *et al.*, 2000; Leutscher *et al.*, 2000; Vennervald *et al.*, 2000). However, use of urinary ECP may not reveal late stage morbidity when the eggs in tissues are dead and fibrosis and calcification have set in. This and the resultant organ deformation are best visualized directly using ultrasonography, which on the other hand, may not be able to accurately reveal early stage urinary tract morbidity unlike ECP (Hatz *et al.*, 1990; Vennervald *et al.*, 2000; Leutscher *et al.*, 2000).

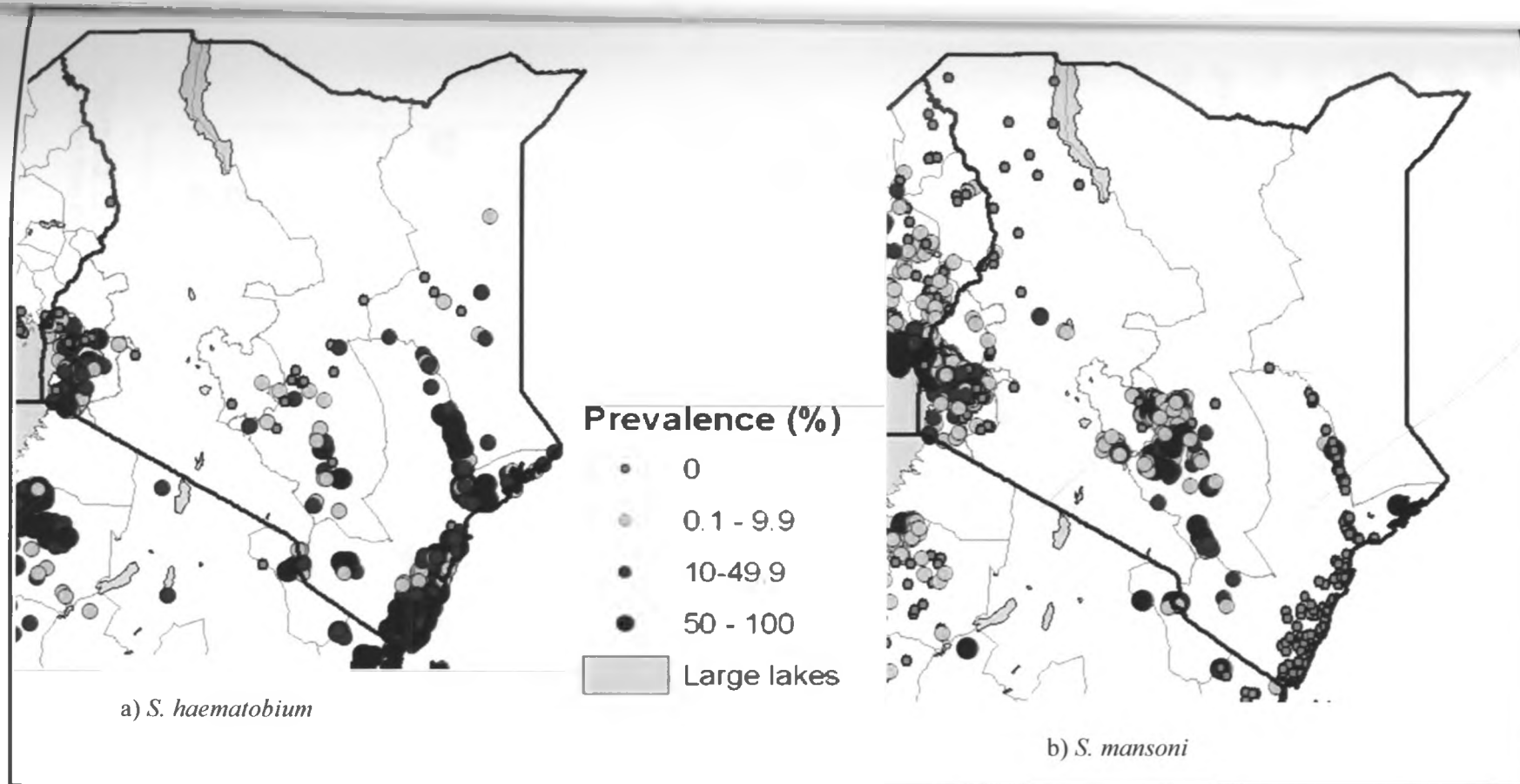
#### **1.2.1.8 Treatment and Control of *S. haematobium* Infections**

Currently, praziquantel is the only drug that is recommended for the treatment of infections with *S. haematobium* (Chitsulo *et al.*, 2000; Danso-Appiah *et al.*, 2009). Until recently, metrifonate was also important in treatment of *S. haematobium* infections but it is no longer in use due to its low efficacy (Danso-Appiah *et al.*, 2009). Other drugs that have previously been used to treat *S. haematobium* infections, but they have been abandoned because of poor effect or adverse events, are such as antimonials, niridazole, lucanthone, hycanthone, oltipraz, cyclosporin A, levamisole, and Oxamniquine (Danso-Appiah *et al.*, 2009). Praziquantel is given as a single standard dose of 40 mg/ kg body weight (Danso-Appiah *et al.*, 2009). The drug is highly effective against *S. haematobium* infections but re-infection rates can be high (N'Goran *et al.*, 2001; Danso-Appiah *et al.*, 2009; King *et al.*, 1989; King, 2006).

Chemotherapy has been the mainstay of schistosomiasis control for a long time. In Kenya, praziquantel has been widely used in control of infections with *S. haematobium*

(King, 2006; King *et al.*, 1989; Magnussen, 2003). The drug is highly effective against not only *S. haematobium* but its associated early stage morbidity as well (Koukounari *et al.*, 2007; King *et al.*, 1989). However, for reasons that are not yet clear the outcomes of mass treatment in endemic populations are highly heterogeneous and even long-term mass drug administration may not stop transmission in highly endemic areas (Bethony *et al.*, 2006; King, 2006).

The chronology of early efforts to control schistosomiasis is highlighted in the treatise by Davis (2000). These activities mainly involved vector snail control, with chemotherapy using trivalent antimonials playing a supplementary role. Using these methods, Japan and the Caribbean Islands of St. Lucia managed to control the disease and some other countries like the Dominican Republic, Iran, Puerto Rico, Venezuela, and the Philippines also managed to reduce schistosomiasis to levels of low public health importance (Davis, 2000). Trivalent antimonials were highly toxic which made chemotherapy unpopular but in the late 1970s, schistosomiasis control received a major boost by the discovery of praziquantel as a safe and effective schistosomicide (McMahon & Kastrup, 1979). It was demonstrated that treatment of school age children reduced *S. haematobium* egg output by over 90% within a short time (King, 1988; Warren, 1982) and attention was subsequently turned on chemotherapy as the mainstay of schistosomiasis control. However, little success was achieved in transmission control although it was demonstrated that multiple treatments during childhood are associated with lower levels of *S. haematobium*-associated morbidity in adulthood for several years despite intervening re-infection (Ouma *et al.*, 2005). This led to a change of paradigm and more efforts were subsequently focused on morbidity control, especially in schoolchildren (Stothard *et al.*, 2009; WHO, 2002). In 2001 the WHO recommended that by the year 2010, regular treatment be offered to between 75% and 100% of all school-aged children and those at risk of morbidity due to schistosomiasis (Stothard *et al.*, 2009).



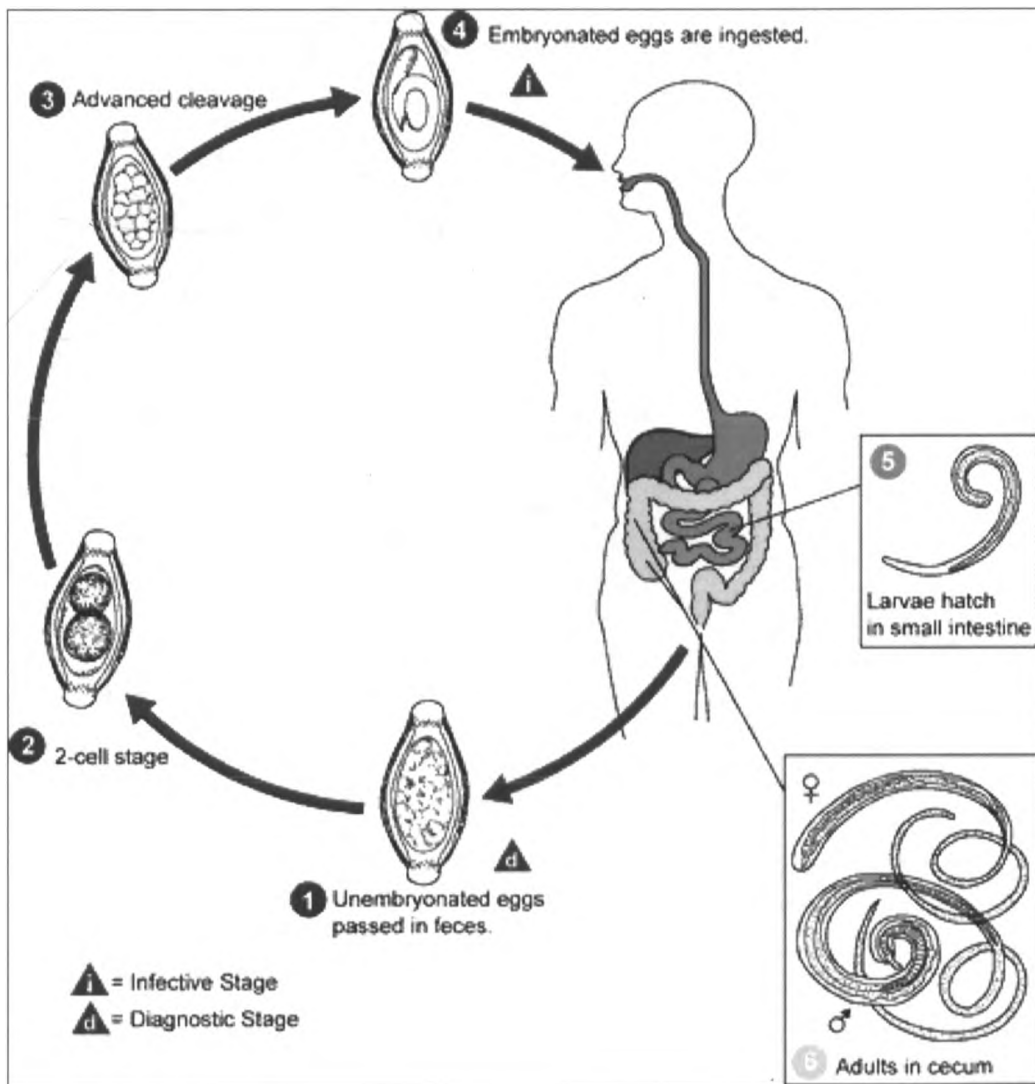
**Figure 1.2:** Map of the distribution of *S. haematobium* and *S. mansoni* infections in Kenya.

Adopted from Brooker *et al.* (2009)

### 1.2.3 Soil Transmitted Helminthiasis

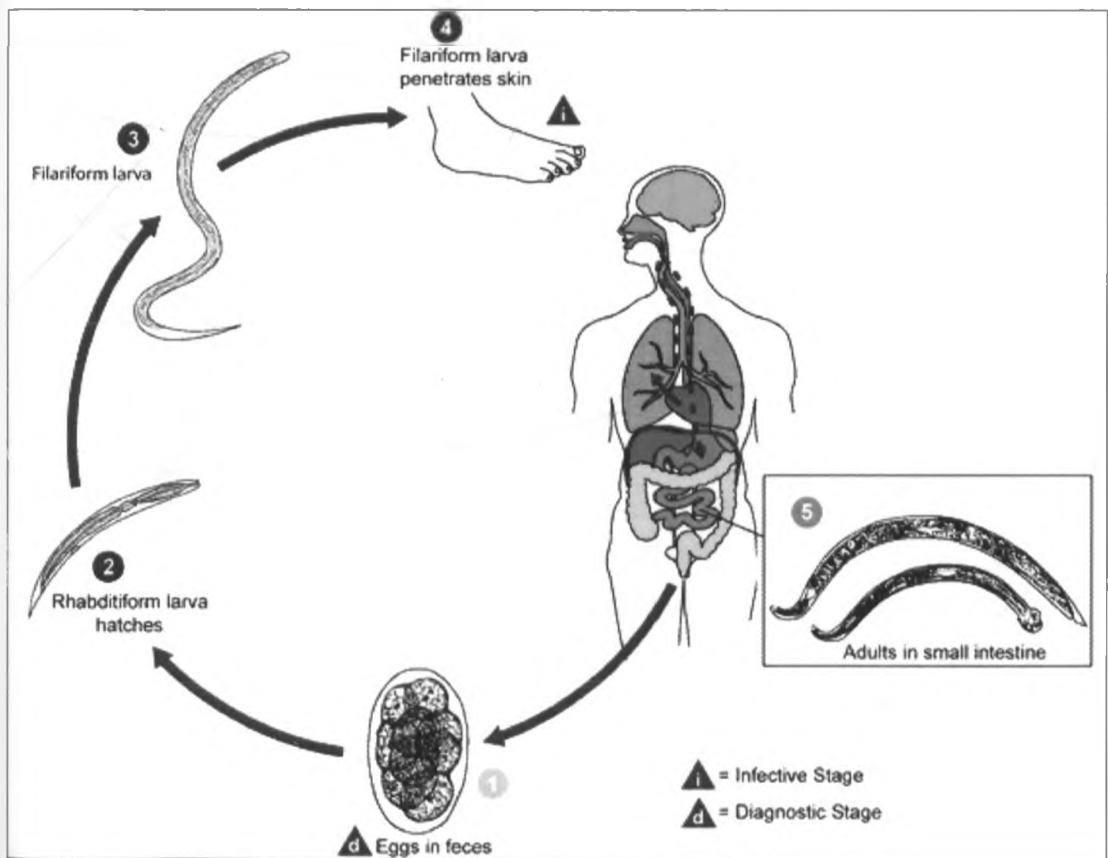
#### 1.2.3.1 Life Cycles of *Trichuris trichiura*, hookworm and *A. lumbricoides*

The life cycles of *T. trichiura* is described in Figure 1.3. After mating, the female worm lays between 3,000 and 5,000 unembryonated characteristically barrel-shaped eggs per day for about 2 years (Bethony *et al.*, 2006). These eggs are voided to the environment together with human faeces. Under ideal conditions such as shaded moist soil and warmth, the eggs are embryonated and become infective within three weeks. Humans are infected after ingesting embryonated eggs. The first-stage larvae hatch and burry themselves in the mucosa of the small intestine before moving to the caecum, where they grow and mature to adults. This cycle takes 30 to 90 days and the adult worms may live in the human host for several years (Stephenson *et al.*, 2000b; Bethony *et al.*, 2006).



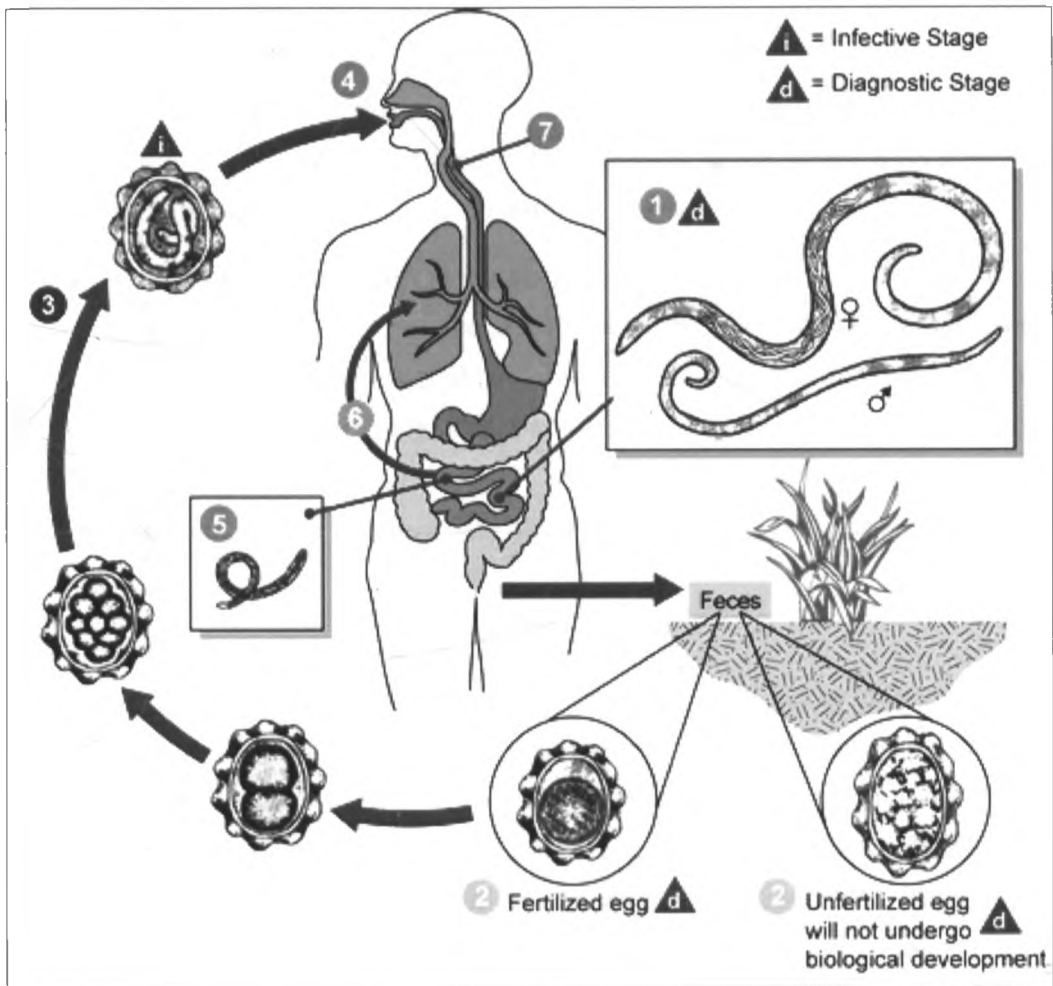
**Figure 1.3:** Life cycle of *T. trichiura* (adopted from CDC; <http://www.dpd.cdc.gov/dpdx/html/Trichuriasis.htm>; accessed on 25<sup>th</sup> June 2011)

The life cycle of hookworms is shown in Figure 1.4. The female worm releases eggs in the intestines of the host. The eggs hatch within 24 to 48 hours and larvae moult twice in warm, shaded, moist, and well-aerated soil. The resultant 3<sup>rd</sup> stage larvae penetrate the skin of humans on contact with infested soil. The larvae access the blood circulation system via sub-cutaneous venules and lymphatic vessels. Humans can also be infected with *A. duodenale* through ingestion of the 3<sup>rd</sup> stage larvae. They are transported by blood to the right side of the heart and then to the pulmonary vasculature. They subsequently rupture the capillaries and access the parenchyma through which they ascend the respiratory tree to the trachea. From the trachea, they are coughed and swallowed thus entering gastrointestinal tract (Hotez *et al.*, 2004). In the intestines, the worms mature to adults in between 5 and 9 weeks. After mating, the female starts laying eggs which are voided together with faeces by the host. It is estimated that one *A. duodenale* female worm can lay up to up to 30,000 eggs whereas *N. americanus* can lay up to 10,000 eggs every day for up to 7 years (Bethony *et al.*, 2006).



**Figure 1.4:** Life cycle of hookworm.  
 (adopted from CDC; <http://www.dpd.cdc.gov/dpdx/html/Hookworm.htm>, accessed on 25<sup>th</sup> June 2011)

The life cycle of *A. lumbricoides* is shown in Figure 1.5. Human infection with *A. lumbricoides* occurs when they ingest fully developed eggs. The eggs hatch in the intestine and the larvae penetrate the intestinal mucosa from where they access the blood circulation. They enter the liver and migrate through blood to the lungs. They subsequently break the lung capillaries into alveoli. They ascend the trachea and pass over the epiglottis where they are coughed and swallowed. They move down the oesophagus to return to the intestine where they mature into adult worms and, after mating, the female worm starts laying eggs (Mahmoud, 1990; Surendran & Paulose, 1988; Markell *et al.*, 1992). One female worm can lay as many as 200,000 eggs per day for 1 year (Bethony *et al.*, 2006).



**Figure 1.5:** Life cycle of *A. lumbricoides*  
 (Adopted from CDC; <http://www.dpd.cdc.gov/dpdx/html/Ascariasis.htm>; accessed on 25<sup>th</sup> June 2011).

### 1.2.3.2 Pathology Associated with *T. trichiura*

Light infections with *T. trichiura* are usually asymptomatic but moderate and heavy infections can result in Trichuris dysentery syndrome, characterized by mucoid diarrhea,



rectal bleeding, rectal prolapse, iron deficiency anaemia and finger clubbing (WHO, 2002; de Silva, 2003). Other subtle health problems related to infections with *T. trichiura* are growth retardation and reduced cognitive abilities (WHO, 2002; Bethony *et al.*, 2006; Stephenson *et al.*, 2000b). Most of this morbidity is as a result of host intestinal inflammatory reaction to the anterior end of the adult *T. trichiura* embedded in the epithelial tunnels of the mucosa (Gilles, 1996; Stephenson *et al.*, 2000b).

### **1.2.3.3 Soil Transmitted Helminthiasis in Kenya**

The distribution of infections with *T. trichiura* and hookworm in Kenya is shown in Figure 1.6 and that of infections with *A. lumbricoides* is shown in Figure 1.7. Previous studies indicate that STH infections are widely distributed throughout the country from Western to Coastal Kenya where they have been associated with considerable anaemia among the residents (Hall *et al.*, 1982; Olsen, 1998; Magnussen *et al.*, 1997; Koukounari *et al.*, 2008; Brooker *et al.*, 2009). However, except for the prevalence data presented by Brooker *et al.* (2009), not much information is available to the scientific world about STH infections and related effects on health in northern part of coastal Kenya.

### **1.2.3.4 Pathology Associated with Hookworms**

Hookworms cause considerable host tissue inflammation during larval migration through tissues. Adult hookworm in the lumen of the intestines lacerate the intestinal mucosa resulting in bleeding thus causing significant anaemia, especially iron deficiency anaemia, (Crompton, 2000; Stephenson *et al.*, 2000a). They also cause enzymatic damage to the intestinal mucosa which may interfere with absorption resulting malnutrition (MacLeod, 1988).

### **1.2.3.5 Pathology Associated with *A. lumbricoides***

Like hookworms, *A. lumbricoides* cause considerable host tissue inflammation during larval development. The adult *A. lumbricoides* reside in the small intestine of the human host and depending on host background health status, intensity and duration of infection the worms may not cause any serious disease (O'Lorcain & Holland, 2000). However, when the environment in the intestines becomes unfavourable, such as during inflammation and obstruction, the adult worms can migrate to other less hostile parts leading to serious intra-abdominal complications such as biliary obstruction, cholangiohepatitis, liver abscess, acute appendicitis, pancreatitis, intestinal perforation, and granulomatous peritonitis (Mahmoud, 1990; Surendran & Paulose, 1988; Markell *et al.*, 1992). Heavy infections with *A. lumbricoides* have also been associated with significant malnutrition, especially in children (Bethony *et al.*, 2006; Mahmoud, 1990; Stephenson *et al.*, 2000a).

#### **1.2.3.6 Immune Responses and Inflammation Soil-Transmitted Helminths**

Infections with STH result in evidently strong host cytokine responses especially the strongly anti-inflammatory IL-10. Elevated Th<sub>2</sub> and IL-10 responses have been found to occur in individuals infected with *A. lumbricoides* as opposed to non-infected individuals in endemic areas (Cooper *et al.*, 2008; 2000). The importance of these cytokine responses is suggested by the findings that weak Th<sub>2</sub> responses predispose individuals to persistent infections and re-infections with *T. trichiura*, *A. lumbricoides* and hookworms (Jackson *et al.*, 2004). However, like in infections with schistosomes, at times there is a lack of clear Th<sub>1</sub> and Th<sub>2</sub> cytokine responses dichotomy during infections with STH. For instance, one *in vitro* study showed that stimulation of peripheral blood mononucleocytes from individuals infected with *T. trichiura* can result in mixed Th<sub>1</sub> and Th<sub>2</sub> cytokine responses (Faulkner *et al.*, 2002). A similar phenomenon has been observed in some individuals infected with hookworms (Quinnell *et al.*, 2004). From the foregoing, it can conceivably be stated that co-infections with other helminths may play an important role in co-endemic populations.

#### **1.2.3.7 Risk Factors for Infection with Soil Transmitted Helminths**

Like schistosome infections, STH infections are more prevalent among socio-economically deprived communities with low hygiene standards (Stephenson *et al.*, 2000a; Crompton, 2000; O’Lorcain & Holland, 2000). The transmission of *T. trichiura* and *A. lumbricoides* depends on the host ingesting embryonated eggs from the environment. As a result, some hygiene behaviours such as not washing hands before eating and not washing foodstuffs like fruits or vegetables with clean water promote the transmission of the two helminths (Idowu & Rowland, 2006; Olsen, 1998). Humans contract hookworm infections through percutaneous infection by the infective larval stage when their skin come into contact with soil harbouring infective larvae and environmental factors such as the type of soil, moisture and temperatures are known to play a significant role in determining the transmission of STH (Mabaso *et al.*, 2003; Lilley *et al.*, 1997).

One common starting point in the transmission of both schistosomes and STH is that eggs from infected individuals must first reach the environment. This means the absence of or improper use of latrines in a community can significantly increase transmission of both schistosomes and STH.

#### **1.2.3.8 Assessment of STH-related Morbidity**

Infections with STH are usually diagnosed by microscopic examination of stool for eggs. There are different methods such as direct smear or egg concentration (centrifugation) techniques that are preferred for routine laboratory diagnosis (Cheesbrough, 1998). In addition, since morbidity of STH infections is related to the intensities of infections,

quantitative examination of STH eggs in stool is also used to assess STH-related morbidity (Tarafder *et al.*, 2010).

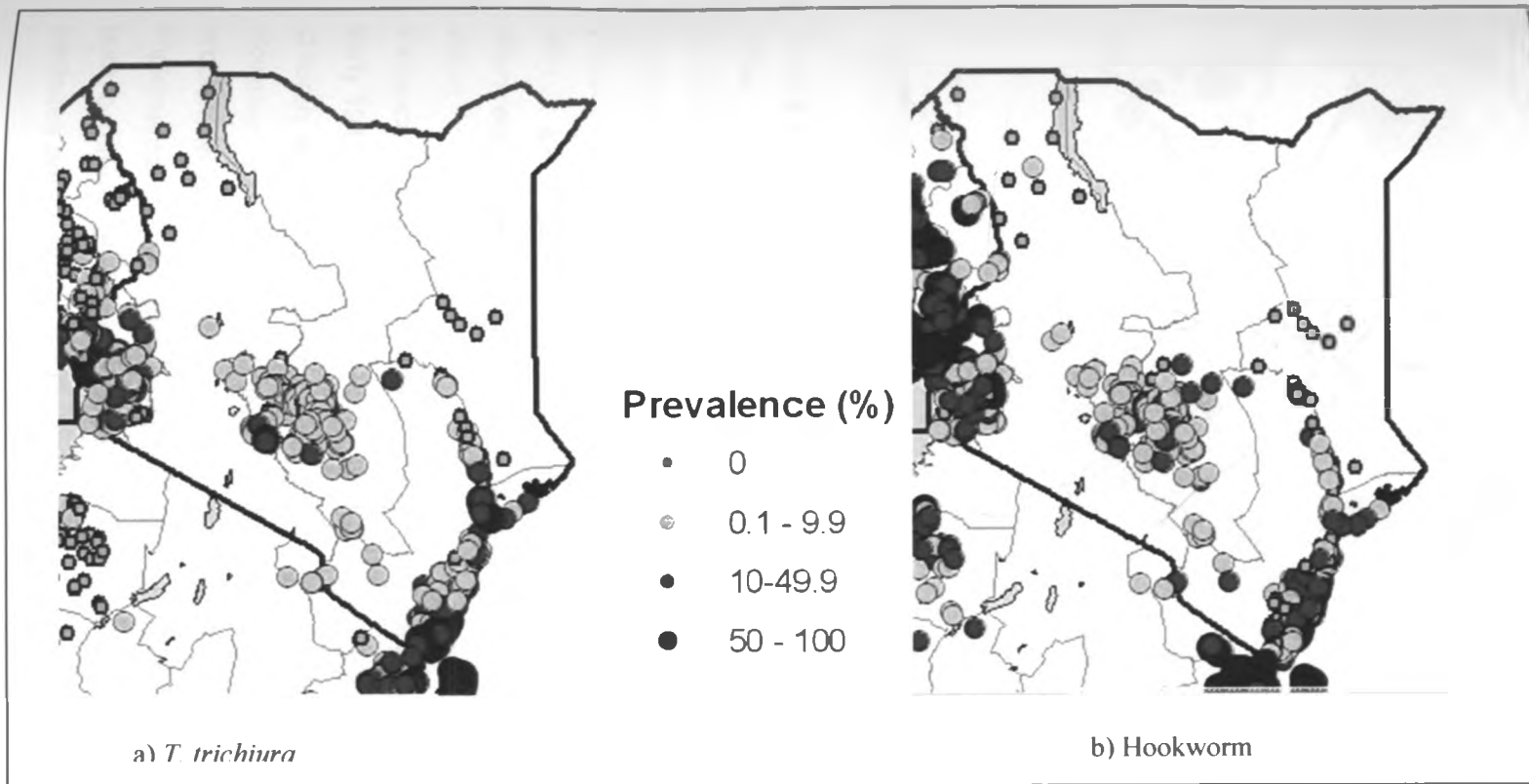
Kato-Katz technique, a quantitative technique in which a measured amount of stool is smeared on a microscope slide, covered with a cellophane cover slip and examined microscopically and eggs counted, is preferred for field studies and evaluation of control programmes (Cheesbrough, 1998; WHO, 1998; Tarafder *et al.*, 2010).

STH infections are mainly characterised by insidious non-specific morbidity. As a result, no morbidity assessment tools have been developed specifically for STH-related morbidity. STH-related morbidity is usually dependent on, among other factors, infection intensity (O'Lorcain & Holland, 2000; WHO, 2002). Egg counts can therefore be an indirect measure of infection intensity and morbidity in general although the interpretation needs to be done with caution as sometimes, such as during heavy infections, egg counts can significantly under-estimate the worm load (O'Lorcain & Holland, 2000). STH-related anaemia can be assessed by the usual methods such as use of a portable haemoglobinometer (Marino *et al.*, 2011; Koukounari *et al.*, 2008). Growth retardation and lower body weight due to STH infections have previously been assessed by taking anthropometrical measures using a stadiometer for height and weighing scale for weight (Koukounari *et al.*, 2008).

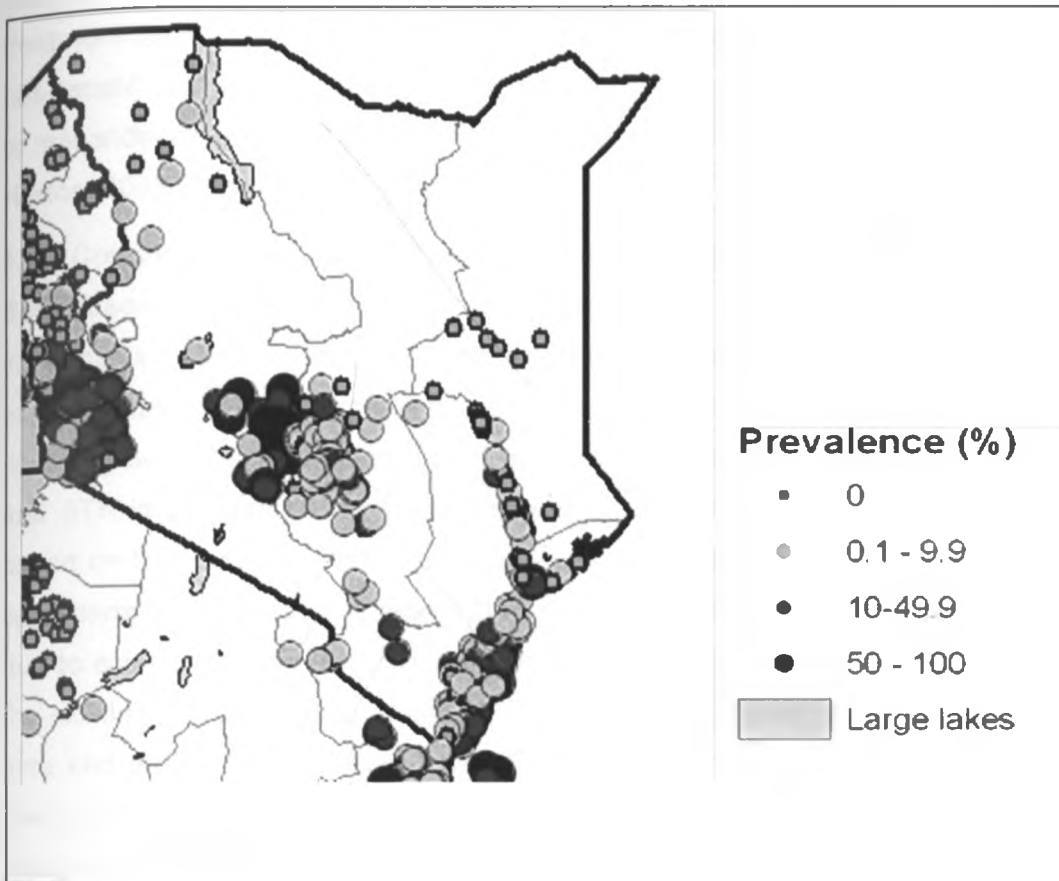
Endoscopy has been used to visualize intestinal inflammation due to *T. trichiura* infections (Khuroo *et al.*, 2010) and may be useful in infections with other STH, particularly hookworm. This method is, however, laborious and expensive in terms of skilled personnel and equipment in addition to being highly invasive. It is therefore only suitable for hospital-based investigations and not for field studies. Owing to these technical difficulties, among others, intestinal inflammation due to STH infections has not been extensively studied.

Intestinal inflammation due to infections with *S. mansoni* has been previously assessed using biochemical markers of inflammation such as eosinophil-derived ECP and eosinophil protein X (EPX) (Reimert *et al.*, 2008). Other intestinal helminths such as hookworm have also been shown to induce high levels of serum ECP and EPX and urinary EPX (Tischendorf *et al.*, 2000). Use of faecal ECP and EPX as biochemical markers of intestinal inflammation due to STH infections may therefore improve our understanding of the less well-studied STH-related intestinal morbidity.

It is clear that STH infections may result in considerable intestinal inflammation, which in turn influences the host's nutrition status (Stephenson *et al.*, 2000b). However, this aspect of the infections has not been given much prominence compared to the anaemia and generalized malnutrition caused by STH infections.



**Figure 1.6:** Map of the distribution of *T. trichiura* and hookworm infections in Kenya.  
Adopted from Brooker *et al.* (2009)



**Figure 1.7:** Map of the distribution of *A. lumbricoides* and hookworm infections in Kenya  
 Adopted from Brooker *et al.* (2009)

### 1.2.3.9 Treatment and Control of Soil transmitted Helminth Infections

Albendazole and mebendazole are the two drugs of choice for treatment of infections with the common STH (Bethony *et al.*, 2006). Albendazole is given as a single 400 mg dose to individuals aged two years and above whereas mebendazole is given as a 500 mg dose (Olsen, 2007; Keiser & Utzinger, 2008). These drugs are highly effective against infections with *A. lumbricoides* and hookworms but infections with *T. trichiura* are less tractable, often requiring more than one annual dosage (de Silva, 2003; Olsen, 2007; Adams *et al.*, 2004).

Evidence of efforts to control STH as a public health problem in Africa dates back to the early 1913 when attempts to examine and treat all infected primary and secondary school children against hookworm infection in Egypt were made (Davis, 2000; WHO, 2002). However, soil transmitted helminthiasis has for decades remained a neglected health problem in many countries of sub-Saharan Africa. One way of controlling STH is by implementation of hygiene measures such as proper sewage disposal but this is difficult in poor communities without clean water supplies and sanitary facilities and where the parasites are endemic (WHO, 2002). The other option is treatment of infected individuals

with appropriate drugs. A compelling wealth of information demonstrating the benefits of treatment against STH infections among children has accumulated over several years and has recently led to the advocacy for mass treatment programmes based on albendazole or mebendazole in endemic areas in order to alleviate STH-related morbidity (WHO, 2002; Horton, 2003; Bethony *et al.*, 2006).

#### **1.2.4 Combined Mass Administration of Praziquantel and Albendazole in Control of Schistosomiasis and Soil Transmitted Helminths**

Implementing and sustaining large-scale mass drug administration programmes is expensive and exerts enormous demands on weak economies of endemic countries. This cost can be considerably reduced if treatment programmes that target schistosomiasis and STH in co-endemic areas are integrated. School-based treatment programmes are based on four cardinal points namely: 1) schistosomiasis and STH are co-endemic over large parts of the developing world; 2) school-aged children are at highest risk of morbidity due to schistosomiasis and STH; 3) school-age children can readily be reached through the existing education system and; 4) praziquantel and albendazole or mebendazole are safe and effective drugs (both when given individually and in combination) that can be easily administered in large-scale with the assistance of school teachers. Owing to these observations, WHO in 2001 endorsed the World Health Assembly (WHA) Resolution 54.19 urging member states to attain a minimum target of regular mass administration of praziquantel and albendazole/ mebendazole to between 75% and 100% of all school-age children at risk of morbidity due to infections with schistosomes and STH by the year 2010 (WHO, 2002).

The control efforts are likely to be long-term and will exert enormous demands on limited national resources. Information that will allow the design of control programmes and optimization of the benefits to the endemic communities while reducing the cost is therefore very important (Utzing *et al.*, 2009). For example, heterogeneities in the distribution of and response to treatment by helminthes have been observed during different studies (Clennon *et al.*, 2004; Clennon *et al.*, 2006; Howard *et al.*, 2002; WHO, 2002). Unraveling the causes of these heterogeneities is key to the design of targeted control measures that will help circumvent the drawbacks posed by such heterogeneities in optimizing the results of mass drug administration programmes. Identification of the most highly afflicted communities in a country is an important step towards realization of this goal. Secondly, owing to the extensive co-occurrence of schistosomes and STH, and co-infections with these worms, there is a need to understand the interaction between them, and how these interactions influence disease distribution. Thirdly, it is important to understand how these parasite interactions influence their response to combined praziquantel-albendazole treatment.

### 1.2.5 Effects of Co-infection with Helminth on Infection Intensities

Several field studies have been carried out on different aspects of infections such as distribution of infections, infection intensities, immune responses to parasites, and morbidity due to infections with a single parasite species in isolation (Supali *et al.*, 2010). As a result, many individuals are usually co-infected with more than one species of parasites in co-endemic areas but the resultant inter-specific interactions have not been considered.

A few studies that have considered inter-helminth species interactions have mainly focused on infection intensities, as measured by egg output, in co-infected individuals. For example, Chamone *et al.* (1990) reported increased *S. mansoni* egg output in subjects co-infected with *S. mansoni* and hookworm among adults in a rice farming community in Brazil. The same study reported increased egg output in individuals co-infected with only two STH species, in the absence of *S. mansoni*, but a reduced egg output in individuals co-infected with the three STH species (*T. trichiura*, *Ancylostoma duodenale* and *Ascaris lumbricoides*). Another study among school children in Brazil reported increasing prevalence and intensity of *S. mansoni* infections with increasing number of STH species in concurrently infected schoolchildren aged between 7 and 17 years (de Cassia Ribeiro Silva *et al.* (2007). However, it is noteworthy that except for the 65% prevalence of *A. lumbricoides* infections reported by Chamone *et al.* (1990), the prevalence of the *S. mansoni* and the other STH reported in these two Brazilian studies were low (below 50%). Another study by Booth *et al.* (1998a) among schoolchildren in an area that is co-endemic for *S. mansoni*, *S. haematobium* and STH, in Tanzanian, found little or no relationship in infection intensities between *S. mansoni* and STH infections. The infection intensities among the children were generally low regardless of co-infection status. In support of these findings, another study among children and adults in Brazil indicated that individuals co-infected with *S. mansoni* and *A. lumbricoides* had lower infection intensities than individual infected with only one of the two species, which suggested that there might be some antagonism between *S. mansoni* and *A. lumbricoides* (Fleming *et al.*, 2006).

Not many studies have investigated the relationship between *S. haematobium* and STH infections as compared to the relationship between *S. mansoni* and STH. Tchuem-Tchuente *et al.* (2003) reported a positive but weak correlation in prevalence of *S. haematobium* and *A. lumbricoides* but not *T. trichiura* infections among school children in one region of Cameroon. However, in this study analyses for the association between *S. haematobium* and hookworm were not carried out although hookworm infections were detected in a few children. In Tanzania, no significant associations in infection prevalence and intensities were observed between *S. haematobium* and STH among children (Booth *et al.*, 1998a). In the southern coastal Kenya, it was suggested that the observed

associations between *S. haematobium* and STH, with regard to prevalence or intensities of infections among children and adults, are due to environmental and epidemiological coincidence rather than due to positive or negative biological influence of infection with one parasite on another (Ashford *et al.*, 1992).

Available evidence suggests that associations between parasites in co-endemic areas vary widely from one area to another and from one age group to another. This may have important implications for control programmes. It is therefore important to gather enough evidence about inter-specific parasite interactions in an area before implementing control programmes.

### 1.2.6 Micro-geographical Distribution of Helminth Infections

STH infections in East Africa are homogeneously distributed over large regions (Howard *et al.*, 2002). Infections with schistosomes, on the other hand, are usually focal and their spatial distribution patterns vary from one region to another (Clennon *et al.*, 2006; Clements *et al.*, 2010). Even within endemic foci, the infections are usually not homogeneously distributed but exhibit marked micro-geographical heterogeneities. Studies have, for example, demonstrated that *S. haematobium* infections are clustered in endemic areas of countries such as Kenya, Cameroon, Zanzibar and Zambia (Clennon *et al.*, 2006; Tchuem Tchuente *et al.*, 2003; Rudge *et al.*, 2008; Simoonga *et al.*, 2008). Similar observations were made for *S. mansoni* elsewhere in Kenya and Uganda (Kloos *et al.*, 1997; Booth *et al.*, 2004; Yiannakoulis *et al.*, 2010; Clements *et al.*, 2010). In all these studies, the distance from household to the source of infection, age and socio-economic status have been proposed as the most important factors for the observed infection clustering.

A few studies have investigated other possible causes of micro-geographical heterogeneities of infections with helminths. Ashford *et al.* (1992) suggested that the association between *S. haematobium* and STH infections among villages in Kilifi District of Kenya were almost wholly explained by environmental heterogeneity and epidemiological co-occurrence rather than biological interactions between the helminths. In Brazil, two studies have suggested that there could be significant biological interactions between *S. mansoni* and STH, and among STH that could influence their infection intensities (Chamone *et al.*, 1990; Fleming *et al.*, 2006). A study using animal models demonstrated that a pre-established infection with *S. mansoni* in mice can induce the expulsion of a subsequent *T. muris* infection from the host small intestine (Curry *et al.*, 1995). This underlines the importance of investigating whether the interactions between schistosome and STH influence the observed micro-geographical distribution in co-endemic areas.



### 1.2.7 Problem Statement

*S. haematobium* and STH infections are of public health importance in Kenya but their morbidity patterns, distribution and the responsible factors in Tana Delta are not clearly understood making it difficult to effectively control the infections in the areas.

### 1.2.8 Research Questions

1. What are *S. haematobium* & STH-related morbidity patterns among schoolchildren in Tana Delta District?
2. How are *S. haematobium* and STH infections related to morbidity markers and urinary cytokines?
3. What are the effects of combined praziquantel-albendazole treatment on *S. haematobium* and STH in the area?
4. What are the socio-economic and environmental factors related to *S. haematobium* and STH infections in the area?

### 1.2.9 Study Justification

Combined praziquantel and albendazole mass administration to control schistosomiasis and STH infections among children in endemic areas is a cost-effective means of controlling morbidity due to these infections (WHO, 2002; van de Werf *et al.*, 2003). However, even long-term mass administration of single doses of praziquantel and albendazole is unlikely to stop transmission of *S. haematobium* and STH, particularly *T. trichiura*, in highly endemic areas meaning that mass drug administration (MDA) will be a long-term activity (Bethony *et al.*, 2006; King, 2006). This may exert enormous demands on the endemic countries' resources. This drawback can be alleviated if the most highly affected strata of the endemic communities are identified and prioritized during the MDA. Among endemic populations and even within a single endemic community, intensities and morbidity due to infections with *S. haematobium* and STH in co-endemic areas are spatially and demographically highly heterogeneous with only a small percentage of infected individuals carrying very high worm burdens and suffering related morbidity (Booth & Dunne, 2004; Clennon *et al.*, 2004; Handzel *et al.*, 2003). Effective focusing of the MDA therefore requires clear *a posteriori* knowledge of the inherent small-scale characteristic heterogeneities of *S. haematobium* and STH infections and, the factors that influence them in specific endemic foci. Characteristics like infection intensities, morbidity, spatial distribution of *S. haematobium* and STH infections and the factors determining them in many important endemic areas however remain unknown. Tana Delta District in Kenya is one such area.

Available information suggests that mixed infections with *S. haematobium* and STH may influence infection intensities, morbidity, the outcome of treatment and thus, their spatial distribution in co-endemic areas. It is therefore imperative to analyse morbidity patterns and spatial distribution of infections with *S. haematobium* and STH among children in Tana Delta District of Kenya and determine how the co-occurrence of these worms influence the outcome of treatment with praziquantel and albendazole. The findings of this study will provide important information for focusing and improving combined schistosomiasis and STH control, and a basis for future evaluation of the control program, in Tana Delta and other similar endemic settings in Kenya as well as other regions.

## **1.2.10 Objectives**

### **1.2.10.1 General objective**

To assess morbidity patterns, response to treatment and spatial distribution of *S. haematobium* and STH infections in primary school children in Tana Delta District, coastal Kenya

### **1.2.10.2 Specific objectives**

1. To determine the prevalences and intensities of *S. haematobium* and soil transmitted helminth (STH) infections among primary school children in two isolated villages of Tana Delta District, Kenya.
2. To compare *S. haematobium*- and STH-related morbidity patterns using eosinophil cationic proteins (ECP), eosinophil protein X (EPX), haematuria, haemoglobin levels, ultrasound patterns and anthropometry in primary school children with different intensities of co-infection.
3. To evaluate the relationship between selected serum and urinary cytokines [interleukin 6 (IL-6), interferon- $\gamma$  (IFN- $\gamma$ ), tumour necrosis factor  $\alpha$  (TNF- $\alpha$ ), and interleukin 10 (IL-10)] and infections with *S. haematobium* and STH in primary school children with different intensities of co-infection.
4. To evaluate the effects of combined praziquantel-albendazole treatment on *S. haematobium* and STH in primary school children with different intensities of co-infection.
5. To determine the relationship between socio-economic, environmental factors and geospatial distributions, and infections with *S. haematobium* and STH in primary school children from the two villages.

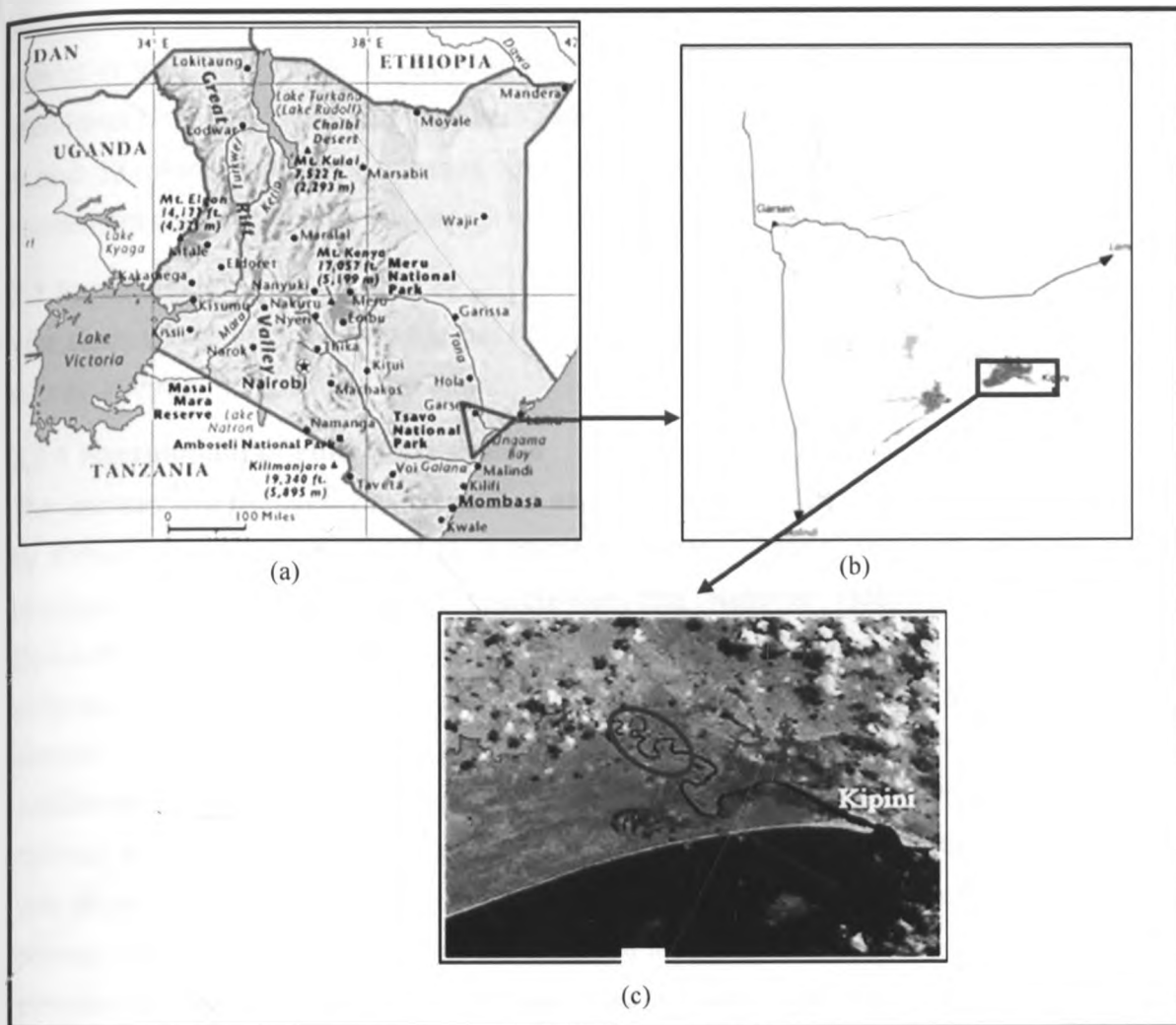
## **CHAPTER TWO: GENERAL MATERIALS AND METHODS**

### **2.1 Study area and study population**

#### **2.1.1 Study area**

The study was carried out in the Tana River Delta District area near the mouth of Tana River in Tana River County, coastal Kenya (Figure 2.1a). This area is roughly triangular in shape, with its apex at Lake Bilisa (north of Garsen) and its base, a 50 km stretch of beach along Ungwana (Formosa) Bay, stretching from Kipini in the north-east to Malindi in the south (Figure 2.1b &c). This is a low-lying area bounded by higher land to the west and south and, to the east by a sand dune system bordering the Indian Ocean. The entire floodplain in the lower parts is covered by alluvial sediments, transported and deposited during the annual flooding of the Tana River. Starting a few metres from the sand dunes and moving inland, there are saline grasslands and wetlands, and succession stages of forest and woodland on the riverbanks (Robertson & Luke, 1993).

The area is characterised by high temperatures and humidity. The rainfall ranges from around 1,000 mm per year at Kipini to less than 600 mm at Garsen. Despite the relatively low rainfall, the area is prone to seasonal flooding which happens not as a result of local precipitation but due to rainfall further inland in the river's catchments on Mt. Kenya and the Aberdare Mountains. Normally the major floods occur in April-May and the more limited short-rains flooding occurs in October–November with the timing, extent and duration varying greatly from year to year.



**Figure 2.1:** Maps of the study area.

(a) Map of Kenya showing the Tana Delta District in red triangle, (b) A sketch of Tana River delta area showing the study area in red rectangle (<http://www.tanariverdelta.org/tana/4390-DSY.html?language=default>; accessed on 18<sup>th</sup> January 2012) and, (c) An aerial photograph of study area showing the area covered by the two study villages in red ellipse (<http://www.vub.ac.be/ANCH/CV/tana.html>; accessed on 18<sup>th</sup> January 2012)

### 2.1.2 Study population

The study focused on children and parents in Kau and Ozi primary schools in Kipini and Garsen Divisions, respectively. The former primary school serves Kau village and the latter serves Ozi village. These two villages are situated on the banks of Tana River and they are separated by a distance of less than 5 km from one another. Kau has predominantly black cotton (clay) soil and has sparse vegetation cover compared to Ozi which has predominantly fine sandy soil with more vegetation cover. In addition, Kau is located on a more or less flat ground and flood water gets very close to the houses whereas Ozi is on a higher ground and flood water mainly accumulates at the peripheries of the village, a distance away from the houses during the wet season. Kau Primary

School starts from nursery and goes to class five and is smaller than Ozi Primary School which starts from nursery and goes to class eight. Children from each village attend school in their own village. In each of the two schools five classes namely nursery and standards one, two, three and four were requested to participate. Children aged between 5 and 12 years from these classes were subsequently selected for the study using a convenient sampling method where all eligible children were targeted.

## **2.2 Study Design**

The study comprised of a detailed baseline survey, followed by treatment and a follow-up survey at three months after treatment.

### **2.2.1 Recruitment of study Participants**

The sample size required was calculated as shown in Appendix I using the formula given by Kirkwood and Sterne (2003) for a single proportion. Prior to the start, the study was approved by the University of Nairobi-Kenyatta National Hospital Ethical Review Committee. Two meetings were held with the teachers in each school during which the purpose and the nature of the study were explained. One meeting was also held with the parents in each village during which the purpose and the nature of the study were explained. In addition, the parents were requested to give written consent before their children were recruited. Using the class registers and with the help of the class teacher, only children aged between 5 and 12 years from nursery school to class 4 in Kau and Ozi primary schools were recruited into the study. At first, they were examined physically by a clinician for any underlying ailment that could interfere with their participation in the research such as signs of immunosuppression. Two hundred and sixty-two children were enrolled and screened for *S. haematobium* and STH infections. The children were also examined for skin and eye infections for which they were treated during the study.

### **2.2.2 Baseline survey**

On the first day of the baseline survey, samples of urine and stool from each child were collected and examined for *S. haematobium* and STH eggs, respectively. The urine sample was also examined visually and by use of a dipstick for haematuria. These exercises were repeated on the second and third day of the baseline survey. On the third day, anthropometric measurement (height and weight) was carried out following which each child was bled to obtain a sample of venous blood. Ultrasound examination for *S. haematobium* infection-related morbidity was also done. A questionnaire was administered to obtain information on behaviour in relation to infection with *S. haematobium* and STH as well as treatment history. Immediately after the baseline survey each infected child was given standard oral doses of praziquantel (40 mg/ kg body weight) and albendazole (400 mg).

### **2.2.3 Follow-up survey**

A follow-up survey was conducted three months after treatment. Three stool and urine samples were collected and examined for *S. haematobium* and STH eggs as in the baseline. One of the three urine samples from each child was also examined for haematuria. Anthropometric measures of each child were also taken. A household survey was also conducted to elucidate the socio-economic conditions which could be predisposing to the infections. Homes from which the children came were visited and the households assessed with regard to presence and conditions of sanitary facilities. The parents were interviewed with regard to their socioeconomic status and the general health conditions of their children. In addition, geographical co-ordinates of the main house in the households as well as the local water contact points were recorded.

### **2.3 Specimen Collection, Field Processing and Examination**

Urine, stool and blood samples were collected in the field, within the school compound. Urine was examined for haematuria in the field. The samples were transported to the laboratory of Mpeketoni sub-District Hospital (Lamu West District) for further processing and examination for *S. haematobium* eggs and STH eggs. To increase the sensitivity of the helminth egg detection methods, on each of three different days a urine and stool sample was collected from each child and examined for *S. haematobium* and STH eggs, respectively. An average egg count was calculated for each of the four helminth species based on the three samples (Webster *et al.*, 2009). Blood samples were also examined for malaria parasites and tested for *W. bancrofti* antigenaemia in the field as the two parasites could be important potential confounder especially on immune responses.

#### **2.3.1 Urine**

At each time of urine specimen collection, each subject was given a clean, detergent-free 100 ml plastic container and requested to give at least 50 ml of urine in the container. During the first day, 10 ml of each urine sample was examined for *S. haematobium* eggs using filtration method (Cheesbrough, 1998; WHO, 1994). About 10 ml of the urine samples was stored in screw-capped Nunc tubes. These urine samples were frozen and stored at  $-20^{\circ}\text{C}$  for 3 months, due to logistical reasons, at the main laboratory in Nairobi before being shipped, under frozen conditions, to DBL-CHRD, University of Copenhagen in Denmark, for ECP and cytokine analysis.

##### **2.3.1.1 Urine examination for haematuria**

The urine samples were examined visually for the presence of blood (macro-haematuria). The samples were also tested for presence or absence of occult blood (micro-haematuria) with dipstix (URISCAN<sup>®</sup>, YD Diagnostics, Korea) according to the manufacturer's instructions. Briefly, a dipstix strip test was dipped in a urine specimen such that the test

pads came in contact with the urine, in a urine cup, and removed. Change in colour of the test pad was noted within 1 minute and interpreted according to a chart provided by the manufacture of the dipstix. The results were recorded as haematuric or non-haematuric.

### **2.3.1.2 Urine examination for *S. haematobium* eggs**

Ten millilitre (10 ml) of each of the three urine samples from each participating child was filtered through a 12 µm-pore polycarbonate filter using the filtration technique and examined microscopically for *S. haematobium* eggs (Cheesbrough, 1998; WHO, 1998). The results, based on mean egg count from each participant, were classified as light (1 – 50 eggs/ 10 ml urine) or heavy ( $\geq$  50 eggs/ 10 ml urine) infections according to WHO classification (WHO, 1998; Bergquist *et al.*, 2009).

### **2.3.2 Stool**

For three consecutive days each child was given a clean detergent-free plastic stool container and requested to give about 300 mg of stool. Only one sample was collected from each subject on each day. Part of the sample was measured using a 41.7 mg Kato template and examined for the presence of STH eggs using the standard Kato-Katz technique (WHO, 1994). Briefly, a part of each stool specimen was sieved and 41.7 mg of it measured using a Kato template and applied on a glass microscope slide. The preparation was covered with a cellophane slide soaked in malachite green and pressed to spread the specimen on the glass slide. The preparation was allowed about 20 minutes and the examined for helminth eggs within 1 hour under a light microscope. Another 41.7 mg of the third day stool sample from each child was measured using the 41.7 mg Kato template and stored in screw-capped Nunc tubes. These stool samples were frozen and stored at  $-20^{\circ}\text{C}$  for 3 months, due to logistical reasons, before being shipped, under frozen conditions, to DBL-CHRD in Denmark, for ECP and EPX analysis.

#### **2.3.2.1 Stool examination for soil transmitted helminth eggs**

From each of the three stool samples obtained from the child, 41.7 mg was examined microscopically for the presence of *T. trichiura*, *A. lumbricoides* and hookworm eggs using the standard Kato-Katz technique (WHO, 1994; Engels *et al.*, 1997). Each slide was examined within one hour of preparation to avoid missing hookworm eggs in the prepared slide through clearing. Individual infections with STH were classified into three classes based on mean infection intensity according to WHO classification (WHO, 1998; Bergquist *et al.*, 2009). Infections with *T. trichiura* were classified as negative, light (1– 999 eggs/ g stool) and moderate to heavy infections ( $\geq$  1,000 eggs/ g stool). Infections with hookworm were classified as negative, light (1 – 1,999 eggs/ g stool) and moderate to heavy ( $\geq$  2,000 eggs/ g stool) whereas those of *A. lumbricoides* were classified as negative, light (1 – 4,999 eggs/ g stool) and moderate to heavy infections ( $\geq$  5,000 eggs/ g stool).

### **2.3.3 Blood**

Venous blood (2 ml) was collected in plain Nunc tubes during the baseline survey by venepuncture. A small proportion of the blood (300 µl) was used for *W. bancrofti* antigen test (ICT), blood smear preparation, haemoglobin test and cell count in the field. The rest was used to prepare serum. The serum samples were frozen and transported to Nairobi where they were stored at  $-20^{\circ}\text{C}$  for 3 months, due to logistical reasons, before being shipped, under frozen conditions, to DBL-CHRD in Denmark, for cytokine assay (ELISA).

#### **2.3.3.1 Blood examination for circulating filarial antigen**

About 100 µl of blood was used for rapid diagnosis of *W. bancrofti* infection in the field. An immunochromatographic card (NOW<sup>®</sup> Filariasis, Binax Inc., USA) which detects the presence of *W. bancrofti* circulating antigens in whole blood was used for this purpose (Simonsen & Dunyo, 1999). A new card was used for each child. Whole blood was applied on to the sample pad of the card. The card was then closed and read within 10 minutes after preparation (Simonsen & Magesa, 2004). Cards exhibiting the control line were considered valid. Reading and interpretation of the results followed the manufacturer's instructions. Briefly, if a card exhibited both the control and test line, the blood sample was considered positive for *W. bancrofti* antigen.

#### **2.3.3.2 Haemoglobin Estimation**

About 10 µl of venous blood was used to estimate haemoglobin concentration using a portable haemoglobinometer, which gives readings in g/dl, according to manufacturer's instructions (HemoCue Hb 301, HemoCue<sup>®</sup>, Sweden). A new standard cuvette supplied together with the haemoglobinometer was used every day for quality control. Haemoglobin concentrations were expressed in g/dl. The results were recorded and categorised into two: normal ( $\geq 11.5$  g/dl of blood) or anaemia ( $< 11.5$  g/dl of blood) (WHO, 2008).

#### **2.3.3.3 Blood cell count**

A thin smear blood film was prepared using a part of each venous blood sample from each child and stained with Giemsa as described by Cheesbrough (1998). Differential cell count was performed on 100 white blood cells in each film and the numbers of each type of white blood cells expressed as a percentage of the total 100 white blood cells. Eosinophilia was defined as eosinophils above 7% of white blood cells on the blood film (<http://pathcuric1.swmed.edu/PathDemo/nrrt.htm>; accessed on 26<sup>th</sup> June 2010).

#### **2.3.3.4 Blood smear examination for malaria parasites**

The thin blood smear films prepared from venous blood samples for white blood cell count were also examined microscopically for any malaria parasite which could be a confounder



of the results on haemoglobin and for informing appropriate treatment of those with clinical malaria. One hundred microscope fields were examined per smear.

### **2.3.4 Ultrasonography**

The children were given soft drinks at least half an hour before ultrasound examination of their urinary tracts was performed. They were advised not to empty their bladders before ultrasound examination in order to ensure adequate bladder filling. Full ultrasound examination of the urinary tract was performed by an experienced ultrasonographer using a portable convex sector scanner (SSD-500®; Aloka, Tokyo, Japan) according to the Niamey protocol (WHO, 2000). The bladder shape and wall thickness, renal pelvis depth and ureters were measured. When a distended renal pelvis was observed, the child was advised to go, empty their bladder and come back for re-examination of the empty bladder. Abnormalities were recorded based on bladder shape, wall thickness, bladder wall irregularities, bladder masses and polyps, ureter dilatation and pyelon dilatation. Pathology was categorised as: 1) overall pathology; 2) bladder pathology and; 3) upper urinary tract pathology.

### **2.3.5 Anthropometry**

The weight of each participant was measured to the nearest 0.5 kg using an electronic scale (Salter Electronic®), with the participant wearing only light clothes and no shoes. The height was taken to the nearest 1cm using a portable stadiometer with the participants not wearing shoes. Body mass index (BMI) was calculated as weight (kg)/height (m)<sup>2</sup> and categorised into two: normal BMI ( $\geq 18.5$  kg/m<sup>2</sup>) or low BMI ( $< 18.5$  kg/m<sup>2</sup>) ([http://apps.who.int/bmi/index.jsp?introPage=intro\\_3.html](http://apps.who.int/bmi/index.jsp?introPage=intro_3.html); accessed on 26<sup>th</sup> May 2011)).

### **2.3.6 Medical history questionnaire**

A questionnaire on medical history was administered to children who could understand and respond to the questions appropriately, aged about seven years and above, in Swahili language in the presence of the class teacher (Appendix II). The children were asked about their frequency of sickness within the previous one month and what the symptoms were. Among other things, they were asked whether they had previously seen blood in their urine and whether they went for medical attention. If they went for medical attention, they were asked about the samples they submitted for examination. The children were also interviewed with regard to their behavioural exposure to *S. haematobium*. They were asked where they fetched water for bathing and domestic use and, if and where they went swimming. They were also asked whether they regularly walked through water. Regarding exposure to STH infections and environmental contamination, the children were asked if they used a latrine at home and if they collected and ate fruits such as mangoes in the fields before washing them.

## **2.3.7 Household Surveys**

### **2.3.7.1 Micro-geographical mapping**

The households where the children lived were visited, with help of a local guide who was identified by the community members. Geographical co-ordinates of the main house in each household were recorded using a hand-held geographical positioning system (e-trex Venture HC, Garmin®, Canada) unit with an accuracy of  $\pm 3$  m, according to the manufacturer. The co-ordinates were taken with the reader standing about 5 m from the door of the house. The co-ordinates of the local fresh water bodies were also recorded. The household co-ordinates were linked to *S. haematobium* and STH infection data and then exported to ArcGIS programme, which was used to draw spatial distribution maps of household and infections among children in Kau and Ozi.

### **2.3.7.2 Socio-economic observations and questionnaires**

When households where the children came from were visited for micro-geographical mapping, observations were also made about some of the socio-economic factors in the families that are relevant to *S. haematobium* and STH infections. Observations were made on the type of houses the families lived in, and these were ranked arbitrarily based on perceived importance in the community. A house made of straw walls and grass thatched roof was given one point, and one made of mud walls and grass thatched roof was given two points. If a house was made of mud walls and iron sheet roof it was given three points. Observations were also made on whether or not a pit latrine was present in the household.

The parents were interviewed about their education levels and economic activities through a questionnaire with close-ended questions. They were also asked about the possession of a few selected items and livestock which were ranked based on the minimum market value of such item or animal in Kenya. A radio and a bicycle were equated to KES 1,000 and 3,000, respectively. A cell phone and a canoe were equated to KES 1,500 and 6,000, respectively. A goat was equated to KES 2,000 and a cow was equated to KES 6,000. Depending on which among these items the family owned, these monetary values were added up and used to rank the socio-economic status of the family. There were three socio-economic classes namely class 1 with items valued at between KES 0 and 10,000, class 2 with items valued between KES 10,001 and 100,000 and, class 3 with items valued above KES 100,000. Regarding health, they were asked whether and how often they dewormed their children and whether any of their children had fallen sick in the previous three months. Regarding diet, the parents were asked about the number of meals the children took and how many among a few selected foodstuffs the children ate at least once in a week. These were fish, beans, milk, maize, rice and leafy vegetables.

Each of these foodstuffs was arbitrarily given one point such that the maximum level of diversity of 6 was recorded.

## **2.4 Examination for Inflammatory Markers and Cytokines**

### **2.4.1 Examination for ECP in Urine**

Non-filtered urine samples (10 ml) were shipped to DBL-CHRD in Denmark, in frozen conditions and immediately stored at  $-20^{\circ}\text{C}$  until use for ECP analysis. The samples were thawed, re-suspended, ECP extracted and analysed using ELISA as described by others (Reimert *et al.*, 1991a; 1993). One hundred microlitres of each urine sample was mixed with 100  $\mu\text{l}$  of extraction buffer of 1% *N*-cetyl- *N,N,N*-trimethyl-ammonium-bromide (CTAB), in 0.15MNaCl, mixed vigorously and left at room temperature for 1 hour. The preparation was mixed with 0.8 ml of sample buffer (0.1% Tween 20, 0.1% CTAB, 20mM EDTA, 0.2% HSA and 0.1% NaN<sub>3</sub> in PBS, pH 7.4) and frozen at  $-20^{\circ}\text{C}$  until required for analysis. Frozen extracts were thawed, centrifuged (3000 $\times$ *g*, for 10 min) and the resulting supernatants used for ECP measurements. ECP in the urine extracts was measured by a polyclonal antibody sandwich ELISA with a biotin–avidin–peroxidase amplification step, effective in the ranges of 15–1000 pg/ml. ECP purified from extracts of human blood eosinophils was used as standards. Before measurement, the standards and test samples were diluted in sample buffer. The standard ECP concentrations used were in the ranges of 15 – 1000 pg/ml. Reading of the ECP concentrations was done using Reading Microplate Manager 4.0 ® (Bio-Rad Laboratories, Inc.) software at a measurement wavelength of 490 nm and reference wavelength of 595 nm.

### **2.4.2 Examination for ECP and EPX in stool**

Stool samples were frozen at  $-20^{\circ}\text{C}$  until use for protein extraction for the analysis of faecal concentrations of ECP and EPX (Reimert *et al.*, 1991a,b; Reimert *et al.*, 2008) in Demark. Two millilitres of stool extraction buffer (0.1% EDTA, 0.1% Triton X-100 and 0.05% NaN<sub>3</sub> in 0.3M phosphate-buffered saline) were added to each tube containing the stool pellet, and the pellet was dissolved by vortex mixing and two cycles of freeze thawing. One hundred microlitres of the resulting slurry was then mixed with one volume of 1% *N*-cetyl-*N,N,N*-trimethyl-ammonium-bromide (CTAB), in 0.15MNaCl, mixed vigorously and left at room temperature for 1h, before being mixed with 0.8 ml of sample buffer (0.1% Tween 20, 0.1% CTAB, 20mM EDTA, 0.2% HSA and 0.1% NaN<sub>3</sub> in PBS, pH 7.4) and frozen at  $-20^{\circ}\text{C}$  until required for analysis. Frozen extracts were thawed, centrifuged (3000 $\times$ *g*, for 10 min) and the resulting supernatants were used for ECP and EPX measurements.

ECP or EPX concentrations in the stool extracts were measured by means of a polyclonal antibody sandwich ELISA with a biotin–avidin–peroxidase amplification step, effective in

the ranges of 15–1000 pg/ml. ECP and EPX purified from extracts of human blood eosinophils were used as standards.

The standard ECP or EPX concentrations used were in the ranges of 15 – 1000 pg/ml. Reading of the ECP and EPX concentrations was done using Reading Microplate Manager® 4.0 (Bio-Rad Laboratories, Inc.) software at measurement wavelength of 490 nm and reference wavelength of 595 nm.

#### **2.4.3 Examination for cytokines in urine and serum**

Serum and urine samples collected from the primary school children were analysed for the concentrations of IL-6, IL-10, TNF- $\alpha$  and IFN- $\gamma$  through solid phase sandwich ELISA using the BD OptEIA™ ELISA Kit II format (BD Biosciences, United States) according to the manufacturer's instructions. For each cytokine, a specific BD OptEIA™ ELISA Kit was used.

The assays were performed at room temperature with all the standards and samples being run in duplicate. Fifty microlitres of the provided ELISA diluents were pipetted in each well of a microtitre plate, coated with specific anti-human cytokine monoclonal antibody, before 100  $\mu$ l of each standard (lyophilized recombinant human IL-6, IL-10, TNF- $\alpha$  or IFN- $\gamma$ ) and sample (serum or urine) was added into appropriate wells. The mixture was gently shaken for 5 seconds to mix. Each of the 96-well microtitre plates was covered with a plate sealer and incubated for 2 hours. Forty-eight microlitres of detection antibody (biotinylated anti-human cytokine monoclonal antibody with ProClin™-150 as preservative) was added to a 12 ml enzyme concentrate (250x concentrated Streptavidin-horseradish peroxidase conjugate with Bovine Serum Antigen and ProClin™-300 as preservative) in a clean flask, mixed well and used within 15 minutes. The contents of the wells were decanted and wells washed five times by filling each well with 300  $\mu$ l of wash buffer four times. After the last wash, plates were blotted on absorbent paper to remove any residual buffer. One hundred microlitres of the detection antibody (biotinylated anti-human IL-6, IL-10, TNF- $\alpha$  or IFN- $\gamma$  monoclonal antibody) were added to each well and covered with a plate sealer before being incubated for 1 hour at room temperature. Wells were then washed seven times as described above. One hundred microlitres of 3,3',5,5'-tetramethylbenzidine (TMB) one-step substrate reagent was added to each well and incubated for 30 minutes at room temperature in the dark. Fifty microlitres of stop solution (1 M phosphoric acid) was added to each well. The absorbance was read at 450 nm and reference wavelength of 570 nm within 30 minutes of stopping the reaction.

## 2.5 Ethical Considerations

Prior to the study, the proposal was approved by the University of Nairobi–Kenyatta National Hospital Ethics and Research Committee. It was also approved by the Danish Central Medical Ethics Committee in Copenhagen, Denmark.

After the field sample collection, all children were treated with praziquantel (40 mg/kg) and albendazole (400 mg) in accordance with the national guidelines. Those who had not cleared the infections after the initial treatment were re-treated appropriately. Those presenting with other minor clinical manifestations were also offered free treatment from the study team and those with clinical manifestations beyond the ability of the team were referred to the local health centre for medical attention.

Only minimum and absolutely necessary invasive procedures (i.e. venepuncture) were carried out on the human subjects during this study. A minimum amount of blood (2 ml) was collected. This was no more than would be done during a normal routine examination. Standard operating procedures of handling potentially infectious biological samples were strictly followed during the study. All biological wastes were disposed of according to the national guidelines.

Privacy and confidentiality of the study participants was upheld at all stages of the study. All the data collected were stripped of all personal identifiers. Informed consent was obtained from each participant's parent who was also informed that they could freely withdraw their children from the study for the best interests of their children without any loss of benefits or penalty.

## 2.6 Data analyses

Data were entered in an Excel spread sheet and then exported to Stata 10 for statistical analyses. Before analyses, non-normally distributed continuous variables such as intensities of *S. haematobium* and STH eggs, cytokines, ECP or EPX were log-transformed, after adding 1 to each value, using the formula:  $\log_{10}(x+1)$ . Geometric means (GMI) were calculated using the formula:  $\text{antilog}_{10}[(\log_{10}(x+1)/n)-1]$ , where  $x$  = the number of *S. haematobium* or STH eggs or, level of ECP, EPX or cytokines, and  $n$  = the total number of children. Standard errors (S.E.) on geometric means were calculated as:  $\text{antilog}[\text{mean of log-transformed}(x+1) \pm \text{S.E. on log transformed}(x+1)]-1$ .

Log-transformed variables were compared between various groups using t-test or one-way analysis of variance (ANOVA), as appropriate. Proportions such as prevalences were compared between groups by using Pearson  $\chi^2$  test. The linear relationship between log-transformed variables was assessed using Pearson correlation analysis.

Analysis of the association between *S. haematobium* egg counts and cytokine levels was done using multiple variable regression models while controlling for age and sex. To deduce how STH influenced this association, each of *T. trichiura*, hookworm and *A. lumbricoides* egg counts were separately introduced in the models and the different correlation coefficient (the proportion of the observed variation in urinary ECP level explained by *S. haematobium* egg count), regression coefficient (the change in urinary ECP level with a change of one *S. haematobium* egg per ml of urine). If the *p*-value changed from  $\geq 0.05$  to  $\leq 0.05$  or vice versa following introduction of STH egg count in the model, the STH in question was regarded as having significant influence on the association. Multivariate regression analysis was used to assess the effects of *S. haematobium* and STH infections on eosinophilia, haemoglobin levels and body mass index while controlling for age and sex.

Post treatment cure rates, based on *S. haematobium* or STH egg counts, were calculated as  $(P_b - P_f)/P_b \times 100\%$  where  $P_b$ = prevalence at baseline and  $P_f$ = prevalence at follow-up. Overall GMI reduction was calculated as  $(GMI_b - GMI_f)/GMI_b \times 100\%$  where  $GMI_b$ = GMI at baseline and  $GMI_f$ = GMI at follow up. Paired *t*-test on log-transformed values was used to compare means between baseline and follow-up. Prevalences were compared between baseline and follow-up studies using McNemar's  $\chi^2$ -test. *p*-values less than 0.05 were considered statistically significant in all tests.

# **CHAPTER THREE: *SCHISTOSOMA HAEMATOBIMUM* AND SOIL TRANSMITTED HELMINTHIC INFECTIONS IN PRIMARY SCHOOL CHILDREN FROM TWO VILLAGES OF TANA DELTA DISTRICT**

## **3.1 Introduction**

Schistosomiasis due to infections with either *S. haematobium* or *S. mansoni* is endemic in all eight administrative Counties of Kenya with the Tana River County having the highest prevalence of *S. haematobium* (WHO, 1987; Brooker *et al.*, 2009). According to these authors, transmission of *S. mansoni* does not occur in the coastal zone of Kenya except for a small focus in Taveta District. Several studies on *S. haematobium* infections have been carried out in coastal Kenya, spanning from Msambweni District in the south to Malindi District in the north. This has resulted in a wealth of information regarding transmission patterns (Clennon *et al.*, 2004; Clennon *et al.*, 2006), morbidity (Kahama *et al.*, 1999a; King *et al.*, 2004; Wamachi *et al.*, 2004) and the effects of treatment (King, 2006). Comprehensive and systematic studies on *S. haematobium* infections in the three districts to the north of Malindi namely; Tana Delta, Lamu West and Ijara have, so far, not been documented.

High prevalences of STH infections have been reported in southern coastal Kenya (Brooker *et al.*, 2009; Magnussen *et al.*, 1997) but STH infections and related effects on health in coastal Kenya have been studied mostly as confounders in the study of other parasitic infections such as malaria or schistosomiasis (Bejon *et al.*, 2008; Pullan & Brooker, 2008). Like for *S. haematobium* infections, information on STH infections in the three districts to the north of Malindi District is scanty. In addition, it is mainly limited to unpublished reports of routine examination of urine and stool specimens from small samples of schoolchildren by the Ministry of Health Division of Vector Borne Diseases personnel's (WHO, 1987; Brooker *et al.*, 2009).

Quite often in endemic areas, individuals are infected with more than one of the common species of helminths (Booth *et al.*, 1998; Tchuem Tchuente *et al.*, 2003). Animal experiments strongly suggest that one helminth species may cause increased or reduced intensity and morbidity of infection with another species (Bickle *et al.*, 2008; Geiger, 2008).

In humans, studies of infection intensities based on helminth egg counts, have reported varying and sometimes contradictory types of relationships among different helminth species during co-infections. Chamone *et al.* (1990) in Brazil observed a positive correlation between the intensities of *S. mansoni* and hookworm infections, leading to increased *S. mansoni* egg counts, but a negative correlation between *S. mansoni* and *A. lumbricoides* or *T. trichiura*. Later on de Cassia Ribeiro Silva *et al.* (2007) in Brazil

reported increasing prevalence and intensity of *S. mansoni* infections with increasing number of STH species in concurrently infected schoolchildren. Although weak, a positive correlation in prevalence between *S. haematobium* and *A. lumbricoides* that depended on sex and age of the host was demonstrated in schoolchildren in one region of Cameroon (Tchuem Tchuente *et al.*, 2003). Little or no relationship between *S. mansoni* and STH could be demonstrated among schoolchildren in a co-endemic area in Tanzania (Booth *et al.*, 1998b). According to these authors, the infection intensities were generally low regardless of co-infection status. Similarly, in Kenya, low infection intensities were observed during co-infections with *S. mansoni* and hookworms (Thiongo *et al.*, 2001). A study by Fleming *et al.* (2006) in Brazil demonstrated that there may even be some antagonism between *S. mansoni* and *A. lumbricoides* resulting in lower infection intensities for each species in co-infected individuals than in individuals infected with only one of the species.

These studies indicate that the interactions between schistosomal and STH infections may vary widely probably which may be due to factors like age and sex of the hosts (Tchuem-Tchuente *et al.*, 2003), socio-economic status, and micro-geographical characteristics of the endemic foci (Satayathum *et al.*, 2006; Rudge *et al.*, 2008; Pullan *et al.*, 2008).

The present study was conducted in order to document *S. haematobium* and STH infection patterns in two rather isolated communities in Tana Delta District of Kenya and to analyse for interactions between *S. haematobium* and STH infections, as a background for further studies on the effects of the interactions on morbidity, micro-geographical distribution and effect of treatment.

## **3.2 Materials and Methods**

### **3.2.1 Study area and study design**

#### **3.2.1.1 Study area**

The study was carried out in Tana Delta District, in the north of coastal Kenya, in two village primary schools: Kau in Kipini Division and Ozi in Garsen South Division. These are the two eastern-most of the ten administrative divisions in Tana Delta District. The two villages are located on the banks of River Tana, about 5 km apart, and are most often reached by boat only. The main fresh water body in the area is River Tana. The area is highly prone to seasonal flooding and, during the dry season, numerous swamps are scattered all over the area. The vegetation in the area comprises of natural woodlands, bushes and grasslands.

The residents of the two villages are the Pokomo people who practice rice farming in the flood plains in the delta of Tana River. They also do fishing along the River Tana and in



the numerous swamps scattered all over the plains. A few of them also keep a few goats and cattle but animal keeping is not a major occupation in the two communities. All the residents in the two villages are Muslim.

The two villages are very similar in terms of housing structure with two main types of houses. At the centre of each village, there are mud-walled houses with thatched roofs. Each of these houses usually has two or more rooms with some housing two families. The densely populated part of Kau village is about 200 m in diameter whereas that of Ozi is about 1 km in diameter. At the peripheries of the densely populated part of each village centre, there are rice paddies spanning a radius of more than 2 km, interspersed with grasslands and natural bushes. Scattered all over the rice paddies, are houses mainly made of straw-walls and thatched roofs. These straw houses are smaller and each usually house only one family. The two villages are characterised by poor hygiene conditions such as lack of clean water supply, latrines or proper sewage disposal facilities.

Within each village there is one primary school. The school in Kau is smaller and starts from nursery to class five whereas Ozi School is larger and starts from nursery to class eight. Children from each village attend school in their own village. School enrolment in the two villages is very high (about 90%) but absenteeism is equally high depending on the season. Reason for absenteeism is because the area is prone to seasonal food shortages.

At the centre of each village, there is a small health clinic. The nearest fulltime operational health facility is Kipini Health Centre situated more than 10 km by river from the centre of each village.

### **3.2.1.2 Study design**

The current study was cross-sectional, and provided the baseline for further studies investigating epidemiology, morbidity and effects of treatment of *S. haematobium* and STH infections among children in the two schools. Three urine and stool samples were collected from each study participant. The samples were collected on a different day, and examined for *S. haematobium* and STH eggs, respectively. One venous blood sample was collected, in a plain Nunc tube, from each participant and part of it used for haemoglobin level estimation as well as differential cell count. Urinary system ultrasound examination for each participating child was carried out in addition to obtaining anthropometric parameters.

### **3.2.2 Parasitological Examinations**

Detection and quantification of *S. haematobium* eggs in urine or STH eggs in stool was crucial in the present study and to increase the sensitivity of the helminth detection method three urine and stool samples from the same child were collected and examined

microscopically on three consecutive days. The average egg counts were calculated based on the three samples (Webster *et al.*, 2009; van Etten *et al.*, 1997; Engels *et al.*, 1997).

### **3.2.2.1 Urine Examination for *S. haematobium* eggs**

Ten millilitres (10 ml) of each of the three urine samples from each participating child was filtered through a 12 µm-pore polycarbonate filter using the Nucleopore filtration technique and examined microscopically for *S. haematobium* eggs (Cheesbrough, 1998). The results, based on mean egg count from each participant, were classified as light (1 – 50 eggs/ 10 ml urine) or heavy ( $\geq$  50 eggs/ 10 ml urine) infections according to WHO (WHO, 1998; Bergquist *et al.*, 2009).

### **3.2.2.2 Stool examination for helminth eggs**

From each of the three stool samples, 41.7 mg was examined microscopically for the presence of *T. trichiura*, *A. lumbricoides* and hookworm eggs using the standard Kato-Katz technique (WHO, 1994; Engels *et al.*, 1997). The slides were examined within one hour of preparation before hookworm eggs in the prepared slide cleared. Infections, based on the mean egg count were divided into light and moderate to heavy infections based on the WHO classification (WHO, 1998) as *T. trichiura* light infections (1 – 999 eggs/g of stool) and moderate to heavy infections ( $\geq$  1,000 eggs/g of stool); hookworm light infections (1 – 1,999 eggs/g of stool) and moderate to heavy infections ( $\geq$  2,000 eggs/g of stool) and; *A. lumbricoides* light infections (1 – 4,999 eggs/g of stool) and moderate to heavy infections ( $\geq$  5,000 eggs/g of stool).

## **3.2.3 Morbidity Markers and Urinary Tract Pathological Examinations**

### **3.2.3.1 Test for Haematuria**

Urine samples were examined visually for the presence of blood (macro-haematuria) by two experienced laboratory technicians. When a disagreement arose regarding presence or absence of macrohaematuria, opinion was sought from a third technician. The samples were also examined for occult blood (micro-haematuria) with dipstix (URISCAN<sup>®</sup>, YD Diagnostics, Korea) according to the manufacturer's instructions. History of haematuria was obtained through a questionnaire administered at the time of examination, during which each participant was asked whether they had seen blood in their urine.

### **3.2.3.2 Urinary Tract Pathology Examination**

Ultrasound examination of the urinary tract was performed by an experienced ultrasonographer using a portable convex sector scanner (SSD-500<sup>®</sup>; *Aloka*, Tokyo, Japan). The urinary tract pathology was graded according to the Niamey protocol (WHO, 2000). Overall pathology including any lesion of the urinary bladder, the ureters, and kidneys was recorded. Bladder pathology was graded as follows; Normal – no lesions

recorded; Mild – a single wall enlargement ( $\geq 5$  mm) or wall irregularities on multiple sites, and/ or 1 mass or 1 polyp; Severe – multiple masses and/or polyps.

### 3.2.3.3 Haemoglobin Measurement

About 20  $\mu$ l of venous blood was used to estimate haemoglobin concentrations (g/dl of blood) using a portable haemoglobinometer (HemoCue Hb 301, HemoCue<sup>®</sup>, Sweden) according to manufacturer's instructions. The results were recorded and categorised into two: normal ( $\geq 11.5$  g/dl of blood) or anaemia ( $< 11.5$  g/dl of blood) (WHO, 2008).

### 3.2.3.4 Body Mass Index

The weight of each participant was measured to the nearest 0.5 kg using an electronic scale, with the participant wearing only light clothes and no shoes. The height was taken to the nearest centimetre using a portable stadiometer with the participants not wearing shoes. Body mass index (BMI) was calculated as weight (kg)/height (m)<sup>2</sup> and categorised into two: normal BMI ( $\geq 18.5$  kg/m<sup>2</sup>) or low BMI ( $< 18.5$  kg/m<sup>2</sup>) (WHO, 1995; WHO, 2000; WHO, 2004).

### 3.2.3.5 White Blood Cell Counts

A thin blood film was prepared from about 20  $\mu$ l of each venous blood sample and stained with Giemsa. Differential cell count was performed on 100 white blood cells (WBCs) per film and the numbers of each type of white blood cells expressed as a percentage of the total 100 WBCs. Eosinophilia was defined as eosinophils above 7% (<http://pathcuric1.swmed.edu/PathDemo/nrrt.htm>; accessed on 26<sup>th</sup> June 2010) of 100 WBCs on blood film.

## 3.2.4 Statistical Analysis

Data were entered in excel spread sheet and then exported to Stata (version 10) which was used to perform statistical analysis. The overall geometric mean intensities (GMI) of *S. haematobium* and STH egg counts were calculated as anti-log  $[(\sum \log x + 1)/n]-1$ , where  $x$  = the number of eggs/10 ml of urine for *S. haematobium* or number of eggs per gram of stool for STH and  $n$  = the total number of children examined when calculating the overall intensities or the number of those positive for helminth eggs when calculating GMI for those positive only. Multivariate regression analysis was used to assess the effects of *S. haematobium* and STH infections on eosinophilia, haemoglobin levels and body mass index. The GMI were compared between schools using *t*-test on log-transformed values. The prevalences of *S. haematobium* or STH infections and markers of morbidity were compared between schools, sexes or age groups using  $\chi^2$ -test. Prevalences of heavy *S. haematobium* infection or moderate-to-heavy STH infection and markers of morbidity were compared between schools, sexes or age groups using  $\chi^2$ -test. The prevalence of

anaemia and low BMI levels were compared between schools, sexes or age groups using  $\chi^2$ -test.

### 3.3 Results

#### 3.3.1 Study Population

Two hundred and ninety (290) children aged 5–12 years were registered from nursery to class 4 in the two schools (77 from Kau and 213 from Ozi). These formed the target population of the current study. Two hundred and sixty-two of these children were recruited for the baseline study; 67 from Kau (87.0% of target) and 195 from Ozi (91.5% of target).

Age and sex distribution of the study population in the two schools is shown in Table 3.1. The 67 children from Kau comprised of 29 girls (43.3%) with a mean age of 9.0 years and 38 boys (56.7%) with a mean age of 10.1 years. The 195 children from Ozi comprised of 105 girls (53.8%) and 90 boys (46.2%) with mean ages 9.7 and 9.8 years, respectively. The ratio of girls to boys in Kau was 0.74 and in Ozi it was 1.17. There was no significant difference in the ratio of girls to boys between the two schools ( $p = 0.14$ ,  $\chi^2$ -test). There was also no significant difference in the mean age of the study participants between the two schools ( $p = 0.71$ ;  $t$ -test). For the purpose of the analyses of parasitology and morbidity data, the study population was divided into two age groups as shown in Table 3.1.

**Table 3.1:** Age and sex distribution of the study populations in Kau and Ozi primary schools

Age group	Kau				Ozi			
	Girls	Boys	Total	Mean age	Girls	Boys	Total	Mean age
5–9 years	16	9	25	7.2	36	35	71	7.5
10–12 years	13	29	42	11.1	69	55	124	11.0
Total	29	38	67	9.6	105	90	195	9.7

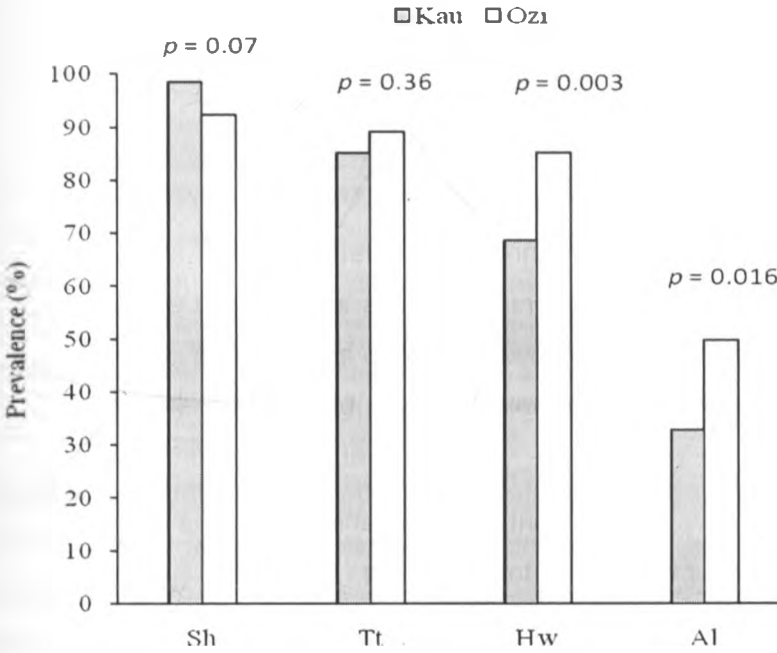
#### 3.3.2 *S. haematobium* and Soil Transmitted Helminth Infections

##### 3.3.2.1 Infections at School Level

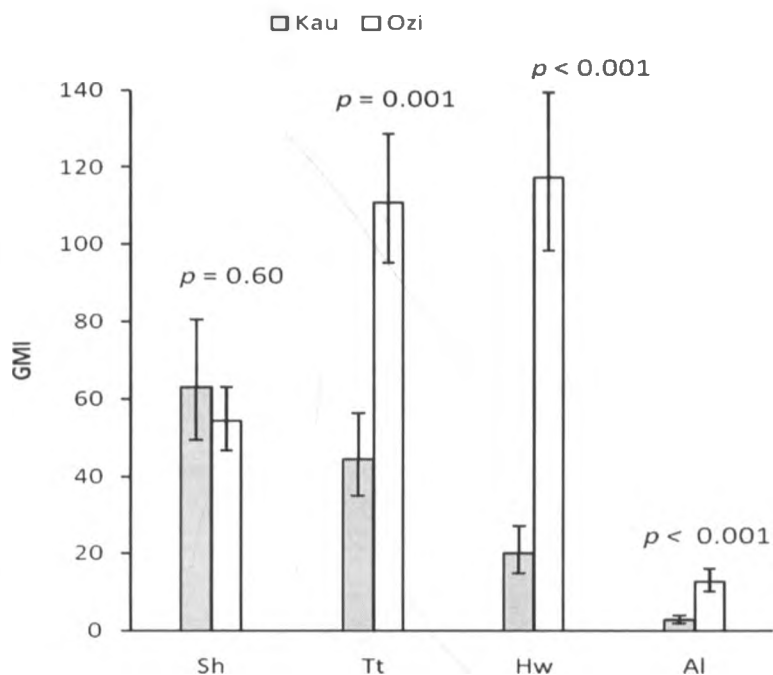
Out of the 262 enrolled children, 242 provided three urine samples, each sample on a different day. The other 20 provided two urine samples (5 from Kau and 15 from Ozi). All those who provided two samples only were positive for *S. haematobium* eggs and were therefore included in the analysis. Two hundred and twenty-nine (229) children provided three stool samples, each on a different day, whereas the other 33 (6 from Kau and 27 from Ozi) provided two stool samples. Of those who provided two stool samples, 1 from Kau and 6 from Ozi tested negative for STH eggs.

The prevalence and geometric mean intensity (GMI) of *S. haematobium* and STH infections in the two schools are shown in Figures 3.1 and 3.2. There was no significant difference in the prevalences of *S. haematobium* or *T. trichiura* infections between the two schools. On the other hand, the prevalences of hookworm and *A. lumbricoides* were significantly higher in Ozi than in Kau.

There were no significant differences in the GMIs of *S. haematobium* infections between the two schools. However, the GMIs of *T. trichiura*, hookworm and *A. lumbricoides* infections were significantly higher in Ozi than in Kau.



**Figure 3.1:** Prevalence of helminth infections among children in Kau and Ozi primary schools. Sh=*Schistosoma haematobium*, Tt = *Trichuris trichiura*, Hw = Hookworm, Al = *Ascaris lumbricoides*. p-values above the bars indicate the significance level of differences between the two schools.



**Figure 3.2:** Geometric mean intensities of helminth infections among children in Kau and Ozi primary schools.

Sh = *Schistosoma haematobium*, Tt = *Trichuris trichiura*, Hw = Hookworm, Al = *Ascaris lumbricoides*. GMI = geometric mean intensity (eggs/10 ml urine for Sh and eggs/g stool for intestinal worms). *p*-values above the bars indicate the significance level of differences between the two schools.

### 3.3.2.2 *S. haematobium* Infections in Relation to Age and Sex

The prevalence of *S. haematobium* infections in Kau and Ozi in relation to sex and age groups is shown in Table 3.2. The prevalence was high in all groups. There was no significant difference in prevalence between girls in the two schools or between boys in the two schools. Similarly, there was no significant difference in prevalence between girls and boys in any of the schools. Within the age groups there was no significant difference in prevalence between the two schools. There was no significant difference in prevalence between the age groups in Kau, but in Ozi the prevalence in the 10–12 years group was significantly higher than in the 5–9 years group.

**Table 3.2:** Prevalence of *S. haematobium* infections in relation to sex and age group in Kau and Ozi primary schools.

N = total number of children examined in each school; Column *p*-values show the significance levels of differences between groups whereas row *p*-values show the significance levels of the differences between schools.

		Kau		Ozi		<i>p</i> -value
		Examined (N=67)	Prevalence (%)	Examined (N=195)	Prevalence (%)	
Sex	Girls	29	96.6	105	91.4	0.35
	Boys	38	100.0	90	93.3	0.10
	<i>P</i> -value		0.25		0.62	
Age	5–9 yrs	25	96.0	71	85.9	0.17
	10–12 yrs	42	100.0	124	96.0	0.19
	<i>P</i> -value		0.19		0.011	

The prevalence of heavy *S. haematobium* infections ( $\geq 50$  eggs/10 ml urine) (WHO, 1998) in Kau and Ozi in relation to sex and age is shown in Table 3.3. There was no significant difference in prevalence of heavy infection between girls or between boys in the two schools. There was no significant difference between girls and boys in Kau, but in Ozi the prevalence was significantly higher in boys than in girls. Within the age groups there was no significant difference in prevalence of heavy infections between the two schools. There was no significant difference in prevalence of heavy infections between the age groups in Kau, but in Ozi it was significantly higher in the 10–12 years than in the 5–9 years group.

**Table 3.3:** Prevalence of heavy *S. haematobium* infections ( $\geq 50$  eggs/10 ml urine) in relation to sex and age group in Kau and Ozi primary schools.

N = total number of children examined in each school; n = number of children examined per group. Column *p*-values show the significance levels of differences between groups whereas row *p*-values show the significance levels of the differences between schools.

		Kau		Ozi		<i>p</i> -value
		Examined (N = 67)	Prevalence (%)	Examined (N = 195)	Prevalence (%)	
Sex	Girls	n = 29	55.2	n = 105	49.5	0.59
	Boys	n = 38	52.6	n = 90	65.6	0.17
	<i>p</i> -value		0.84		0.024	
Age	5–9 yrs	n = 25	56.0	n = 71	45.1	0.35
	10–12 yrs	n = 42	52.4	n = 124	63.7	0.19
	<i>p</i> -value		0.77		0.011	

### 3.2.3 Soil Transmitted Helminth Infections in Relation to Age and Sex

The prevalence of STH infections in Kau and Ozi in relation to sex and age group is shown in Table 3.4, and the prevalence of moderate to heavy STH infections (WHO, 1998; Bergquist et al., 2009) in Kau and Ozi in relation to sex and age group is shown in Table 3.5.

The prevalence of *T. trichiura* infections was high in all groups (Table 3.4). There was no significant difference in prevalence between girls in the two schools but it was significantly higher among boys in Ozi than in Kau. There was no significant difference in prevalence between girls and boys in the two schools. Within the age groups there was no significant difference in prevalence between the two schools or within any of the schools.

There was no significant difference in prevalence of moderate to heavy *T. trichiura* infections between girls in the two schools or between boys in the two schools. There was no significant difference between girls and boys in the two schools. Within age groups there was no significant difference in the prevalence of moderate to heavy *T. trichiura* infections between the two schools or between the two age groups within any of the two schools.

**Table 3.4:** Prevalence of STH infections in relation to sex and age group in Kau and Ozi primary schools.

N = total number of children examined in each school. Column *p*-values show the significance levels of differences between groups whereas row *p*-values show the significance levels of the differences between schools.

		Kau		Ozi		<i>p</i> -value
		Examined (N=67)	Prevalence (%)	Examined (N=195)	Prevalence (%)	
<b><i>T. trichiura</i></b>						
Sex	Girls	29	89.7	105	85.7	0.58
	Boys	38	81.6	90	93.3	0.044
	<i>P</i> -value		0.36		0.09	
Age	5–9 yrs	25	88.0	71	90.1	0.76
	10–12 yrs	42	83.3	124	88.7	0.37
	<i>P</i> -value		0.60		0.76	
<b>Hookworm</b>						
Sex	Girls	29	55.2	105	79.1	0.010
	Boys	38	79.0	90	92.2	0.033
	<i>P</i> -value		0.04		0.010	
Age	5–9 yrs	25	56.0	71	84.5	0.004
	10–12 yrs	42	76.2	124	85.5	0.16
	<i>P</i> -value		0.09		0.85	
<b><i>A. lumbricoides</i></b>						
Sex	Girls	29	20.7	105	47.6	0.009
	Boys	38	42.1	90	52.2	0.30
	<i>P</i> -value		0.06		0.52	
Age	5–9 yrs	25	28.0	71	52.1	0.037
	10–12 yrs	42	35.7	124	48.4	0.15
	<i>P</i> -value		0.52		0.62	

The prevalence of hookworm infections was lower than that seen for *T. trichiura*. This was reflected in all groups (Table 3.4). In Ozi, both girls and boys had significantly higher prevalence of hookworm infections than in Kau. Moreover, in both schools boys had significantly higher prevalence than girls. In both age groups, the prevalence was higher in



Ozi than in Kau, but this difference was only significant for the 5 – 9 years group. There was no significant difference in prevalence between the two age groups within any of the two schools.

The prevalence of moderate to heavy hookworm infections was low (Table 3.5). There was no significant difference in the prevalence in girls between Kau and Ozi. However, there was a significant difference in prevalence in boys between Ozi and Kau. There were no moderate to heavy hookworm infections in Kau and therefore statistical comparison was not done between girls and boys in the school. Boys in Ozi had significantly higher prevalence of moderate to heavy hookworm infections than girls. Within age groups, the prevalence in both age groups was not significantly different between the two schools.

**Table 3.5:** Prevalence of moderate to heavy *T. trichiura* infections ( $\geq 1,000$  eggs/g of stool), hookworm infections ( $\geq 2,000$  eggs/g of stool) and, *A. lumbricoides* infections ( $\geq 5,000$  eggs/g of stool) in relation to sex and age group in Kau and Ozi primary schools. N = total number of children examined in each school. Column *p*-values show the significance levels of differences between groups whereas row *p*-values show the significance levels of the differences between schools.

		Kau		Ozi		<i>p</i> -value
		Examined (N = 67)	Prevalence (%)	Examined (N = 195)	Prevalence (%)	
<b><i>T. trichiura</i></b>						
Sex	Girls	29	0.0	105	10.5	0.07
	Boys	38	2.63	90	6.7	0.36
	<i>p</i> -value		0.38		0.35	
Age	5–9 yrs	25	4.0	71	9.9	0.36
	10–12 yrs	42	0.0	124	8.1	0.06
	<i>p</i> -value		0.19		0.67	
<b>Hookworm</b>						
Sex	Girls	29	0.0	105	2.9	0.36
	Boys	38	0.0	90	13.3	0.018
	<i>p</i> -value		-		0.006	
Age	5–9 yrs	25	0.0	71	8.8	0.13
	10–12 yrs	42	0.0	124	7.3	0.7
	<i>p</i> -value		-		0.76	
<b><i>A. lumbricoides</i></b>						
Sex	Girls	29	0.0	105	3.8	0.29
	Boys	38	2.6	90	2.2	0.89
	<i>p</i> -value		0.38		0.52	
Age	5–9 yrs	25	0.0	71	4.2	0.30
	10–12 yrs	42	2.4	124	2.4	0.99
	<i>p</i> -value		0.44		0.48	

The prevalence of *A. lumbricoides* infections was the lowest for the STH infections. Girls in Ozi had significantly higher prevalence of *A. lumbricoides* infections than girls in Kau. However, there was no significant difference in prevalence between boys in Kau and boys in Ozi. In either of the two schools, there was no significant difference in the prevalence between girls and boys. Within age groups, the 5–9 years age group Ozi had significantly

higher prevalence than the 5–9 years age group in Kau. However, there was no significant difference in prevalence in the 10–12 years age group between Kau and Ozi. There was no significant difference in prevalence between the two age groups within Kau or Ozi.

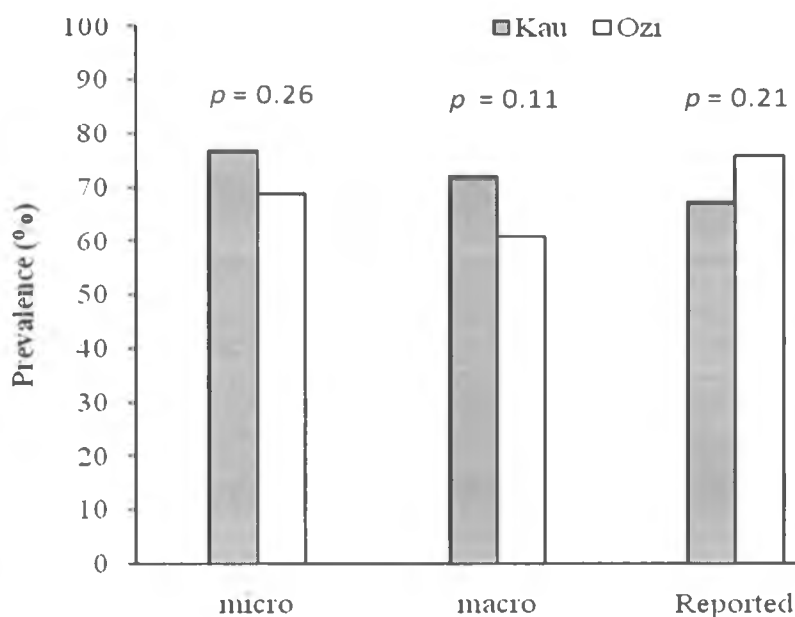
The prevalence of moderate to heavy *A. lumbricoides* infections was also low (Table 3.5). There were no moderate to heavy *A. lumbricoides* infections in girls in Kau but low prevalence was observed in girls in Ozi although the difference was not statistically significant. There was no significant difference in boys between the two schools. There was no significant difference in prevalence between girls and boys in either of the two schools. Within age groups, no significant difference in prevalence was observed in the 5–9 years age group or in the 10–12 years age group between the two schools. There was no significant difference between the two age groups in Kau or Ozi.

### **3.3.3 Markers of Morbidity and Urinary Tract Pathological Examinations**

#### **3.3.3.1 Haematuria**

The prevalence of haematuria in the two schools is shown in Figure 3.3 and that of haematuria in relation to sex and age groups in the two schools in Table 3.6. From the figure and table, the prevalence of haematuria in the study area was high although there was no significant difference in prevalences of macrohaematuria, microhaematuria, or history of haematuria between Kau and Ozi.

There were no significant differences in the prevalence of macrohaematuria or microhaematuria between the sexes or age groups in Kau but boys and the 10–12 years age group in Ozi had significantly higher prevalence of macrohaematuria and microhaematuria than girls and the 5–9 years age group, respectively. In addition, the 5–9 years age group had significantly higher prevalence of microhaematuria in Kau than in Ozi.



**Figure 3.3:** Prevalence of haematuria among children in Kau and Ozi primary schools. Micro = microhaematuria (detected by dipstix), Macro = macrohaematuria (visible haematuria), History = subject reported ever having macrohaematuria. *p*-values above the bars indicate the significance level of differences between the two schools.

**Table 3.6:** Prevalence of macrohaematuria (visible haematuria) and microhaematuria (detected by dipstix) in relation to sex and age group in Kau and Ozi primary schools  
N = total number of children examined in each school. Column *p*-values show the significance levels of differences between groups whereas row *p*-values show the significance levels of the differences between schools.

		Kau		Ozi		<i>p</i> -value
		Examined (N = 64)	Prevalence (%)	Examined (N = 194)	Prevalence (%)	
<b>Macrohaematuria</b>						
Sex	Girls	27	63.0	104	53.9	0.40
	Boys	37	78.4	90	68.9	0.28
	<i>p</i> -value		0.18		0.032	
Age	5–9 yrs	23	69.6	70	50.0	0.10
	10–12 yrs	41	73.2	124	66.9	0.46
	<i>p</i> -value		0.76		0.020	
<b>Microhaematuria</b>						
Sex	Girls	28	75.0	104	62.5	0.22
	Boys	35	77.2	88	76.1	0.91
	<i>p</i> -value		0.84		0.042	
Age	5–9 yrs	24	79.26	68	55.9	0.043
	10–12 yrs	39	74.4	124	75.8	0.86
	<i>p</i> -value		0.66		0.004	

### 3.3.3.2 Anaemia

There was no significant difference in the prevalence of anaemia between Kau (51.7%) and Ozi (64.5%) ( $p=0.08$ ). The prevalence of anaemia in relation to sex and age group in the two schools is shown in Table 3.7. No significant difference was observed in

prevalence of anaemia in girls although boys in Ozi had significantly higher prevalence of anaemia than boys in Kau ( $p=0.040$ ).

**Table 3.7:** Prevalence of anaemia (haemoglobin level < 11 g/dl of blood) in relation to sex and age group in Kau and Ozi primary schools

N = total number of children examined in each school. Column  $p$ -values show the significance levels of differences between groups whereas row  $p$ -values show the significance levels of the differences between schools.

		Kau		Ozi		$p$ -value
		Examined (N = 58)	Prevalence (%)	Examined (N = 172)	Prevalence (%)	
Sex	Girls	26	57.7	97	61.9	0.70
	Boys	32	46.9	75	68.0	0.04
	$p$ -value		0.41		0.40	
Age	5–9 yrs	22	63.6	59	72.9	0.42
	10–12 yrs	36	44.4	113	64.5	0.10
	$p$ -value		0.16		0.10	

### 3.3.3.3 Body Mass Index

The prevalence of low BMI was high in the study area but there was no significant difference between Kau (98.3%) and Ozi (94.3%) ( $p=0.22$ ). The prevalence of low BMI (BMI < 18.5 kg/m<sup>2</sup>) in relation to sex and age in the two schools is shown in Table 3.8. There were also no significant differences in the prevalence of low BMI between the sexes or two age groups within the two schools.

**Table 3.8:** Prevalence of low body mass index (BMI < 18.5 kg/m<sup>2</sup>) in relation to sex and age group in Kau and Ozi primary schools.

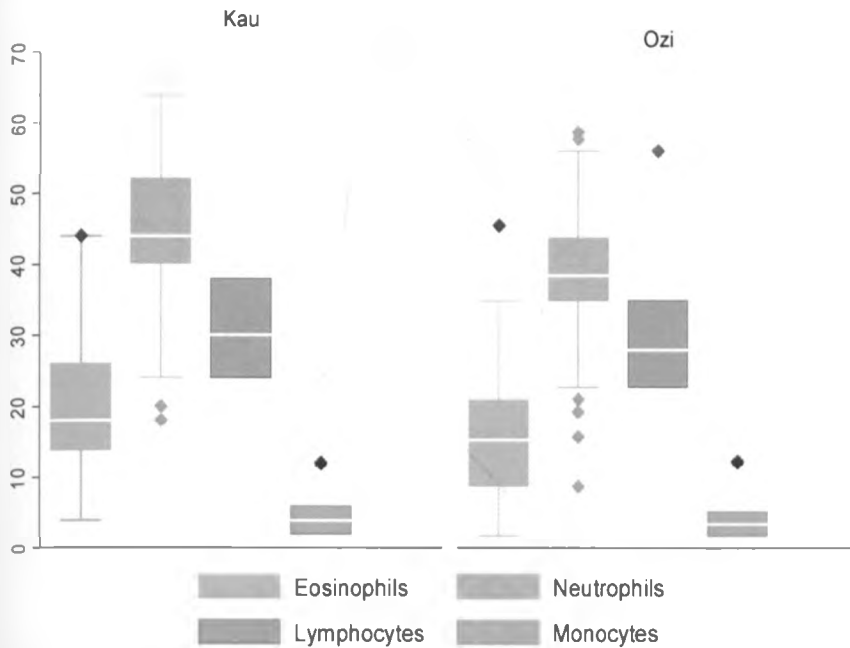
N = total number of children examined in each school. Column  $p$ -values show the significance levels of differences between groups whereas row  $p$ -values show the significance levels of the differences between schools.

		Kau		Ozi		$p$ -value
		Examined (N = 57)	Prevalence (%)	Examined (N = 173)	Prevalence (%)	
Sex	Girls	25	100.0	97	91.8	0.14
	Boys	32	96.9	77	97.4	0.88
	$p$ -value		0.37		0.11	
Age	5 – 9 yrs	21	100.0	61	95.1	0.30
	10– 12 yrs	36	97.2	113	93.8	0.43
	$p$ -value		0.44		0.73	

### 3.3.3.4 Blood cell counts

The results of the overall differential cell count in the study area are shown in Figure 3.5. The prevalence of eosinophilia (eosinophils  $\geq$  7% of total white blood cells) in relation to sex and age in the two schools is shown in Table 3.9. The prevalence of eosinophilia was high in the study area. The overall prevalence of eosinophilia in Kau was 91% and in Ozi it

was 93%. However, there were no significant differences between schools ( $p=0.70$ ,  $\chi^2$ -test), age groups or gender.



**Figure 3.4:** Box plots of blood cell counts (%) of children in the two schools.

The horizontal lines in middle of the boxes represent the medians of the cell counts. The lower boundary of each box represents the 25<sup>th</sup> percentile and the upper boundary represents the 75<sup>th</sup> percentile. The "whiskers" represent the range of the cell counts whereas the diamonds above and below each box represent the outliers. Normal values are: eosinophils (0–7%); neutrophils (35–80%); lymphocytes (20–50%); monocytes (2–12%).

(<http://pathcuric1.swmed.edu/PathDemo/nrrt.htm>; accessed on 26<sup>th</sup> June 2010).

**Table 3.9:** Prevalence of eosinophilia among children in Kau and Ozi primary schools.

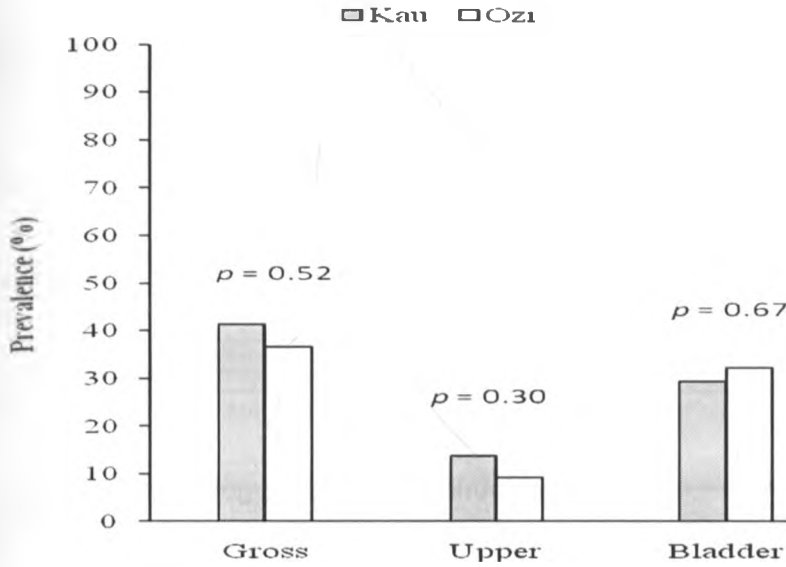
N = total number of children examined in each school. Column  $p$ -values show the significance levels of differences between groups whereas row  $p$ -values show the significance levels of the differences between schools.

		Kau		Ozi		$p$ -value
		Examined (N = 59)	Prevalence (%)	Examined (N = 158)	Prevalence (%)	
Sex	Girls	26	84.6	89	92.1	0.25
	Boys	33	97.0	69	94.2	
	$p$ -value		0.09		0.61	
Age	5–9 yrs	22	95.5	49	85.7	0.23
	10–12 yrs	37	89.2	109	96.3	
	$p$ -value		0.40		0.015	

### 3.3.3.5 Urinary Tract Pathology

Overall, the prevalence of upper urinary tract pathology (kidneys and ureters) was 10.5%, bladder pathology 31.5% of cases. In total, 37.9% of cases had pathological changes involving the upper and lower urinary tract. The prevalence of ultrasound-detectable

urinary tract pathology in the two schools is shown in Figure 3.6 and the prevalence of total ultrasound-detectable urinary tract pathology in relation to sex and age group in the two schools is shown in Table 3.10. There was no significant difference in the prevalence of upper urinary tract, bladder or gross pathology between Kau and Ozi.



**Figure 3.5:** Prevalence of ultrasound-detectable pathology among children in Kau and Ozi primary schools.

Gross = gross urinary tract pathology, Upper = upper urinary tract pathology, Bladder = bladder pathology. *p*-values above the bars indicate the significance level of differences between the two schools.

**Table 3.10:** Prevalence of ultrasound detectable urinary tract pathology in relation to sex and age group in Kau and Ozi primary schools.

N = total number of children examined in each school. Column *p*-values show the significance levels of differences between groups whereas row *p*-values show the significance levels of the differences between schools.

		Kau		Ozi		<i>p</i> -value
		Examined (N = 58)	Prevalence (%)	Examined (N = 161)	Prevalence (%)	
<b>Upper Urinary Tract pathology</b>						
Sex	Girls	26	11.5	88	9.1	0.71
	Boys	32	15.6	73	9.6	0.37
		<i>p</i> -value				0.65
Age	5–9 yrs	22	9.1	55	5.5	0.56
	10–12 yrs	36	16.7	106	11.3	0.41
		<i>p</i> -value				0.42
<b>Bladder pathology</b>						
Sex	Girls	26	23.1	88	28.4	0.59
	Boys	32	34.4	73	37.0	0.80
		<i>p</i> -value				0.35
Age	5–9 yrs	22	36.4	55	25.5	0.34
	10–12 yrs	36	25.0	106	35.9	0.23
		<i>p</i> -value				0.36
<b>Gross Urinary Tract pathology</b>						
Sex	Girls	26	34.6	88	33.0	0.88
	Boys	32	46.9	73	41.1	0.58
		<i>p</i> -value				0.35
Age	5–9 yrs	22	45.5	55	27.3	0.12
	10–12 yrs	36	38.9	106	41.5	0.78
		<i>p</i> -value				0.62

### 3.3.4 The Relationship between *S. Haematobium* Infection Intensity and STH Infections Intensities and the Status of Morbidity Markers

Since there were no significant differences in the overall prevalences and intensities of *S. haematobium* infections in the two schools, it was considered appropriate to analyse for the relationship between *S. haematobium* infections intensity and STH infection intensity as well as morbidity markers for the two schools together.

The relationship between *S. haematobium* infection intensity group and STH infections status is shown in Table 3.11. For *T. trichiura*, there was no significant difference in the overall prevalence, the prevalence of moderate to heavy infections or the GMI for all examined between children with light and those with heavy *S. haematobium* infections. However, when only those positive for *T. trichiura* eggs were considered, children with light *S. haematobium* infections had significantly higher *T. trichiura* GMI than those with heavy *S. haematobium* infections. A similar pattern was seen for hookworms, whereas for *A. lumbricoides* no significant differences were observed between the two *S. haematobium* infection intensity groups in any of these parameters.

**Table 3.11:** Relationship between *S. haematobium* infection intensity group and the status of soil transmitted helminth infections.

*p*-values indicate the difference between the light *S. haematobium* infection group and the heavy *S. haematobium* infection group. *Sh* = *S. haematobium* infection

	Light <i>Sh</i>	Heavy <i>Sh</i>	<i>p</i> -value
<b><i>Trichuris trichiura</i> (N = 262)</b>			
Number examined	115	147	-
No. positive (%)	99 (86.1)	132 (89.8)	0.36*
No. moderate/heavy positive (%)	10 (8.7)	8 (5.4)	0.30*
GMI for all examined	93.2	83.7	0.68**
GMI for positives	195.3	139.3	0.028**
<b>Hookworms (N = 262)</b>			
Number examined	115	147	-
No. positive (%)	91 (79.1)	121 (82.3)	0.52*
No. moderate/heavy positive (%)	4 (3.5)	11 (7.5)	0.17*
GMI for all examined	58.0	92.0	0.14**
GMI for positives	172.1	245.1	0.043**
<b><i>Ascaris lumbricoides</i></b>			
Number examined	115	147	-
No. positive (%)	50 (43.5)	69 (46.9)	0.58*
No. moderate/heavy positive (%)	3 (2.6)	4 (2.7)	0.96*
GMI for all examined	7.9	9.9	0.57**
GMI for positives	150.5	160.0	0.87**

\*) Chi-square test

\*\*) t-test on log transformed values

The relationship between *S. haematobium* infection intensity group and the status of morbidity markers is shown in Table 3.12. Significantly higher proportions of children with heavy *S. haematobium* infections had microhaematuria or macrohaematuria than children with light *S. haematobium* infections. There was no significant difference in the prevalence of upper urinary tract pathology between children with light *S. haematobium* infections and children with heavy *S. haematobium* infections. However, a significantly higher proportion of children with heavy *S. haematobium* infections had bladder pathology or gross pathology than children with light *S. haematobium* infections. A significantly higher proportion of children with heavy *S. haematobium* than of those with light *S. haematobium* infections had anaemia. On the other hand, there was no significant difference in the prevalence of eosinophilia or low BMI between the two *S. haematobium* infection intensity groups.

Multivariate regression analysis while controlling for age and sex showed that the helminth infections had varying effects on eosinophilia, haemoglobin levels and BMI among the children. Only *S. haematobium* and not STH infections significantly affected eosinophilia among the children ( $p = 0.002$ ). Both hookworm and *S. haematobium* infections, and not other STH infections, had significant effect on haemoglobin levels ( $p < 0.001$  and  $p = 0.006$ , respectively). Lastly, both *A. lumbricoides* and *T. trichiura* infections, and not *S. haematobium* or hookworm, had significant effect on BMI ( $p = 0.006$  and  $p = 0.027$ , respectively).



**Table 3.12:** Relationship between *S. haematobium* (Sh) infection intensity group and morbidity markers.

*p*-values indicate the difference between the light *S. haematobium* infection group and the heavy *S. haematobium* infection group ( $\chi^2$ -test)

	Light Sh	Heavy Sh	<i>p</i> -value
Microhaematuria (N = 241)			
Number examined	95	146	-
No. positive	43 (45.2)	137 (93.8)	<0.001
Macrohaematuria (N = 243)			
Number examined	96	147	-
No. positive (%)	31 (32.3)	133 (90.5)	<0.001
Upper UT pathology (N = 206)			
Number examined	81	125	-
No. positive (%)	6 (7.4)	16 (12.8)	0.22
Bladder pathology (N = 206)			
Number examined	81	125	-
No. positive (%)	17 (21.0)	51 (40.8)	0.003
Gross pathology (N = 206)			
Number examined	81	125	-
No. positive (%)	23 (28.4)	59 (47.2)	0.007
Anaemia (N = 218)			
Number examined	86	132	-
No. positive (%)	46 (53.5)	89 (67.4)	0.038
Eosinophilia (N = 205)			
Number examined	81	124	-
No. positive (%)	74 (91.4)	117 (94.4)	0.41
Low BMI (N = 218)			
Number examined	86	132	-
No. positive (%)	81 (94.2)	126 (95.5)	0.68

### 3.4 Discussion

The present study was carried out among 262 pupils, aged between 5 and 12 years old, from two schools (Kau and Ozi) in two adjacent villages located along the River Tana which are inhabited by the Pokomo people. The study population represented approximately 80% of all the children, within the age range, in the two villages. The main types of occupation in the two villages are rice farming and fishing, and the village populations are characterized by low socio-economic status and poor hygiene conditions such as lack of clean water supply, latrines and sewage disposal facilities.

The prevalence of *S. haematobium* and STH among the children was considerably high, confirming the previous reports that the area is one of the most highly endemic for *S. haematobium* and STH in Kenya (WHO, 1987; Brooker *et al.*, 2009). The present study focused on children of age 5–12 years and reported prevalences of *S. haematobium* infections above 90%. Considering that *S. haematobium* infections usually have their peak prevalence among children of age 5–15 years (Chitsulo *et al.*, 2000; Clennon *et al.*, 2004; King *et al.*, 2004; Kahama *et al.*, 1999b), these findings suggest that the prevalence of *S. haematobium* infections in the present study area is high.

There were no significant differences in the prevalence and intensity of *S. haematobium* infections between the two schools. This could be explained by the fact that although schistosomiasis can be highly focal, the two villages are less than 5 km apart and have minimal climatic and environmental differences that can influence the prevalence and intensity of *S. haematobium* infections between them (Clennon *et al.*, 2004; Satayathum *et al.*, 2006; Rudge *et al.*, 2008). For example, both areas are highly prone to seasonal flooding and have no road infrastructure. In addition, the residents are socio-economically and culturally similar suggesting that their children have more or less similar patterns of exposure to infections as observed elsewhere (Muhumuza *et al.*, 2009). Overall, age and sex did not significantly influence the prevalence or intensity of *S. haematobium* infections between the two schools, unlike what has been reported elsewhere in the Kenyan coast (Kahama *et al.*, 1999b; Satayathum *et al.*, 2006; Kihara *et al.*, 2009). This may be because in the present study only pre-adolescent children were involved whereas in these other studies pre-adolescents and adolescents have been studied together. This may be important because apart from behavioural differences between adolescent girls and boys, puberty-related physiological differences could influence gender-specific infection patterns (Nakazawa *et al.*, 1997; Abebe *et al.*, 2003; Kurtis *et al.*, 2006).

Whereas no significant difference in the intensity of *S. haematobium* infections was observed between the two sexes or age groups in Kau, boys in Ozi had significantly higher prevalence of heavy infections than girls. Similarly, in Ozi the 10–12 years age group had significantly higher prevalence of heavy *S. haematobium* infections than the 5–9 years age group. These differences indicated that Ozi could have had inherent factor(s) that predisposed boys more than girls, and older children more than younger ones, to infections. Usually, boys are behaviourally more exposed than girls due to their greater tendency to go out and swim in the rivers or swamps. By the virtue of the same fact, older children may be more exposed than younger ones. It was observed that Kau village, and school, is situated on a rather flat ground with poorly-drained clay soil where water accumulates very close to the houses during the wet season. This forms transmission sites very close to the houses where boys and girls, younger and older children, are equally exposed. On the other hand, Ozi is situated on a slightly elevated ground with well drained sandy soil. Therefore, during the wet season in Ozi water accumulates at the peripheries of the village, a distance from the houses. This suggests that, in contrast to the situation in Kau, in Ozi it is boys and older children who may more often go to the snail-infested water, a distance from the houses, compared to girls and younger children respectively. In support of this argument, in Kau children in the 5–9 years age group had a significantly higher prevalence of *S. haematobium* infections than children of the same age group in Ozi. This corroborates the findings of other studies that micro-geography and

location of water contact points relative to the houses are important factors (Satayathum *et al.*, 2006; Rudge *et al.*, 2008).

The prevalence of STH infections among the children was very high, similar to the report by Brooker *et al.* (2009). The prevalence of moderate to heavy STH infections was very low according to the WHO classification criteria (WHO, 1998; Bergquist *et al.*, 2009). The presently observed low intensities could be multi-factorial and no immediate explanation could be advanced for their cause but probably other factors play a major role. For example, eosinophils are known to kill larval worms and may thus reduce the intensity of the infections (Maizels & Balic, 2004; Maizels *et al.*, 2004). Since high levels of eosinophilia were observed among the children, it is probable that this contributed in reducing the intensities. Other studies elsewhere in the country have also reported low STH infection intensities in areas of Kenya with considerably high prevalences (Magnussen *et al.*, 1997; Olsen *et al.*, 1998; Olsen *et al.*, 2003). However, according to Brooker *et al.* (1999) even hookworm infection intensities as low as 200 eggs per gram of stool, termed low intensity infection, can cause anaemia under some settings. This emphasizes the importance of considering the local settings in determining what intensities are significantly pathological.

Among the three STH, *T. trichiura* had the highest prevalence followed by hookworm and *A. lumbricoides*. There was no significant difference in the prevalence of *T. trichiura* infections between the two schools but girls and the younger age group in Ozi had significantly higher prevalence of hookworm and *A. lumbricoides* than those in Kau. Similarly, Ozi had significantly higher intensities of all three STH infections than Kau. No immediate explanation could be advanced for this. However, it can be speculated that there were some yet unexplained interactions between *S. haematobium* and, *T. trichiura* and hookworm but not *A. lumbricoides* that led to simultaneously higher egg densities (Chamone *et al.*, 1990; de Cassia Ribeiro Silva *et al.*, 2007).

In most cases, Ozi had higher prevalences of moderate to heavy STH infections although the differences between the two schools were, in most cases, not statistically significant. Boys in Ozi could be more behaviourally exposed or more susceptible to hookworm than the rest of the children as they had significantly higher prevalence of moderate to heavy infections compared to those in Kau or their girl counterparts in Ozi. Children in Ozi had significantly higher prevalence of hookworm and *A. lumbricoides* as well as higher intensities (GMI) of all three STH. Since the two schools did not differ significantly in terms of *S. haematobium* infections, the immediate explanation for this difference could probably be environmental. For example, whereas Kau mainly has poorly-drained clay soil with less shade, Ozi is characterised by well-drained sandy soils that are mainly protected from

direct sunlight by bushes and big mango trees thus forming more suitable habitat for larval STH development and survival than in Kau (Mabaso *et al.*, 2003).

*S. haematobium* infections have been strongly associated with haematuria among children in the Kenyan coastal trip (Magnussen *et al.*, 1997; King, 2006). This is confirmed by the findings of the present study where the prevalence of macrohaematuria, microhaematuria and history of macrohaematuria were high and were associated with heavy *S. haematobium* infections. For example, in Ozi boys who had significantly higher prevalence of macrohaematuria and microhaematuria than girls also had significantly higher prevalence of heavy *S. haematobium* infections. Similarly, older children in Ozi had significantly higher prevalence of macrohaematuria, microhaematuria and heavy *S. haematobium* infections compared to younger children.

The prevalence of eosinophilia among the children was noted to be high in the study area. Eosinophilia is associated with infections by a wide spectrum of helminth species including schistosomes and STH (Loukas & Procriv, 2001; Kaminsky *et al.*, 2004; Meltzer *et al.*, 2008). Due to its location in the blood, *S. haematobium* may be a stronger inducer of eosinophilia than STH (Pardo *et al.*, 2006). This is congruent with findings that there was no significant difference in either the prevalence of *S. haematobium* infections or eosinophilia between the two schools. Previously, eosinophilia has been associated with protection against schistosomiasis (Hagan *et al.*, 1985; Butterworth, 1998). The role of eosinophilia in *S. haematobium* infections in the present study population remains unclear as it occurred alongside high *S. haematobium* infection intensities. It is also probable that eosinophilia played a role in keeping the intensities of STH infections low by killing incoming larval stages (Maizels & Balic, 2004; Maizels *et al.*, 2004).

The prevalences of anaemia and low BMI among the children in the study area were high and could be, at least partly, attributed to the high prevalence of *S. haematobium* and STH infections among the children. A combination of schistosomes and STH infections is known to cause high levels of anaemia through several mechanisms (Friedman *et al.*, 2005; Stoltzfus *et al.*, 1997; Brito *et al.*, 2006; Ezeamana *et al.*, 2008). For example, boys in Ozi had significantly higher prevalence of *T. trichiura* and moderate to heavy hookworm infections as well as anaemia. A similar observation has been made with regard to *S. mansoni* and STH infections in Brazil (Parraga *et al.*, 1996). It is also worth mentioning that other factors such as diet could also significantly contribute to the observed prevalence of anaemia (Koski & Scott, 2001; Lutter, 2008; Stoltzfus, 2001). For instance, during the study it was observed that the area had an acute food shortage and the children significantly relied on relief food supplements from charitable organisations. Low BMI, like anaemia, could be attributed to several factors, the major ones being *S.*

*haematobium* and STH infections and as mentioned above, poor or inadequate diet (Jardim-Batelho *et al.*, 2008; Koski & Scott, 2001).

*S. haematobium* infections among children in coastal Kenya and elsewhere are associated with ultrasound-detectable urinary tract pathology (Wamachi *et al.*, 2004; Keita *et al.*, 2005). Similar observations were made during the present study. No significant difference in prevalence and intensity of *S. haematobium* infections was observed between the two schools and, likewise, no significant difference was observed in ultrasound-detectable urinary tract pathology between the two schools. Neither age nor sex seemed to influence the prevalence of pathology among the children in the area. However, despite the high prevalence and intensity of *S. haematobium* infections in the area, the prevalence of urinary tract pathology was lower (37.9%) than most of what has been reported in other studies in coastal Kenya (Kahama *et al.*, 1999b; Wamachi *et al.*, 2004) and elsewhere (Hatz *et al.*, 1998; Brouwer *et al.*, 2004; Keita *et al.*, 2005). Probably this is because the present study only examined children aged 12 years and below whereas it is evident that the prevalence and severity of ultrasound detectable urinary tract pathology due to *S. haematobium* infections increases with age (Hatz *et al.*, 1998).

A comparison of the STH infections GMIs between children with light *S. haematobium* infection intensity and those with heavy *S. haematobium* infection intensity revealed that children with heavy *S. haematobium* infection intensity had significantly higher GMIs of *T. trichiura* and hookworm infections but not *A. lumbricoides*. An association between *S. mansoni* and *T. trichiura* but not *A. lumbricoides* was suggested by a study in Brazil (Parraga *et al.*, 1996) whereas in another study in the same country it was suggested that the association between *S. mansoni* and hookworm was more evident (Fleming *et al.*, 2006). Tchuem-Tchuente *et al.* (2003), however, did not find any convincing associations between *S. haematobium* and *T. trichiura* or *A. lumbricoides* in Cameroon. The findings of the present study and others indicate that associations between schistosomes and STH vary from one study population to another.

The relationship between intensity of *S. haematobium* infections and *S. haematobium*-related morbidity was evaluated by comparing the morbidity markers between those children with light *S. haematobium* infections and those with heavy infections. This revealed a significant relationship between heavy *S. haematobium* infections and haematuria, ultrasound-detectable urinary tract pathology and anaemia but not eosinophilia or BMI. One explanation for these differences is that haematuria and urinary tract lesions in children from *S. haematobium* endemic areas are direct results of eggs passing through or lodged in the urinary tract wall (Cheever, 1985; Hatz *et al.*, 1990).

Eosinophilia can be caused by infection with *S. haematobium* as well as STH, especially in the tissue stage (Maizels *et al.*, 2004; Supali *et al.*, 2010). In the present study, *S. haematobium* was more significantly associated with eosinophilia compared to STH. This is probably because blood and tissue-lodged *S. haematobium* eggs may be more potent inducers of eosinophilia than lumen dwelling STH (Pardo *et al.*, 2006; Tischendorf *et al.*, 2000; Loukas & Prociv, 2001).

*S. haematobium* infections are known to cause anaemia directly through bleeding lesions in the urinary tract and indirectly through chronic inflammation (Friedman *et al.*, 2005). Similarly, hookworm mainly cause anaemia through sucking of blood and bleeding lesions in the intestinal tract (Stoltzfus *et al.*, 1997; Smith & Brooker, 2010) whereas *T. trichiura* and *A. lumbricoides* may not cause as much blood loss although *T. trichiura* can cause dysentery in heavy infections (Khuroo *et al.*, 2010). This is supported by the findings of this study and others that *S. haematobium* and hookworm are more associated with anaemia than *T. trichiura* and *A. lumbricoides* (Brito *et al.*, 2006; Stoltzfus *et al.*, 1997).

Low BMI can be caused by helminth infections as well as other factors like poor diet (Beasley *et al.*, 2002; Lartey, 2008). The findings of the present study show that *A. lumbricoides* and *T. trichiura* were important causes of low BMI among the children. This could be because of these worms competing with the host for nutrients in the intestinal lumen (Stephenson *et al.*, 2000c; O'Lorcain & Holland, 2000). *S. haematobium* and hookworm were not significantly related to low BMI, probably because they are more involved with blood loss than general nutrient uptake from the intestinal lumen.

### 3.5 Conclusion

In conclusion, although Kenya is regarded as a moderate-schistosomiasis prevalence country (Utzing *et al.*, 2009) the findings of the present study indicate that some foci in the country are hyper-endemic and require special attention in terms of control efforts. These infections are associated with significant morbidity among schoolchildren in the study area and there is an urgent need for control measures to be put in place. The findings in the present study suggest that infections with *S. haematobium* in the area may, among other factors, play a role in influencing the prevalence and intensity of STH infections especially *T. trichiura* and hookworms. However, further detailed analyses are necessary in order to determine the levels of the observed interactions. This information may be important in making informed decision when designing control of *S. haematobium* and STH infections in the area.

## **CHAPTER FOUR: *SCHISTOSOMA HAEMATOBIMUM* AND SOIL TRANSMITTED HELMINTH INFECTIONS: MORBIDITY MARKERS AND THEIR RELATIONSHIP TO INFECTIONS**

### **4.1 Introduction**

Primary exposure to *S. haematobium* infection results in a sudden onset of fever, fatigue, myalgia, malaise, non-productive cough, eosinophilia, and appearance of patchy infiltrates on chest radiography, which are mainly observed in non-endemic populations (Gryseels *et al.*, 2006). Severe chronic pathology due to infections with *S. haematobium* is mainly a result of inflammatory responses to tissue-lodged parasite eggs (King *et al.*, 2001; Wamachi *et al.*, 2004). Miracidia in the eggs secrete proteins that provoke inflammatory, granulomatous reactions and ulcerations, which are progressively replaced by fibrotic deposits and pseudopolyposis of the vesical and ureteral walls (Cheever *et al.*, 1978; Gryseels *et al.*, 2006). This results in signs like dysuria, frequent micturition, proteinuria and haematuria. In addition, immune responses to chronic infection with *S. haematobium* may contribute to nutritional problems such as anaemia (Beasley *et al.*, 2002; Friedman *et al.*, 2005).

Infections with STH are commonly associated with nutritional deficits such as anaemia although they may also induce tissue inflammation (Stoltzfus *et al.*, 1997; Stephenson *et al.*, 2000a; Loukas & Prociv, 2001). *T. trichiura* larvae do not migrate through the host tissues but they spend a short time buried in the host intestinal mucosa where they are likely to induce inflammatory responses (Ortega *et al.*, 2010; Crompton, 2000; O'Lorcain & Holland, 2000). In addition, adult *T. trichiura* reside in the lumen of the host intestines with their anterior ends buried in the intestinal epithelium thus causing intestinal inflammation which is an important feature of trichuris dysentery syndrome during heavy infections (Stephenson *et al.*, 2000b; Khuroo *et al.*, 2010; Loukas & Prociv, 2001; de Silva, 2003). It is also thought that overt intestinal inflammation rarely occurs because *T. trichiura* may down-regulate the host inflammatory responses (Faulkner *et al.*, 2002; Jackson *et al.*, 2004). Hookworms evoke host inflammatory responses during percutaneous infection and during larval migration especially in the lungs (Ortega *et al.*, 2010; Reece *et al.*, 2006). Adult hookworms in the intestinal lumen lacerate the host intestinal mucosa and feed on the oozing blood which, apart from causing anaemia, may result in considerable intestinal inflammation (Hsieh *et al.*, 2004). Hookworms are also known to secrete molecules that down-regulate the host inflammatory responses thus reducing intestinal inflammation (Loukas *et al.*, 2005; Hsieh *et al.*, 2004). Larval *A. lumbricoides* induce host inflammatory responses during their migration in the host tissues (da Silva *et al.*, 2008). The adult stage resides in the lumen of the intestines and normally does not invade host tissues but they

are known to induce strong anti-inflammatory immune responses (Ortega *et al.*, 2010; Cooper *et al.*, 2000; Cooper *et al.*, 2008; Jackson *et al.*, 2004).

Eosinophils are a major cellular constituent of helminth induced inflammation and are found in blood and tissues, such as intestinal and urinary tracts mucosae, in large numbers during infections with helminths (Silveira-Lemos *et al.*, 2008; Gleich, 2000). This results in eosinophil-derived proteins such as the granular eosinophil cationic protein (ECP) and the neurotoxin eosinophil protein X (EPX) being released in large amounts from inflamed tissues (Majamaa *et al.*, 1999; Reimert *et al.*, 2000; Reimert *et al.*, 2008). For example, high amounts of these proteins are found in stool and urine of individuals infected with *S. mansoni* and *S. haematobium*, respectively, and these can therefore act as markers of inflammation in these infections (Tischendorf *et al.*, 2000; Reimert *et al.*, 2000; Reimert *et al.*, 2008).

Evidence thus suggests that during the course of an infection, both schistosomes and STH may evoke host inflammatory responses which result in different forms of morbidity (Coutinho *et al.*, 2007; Friedman *et al.*, 2005; Jackson *et al.*, 2006). These worms also have mechanisms of down-regulating the host inflammatory responses (Maizels *et al.*, 2004; Wang *et al.*, 2008). During co-infections, different stimuli from schistosomes and STH may act on the same components of the immune system and may influence the morbidity associated with one another. For example, if there are strong anti-inflammatory responses induced by STH, the tissue inflammation due to tissue-lodged schistosome eggs may be ameliorated during co-infections (King *et al.*, 2001; Wamachi *et al.*, 2004). Conversely, a strong mucosal activation and inflammation due to tissue-lodged schistosome eggs may end up killing the larval forms or expelling adult STH attached on the intestinal mucosa thus reducing STH infection intensity and morbidity (Yoshida *et al.*, 1999; Curry *et al.*, 1995). However, it is not known whether this happens in humans during schistosomes-STH co-infections and the possible outcomes of such co-infections remain poorly understood (Cox, 2001; Moreau & Chauvin, 2010; Pullan & Brooker, 2008). Considering the vast co-endemicity and high prevalences of co-infections this information may be important in detection of communities at greatest risk of exacerbated co-morbidities thus advising authorities involved in morbidity control on where to concentrate control efforts (Brooker *et al.*, 2009; Tchuem Tchuente *et al.*, 2003; Moreau & Chauvin, 2010).

The present study aimed at evaluating relationship between selected markers of urinary tract and intestinal inflammation and *S. haematobium* and STH infections, including co-infections, among children in two isolated villages of Tana Delta District, Kenya.



## **4.2 Materials and Methods**

The study area, study population and study design were as described in Chapter 3. Briefly, a cross-sectional study was carried out whereby urine samples from children in Kau and Ozi Primary schools were examined for *S. haematobium* eggs, ECP levels and haematuria whereas stool samples were examined for STH eggs, ECP and EPX levels. Venous blood samples were subjected to haemoglobin test and full differential cell counts. In addition, urinary tract of each child was subjected to ultrasound examination for pathology.

### **4.2.2 Parasitological examination**

#### **4.2.2.1 Urine examination for *S. haematobium* eggs**

Ten millilitre (10 ml) of each urine sample from each child was filtered through a 12 µm-pore polycarbonate filter, using the urine filtration technique, and examined microscopically for *S. haematobium* eggs as described in Chapter 2 (Cheesbrough, 1998).

#### **4.2.2.2 Stool examination for helminth eggs**

From each of the three stool samples from each participant, 41.7 mg was examined microscopically for the presence of *T. trichiura*, *A. lumbricoides* and hookworm eggs using the standard Kato-Katz technique as described in Chapter 2 (WHO, 1994; Engels *et al.*, 1997).

### **4.2.3 Measurement of Morbidity Markers**

#### **4.2.3.1 Examination for ECP in urine**

Urine samples were assayed for ECP levels according to Reimert *et al.* (2000) as described in Chapter 2. Briefly, the concentrations of ECP in urinary extracts were measured by a polyclonal sandwich type ELISA and reading of the ECP concentrations in pg/ml of urine done using Reading Microplate Manager 4.0<sup>®</sup> (Bio-Rad Laboratories, Inc.) software at a measurement wavelength of 490 nm and a reference wavelength of 595 nm. The amount of ECP in 1 ml urine was expressed as ng/ml by dividing the results by 1000.

#### **4.2.3.2 Examination for ECP and EPX in stool**

Stool samples were assayed for ECP and EPX levels according to Reimert *et al.* (2000) as described in Chapter 2 and elsewhere (Reimert *et al.*, 1991a, b; Reimert *et al.*, 2008). Briefly, the ECP and EPX levels in the stool extracts were measured by means of polyclonal antibody sandwich ELISA techniques and reading of the ECP and EPX concentrations in pg/g of stool was done using Reading Microplate Manager<sup>®</sup> 4.0 (Bio-Rad Laboratories, Inc.) software at measurement wavelength of 490 nm and a reference wavelength of 595 nm. The amount of ECP or EPX in 1 g of stool was expressed as ng/ml by dividing the results by 1000.

#### 4.2.3.3 Differential White Blood Cell Counts

A thin smear blood film was prepared from part of each venous blood sample from each child and stained with Giemsa and differential cell count performed on the blood films as described in Chapter 2. The children were arbitrarily categorised as non-eosinophilic (1 – 7% eosinophils), mildly eosinophilic (8 – 30% eosinophils) and severely eosinophilic (31 – 52% eosinophils).

#### 4.2.3.4 Haemoglobin Measurement

About 20 µl of venous blood was used to estimate haemoglobin concentrations (g/dl of blood) using a portable haemoglobinometer (HemoCue Hb 301, HemoCue®, Sweden) according to manufacturer's instructions. The results were recorded and categorised into two: normal ( $\geq 11.5$  g/dl of blood) or anaemic ( $< 11.5$  g/dl of blood) (WHO, 2008).

#### 4.2.3.5 Test for Haematuria

Urine samples were examined for haematuria using dipstix (URISCAN®, YD Diagnostics, Korea) according to the manufacturer's instructions and as described in Chapter 2.

#### 4.2.3.6 Ultrasonography

Full ultrasound examination of the urinary tracts of the children was performed by an experienced ultrasonographer using a portable convex sector scanner (SSD-500®; Aloka, Tokyo, Japan). The urinary tract pathology was recorded as described in Chapter 2, according to the Niamey protocol (WHO, 2000).

#### 4.2.4 Data Analyses

Data were entered in an Excel spread sheet and then exported to Stata (Version 10) for statistical analysis as explained in Chapter 2. Briefly, log-transformed values of egg count and urinary ECP levels were used for the analysis of the association between *S. haematobium* egg counts and inflammatory markers, while controlling for the effects of age and sex in multiple variable regression models. To deduce how STH influenced this association, each of *T. trichiura*, hookworm and *A. lumbricoides* egg counts were separately introduced in the model and the different correlation coefficient (the proportion of the observed variation in urinary ECP level explained by *S. haematobium* egg count), regression coefficient (the change in urinary ECP level with a change of one *S. haematobium* egg per ml of urine) and *p*-value recorded. If, *p*-value changed from  $\geq 0.05$  to  $\leq 0.05$  or vice versa following introduction of STH egg count in the model, the STH in question was regarded as having significant influence on urinary ECP response to *S. haematobium* infection. *p*-values less than 0.05 were considered statistically significant in all tests.

## 4.3 Results

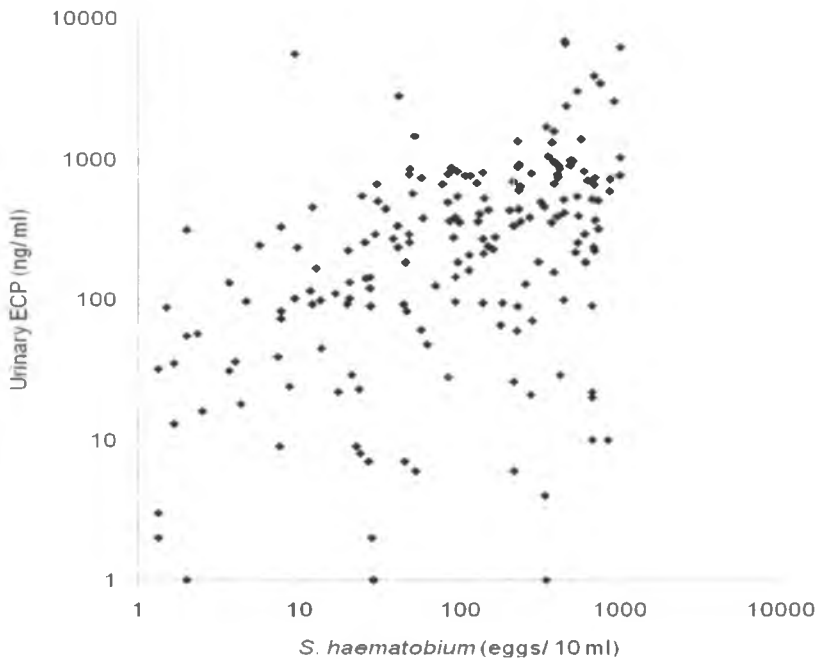
### 4.3.1 Study population

There were 262 children who provided urine and stool samples as described in Chapter 3. Out of these, only urine samples from 211 children were available for ECP analysis and stool samples from 186 children only were available for faecal ECP and EPX analysis. There were no significant differences in age, sex ratio, or *S. haematobium* and STH infections between the initial 262 children and the two groups of children.

### 4.3.2 Urinary ECP

#### 4.3.2.1 Relationship between Urinary ECP and *S. haematobium* Egg Count

A scatter diagram showing the relationship between urinary ECP and *S. haematobium* egg count is shown in Figure 4.1. There was significant positive correlation between urinary ECP and *S. haematobium* egg count ( $r=0.46$ ,  $p<0.001$ ;  $n=211$ ).

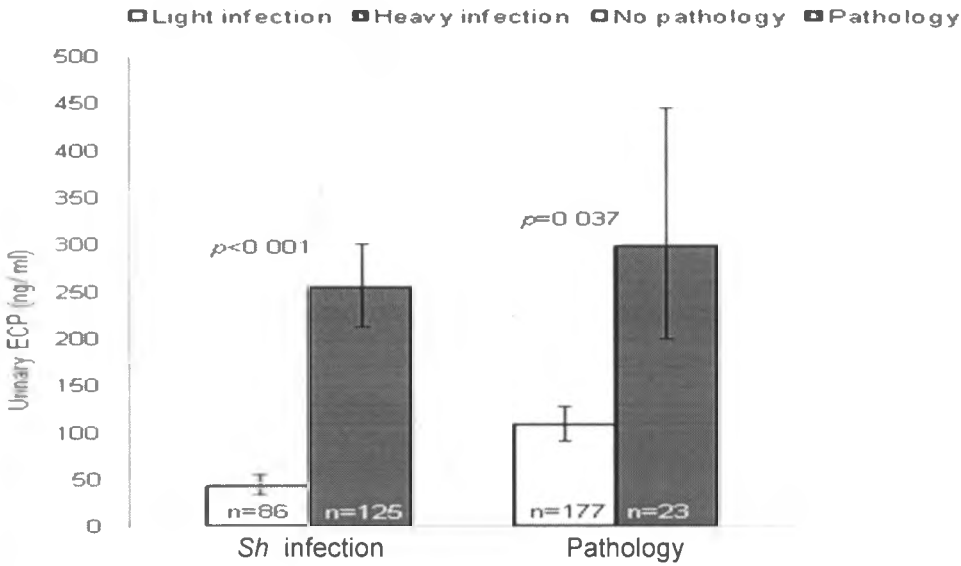


**Figure 4.1:** Relationship between urinary ECP and *S. haematobium* egg count in children in Kau and Ozi primary schools.

#### 4.3.2.2 Relationship between Urinary ECP, *S. haematobium* Infection and other Morbidity Markers

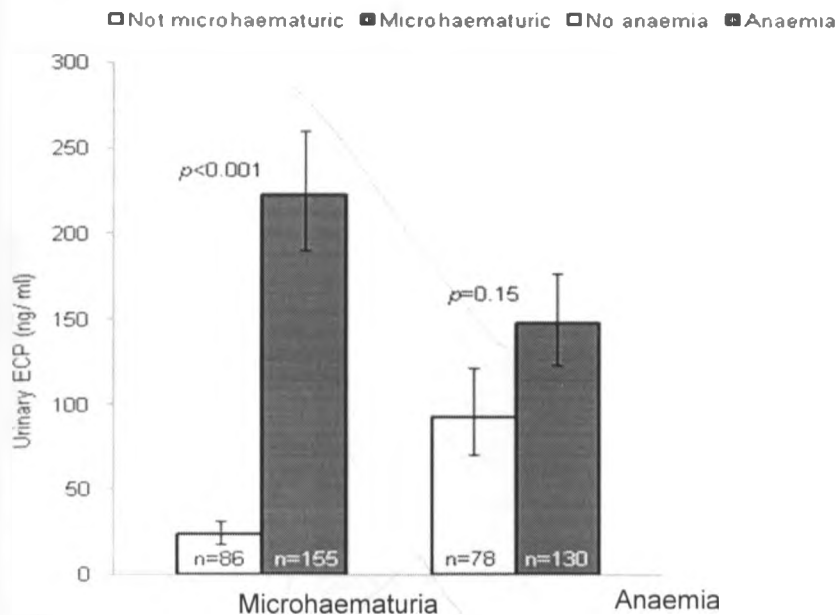
The mean levels of urinary ECP in relation to *S. haematobium* infection intensity group and in relation to upper urinary tract pathology status are shown in Figure 4.2. Geometric means of urinary ECP was significantly higher in children with heavy *S. haematobium* infections than in children with light *S. haematobium* infections. Similarly, the geometric mean of urinary ECP in children with ultrasound-detectable upper urinary tract pathology

was significantly higher than in children without ultrasound-detactable upper urinary tract pathology.



**Figure 4.2:** Geometric mean levels of urinary ECP in relation to *S. haematobium* (Sh) infection intensity and urinary tract pathology (Pathology) among children in Kau and Ozi primary schools. *p*-values above the bars indicate the significance levels of differences between the groups. The error bars indicate the standard errors on the log-transformed values.

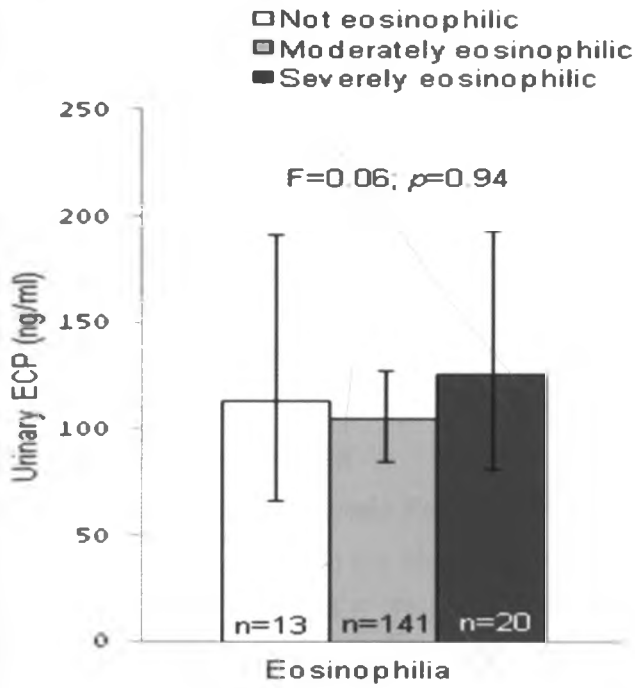
The mean levels of urinary ECP in relation to haematuria and in relation to anaemia status are shown in Figure 4.3. Children with microhaematuria had significantly higher geometric means of urinary ECP than children without microhaematuria. There was no significant difference in geometric means of urinary ECP between children with and without anaemia.



**Figure 4.3:** Geometric mean levels of urinary ECP in relation to microhaematuria and anaemia among children in Kau and Ozi primary schools. *p*-values above the bars indicate the significance levels of differences between the light and heavy infection intensity groups. The error bars indicate the standard errors on the log-transformed values.

#### 4.3.2.3 Relationship between Urinary ECP and Eosinophilia

The mean levels of urinary ECP in relation to eosinophilia status are shown in Figure 4.4. There were no significant differences in geometric means of urinary ECP between the three groups of children with regard to eosinophilia.



**Figure 4.4:** Geometric mean levels of urinary ECP in relation to eosinophilia among children in Kau and Ozi primary schools. *p*-value above the bars indicate the significance level of variation between groups with different levels of eosinophilia. The error bars indicate the standard errors on the log-transformed values.

#### 4.3.2.4 Effects of STH on the Association between Urinary ECP and *S. haematobium* Infection

Log-transformed values of egg count and urinary ECP titres were used for the analysis of the effects of STH on the association between *S. haematobium* egg counts and urinary ECP, while controlling for the effects of age and sex, in a multiple variable regression model. Each of *T. trichiura*, hookworm and *A. lumbricoides* egg counts were introduced in the model separately and the different correlation coefficient ( $r^2$ ), regression coefficient and *p*-value recorded as shown in Table 4.1. Urinary ECP titre was significantly positively associated with *S. haematobium* infection intensity but there were no changes in correlation coefficient, regression coefficient or *p*-value following introduction of any of the three STH and thus none of the STH species studied had significant effect on this association.

**Table 4.1:** The effects of STH on the association between urinary ECP and *S. haematobium* infections, using a regression model controlling for the effects of age and sex.

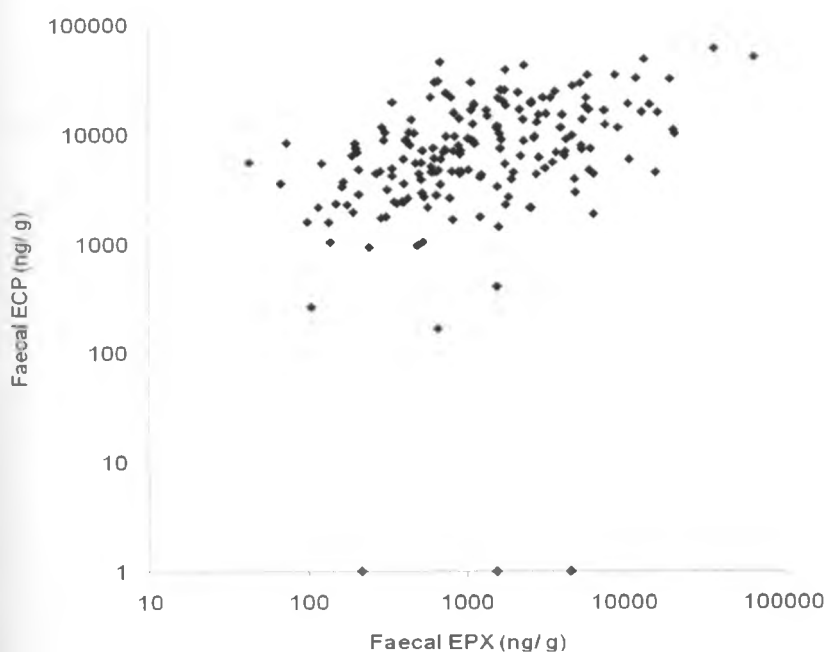
$r^2$  = correlation coefficient.

	Urinary ECP			
	<i>S. haematobium</i> only	<i>T. trichiura</i>	Hookworm	<i>A. lumbricoides</i>
$r^2$	0.22	0.22	0.22	0.22
Regression Coefficient	0.50	0.50	0.50	0.50
p-value	<0.001	<0.001	<0.001	<0.001

### 4.3.3 Faecal ECP and EPX

#### 4.3.3.1 Relationship between Faecal ECP and EPX

A scatter diagram showing the linear relationship between levels of faecal ECP and faecal EPX is shown in Figure 4.5. There was a significant positive correlation between faecal ECP and EPX ( $r=0.32$ ,  $p<0.001$ ;  $n=186$ ).



**Figure 4.5:** Relationship between faecal ECP and EPX

#### 4.3.3.2 Relationship between Faecal ECP, Soil Transmitted Helminths and Other Morbidity Markers

Scatter diagrams of the relationships between levels of faecal ECP and *T. trichiura*, hookworm and *A. lumbricoides* egg counts are shown in Figures 4.6, 4.7 and 4.8, respectively. Both egg positive and negative children were included in these analyses. There was no significant correlation between faecal ECP and *T. trichiura* ( $r=0.07$ ,  $p=0.31$ ;

n=186), *A. lumbricoides* ( $r=0.07$ ,  $p=0.33$ ; n=186) or hookworm egg counts ( $r=0.12$ ,  $p=0.10$ ; n=186).

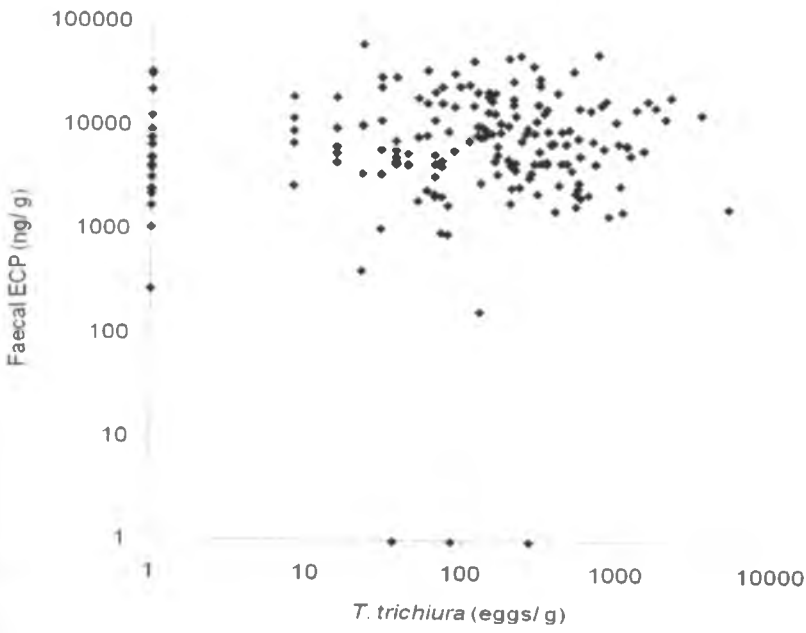


Figure 4.6: Relationship between faecal ECP and *T. trichiura* egg count.

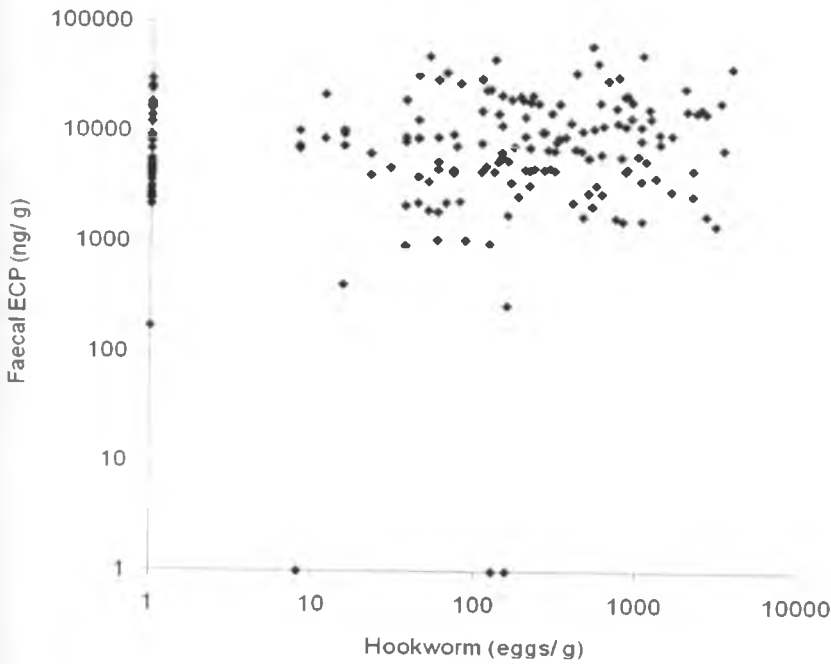
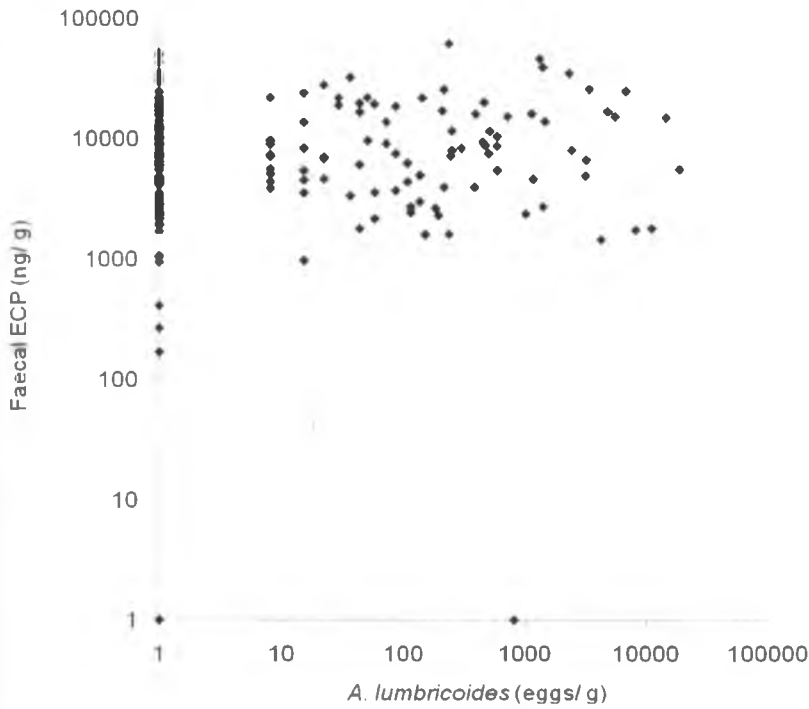


Figure 4.7: Relationship between faecal ECP and hookworm egg count.

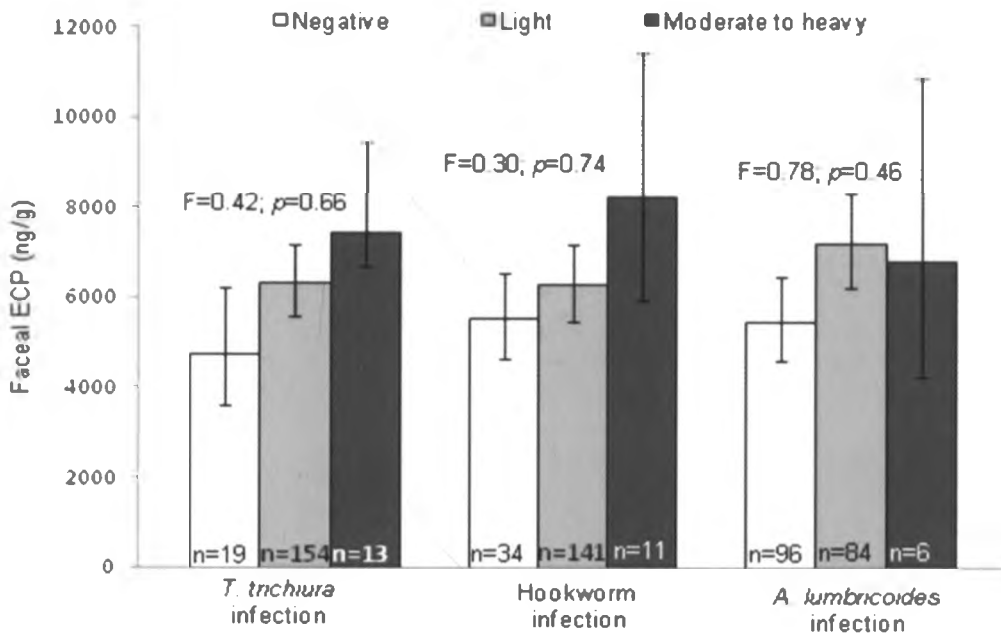




**Figure 4.8:** Relationship between faecal ECP and *A. lumbricoides* egg count.

The mean levels of faecal ECP in relation to STH infection intensity groups is shown in Figure 4.9. From the figure, there were no significant differences in geometric means of faecal ECP between children with no, light or heavy to moderate *T. trichiura*, hookworm or *A. lumbricoides* infections. There was a trend of increase in faecal ECP levels with increasing infection intensity.

A comparison of the geometric means of faecal ECP between children negative and positive for STH eggs was done using t-test. There was no significant difference between children negative and positive for *T. trichiura* eggs (4732 ng/g vs. 6405 ng/g;  $p=0.31$ ), between children negative and positive for hookworm eggs (5510 ng/g vs. 6378 ng/g;  $p=0.50$ ) or between children negative and positive for *A. lumbricoides* eggs (5441 ng/g vs. 7150 ng/g;  $p=0.21$ ).

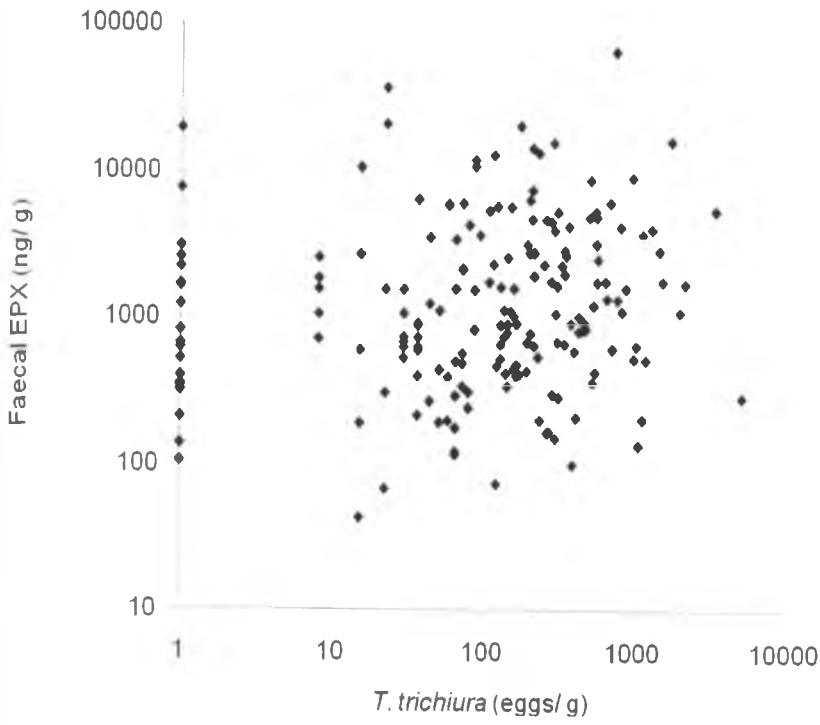


**Figure 4.9:** Geometric mean levels of faecal ECP in relation to STH infection intensity among children in Kau and Ozi primary schools.  $p$ -values above the bars indicate the significance levels of differences between the infection intensity groups. The error bars indicate the standard errors on the log-transformed values)

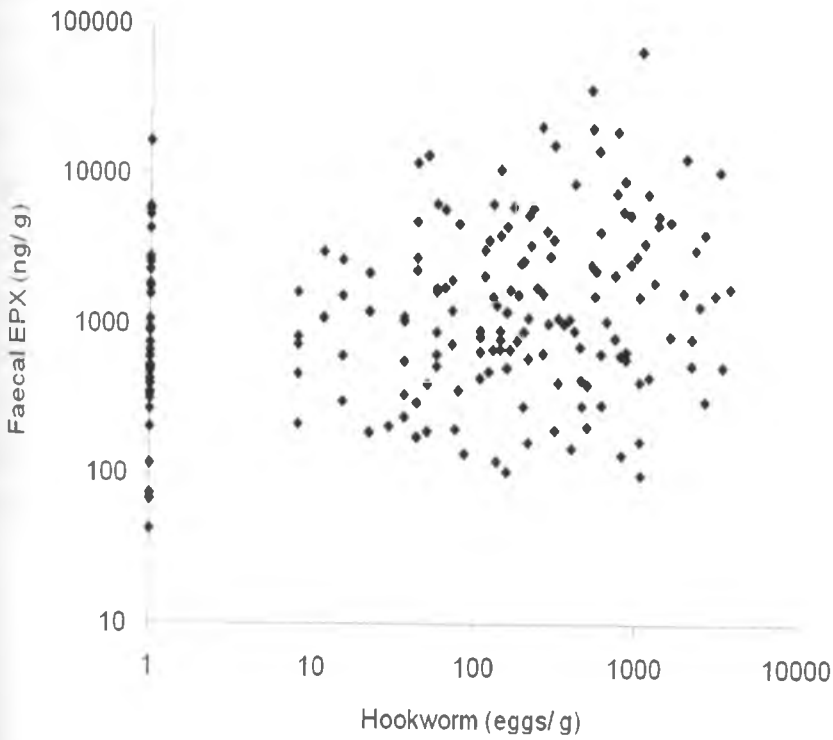
The mean levels of faecal ECP were compared between children with and without anaemia, and between children with the eosinophilia. No significant difference in geometric means of faecal ECP between children with or without anaemia (7,1601 ng/g,  $n=74$  vs. 5,489 ng/g,  $n=107$ ;  $p=0.24$ ; t-test) and eosinophilia was observed ( $F=0.20$ ;  $p=0.94$ ; One-way ANOVA).

#### 4.3.3.3 Relationship between faecal EPX and STH

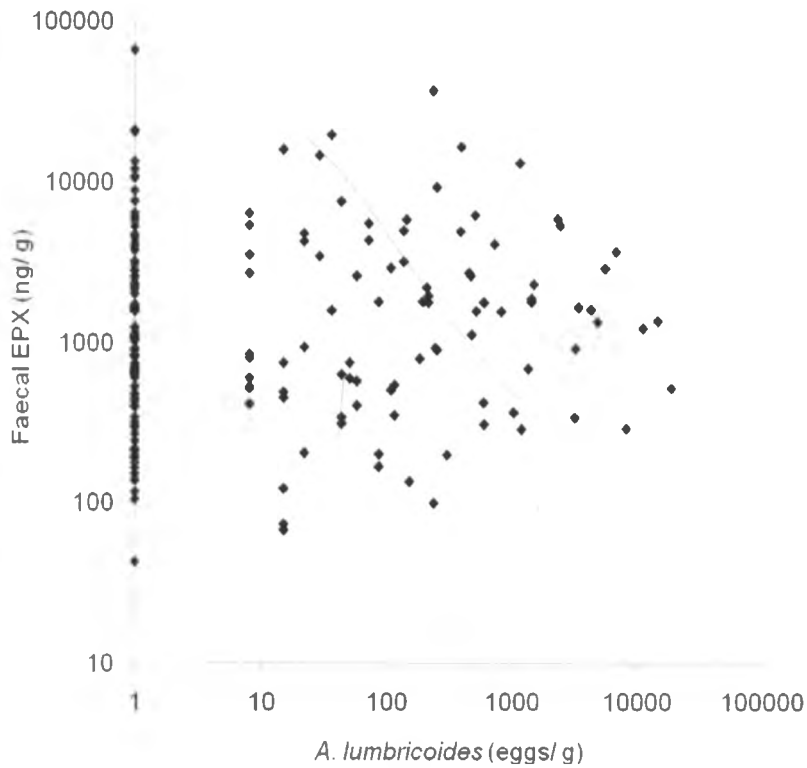
Scatter diagrams of the relationships between levels of faecal EPX and *T. trichiura*, hookworm and *A. lumbricoides* egg counts are shown in Figures 4.10, 4.11 and 4.12, respectively. There was significant positive correlation between levels of faecal EPX and hookworm egg count ( $r=0.24, p=0.001$ ;  $n=186$ ) but only borderline significance in correlation between levels of faecal EPX and *T. trichiura* egg counts ( $r=0.14, p=0.05$ ;  $n=186$ ). There was no significant correlation between levels of faecal EPX and *A. lumbricoides* egg counts ( $r=0.08, p=0.27$ ;  $n=186$ ).



**Figure 4.10:** Relationship between faecal EPX and *T. trichiura* egg count.



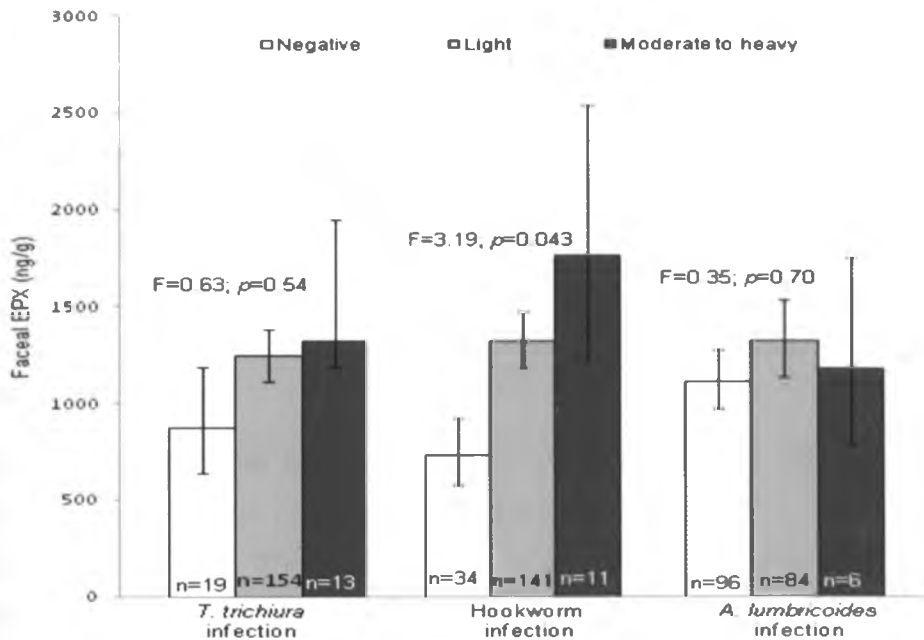
**Figure 4.11:** Relationship between faecal EPX and hookworm egg count.



**Figure 4.12:** Relationship between faecal EPX and *A. lumbricoides* egg count.

The mean levels of faecal EPX in relation to STH infection intensity groups is shown in Figure 4.13. There were no significant differences in the geometric means of faecal EPX between children with no, light or moderate to heavy *T. trichiura* or *A. lumbricoides* infections but there was a trend of increasing levels of faecal EPX with increasing *T. trichiura* infection intensities. There was a significant difference between children with no, light and moderate to heavy hookworm infections.

A comparison of the geometric means of faecal EPX between children negative and positive for STH eggs revealed that there were no significant differences between children negative and positive for *T. trichiura* eggs (869 ng/g vs. 1,247 ng/g;  $p=0.28$ ) or *A. lumbricoides* (1,112 ng/g vs. 1306 ng/g;  $p=0.41$ ) but there was significant difference between children negative and positive for hookworm eggs (729 ng/g vs. 1,343 ng/g;  $p=0.02$ ).



**Figure 4.13:** Geometric mean levels of faecal EPX in relation to STH infection intensity among children in Kau and Ozi primary schools.

*p*-values above the bars indicate the significance levels of differences between the infection intensity groups. The error bars indicate the standard errors on the log-transformed values

The mean levels of faecal EPX were compared between children with and without anaemia, and between children with the different levels of eosinophilia. There was no significant difference in geometric means of faecal EPX between children with and without anaemia (1,274 ng/g (n=74) vs 1,138 ng/g (n=107);  $p=0.59$ ), or normal (1,062 ng/g n=12), moderately eosinophilic (1,190 ng/g; n= 123) and severely eosinophilic (1,101 ng/g; n=19) ( $F=0.06$ ;  $p=0.94$ ).

#### 4.4 Discussion

Blood eosinophilia is a common feature in helminth infections and allergic reactions but mature eosinophils are predominantly tissue-dwelling cells mainly in the mucosae of the gastro-intestinal and urinary tracts (Silveira-Lemos *et al.*, 2008; Oh & Chetty, 2008; Gleich, 2000). Activation of eosinophils in the mucosae results in their degranulation and release of toxic products such as ECP and EPX from specific cytoplasmic granules (Gleich, 2000). These products may kill the offending organism but are also implicated in causing considerable immunopathology characterized by inflammation (Meeusen & Balic, 2000; McLaren *et al.*, 1981; Ackerman *et al.*, 1985; Hamann *et al.*, 1990). In the process, ECP and EPX are excreted in stool and urine and have been suggested as useful markers of early inflammation due to *S. mansoni* and *S. haematobium* infections (Leutscher *et al.*, 2000; Reimert *et al.*, 2000; Reimert *et al.*, 2008).

There was a strong positive correlation between urinary ECP and *S. haematobium* egg

counts. Children with heavy infections and ultrasound-detectable urinary tract morbidity had significantly higher levels of urinary ECP than children without. This corroborates the findings of earlier studies and indicates that urinary ECP reflects *S. haematobium*-related urinary tract pathology in children from endemic areas (Reimert *et al.*, 2000; Leutscher *et al.*, 2000). High levels of urinary EPX have also been reported in *S. haematobium* infected individuals but the fact that this protein has also been reported in urine from healthy and hookworm or onchocerciasis patients (Tischendorf *et al.*, 2000) suggests that it is less specific with regard to *S. haematobium*-related morbidity and was therefore not assessed in the present study.

Haematuria, a cardinal feature of chronic *S. haematobium* infection due to bleeding lesions formed by eggs passing through the urinary tract wall, may result in considerable blood loss and may thus contribute to anaemia (Gryseels *et al.*, 2006). Although inflammation is an important process in formation of these lesions, there was no association between anaemia and urinary ECP level in *S. haematobium* infected children in the present study. This suggests that the observed anaemia may have been multifactorial and not due to haematuria alone. For example, it has been noted that schistosomes-induced systemic pro-inflammatory cytokines, which may not be directly related to egg output, can significantly contribute to anaemia in schistosomiasis (Friedman *et al.*, 2005). Other factors that could have contributed to the observed anaemia include STH infections and iron-deficient diets (Stephenson *et al.*, 2000a; Koski & Scott, 2001).

The data in the present study showed no significant relationship between peripheral blood eosinophilia and urinary ECP but there was a strong positive correlation between urinary ECP and *S. haematobium* egg count that was not influenced by STH infections. This strongly suggests that urinary ECP was secreted by *S. haematobium* egg antigen-activated eosinophils in the urinary tract mucosa. This is consistent with reports that although blood eosinophilia is a common feature of helminth infections, mature and activated eosinophils are predominantly tissue-dwelling cells whose products reflect local eosinophil-mediated inflammatory status (Kato *et al.*, 1998; Reimert *et al.*, 1993; Reimert *et al.*, 2000; Leutscher *et al.*, 2000; Kouriba *et al.*, 2005).

The present study, for the first time, reports on the relationship between STH egg counts and the two inflammatory markers namely, faecal ECP and EPX. Both non-infected and infected children had high levels of faecal ECP and EPX. Due to their close contact with host intestinal tissues, adult *T. trichiura* and hookworm may be expected to cause severe intestinal inflammation whereas adult *A. lumbricoides* does not usually invade the intestinal tissues and may thus not be associated with inflammation (Ortega *et al.*, 2010; Iroo *et al.*, 2010). They, on the other hand, secrete anti-inflammatory proteins that may

substantially reduce this inflammation (Johnston *et al.*, 2009; Maizels *et al.*, 2000; Loukas *et al.*, 2005; Hsieh *et al.*, 2004). As a result, these worms, especially during light or moderate infections, may not cause severe intestinal inflammation. In the present study, most of the STH infections were light (WHO, 2002; Bergquist *et al.*, 2009) which may at least partly explain the lack of significant positive correlation between faecal ECP and STH egg counts although there was an increase in faecal ECP with increasing *T. trichiura* and hookworm egg counts but this was not statistically significant. This sharply contrasts the findings that faecal ECP is positively correlated with *S. mansoni* egg counts (Reimert *et al.*, 2008). This is probably because unlike adult *T. trichiura* and hookworm, *S. mansoni* miracidia in tissue-lodged eggs may lack strong ability to down-regulate inflammatory responses directed against them which suggests that faecal ECP is a better marker of *S. mansoni* intestinal morbidity than *T. trichiura*- or hookworm-related intestinal morbidity.

The lack of a clear-cut relationship between levels of faecal ECP and *T. trichiura*, hookworm or *A. lumbricoides* shows that light or moderately heavy STH infections may not evoke substantial intestinal inflammation. On the other hand, there was a significant positive correlation between levels of faecal EPX, another marker of intestinal inflammation (Reimert *et al.*, 2008; Wagner *et al.*, 2008), and hookworm egg counts. Coupled with the finding of a significant difference in levels of faecal EPX between children with different hookworm infection intensities, this could mean that ECP- and EPX-containing eosinophil granules respond differently to the same stimuli. Majamaa *et al.* (1999) have also reported some differences between faecal ECP and EPX in children with atopic eczema and food allergy. Reimert *et al.* (2008) have also demonstrated that following praziquantel treatment of *S. mansoni*-infected individuals, faecal ECP and EPX fluctuate differently. Taken together, these findings indicate that faecal ECP and EPX may have different values and importance as markers of intestinal inflammation due to *S. mansoni* or STH infection. Further detailed studies on this subject should however be carried out before a concrete conclusion is made. It is also noteworthy that there were substantially high levels of faecal ECP and EPX in both STH-infected and non-infected children. This suggests that faecal ECP and EPX may to some extent be influenced by other conditions, including intestinal infection with other pathogens such as protozoa and bacteria that may cause inflammation (Tischendorf *et al.*, 2000).

As observed for urinary ECP, there was no relationship between faecal ECP or EPX and anaemia. This lends support to the argument that the observed anaemia was not due to schistosome- or STH-induced inflammation alone but may have been due to many other factors in addition to the effects of schistosome and STH infections (Stephenson *et al.*, 2000a; Koski & Scott, 2001). Similarly, there were no significant differences in levels of faecal ECP or EPX between children with different levels of eosinophilia. This is

consistent with the lack of significant relationship between urinary ECP and peripheral blood eosinophilia. Seemingly, faecal ECP and EPX were secreted by activated eosinophils in the intestinal mucosa and may not accurately mirror systemic eosinophil activity.

#### **4.5 Conclusion**

In the present study, urinary ECP was positively associated with *S. haematobium* egg counts in urine, haematuria and ultrasound-detectable urinary tract pathology but not with peripheral blood eosinophilia or anaemia. The data also indicated that urinary ECP was not sensitive to the influence of STH infections. These results suggest that urinary ECP may be a reliable marker of *S. haematobium* related urinary tract morbidity (Leutscher *et al.*, 2000; Reimert *et al.*, 2000). Data on faecal ECP indicated that although it is a useful marker of intestinal morbidity due to *S. mansoni* infection (Reimert *et al.*, 2008), it may be less valuable as a marker of intestinal morbidity due to STH infections. Similarly, the data suggest that faecal EPX may not be a good marker of intestinal morbidity due to *T. trichiura* and *A. lumbricoides* infections as there was no association between it and either *T. trichiura* or *A. lumbricoides* egg count. The finding that faecal EPX was positively associated with hookworm egg counts suggests that this protein may be a useful marker of intestinal morbidity due to hookworm infection. This is the first such report and more studies need to be carried out to evaluate the importance of faecal EPX as a marker for hookworm-related intestinal morbidity.



# CHAPTER FIVE: SERUM AND URINARY CYTOKINE LEVELS IN SCHISTOSOMA HAEMATOBIIUM INFECTION AMONG SCHOOL CHILDREN OF TANA DELTA DISTRICT, KENYA

## 5.1 Introduction

It is well recognized that most of morbidity during infection with schistosomes emanates from immune responses to tissue-lodged schistosome eggs. However, there is no clear consensus about the pattern of cytokine production and regulation that leads to this morbidity (Abath *et al.*, 2006). One of the reasons for this is a lack of appropriate tools and approaches to study human disease in endemic areas as a result of which studies rely heavily on findings generated from experimental models of the disease (Abath *et al.*, 2006). In addition, due to many laboratory animals refractoriness to *S. haematobium*, less is understood about the immunology and pathological mechanisms related to this parasite compared to *S. mansoni* and *S. japonicum* (Rollinson, 2009).

Morbid sequelae of *S. haematobium* infection is characterised by dermatitis, fevers, fatigue, myalgia, malaise, non-productive cough, and eosinophilia in the acute phase of the infection (Ross *et al.*, 2002). This is followed by ulceration and pseudopolyposis in the urinary tract leading to dysuria, frequent micturation, proteinuria, eosinophiluria and haematuria during the chronic phase (Ross *et al.*, 2002). Several experimental studies using *S. mansoni*-animal models of schistosomiasis and a few on humans, mainly *ex vivo*, have shown that this clinical course is primarily orchestrated by the host cytokine responses (Abath *et al.*, 2006).

Currently available information suggest that there is significant variability in the profiles of immune responses in humans characterized by a mixed Th<sub>1</sub> – Th<sub>2</sub> response, with a predominance of Th<sub>1</sub> cytokines in acutely infected patients and a low secretion of Th<sub>2</sub> cytokines as the infection becomes chronic (Abath *et al.*, 2006). Several studies, some using human peripheral blood cells and others using *S. mansoni*-mice models, have shown that increased systemic production of Th<sub>1</sub> pro-inflammatory cytokines such as TNF- $\alpha$  and IL-6 may lead to vigorous circumoval granulomas (Abath *et al.*, 2006). It has also been demonstrated that increased production of IFN- $\gamma$ , another Th<sub>1</sub> cytokine, may protect the host not only against infection but also against severe morbidity by suppressing granuloma formation and even reducing their sizes (de Jesus *et al.*, 2002; Lammie *et al.*, 1986; Wynn *et al.*, 1995; Czaja *et al.*, 1989; Lukacs & Boros, 1993). Information emanating from these and other studies have also led to an almost unanimous agreement that the regulatory IL-10 plays a pivotal role in down-modulating the inflammatory processes that lead to schistosome egg-associated morbidity (Abath *et al.*, 2006; van Stijn *et al.*, 2010).

There have been fewer studies on immunopathology of *S. haematobium* infections compared to *S. mansoni* infections. These studies, mainly by stimulation of peripheral blood cells of infected individuals, have yielded results that are almost similar to those of *S. mansoni* infection in humans and in mice. For example, it has been shown that strong TNF- $\alpha$  response relative to IL-10 leads to increased ultrasound-detectable morbidity in the urinary tracts of children in coastal Kenya (Wamachi *et al.*, 2004; King *et al.*, 2001). However, just like in *S. mansoni* studies, there has been some variations in cytokine profiles among individuals in endemic populations which, according to Wamachi *et al.* (2004), could be due to co-infections with other nematode parasites. In an endemic population in Zimbabwe it was not possible to relate Th<sub>1</sub>/Th<sub>2</sub> cytokine responses dichotomy to susceptibility or resistance to *S. haematobium* infections (Mutapi *et al.*, 2007).

Moreover, infections with STH evidently evoke strong host cytokine responses especially the regulatory and anti-inflammatory IL-10 (Cooper *et al.*, 2008; Maizels *et al.*, 2004; Jackson *et al.*, 2004; Quinnell *et al.*, 2004). It is therefore probable that, if individuals have underlying STH co-infection, they contribute to the variation observed in schistosome egg-directed cytokine responses (Wamachi *et al.*, 2004).

Most of the studies have concentrated on systemic cytokine responses and related them to local inflammatory processes in distant tissues in the urinary or intestinal tracts instead of studying cytokine responses at the target organ. This is complicated by the fact that immune cells may secrete different cytokines in different environments, due to different stimuli (Duitman *et al.*, 2011). Most of the cytokines involved in these inflammatory processes are secreted not only by immune cells but also by non-haematopoietic cells (Hoffmann *et al.*, 2000; Lu *et al.*, 2011; Flower *et al.*, 2003; Guarda *et al.*, 2011; Gray & Kamolrat, 2011). This suggests that studying local cytokine responses in the urinary tract mucosa may yield more accurate information about how cytokines influence morbidity in the target organ during *S. haematobium* infection.

Previous studies on bacterial infections in the urinary tract have demonstrated that there is substantial cytokine secretion in the mucosa indicating that urinary tract mucosa is an immunologically active tissue with significant secretion of cytokines. For example, by deliberately colonizing the urinary tract of volunteers with *Escherichia coli*, Hedges *et al.* (1991) were able to elicit secretion of measurable amounts of IL-6 from the urinary tract mucosa into urine. In another *in vitro* study using epithelial cells from human urinary tract, *E. coli* elicited high levels of IL-6 but not TNF- $\alpha$  whereas human peripheral blood monocytes secreted both IL-6 and TNF- $\alpha$  among other cytokines in response to the same bacterium (Agace *et al.*, 1993). This suggests that there could be differences between

systemic and local mucosa cytokine responses during infections such as with *S. haematobium*. It has also been demonstrated that local IL-6 levels in response to *E. coli* in the urinary tract mucosa are slightly lower than systemic levels and there is no correlation in IL-6 levels between the two compartments (Otto *et al.*, 1999). Urinary cytokine levels relate to disease severity and may therefore be a better reflection of the magnitude of infection-driven inflammation in the urinary tract (Otto *et al.*, 1999). However, no studies of *S. haematobium* have so far been carried out to demonstrate this despite the importance of the pathological mucosal inflammation caused by infection with this parasite.

Based on other studies, it can therefore be hypothesized that urinary cytokines are good indicators of *S. haematobium*-related urinary tract pathology. The present study therefore aimed at assessing and comparing serum and urinary IL-6, IL-10, IFN- $\gamma$  and TNF- $\alpha$  levels, and analyzing their relationship to *S. haematobium* infection in school children from the two villages of Tana Delta District.

## **5.2 Materials and Methods**

### **5.2.1 Study area and study design**

The study area, study population and study design were as described in chapters 2 and 3. Briefly, a cross-sectional study was carried out whereby serum and urine samples were assayed for concentrations of selected cytokines. Urine samples were examined for *S. haematobium* eggs, ECP levels and haematuria whereas stool samples were examined for STH eggs. Venous blood samples were subjected to haemoglobin test. In addition, urinary tract of each child was subjected to ultrasound examination for pathology.

### **5.2.2 Cytokine ELISA**

Serum and urine samples were assayed for concentrations of IL-6, IL-10, TNF- $\alpha$  and IFN- $\gamma$  by monoclonal sandwich ELISA technique using specific cytokine ELISA kits (BD OptEIA™, USA) according to manufacturer's instructions and as described in Chapter 2. Briefly, lyophilized recombinant human IL-6, IL-10, TNF- $\alpha$  or IFN- $\gamma$  was used as standard for respective cytokine assay. Reading of the cytokine concentrations was done using Reading Microplate Manager 4.0® (Bio-Rad Laboratories, Inc.) software at a measurement wavelength of 490 nm and a reference wavelength of 595 nm. The amount of IL-6, IL-10, TNF- $\alpha$  or IFN- $\gamma$  in serum or urine was expressed as pg/ ml.

### **5.2.3 Statistical Analysis**

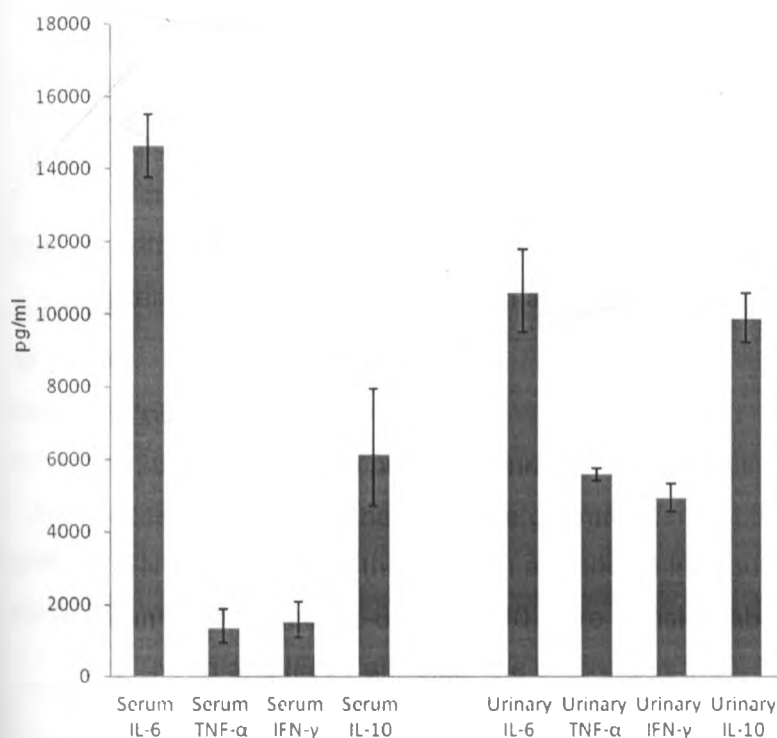
Data were entered in an Excel spread sheet and then exported to Stata (Version 10) for statistical analysis as explained in Chapter 2. Briefly, log-transformed values of egg count and IL-6, TNF- $\alpha$ , IFN- $\gamma$  or IL-10 levels were used for the analysis of the association between *S. haematobium* egg counts and cytokine responses, while controlling for the effects of age and sex in multiple variable regression models. To deduce how STH

influenced this association, each of *T. trichiura*, hookworm and *A. lumbricoides* egg counts were separately introduced in the model and the different correlation coefficient (the proportion of the observed variation in IL-6, TNF- $\alpha$ , IFN- $\gamma$  or IL-10 level explained by *S. haematobium* egg count), regression coefficient (the change in IL-6, TNF- $\alpha$ , IFN- $\gamma$  or IL-10 level with a change of one *S. haematobium* egg per ml of urine) and *p*-value recorded. If *p*-value changed from  $\geq 0.05$  to  $\leq 0.05$  or vice versa following introduction of STH egg count in the model, the STH in question was regarded as having significant influence on IL-6, TNF- $\alpha$ , IFN- $\gamma$  or IL-10 response to *S. haematobium* infection. *p*-values less than 0.05 were considered statistically significant in all tests.

## 5.3 Results

### 5.3.1 Study population

There were 262 children who provided urine as described in Chapter 3 while 158 provided blood samples for cytokine analysis. The geometric mean levels of serum and urinary IL-6, TNF- $\alpha$ , IFN- $\gamma$  and IL-10 in the children are shown in Figure 5.1. Geometric mean levels of both serum and urinary IL-6 and IL-10 were considerably higher than those of serum and urinary TNF- $\alpha$  and IFN- $\gamma$  although the differences were not tested statistically.

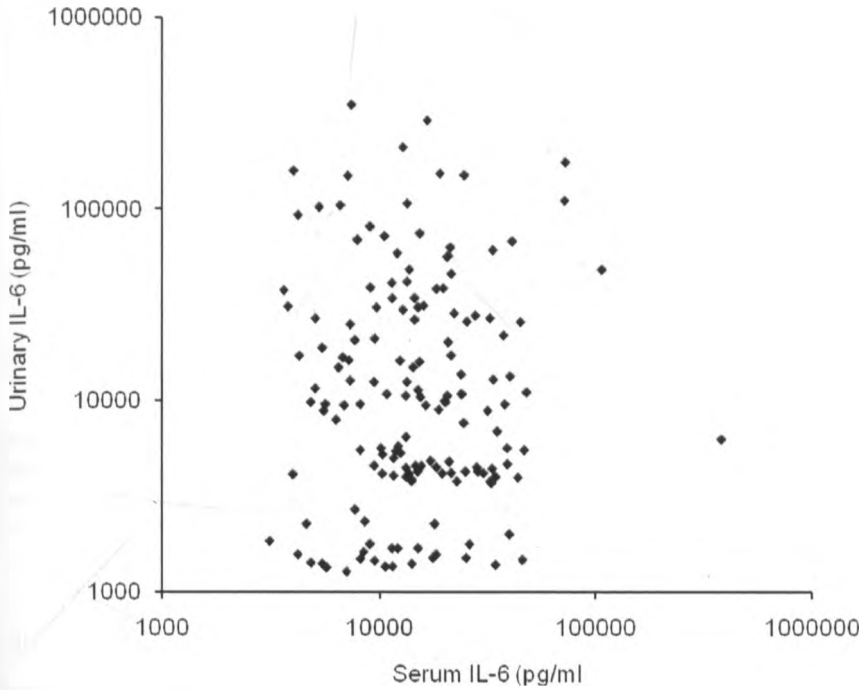


**Figure 5.1:** Geometric mean levels of serum and urinary IL-6, TNF- $\alpha$ , IFN- $\gamma$  and IL-10 in children in Kau and Ozi primary schools. The error bars indicate the standard error on log-transformed values.

### 5.3.2 IL-6 responses

#### 5.3.2.1 Relationship between serum and urinary IL-6

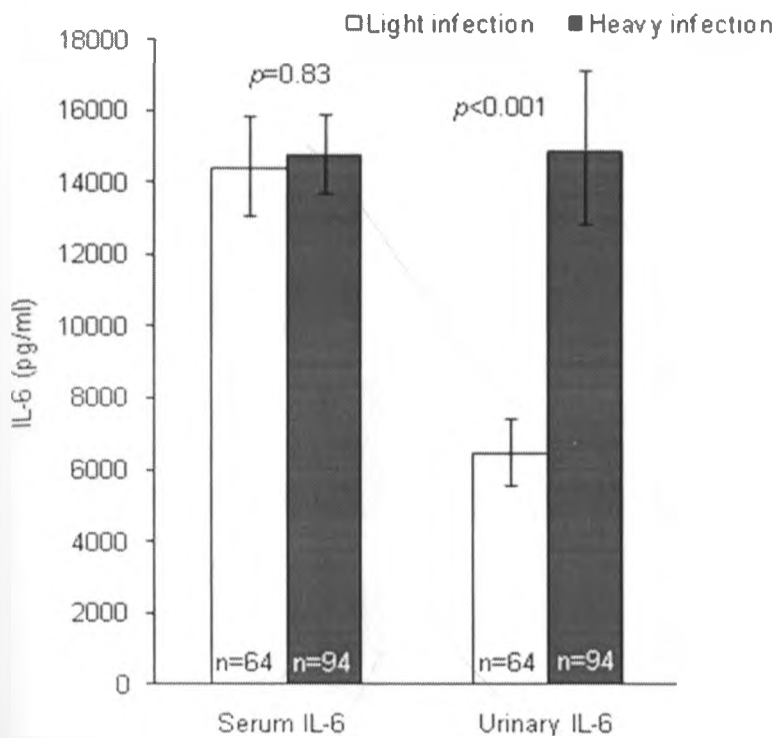
A scatter diagram showing the relationship between serum IL-6 and urinary IL-6 levels in the examined individuals is shown in Figure 5.2. When the linear relationship between geometric mean levels of serum IL-6 and urinary IL-6 was assessed, there was no significant linear correlation between serum IL-6 and urinary IL-6 ( $r=0.01$ ,  $p=0.94$ ;  $n=158$ ).



**Figure 5.2:** Relationship between serum IL-6 and urinary IL-6 among children in Kau and Ozi.

#### 5.3.2.2 Relationship between IL-6 and *S. haematobium* infection

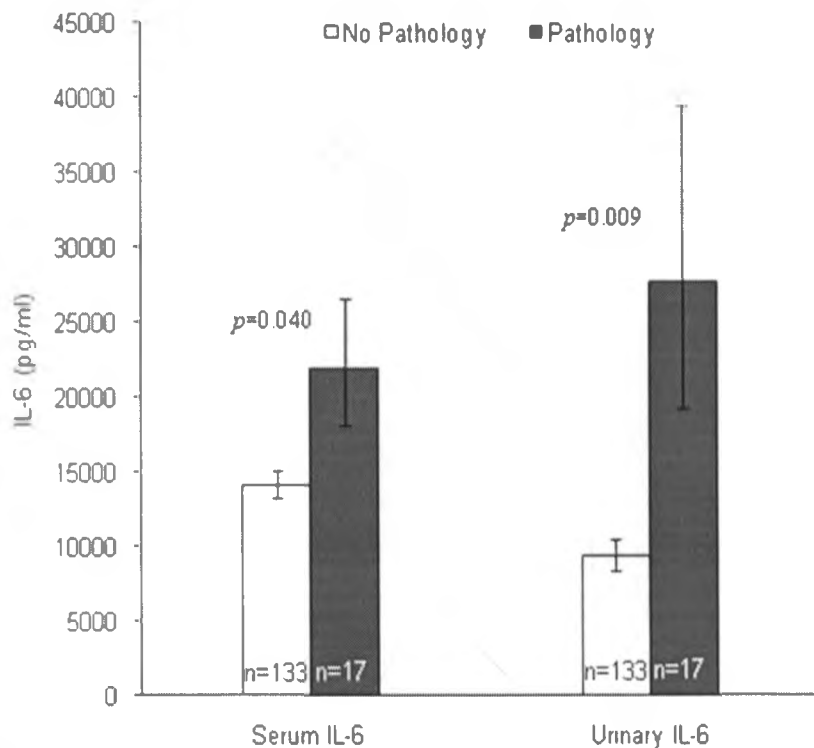
The geometric mean levels of serum IL-6 and urinary IL-6 in relation to *S. haematobium* infection intensity groups is shown in Figure 5.3. There was no significant difference in geometric mean levels of serum IL-6 between children with light and children with heavy *S. haematobium* infections but children with heavy *S. haematobium* infections had a significantly higher geometric mean level of urinary IL-6 than those with light *S. haematobium* infections.



**Figure 5.3:** Geometric mean levels of IL-6 in relation to *S. haematobium* infection intensity among children in Kau and Ozi primary schools.

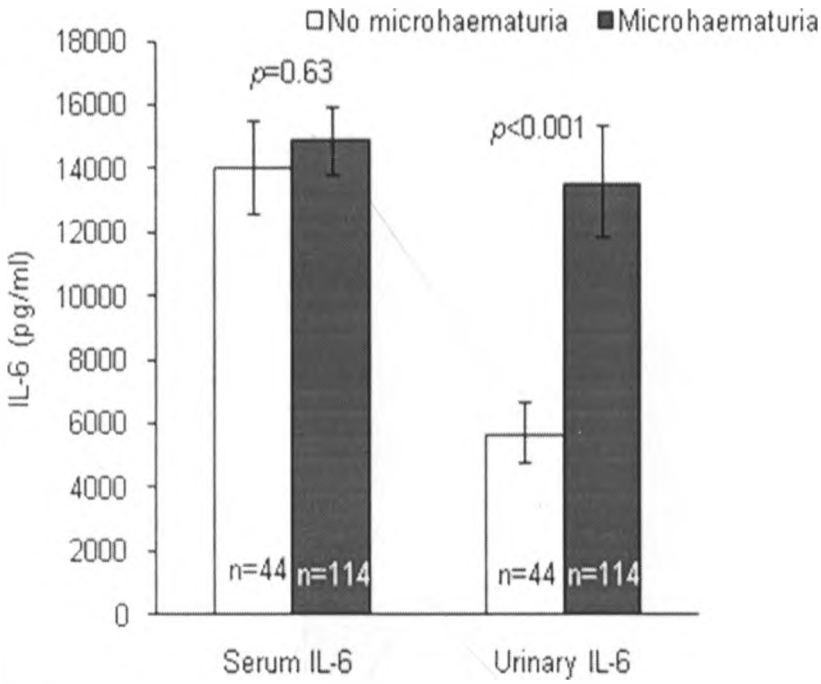
*p*-values above the bars indicate the significance level of differences between the light and heavy *S. haematobium* infection intensity groups. The error bars indicate the standard error on log-transformed values.

The mean levels of IL-6 in relation to ultrasound-detectable upper urinary tract pathology groups is shown in Figure 5.4. Children with pathology had a significantly higher geometric means of serum IL-6 and urinary IL-6 than those without pathology.



**Figure 5.4:** Geometric mean levels of IL-6 in relation to ultrasound-detectable upper urinary tract pathology among children in the two schools. *p*-values above the bars indicate the significance level of differences between the group with pathology and that without pathology. The error bars indicate the standard error on log-transformed values

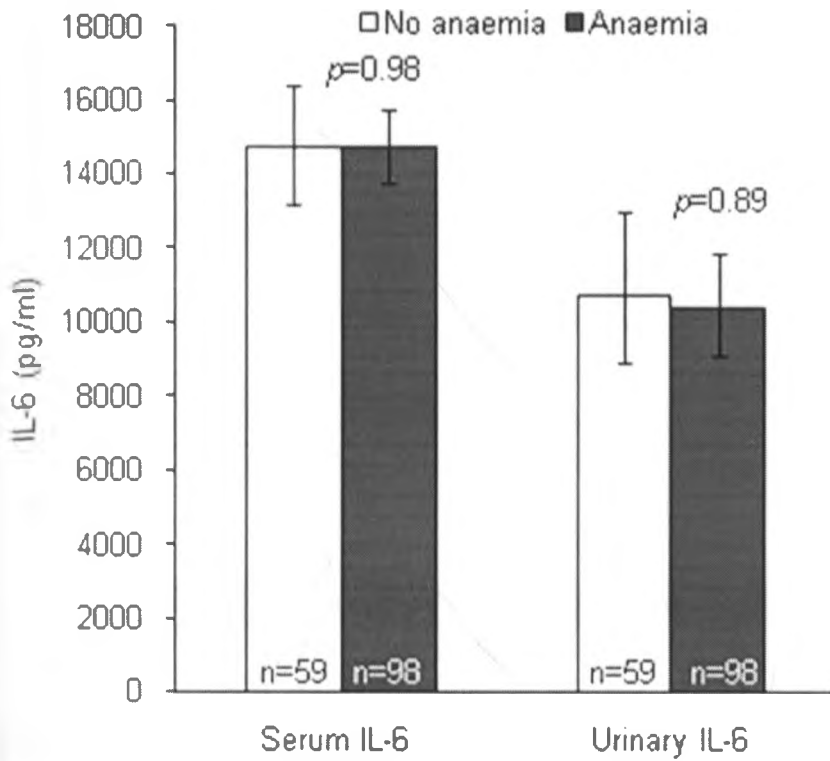
The geometric mean levels of serum IL-6 and urinary IL-6 in relation to microhaematuria are shown in Figure 5.5. From the figure, there was no significant difference in geometric mean levels of serum IL-6 between children with microhaematuria and those without microhaematuria but children with microhaematuria had a significantly higher geometric mean of urinary IL-6 than children without microhaematuria.



**Figure 5.5:** Geometric mean levels of IL-6 in relation to microhaematuria among children in Kau and Ozi primary schools. *p*-values above the bars indicate the significance level of differences between the group with microhaematuria and that without microhaematuria. The error bars indicate the standard error on log-transformed values.

The mean levels of IL-6 in relation to anaemia is shown in Figure 5.6. There was no significant difference in geometric means of serum IL-6 or urinary IL-6 between children with anaemia and those without.

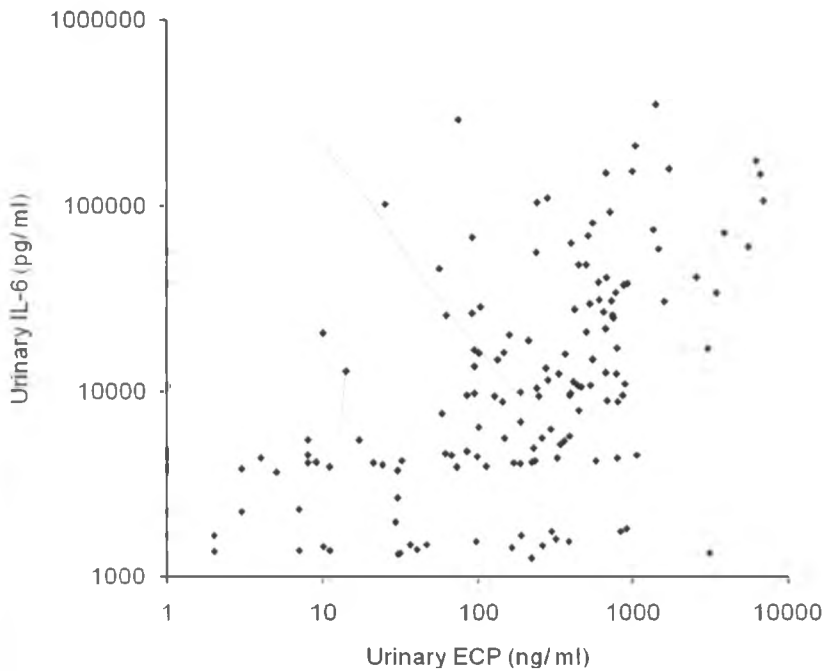




**Figure 5.6:** Geometric mean levels of IL-6 in relation to anaemia.

*p-values* above the bars indicate the significance level of differences between the group with anaemia and that without anaemia. The error bars indicate the standard error on log-transformed values.

Assessment of the linear relationship between levels of serum IL-6 and urinary ECP did not show a significant relationship ( $r = -0.08$ ,  $p = 0.32$ ,  $n = 143$ ). A scatter diagram showing the relationship between urinary IL-6 and urinary ECP is shown in Figure 5.7. There was a significant positive linear relationship between urinary IL-6 and urinary ECP ( $r = 0.54$ ,  $p < 0.001$ ;  $n = 143$ ).



**Figure 5.7:** Relationship between urinary IL-6 and urinary ECP among children in Kau and Ozi primary schools.

### 5.3.2.3 Effect of soil-transmitted helminths on the association between IL-6 and *S. haematobium* infection

The effects of STH egg counts on the association between *S. haematobium* egg counts and levels of serum and urinary IL-6 is shown in Table 5.1. From the table, STH egg counts had no significant effects on the relationship between serum IL-6 or urinary IL-6 and *S. haematobium* eggs counts.

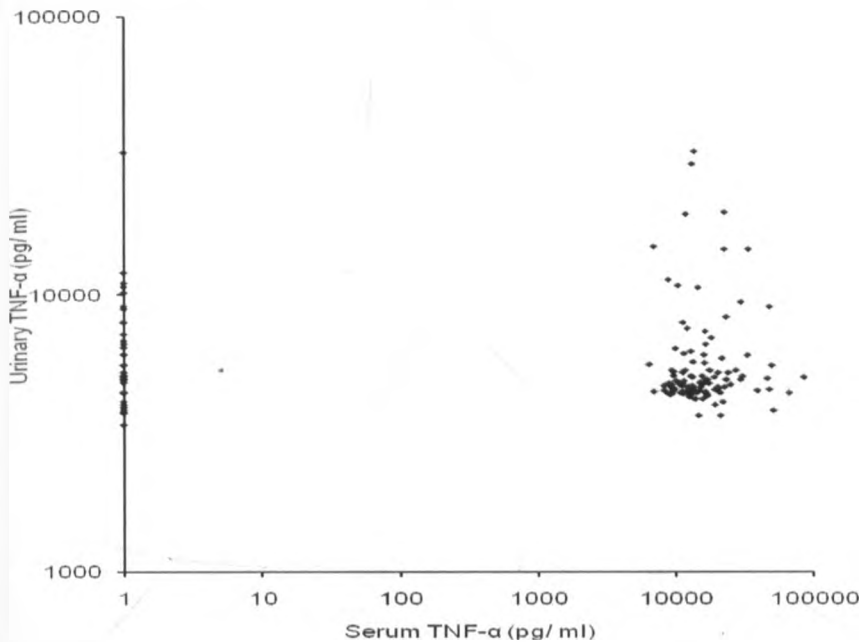
**Table 5.1:** The effects of STH on IL-6 responses to *S. haematobium* infections, using a regression model controlling for the effects of age and sex.  
 $r^2$  = correlation coefficient, Coefficient = regression coefficient.

Serum IL-6				
	<i>S. haematobium</i> only	<i>T. trichiura</i>	Hookworm	<i>A. lumbricoides</i>
$r^2$	0.01	0.03	0.03	0.03
Coefficient	-0.01	-0.02	-0.02	-0.01
p-value	0.63	0.51	0.60	0.67
Urinary IL-6				
	<i>S. haematobium</i> only	<i>T. trichiura</i>	Hookworm	<i>A. lumbricoides</i>
$r^2$	0.15	0.17	0.16	0.16
Coefficient	0.22	0.21	0.22	0.23
p-value	<0.001	<0.001	<0.001	<0.001

### 5.3.3 Tumour Necrosis Factor- $\alpha$ responses

#### 5.3.3.1 Relationship between serum TNF- $\alpha$ and urinary TNF- $\alpha$

A scatter diagram showing the relationship between serum TNF- $\alpha$  and urinary TNF- $\alpha$  levels in the examined individuals is shown in Figure 5.8. There was no significant linear correlation between serum TNF- $\alpha$  and urinary TNF- $\alpha$  ( $r = -0.09$ ,  $p=0.24$ ;  $n=158$ ).



**Figure 5.8:** Relationship between serum TNF- $\alpha$  and urinary TNF- $\alpha$  among children in Kau and Ozi primary schools.

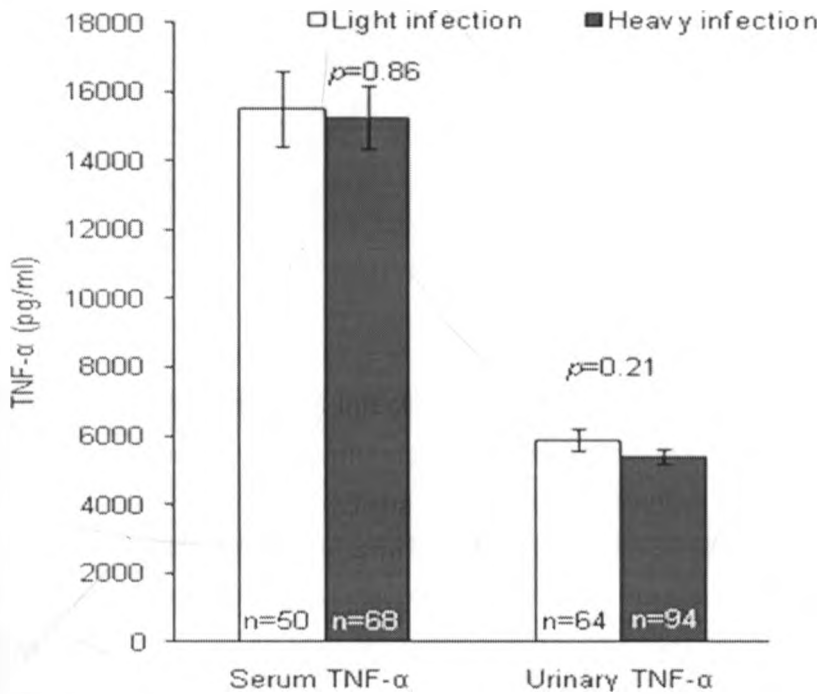
A group of 40 children had levels of serum TNF- $\alpha$  below the lower detection limit of the test and were thus regarded as non-responders. There was a significant difference in mean age between the responders and non-responders (9.6 years vs. 10.6 years,  $p=0.002$ ).

There was no significant difference between the responders and non-responders in terms of *S. haematobium*, *T. trichiura* or *A. lumbricoides* infection intensities ( $p=0.29$ , 0.22 and 0.85, respectively) or prevalences ( $p=0.41$ , 0.17 and 0.19, respectively). Although there was no significant difference in prevalence of hookworm infections between responders and non-responders ( $p=0.17$ ), the non-responders had a significantly higher geometric mean of hookworm egg count than responders ( $p=0.04$ ).

#### 5.3.3.2 Relationship between TNF- $\alpha$ and *S. haematobium* infection and morbidity markers

The group of non-responders could negatively skew the distribution of serum TNF- $\alpha$  data and was therefore excluded in the subsequent analysis. The mean levels of TNF- $\alpha$  in

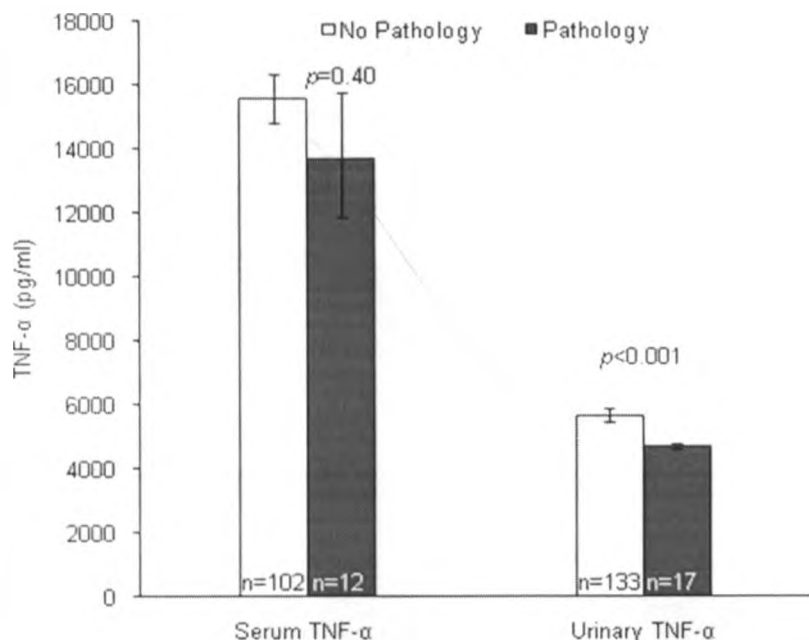
relation to *S. haematobium* infection intensity group is shown in Figure 5.9. There was no significant difference in geometric means of serum or urinary TNF- $\alpha$  between children with light and those with heavy *S. haematobium* infections. The geometric means of urinary TNF- $\alpha$  were lower than those of serum TNF- $\alpha$  in both children with light and those with heavy *S. haematobium* infections.



**Figure 5.9:** Geometric mean levels of TNF- $\alpha$  in relation to *S. haematobium* infection intensity among children in Kau and Ozi primary schools.

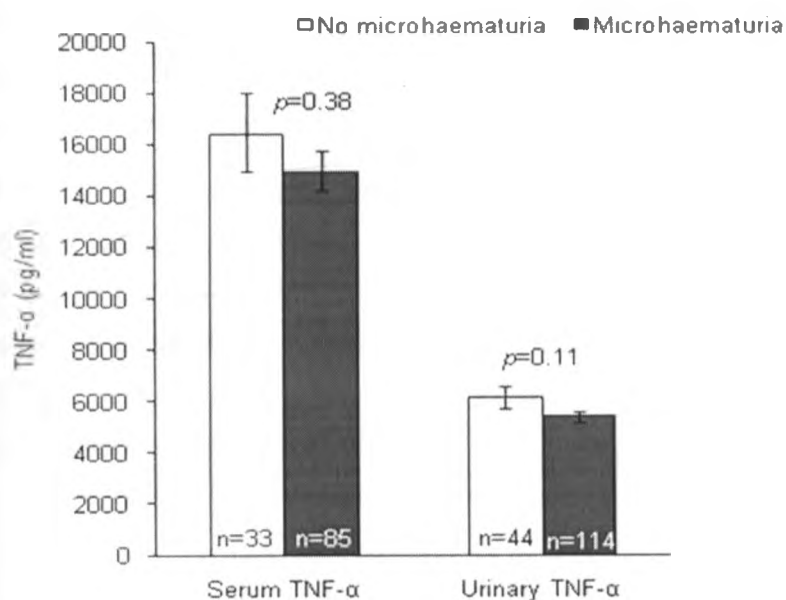
*p*-values above the bars indicate the significance level of differences between the light and heavy *S. haematobium* infection intensity groups. The error bars indicate the standard error on log-transformed values

The mean levels of TNF- $\alpha$  in relation to ultrasound-detectable upper urinary tract pathology group is shown in Figure 5.10. There was no significant difference in the geometric means of serum TNF- $\alpha$  between children with and without pathology. However, children without pathology had a significantly higher geometric mean of urinary TNF- $\alpha$  than those with pathology.



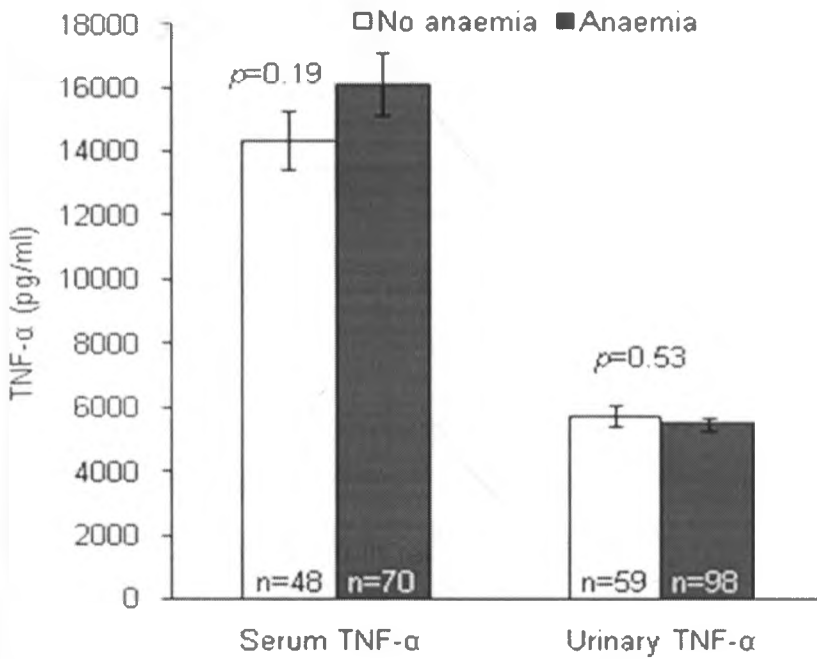
**Figure 5.10:** Geometric mean levels of TNF- $\alpha$  in relation to ultrasound-detectable upper urinary tract pathology among children in Kau and Ozi primary schools. *p*-values above the bars indicate the significance level of differences between the group with pathology and that without pathology. The error bars indicate the standard error on log-transformed values

The mean levels of TNF- $\alpha$  in relations to microhaematuria group is shown in Figure 5.11. There was no significant difference in geometric means of serum TNF- $\alpha$  or urinary TNF- $\alpha$  between children with microhaematuria and those without microhaematuria.



**Figure 5.11:** Geometric mean levels of TNF- $\alpha$  in relation to microhaematuria among children in Kau and Ozi primary schools. *p*-values above the bars indicate the significance level of differences between the group with microhaematuria and that without microhaematuria. The error bars indicate the standard error on log-transformed values

The mean levels of TNF- $\alpha$  in relation to anaemia group is shown in Figure 5.12. There was no significant difference in geometric means of serum TNF- $\alpha$  or urinary TNF- $\alpha$  between children with anaemia and those without.



**Figure 5.12:** Geometric mean levels of TNF- $\alpha$  in relation to anaemia among children in Kau and Ozi primary schools.

$p$ -values above the bars indicate the significance level of differences between the group with anaemia and that without anaemia. The error bars indicate the standard error on log-transformed values

Assessment of the linear relationship between TNF- $\alpha$  and urinary ECP revealed no significant relationship between serum TNF- $\alpha$  and urinary ECP ( $r = -0.11$ ,  $p = 0.29$ ,  $n = 105$ ) or between urinary TNF- $\alpha$  and urinary ECP ( $r = 0.02$ ,  $p = 0.80$ ,  $n = 143$ ).

### 5.3.3.3 Effect of soil-transmitted helminths on the association between TNF- $\alpha$ and *S. haematobium* infection

The effects of STH on the association between *S. haematobium* egg counts and levels of serum and urine TNF- $\alpha$  is shown in Table 5.2. From the table, STH egg counts had no significant effects on the relationship between serum TNF- $\alpha$  or urinary TNF- $\alpha$  and *S. haematobium* eggs counts.

**Table 5.2:** The effects of STH on TNF- $\alpha$  responses to *S. haematobium* infections, using a regression model controlling for the effects of age and sex.  
 $r^2$  = correlation coefficient, Coefficient = regression coefficient

Serum TNF- $\alpha$				
	<i>S. haematobium</i> only	<i>T. trichiura</i>	Hookworm	<i>A. lumbricoides</i>
$r^2$	0.01	0.01	0.02	0.03
Coefficient	-0.01	-0.01	-0.01	-0.02
p-value	0.53	0.55	0.61	0.49
Urinary TNF- $\alpha$				
	<i>S. haematobium</i> only	<i>T. trichiura</i>	Hookworm	<i>A. lumbricoides</i>
$r^2$	0.02	0.25	0.04	0.02
Coefficient	-0.03	-0.02	-0.02	-0.02
p-value	0.17	0.21	0.21	0.18

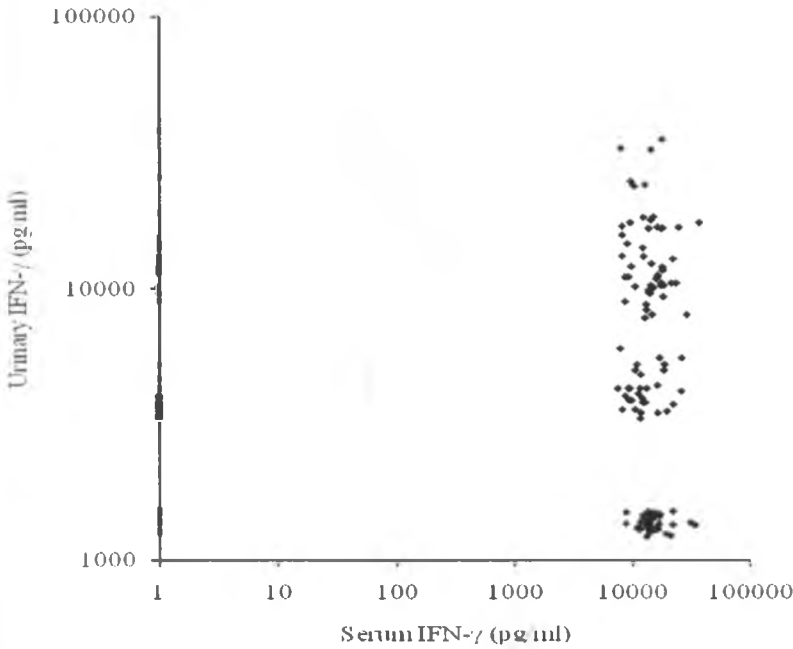
### 5.3.4 IFN- $\gamma$ responses

#### 5.3.3.4 Relationship between Serum IFN- $\gamma$ and Urinary IFN- $\gamma$

A scatter diagram showing the relationship between serum IFN- $\gamma$  and urinary IFN- $\gamma$  levels in the examined individuals is shown in Figure 5.13. There was no linear relationship between levels of serum IFN- $\gamma$  and urinary IFN- $\gamma$  ( $r = -0.07$ ,  $p = 0.36$ ).

A group of 39 children had very low levels of serum IFN- $\gamma$ , below the lower detection limit of the test (non-responders). There was no significant difference in mean age between the responders and non-responders (9.7 years vs. 9.9 years,  $p = 0.69$ ).

There was no significant difference between responders and non-responders in terms of *S. haematobium*, *T. trichiura*, hookworm or *A. lumbricoides* infection intensities ( $p = 0.39$ , 0.84, 0.11 and 0.08, respectively) or prevalences of *S. haematobium*, *T. trichiura* and hookworm ( $p = 0.33$ , 0.85 and 0.53, respectively). However, a significantly higher proportion of the responders (52.9%) than that of non-responders (33.3%) had *A. lumbricoides* infections ( $p = 0.03$ ;  $n = 158$ ).

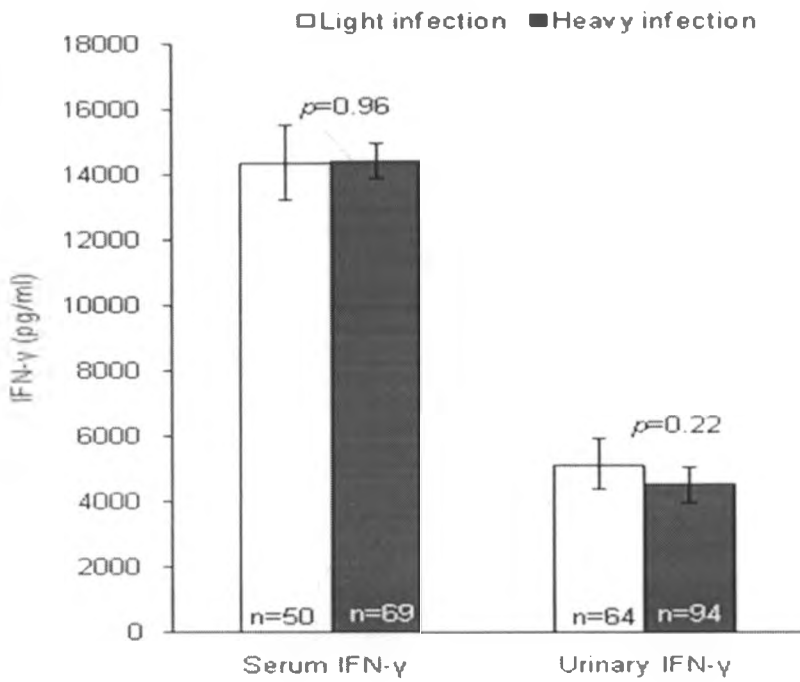


**Figure 5.13:** Relationship between serum IFN- $\gamma$  and urinary IFN- $\gamma$  among children in Kau and Ozi primary schools.

#### 5.3.4.2 Relationship between IFN- $\gamma$ And *S. haematobium* infection and Morbidity Markers

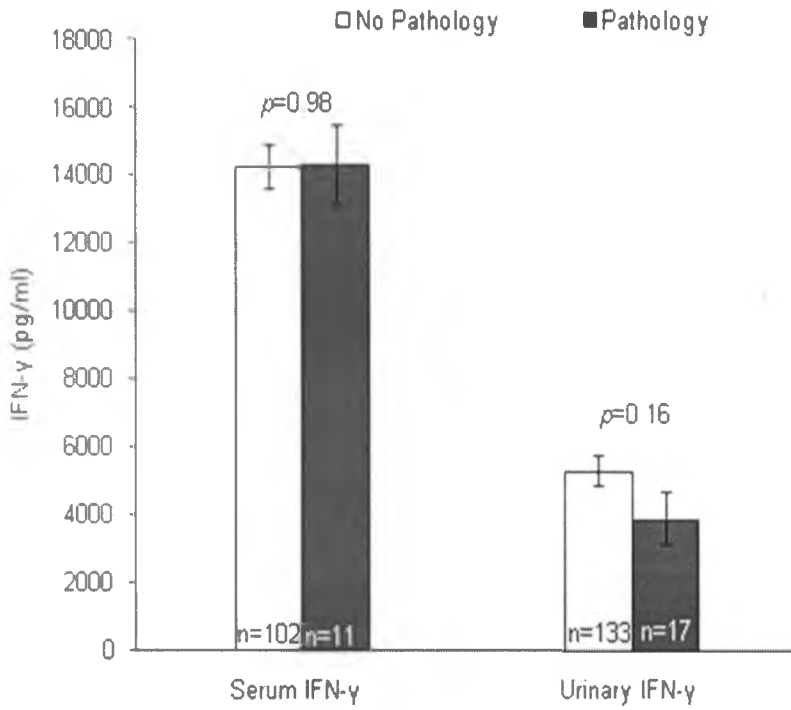
The group of serum IFN- $\gamma$  non-responders could negatively skew the distribution of serum IFN- $\gamma$  data and was therefore excluded in the subsequent analysis. The mean levels of IFN- $\gamma$  in relation to *S. haematobium* infection intensity group is shown in Figure 5.14. There was no significant difference in geometric means of serum IFN- $\gamma$  or urinary IFN- $\gamma$  between children with light and those with heavy *S. haematobium* infections. In both light and heavy *S. haematobium* infection groups, geometric mean of urinary IFN- $\gamma$  were lower than those of serum IFN- $\gamma$ .





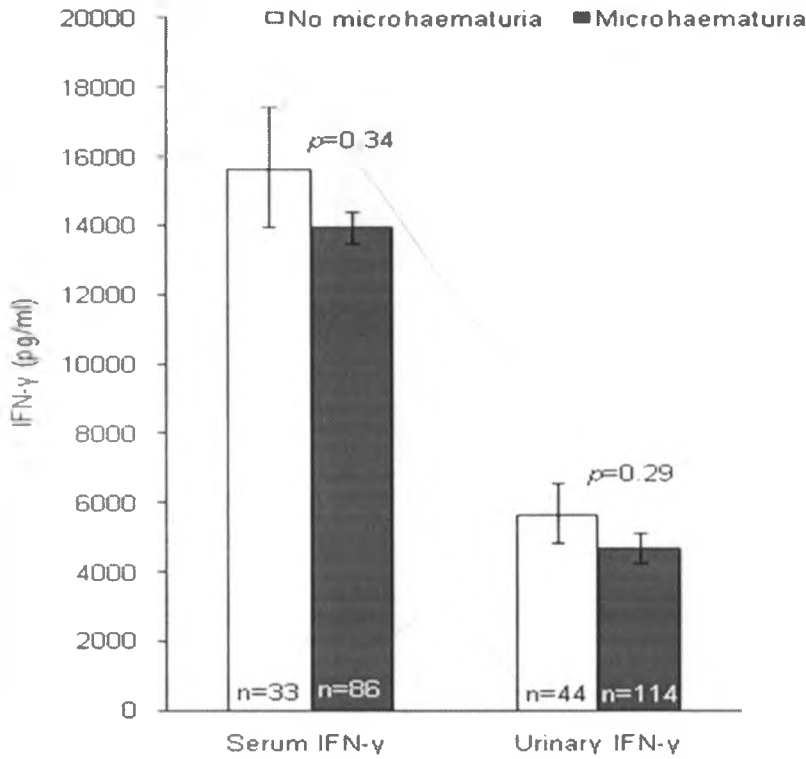
**Figure 5.14:** Geometric mean levels of IFN- $\gamma$  in relation to *S. haematobium* infection intensity among children in Kau and Ozi primary schools. *p*-values above the bars indicate the significance level of differences between the light and heavy *S. haematobium* infection intensity groups. The error bars indicate the standard error on log-transformed values

The mean levels of IFN- $\gamma$  in relation to ultrasound-detectable upper urinary tract pathology group is shown in Figure 5.15. There was no significant difference in geometric means of serum IFN- $\gamma$  or urinary IFN- $\gamma$  between children with and without ultrasound-detectable upper urinary tract pathology. In both groups of children, the geometric means of urinary IFN- $\gamma$  were lower than those of serum IFN- $\gamma$ .



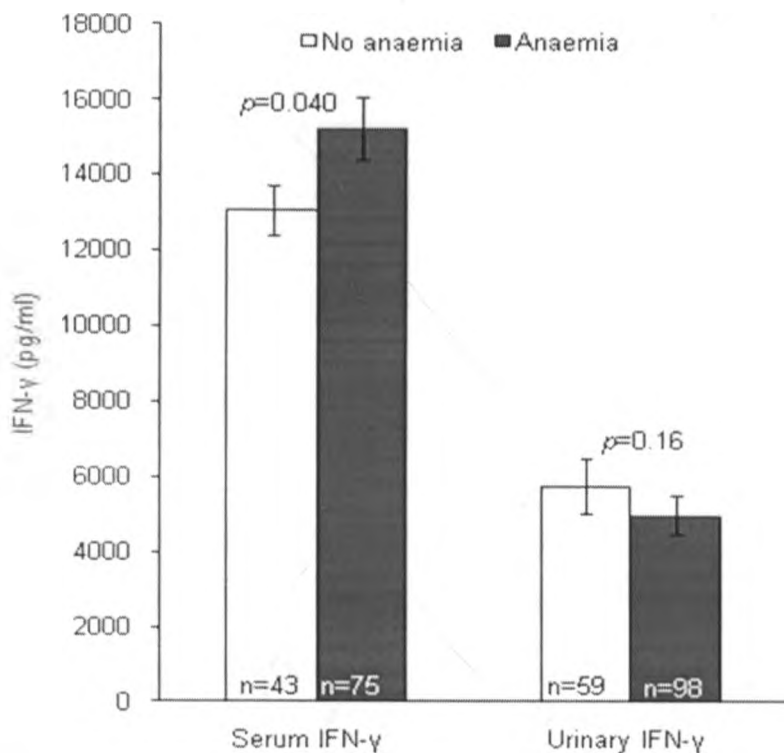
**Figure 5.15:** Geometric mean levels of IFN- $\gamma$  in relation to ultrasound-detectable upper urinary tract pathology among children in Kau and Ozi primary schools. *p*-values above the bars indicate the significance level of differences between the group with pathology and that without pathology. The error bars indicate the standard error on log-transformed values

The mean levels of IFN- $\gamma$  in relation to microhaematuria group is shown in Figure 5.6. There was no significant difference in geometric means of serum IFN- $\gamma$  or urinary IFN- $\gamma$  between children with and without microhaematuria. In both groups of children, the geometric means of urinary IFN- $\gamma$  were lower than those of serum IFN- $\gamma$ .



**Figure 5.16:** Geometric mean levels of IFN- $\gamma$  and microhaematuria among children in Kau and Ozi primary schools.  $p$ -values above the bars indicate the significance level of differences between the group with microhaematuria and that without microhaematuria. The error bars indicate the standard error on log-transformed values.

The mean levels of IFN- $\gamma$  in relation to anaemia group is shown in Figure 5.17. Children with anaemia had a significantly higher geometric mean of serum IFN- $\gamma$  than children without anaemia but there was no significant difference in geometric means of urinary IFN- $\gamma$  between children with anaemia and those without anaemia. In both groups of children, the geometric means of urinary IFN- $\gamma$  were lower than those of serum IFN- $\gamma$ .



**Figure 5.17:** Geometric mean levels of IFN- $\gamma$  in relation to anaemia among children in Kau and Ozi primary schools.

*p*-values above the bars indicate the significance level of differences between the group with anaemia and that without anaemia. The error bars indicate the standard error on log-transformed values

Assessment of the linear relationships between IFN- $\gamma$  and urinary ECP revealed no significant relationships between serum IFN- $\gamma$  and urinary ECP ( $r = -0.11$ ,  $p = 0.26$ ,  $n = 107$ ) or between urinary IFN- $\gamma$  and urinary ECP ( $r = 0.02$ ,  $p = 0.85$ ,  $n = 143$ ).

### 5.3.4.3 Effect of soil-transmitted helminths on the association between IFN- $\gamma$ and *S. haematobium* infection

The effects of STH on the association between *S. haematobium* egg count and levels of serum and urine IFN- $\gamma$  is shown in Table 5.3. From the table, STH egg counts had no significant effects on the relationship between serum IFN- $\gamma$  or urinary IFN- $\gamma$  and *S. haematobium* eggs counts.

**Table 5.3:** The effects of STH on IFN- $\gamma$  responses to *S. haematobium* infections, using a regression model controlling for the effects of age and sex.

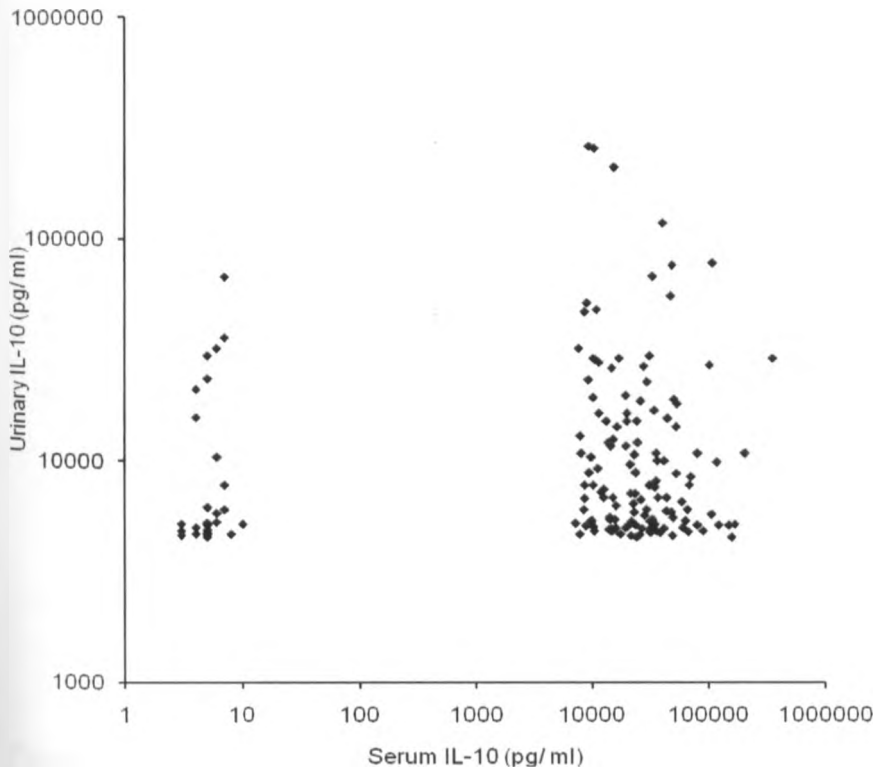
$r^2$  = correlation coefficient, Coefficient = regression coefficient.

Serum IFN- $\gamma$				
	<i>S. haematobium</i> only	<i>T. trichiura</i>	Hookworm	<i>A. lumbricoides</i>
$r^2$	0.02	0.03	0.03	0.03
Coefficient	-0.03	-0.03	-0.03	-0.03
<i>p</i> -value	0.19	0.17	0.19	0.18
Urinary IFN- $\gamma$				
	<i>S. haematobium</i> only	<i>T. trichiura</i>	Hookworm	<i>A. lumbricoides</i>
$r^2$	0.02	0.04	0.03	0.06
Coefficient	-0.06	-0.07	-0.06	-0.06
<i>p</i> -value	0.13	0.10	0.12	0.14

### 5.3.5 Interleukin-10 responses

#### 5.3.5.1 Relationship between serum IL-10 and urinary IL-10

A scatter diagram showing the relationship between serum IL-10 and urinary IL-10 levels in the examined individuals is shown in Figure 5.18. There was no significant linear relationship between levels of serum IL-10 and urinary IL-10 ( $r = 0.06$ ,  $p=0.43$ ;  $n=158$ ).

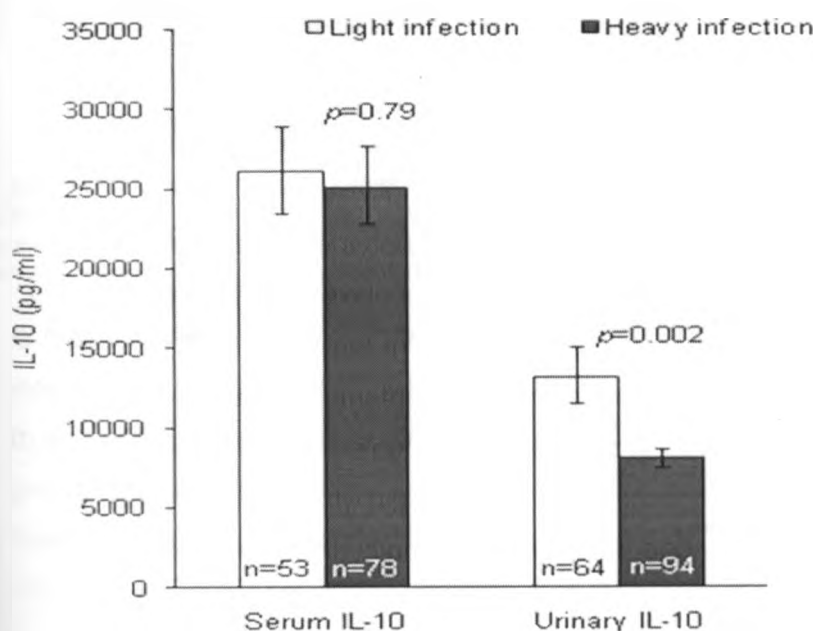


**Figure 5.18:** Relationship between serum IL-10 and urinary IL-10 among children in Kau and Ozi primary schools.

A group of 27 children had considerably low levels of serum IL-10 and were thus regarded as non-responders. There was no significant difference in mean age between the responders and non-responders (9.7 years vs. 10.4 years,  $p=0.09$ ). Also, there were no significant differences between responders and non-responders in terms of *S. haematobium*, *T. trichiura*, hookworm or *A. lumbricoides* infection intensities ( $p=0.78$ , 0.12, 0.66 and 0.07, respectively) or prevalences ( $p=0.16$ , 0.69, 0.37 and 0.40, respectively).

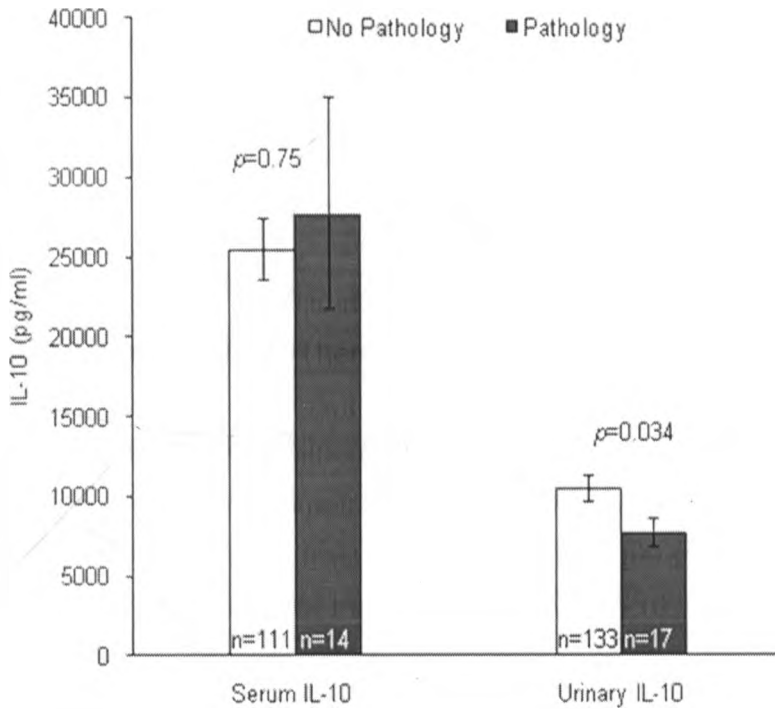
### 5.3.5.2 Relationship between IL-10 and *S. haematobium* infection and morbidity markers

The group of non-responders could negatively skew the distribution of serum IL-10 data to the left and was therefore excluded in the subsequent analysis. The mean levels of IL-10 in relation to *S. haematobium* infection intensity is shown in Figure 5.19. There was no significant difference in geometric means of serum IL-10 between children with light and those with heavy *S. haematobium* infections but children with light *S. haematobium* infections had a significantly higher geometric mean of urinary IL-10 than children with heavy *S. haematobium* infections. In both light and heavy *S. haematobium* infection groups, geometric mean of urinary IL-10 were lower than those of serum IL-10.



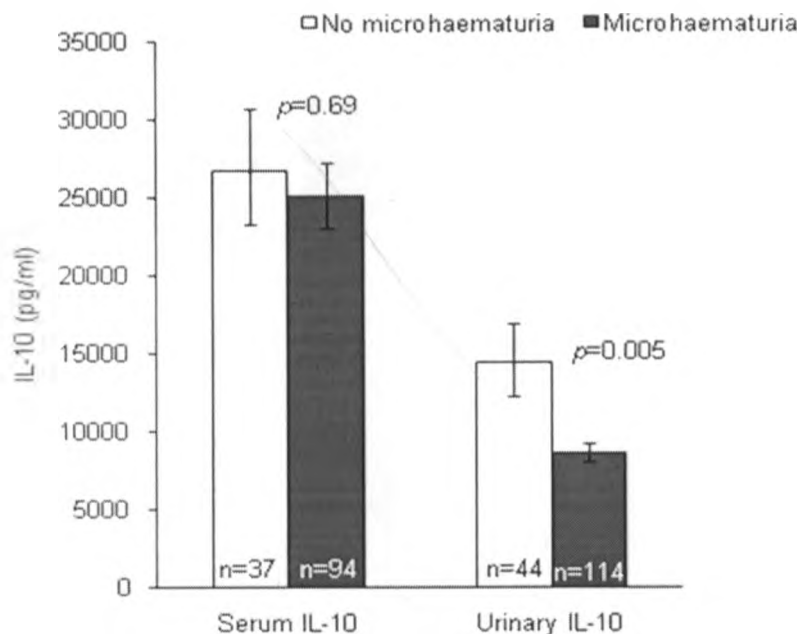
**Figure 5.19:** Geometric mean levels of IL-10 in relation to *S. haematobium* infection intensity among children in Kau and Ozi primary schools. *p*-values above the bars indicate the significance level of differences between the light and heavy *S. haematobium* infection intensity groups. The error bars indicate the standard error on log-transformed values

The mean levels of IL-10 in relation to ultrasound-detectable upper urinary tract pathology is shown in Figure 5.20. There was no significant difference in geometric means of serum IL-10 between children with and without ultrasound-detectable upper urinary tract pathology but children without ultrasound-detectable upper urinary tract pathology had a significantly higher geometric mean of urinary IL-10 than children with ultrasound-detectable upper urinary tract pathology. In both groups of children, the geometric means of urinary IL-10 were lower than those of serum IL-10.



**Figure 5.20:** Geometric mean levels of IL-10 in relation to ultrasound-detectable upper urinary tract pathology among children in Kau and Ozi primary schools. *p*-values above the bars indicate the significance level of differences between the group with pathology and that without pathology. The error bars indicate the standard error on log-transformed values

The mean levels of IL-10 in relation to microhaematuria group is shown in Figure 5.21. There was no significant difference in geometric means of serum IL-10 between children with and without microhaematuria but children without microhaematuria had a significantly higher geometric mean of urinary IL-10 than children with microhaematuria. In both groups of children, the geometric means of urinary IL-10 were lower than those of serum IL-10.

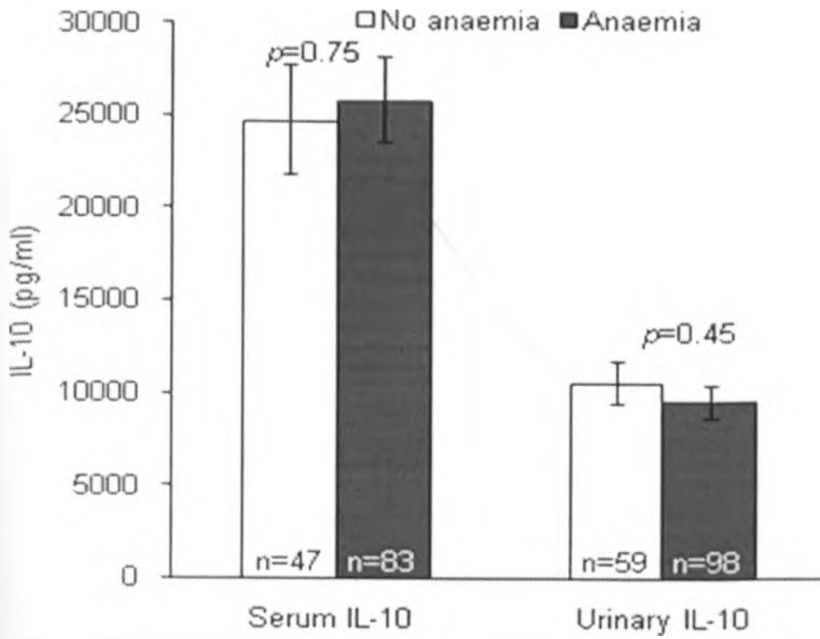


**Figure 5.21:** Geometric mean levels of IL-10 in relation to microhaematuria among children in Kau and Ozi primary schools.

*p*-values above the bars indicate the significance level of differences between the group with microhaematuria and that without microhaematuria. The error bars indicate the standard error on log-transformed values

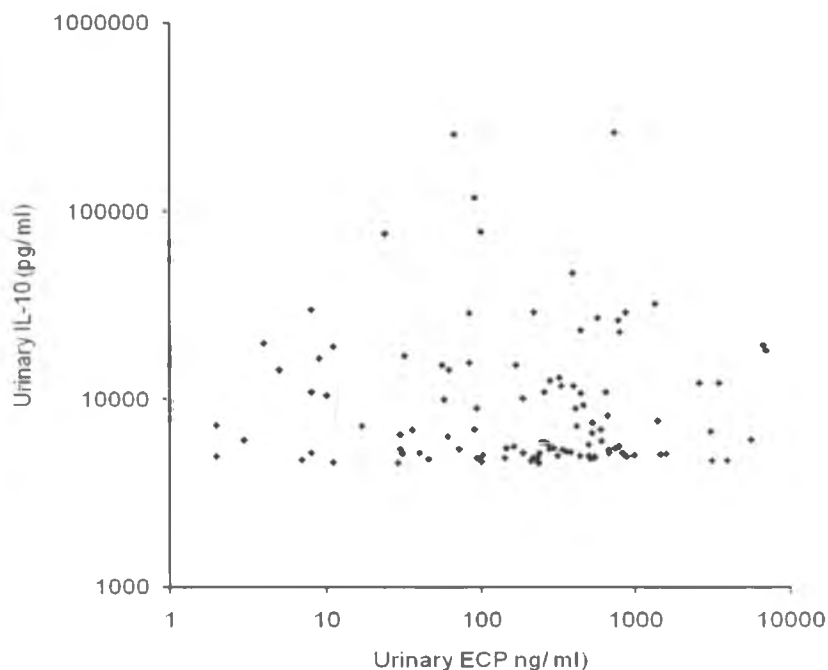
The mean levels of IL-10 in relation to anaemia group are shown in Figure 5.22. There was no significant difference in geometric means of serum IL-10 or urinary IL-10 between children with anaemia and those without anaemia. In both groups of children, the geometric means of urinary IL-10 were lower than those of serum IL-10.





**Figure 5.22:** Geometric mean levels of IL-10 in relation to anaemia among children in Kau and Ozi primary schools. *p*-values above the bars indicate the significance level of differences between the group with anaemia and that without anaemia. The error bars indicate the standard error on log-transformed values.

Assessment of the linear relationship between levels of serum IL-10 and urinary ECP revealed no significant relationship ( $r = -0.13$ ,  $p = 0.17$ ;  $n = 118$ ). A scatter diagram showing the relationship between urinary IL-10 and urinary ECP is shown in Figure 5.23. There was a significant negative linear relationship between urinary IL-10 and urinary ECP ( $r = -0.21$ ,  $p = 0.012$ ;  $n = 143$ ).



**Figure 5.23:** Relationship between urinary IL-10 and urinary ECP among children in Kau and Ozi primary schools.

### 5.3.5.3 Effect of soil-transmitted helminths on the association between IL-10 and *S. haematobium* infection

The effects of STH on the association between *S. haematobium* egg counts and levels of IL-10 is shown in Table 5.4. From the table, STH egg counts had no significant effects on the relationship between serum IL-10 or urinary IL-10 and *S. haematobium* eggs counts.

**Table 5.4:** The effects of STH on IL-10 responses to *S. haematobium* infections, using a regression model controlling for the effects of age and sex.

$r^2$  = correlation coefficient, Coefficient = regression coefficient

Serum IL-10				
	<i>S. haematobium</i> only	<i>T. trichiura</i>	Hookworm	<i>A. lumbricoides</i>
$r^2$	0.05	0.07	0.07	0.05
Coefficient	-0.04	-0.04	-0.04	-0.04
p-value	0.29	0.23	0.27	0.30
Urinary IL-10				
	<i>S. haematobium</i> only	<i>T. trichiura</i>	Hookworm	<i>A. lumbricoides</i>
$r^2$	0.11	0.12	0.12	0.13
Coefficient	-0.14	-0.14	-0.14	-0.13
p-value	<0.001	<0.001	<0.001	<0.001

## 5.4 Discussion

To the best of the author's knowledge, the present study for the first time reports serum and urinary cytokine responses in relation to *S. haematobium* and STH infections in chronically infected primary school children. There was no correlation between levels of serum and urinary IL-6, IFN- $\gamma$ , TNF- $\alpha$  and IL-10 among the children. A few other studies have also demonstrated a lack of correlation between serum and urinary cytokine levels during different disease states in the urinary system. For example, Otto *et al.* (1999) showed that serum IL-6 levels do not correlate with urinary IL-6 levels during urinary tract infection with *E. coli*. Similar findings were also recently reported in patients with cryoglobulinaemic glomerulonephritis associated with chronic hepatitis C (Korotchaeva *et al.*, 2011). The findings of the present study suggest that in *S. haematobium* infections, urinary cytokines have a local origin and reflect cytokine responses in the urinary tract.

In all cases, cytokines levels were higher in serum than in urine. This could be due to a number of reasons. During chronic infections with *S. haematobium*, cytokine responses may occur both in blood vascular system, due to antigens released by naturally dying worms and eggs, and in the mucosa due to eggs lodged in the tissues. The data suggest that cytokine responses in blood vascular system are more vigorous than in the urinary tract mucosa. It can also be argued that more rapid cytokine degradation in the urinary system led to lower concentration in urine than in serum as suggested by Ke and Ho (1967) who observed important differences between rabbit serum and urinary interferons. In addition, the observed lower urinary cytokine levels as compared to serum levels may have been due to further dilution in water as excess water is excreted in urine.

Some children had very low levels of serum TNF- $\alpha$ , IFN- $\gamma$  or IL-10. No immediate explanation could be advanced for this. These children belonged to different groups for each of these cytokines but had no major differences between them and the rest of the children with regard to other important parameters such as infection, age or sex. None of these children had neutrophilia (>80% neutrophils) as shown in Chapter 3 and it was therefore unlikely that the differences in cytokine levels were due to bacterial infections. To avoid skewing of the data, this group of children was excluded from the subsequent analyses.

Previous studies have reported a correlation between *ex vivo* cytokine secretion by schistosome antigen-stimulated peripheral blood cells and schistosome-related morbidity (Amiri *et al.*, 1992; Wamachi *et al.*, 2004; King *et al.*, 2001; Abath *et al.*, 2006; Friedman *et al.*, 2005). On the contrary, the present study indicated no correlation between serum levels of IL-6, TNF- $\alpha$ , IFN- $\gamma$  or IL-10 and some markers of *S. haematobium*-related morbidity such as egg counts, microhaematuria or urinary ECP. In addition, although there

was a correlation between serum levels of IL-6 and ultrasound-detectable urinary tract pathology, there was no correlation between this pathology and serum levels of TNF- $\alpha$ , IFN- $\gamma$  or IL-10. As the present study assessed serum cytokine levels, the differences may be explained by the conditions under which cytokine secretion was assessed in the present study and the previous ones (Moore *et al.*, 2001). Serum cytokine levels therefore may not accurately reflect *S. haematobium*-related urinary tract morbidity (Mutapi *et al.*, 2007).

Significantly higher levels of urinary IL-6 were observed in children with heavy *S. haematobium* infections, upper urinary tract pathology and microhaematuria than in children with light *S. haematobium* infections, and those without upper urinary tract pathology or microhaematuria. In addition, there was a significant positive correlation between levels of urinary IL-6 and urinary ECP. These findings suggest that the observed levels of urinary IL-6 were a result of local inflammation evoked by *S. haematobium* eggs in the urinary tract. Macrophages and eosinophils are major cellular constituents of schistosome egg-driven granulomas that are known to produce IL-6 (Magalhaes *et al.*, 2009; Magalhaes *et al.*, 2010; Gleich, 2000). This suggests that the inflammatory cells in the urinary tract walls locally secreted IL-6 that was subsequently excreted in urine. This is supported by the findings of previous studies of bacterial infections in the urinary tract and in patients with cryoglobulinaemic glomerulonephritis associated with chronic hepatitis C that levels of urinary IL-6 reflect local inflammation (Otto *et al.*, 1999; Agace *et al.*, 1993; Hedges *et al.*, 1991; Korotchaeva *et al.*, 2011).

There was no significant difference in levels of urinary TNF- $\alpha$  between children with light and those with heavy *S. haematobium* infections or between those with and without microhaematuria or anaemia. Children without urinary tract pathology had significantly higher levels of urinary TNF- $\alpha$  than those with pathology. Although urinary tract cells may not secrete a lot of soluble TNF- $\alpha$  in the face of active inflammation (Agace *et al.*, 1993), there could still be sufficient amounts of cell membrane-bound TNF- $\alpha$  in the mucosal granulomas to mediate development of urinary tract pathology. This is more so bearing in mind that membrane-bound TNF- $\alpha$  is known to be more cytotoxic than soluble TNF- $\alpha$  (Duitman *et al.*, 2011).

IFN- $\gamma$  is another multifunctional cytokine mainly secreted by activated T cells including during infections with schistosomes (Duitman *et al.*, 2011; van Stijn *et al.*, 2010). In the present study, children without ultrasound-detectable urinary tract pathology or microhaematuria had higher levels of urinary IFN- $\gamma$  although the differences between them and those without these conditions were not statistically significant, probably because the sample was too small to detect significant differences. These findings suggest that urinary

IFN- $\gamma$  could be associated with reduced inflammatory processes and pathology in the urinary tract mucosa during infections with *S. haematobium*. A few studies have reported findings that are supportive of this. For instance, in *S. mansoni* infections, secretion of high levels of IFN- $\gamma$  by peripheral blood cells was associated with reduced risk of periportal fibrosis and portal hypertension (Abath *et al.* 2005). IFN- $\gamma$  has also been shown to be important in limiting the number and sizes of granulomas formed in an *in vivo* pulmonary *S. mansoni* egg infection model and the extent of fibrous tissue deposition (Lammie *et al.*, 1986; Wynn *et al.*, 1995; Czaja *et al.*, 1989; Lukacs & Boros, 1993).

IL-10 is a highly potent anti-inflammatory cytokine produced by lymphocytes (Bouaziz *et al.*, 2010; Moore *et al.*, 2001; Chiaramonte *et al.*, 1999). Several studies have demonstrated that IL-10 is probably the most important cytokine in down-modulating pathological inflammatory responses in schistosomiasis (Hoffmann *et al.*, 2000; Sadler *et al.*, 2003; Abath *et al.*, 2006). The present finding of a significant relationship between urinary IL-10 and *S. haematobium* infections were therefore intriguing. Children with light *S. haematobium* infections had significantly higher urinary levels of IL-10 than those with light infections. Similarly, children without ultrasound-detectable urinary tract pathology or microhaematuria had significantly higher levels of urinary IL-10 than children with these conditions. In addition, levels of urinary IL-10 were significantly negatively correlated with levels of urinary ECP. As these are markers of urinary tract pathology (Vennervald *et al.*, 2004), the data from the present study indicate that IL-10 is significantly involved in down-modulating inflammatory processes due to tissue lodged *S. haematobium* eggs in the urinary tract. This is in agreement with the findings of other studies suggesting that IL-10 is important in reducing urinary tract pathology due to *S. haematobium* infections among children in coastal Kenya (Wamachi *et al.*, 2004; King *et al.*, 2001).

Although IL-6 responses to schistosome infections are associated with anaemia (Friedman *et al.*, 2005), there was no significant difference in levels of serum IL-6 between children with and without anaemia. Children with anaemia had higher levels of serum TNF- $\alpha$  and IFN- $\gamma$  than children without anaemia although this was statistically significant only for IFN- $\gamma$ . Both cytokines have been cited as major players in aplastic anaemia (Li *et al.*, 2011) which supports the argument that anaemia observed in chronic schistosomiasis infection is at least partly mediated by cytokines induced by the worms (Friedman *et al.*, 2005). Although food intake was not assessed in the present study, it is possible that TNF- $\alpha$  contributes to this anaemia by inducing anorexia as demonstrated in rats (de Kloet *et al.*, 2011). Moreover, both TNF- $\alpha$  and IFN- $\gamma$  have also been implicated in causing anaemia by mediating apoptosis or inhibition of proliferation and differentiation of erythroid progenitor cells as well as suppression of erythropoiesis in bone marrow (Kheansaard *et*

*al.*, 2011; Dufour *et al.*, 2003; Bertero *et al.*, 1997). This suggests that the observed high prevalence of anaemia among the children was due to many factors including helminth-induced cytokines (Lutter, 2008).

Previous field and experimental studies suggest that, by modulating cytokine responses, different helminth species may influence the outcome of infection with other incoming helminth species resulting in varying worm burdens and disease outcomes (Booth *et al.*, 1998a,b; Cox, 2001; Bickle *et al.*, 2008). Contrary to this, multiple variable analysis did not detect any significant influence of *T. trichiura*, hookworm or *A. lumbricoides* on the relationship between *S. haematobium* infection and IL-6, TNF- $\alpha$ , IFN- $\gamma$  or IL10 in the present study. This supports the reports from other field studies suggesting that worm burdens in co-infected individuals may be due to epidemiological coincidence rather than biological interaction between helminth species in co-endemic areas (Ashford *et al.*, 1992; Ashford *et al.*, 1993; Booth *et al.*, 1998a). From the findings of the present study it appears that STH infections may not have major effects on cytokine responses to *S. haematobium* in co-infected individuals.

## 5.5 Conclusion

Based on the findings of the present study it appears that in *S. haematobium* infections, systemic cytokine responses do not correlate with local cytokine responses in the urinary tract mucosa. The data also show that urinary cytokines, particularly IL-6 and IL-10, are involved in and are better indicators of urinary tract morbidity during *S. haematobium* infections compared to serum cytokines. In addition, the data indicate that during co-infections with *S. haematobium* and STH, the latter do not appear to markedly influence cytokine responses to *S. haematobium* and may as such not be major confounders in studies of cytokine responses to *S. haematobium*. In conclusion, urinary IL-6 and IL-10 are good indicators of urinary tract morbidity due to *S. haematobium* and thus a potential tool for monitoring *S. haematobium*-related morbidity. However, additional longitudinal studies are needed to elucidate how levels of these cytokines change following treatment with praziquantel before they can be adopted for monitoring the performance of large-scale morbidity control programmes.

# **CHAPTER SIX: THE EFFECT OF COMBINED PRAZIQUANTEL-ALBENDAZOLE TREATMENT ON *SCHISTOSOMA HAEMATOBIMUM* AND SOIL TRANSMITTED HELMINTH INFECTION**

## **6.1 Introduction**

Praziquantel is the drug of choice for treatment of infections with all schistosome species (Chitsulo *et al.*, 2000). The drug is highly effective against *Schistosoma haematobium* infections and has been widely used against infections with this parasite in Kenya and elsewhere (Koukounari *et al.*, 2007; King, 2006; Magnussen, 2003). Albendazole, the drug of choice against STH in Kenya, and mebendazole are the two drugs commonly used for the treatment of infections with the common STH (Bethony *et al.*, 2006). These drugs are highly effective against infections with *A. lumbricoides* and hookworms but less effective against infections with *T. trichiura*, often requiring several treatments (de Silva, 2003; Olsen, 2007).

Co-infections with schistosomes and one or more of the three intestinal nematode species are very common in endemic countries especially among primary schoolchildren (Booth *et al.*, 1998a; Bethony *et al.*, 2006). As a result, following observations that a combination of praziquantel and albendazole or mebendazole is safe, effective and easy to administer, mass co-administration of praziquantel and albendazole or mebendazole in control of morbidity due to schistosomes and STH in endemic areas is encouraged (Olsen, 2007; Savioli *et al.*, 2009).

However, understanding of the effects of the extensive co-occurrence of schistosomes and STH on the course of the individual infections, and on the outcome of treatment is still limited. For example, the effects of *S. haematobium* infection on the response of STH to combined praziquantel-albendazole/ mebendazole treatment remain largely unknown. Such co-occurrence may have important implications for combined mass drug administration in control programs. It is, however, not known how schistosomes, which have strong immunomodulatory effects (Araujo *et al.*, 1996; Corrêa-Oliveira *et al.*, 1998), may influence the effects of albendazole on co-infecting STH.

The present study was carried out to investigate the effects of combined praziquantel-albendazole treatment on *S. haematobium* and STH infections, and the influence of *S. haematobium* infection intensity on the treatment effect on the STH infections among primary schoolchildren in two schools of Tana Delta District.

## **6.2 Materials and Methods**

### **6.2.1 Design**

This was a longitudinal study involving a baseline survey and a follow-up study three months after treatment as described in detail in Chapter 2. At baseline, three urine and stool specimens were collected from each participant and examined for *S. haematobium* and STH eggs, respectively. Each participant was then treated with a standard praziquantel dose (40 mg/kg body weight) and albendazole (400 mg). Follow-up urine and stool specimens were collected from the same participants three months after treatment and examined for *S. haematobium* and STH eggs, respectively.

### **6.2.3 Parasitological Examinations**

#### **6.2.2.1 Urine examination for *S. haematobium* eggs**

One urine specimen was collected from each participant on each of three different days and examined for *S. haematobium* eggs using urine filtration technique as described in detail in Chapter 2.

#### **6.2.2.2 Stool examination for helminth eggs**

One stool specimen was collected from each participant on each of three different days and examined for STH eggs using Kato-Katz technique as described in detail in Chapter 2.

### **6.2.3 Data analysis**

Data entry and analyses were carried out as explained in Chapter 2. In brief, cure rates were calculated as  $(P_b - P_f)/P_b \times 100\%$  where  $P_b$ = prevalence at baseline and  $P_f$ = prevalence at follow-up. Overall GMI reduction was calculated as  $(GMI_b - GMI_f)/GMI_b \times 100\%$  where  $GMI_b$ = GMI at baseline and  $GMI_f$ = GMI at follow up. Paired *t*-test on log-transformed values was used to compare means between baseline and follow-up egg counts. Prevalences of the infections were compared between baseline and follow-up studies using McNemar's  $\chi^2$ -test. *p*-values less than 0.05 were considered statistically significant in all tests.

## **6.3 Results**

### **6.3.1 Study Population**

One hundred and seventy-one of the 262 children from Kau and Ozi primary schools who participated in the baseline study, and were treated with praziquantel and albendazole after the baseline study, participated in the follow-up study. These 171 children formed the cohort population for the follow-up analysis of the effects of treatment on *S. haematobium* and STH infections. A comparison of the cohort group of children to the initial group of 262 children is shown in Table 6.1. Generally, the two groups of children were very similar in



terms of sex ratio, mean age and *S. haematobium*, *T. trichiura*, hookworm and *A. lumbricoides* infection prevalence.

**Table 6.1:** Comparison of the study cohort of 171 children to the initial study population of 262 children at baseline

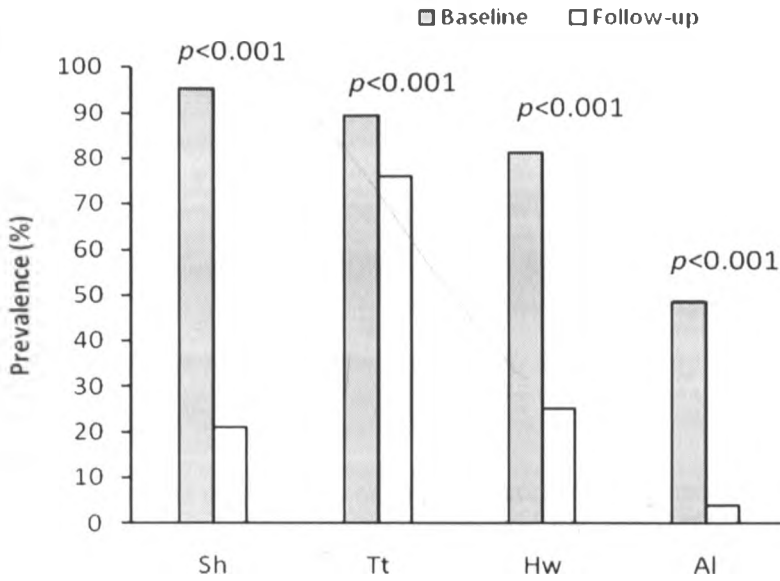
N	Girls:boys ratio	Mean age	<i>S. haematobium</i> prevalence	<i>T. trichiura</i> prevalence	Hookworm prevalence	<i>A. lumbricoides</i> prevalence
262	1.0	9.7 years	93.9%	88.2%	80.9%	45.4%
171	1.2	9.6 years	95.3%	89.5%	81.3%	48.5%

### 6.3.2 Reactions to Treatment

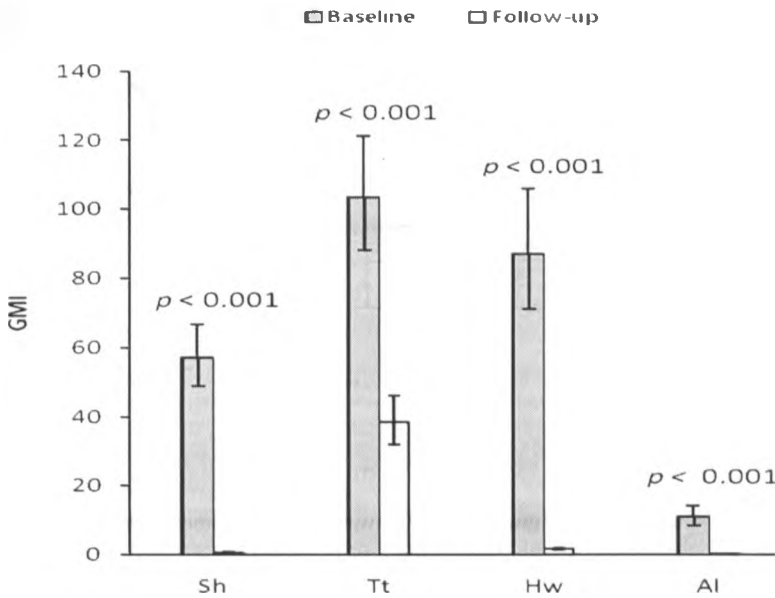
Five children (2.9%) vomited within one hour after treatment. They were allowed to go home and were treated again the following day. On the second day after treatment, 79 (46.2%) of the children reported experiencing transient headaches in the evening after treatment. Eighty-six (52%) of the children reported experiencing transient lower abdominal pains after treatment. None reported vomiting or severe reactions that required special treatment.

### 6.3.3 Effect of Treatment on *S. haematobium* and STH Infections

The prevalences and GMI of *S. haematobium* and STH infections in the study cohort before and after treatment are shown in Figures 6.1 and 6.2. There was significant reduction in the prevalences of *S. haematobium* and STH infections among the children after treatment but, although the prevalence of *T. trichiura* infections was significantly reduced, it remained relatively high as opposed to that of other helminth species.



**Figure 6.1:** Effect of treatment on prevalence of helminth infections in the study cohort of children from Kau and Ozi primary schools.  
 n = 171, Sh=*Schistosoma haematobium*, Tt = *Trichuris trichiura*, Hw = Hookworm, Al = *Ascaris lumbricoides*.  
 p-values above the bars indicate the significance level of differences between pre-treatment and post-treatment prevalences



**Figure 6.2:** Effect of treatment on geometric mean intensities of helminth infections in the study cohort of children from Kau and Ozi primary schools.  
 n = 171, Sh = *Schistosoma haematobium*, Tt = *Trichuris trichiura*, Hw = Hookworm, Al = *Ascaris lumbricoides*.  
 GMI = geometric mean intensity (eggs/10 ml urine for *S. haematobium* and eggs/g stool for intestinal worms).  
 p-values above the bars indicate the significance level of differences between baseline and follow-up GMI.  
 Error bars represent the standard error lower and upper limits.

Table 6.2 shows the baseline and follow-up prevalences of *S. haematobium* and STH. The highest reduction in prevalence after treatment was observed for *A. lumbricoides* followed by *S. haematobium* and hookworm. The lowest reduction was observed for *T. trichiura* infections.

Table 6.3 shows the baseline and follow-up GMIs of *S. haematobium* and STH as well as the GMI reductions. A slightly different trend, from that observed in the cure rates, was observed in the GMIs reduction where *S. haematobium* infections had the highest reduction followed by hookworm and *A. lumbricoides*. The least reduction was observed for *T. trichiura* infections.

**Table 6.2:** Effect of treatment on prevalence of *S. haematobium* and STH infections, as well as cure rates, in the study cohort of children from Kau and Ozi primary schools. n=171, *p*-values show the significance levels of differences between pre-treatment and post-treatment prevalences

	Baseline	Follow-up	<i>p</i> -value	Prevalence
	Prevalence (%)	Prevalence (%)		Reduction (%)
<i>S. haematobium</i>	95.3	21.1	< 0.001	77.9
<i>T. trichiura</i>	89.5	76.0	< 0.001	15.1
Hookworm	81.3	25.2	< 0.001	69.0
<i>A. lumbricoides</i>	48.5	4.1	< 0.001	91.5

**Table 6.3:** Effect of treatment on GMI (eggs/10 ml urine for *S. haematobium* and eggs/g stool for intestinal worms), as well as the GMI reduction, in the study cohort of children from Kau and Ozi primary schools. n=171, *p*-values show the significance levels of differences between pre-treatment and post-treatment GMI

	Baseline	Follow-up	<i>p</i> -value	GMI
	GMI	GMI		Reduction (%)
<i>S. haematobium</i>	57.2	0.6	< 0.001	99.0
<i>T. trichiura</i>	103.5	38.4	< 0.001	62.9
Hookworm	86.9	1.7	< 0.001	98.0
<i>A. lumbricoides</i>	11.1	0.3	< 0.001	97.3

The effect of treatment on the prevalence of heavy *S. haematobium* and moderate to heavy STH infections as well as the prevalence reductions are shown in Table 6.4. There were significant reductions in all prevalences after treatment. The highest reductions were observed for *A. lumbricoides* and hookworm followed by *S. haematobium* infections. *T. trichiura* infections had the least reduction.

**Table 6.4:** Effect of treatment on prevalence of heavy *S. haematobium* and moderate to heavy *T. trichiura* infections, hookworm infections and, *A. lumbricoides* infections in the study cohort of children from Kau and Ozi primary schools.

Heavy *S. haematobium* ( $\geq 50$  eggs/10 ml urine), moderate to heavy *T. trichiura* infections ( $\geq 1,000$  eggs/g of stool), hookworm infections ( $\geq 2,000$  eggs/g of stool), *A. lumbricoides* infections ( $\geq 5,000$  eggs/g of stool).

	Baseline	Follow-up	Prevalence	
	Prevalence (%)	Prevalence (%)	<i>p</i> -value	reduction rate (%)
<i>S. haematobium</i>	56.1	5.9	< 0.001	89.5
<i>T. trichiura</i>	8.8	4.1	0.033	53.4
Hookworm	7.6	0.0	< 0.001	100.0
<i>A. lumbricoides</i>	2.3	0.0	0.046	100.0

### 6.3.4 Effect of *S. haematobium* Infection Intensity on the Reduction of STH Infection after Treatment

In order to analyse for the effect of *S. haematobium* infection intensity on the reduction of STH infection prevalence and GMIs, the children were divided into two groups: one of children with light *S. haematobium* infection (0–49 eggs/ 10 ml urine) and the other one of children with heavy *S. haematobium* infection ( $\geq 50$  eggs/ 10 ml urine). There was no significant difference in the mean ages of the light infection and heavy infection groups of children (9.3 vs 9.9 years;  $p=0.09$ ). Baseline and follow-up prevalence and GMIs of STH infections were then compared between the two groups to elucidate the effects of *S. haematobium* infection intensity on STH infection prevalence and GMI reduction after treatment.

Table 6.5 shows the effects of *S. haematobium* infection intensity on STH infection prevalence and GMIs reductions after treatment. There was about twice as much reduction of the prevalence of trichuris infection in the heavy as in the light *S. haematobium* infection intensity group. On the other hand, the reduction of the prevalence of hookworm and *A. lumbricoides* infections was almost similar between the light and heavy *S. haematobium* infection intensity groups. No major differences were observed in the GMI reductions for any of the STH between the light and heavy *S. haematobium* infection intensity group.

Table 8.5. Effects of *S. haematobium* infection intensity on STH infection prevalence and GMIs reduction after treatment

		Light <i>S. haematobium</i> infection			Heavy <i>S. haematobium</i> infection		
		Baseline	Follow-up	Reduction (%)	Baseline	Follow-up	Reduction (%)
<i>T. trichiura</i>	No. examined	75	75		96	96	
	No. positive (%)	67(89.3)	61(81.3)	9.0	86(89.6)	69(71.9)	19.8
	GMI for all examined	123.2	46.5	62.3	90.3	33.1	63.3
	GMI for positives	219.9	52.6	76.1	153.2	46.6	69.6
Hookworm	No. examined	75	75		96	96	
	No. positive (%)	59(78.7)	17(22.7)	71.2	80(83.3)	26(27.1)	67.5
	GMI for all examined	58.4	1.2	97.9	118.5	2.1	98.2
	GMI for positives	178.8	1.5	99.2	310.0	2.4	99.2
<i>A. lumbricoides</i>	No. examined	75	75		96	96	
	No. positive (%)	36(48.0)	4(5.3)	89.0	47(49.0)	3(3.1)	93.7
	GMI for all examined	11.8	0.3	97.5	10.5	0.2	98.1
	GMI for positives	202.2	0.6	99.7	146.5	0.4	99.7

## 6.4 Discussion

Mass co-administration of praziquantel and albendazole/ mebendazole has been advocated to control co-morbidity due to infection with schistosomes and STH in sub-Saharan countries including Kenya (WHO, 2002; Danso-Appiah *et al.*, 2009). As a result, many countries undertook large mass chemotherapy programmes using these drugs even though some important information was still lacking (WHO, 2002). The present study aimed at elucidating the effects of combined praziquantel-albendazole treatment against *S. haematobium* and STH infections, and the influence of *S. haematobium* infection intensity on the effect of the treatment on STH infections.

In the present study, combined praziquantel-albendazole treatment against *S. haematobium* realized 77.9% reduction in prevalence and 99.3% egg reduction in primary school children. Elsewhere, treatment with praziquantel alone has been shown to be highly effective against *S. haematobium* infections often giving cure rates above 60% and over 90% egg reduction (Tchuem Tchuente *et al.*, 2004; Danso-Appiah *et al.*, 2009; Cioli & Pica-Mattocchia, 2003). This suggests that combined praziquantel-albendazole treatment may be superior to treatment with praziquantel alone (Danso-Appiah *et al.*, 2009). It has been shown that mebendazole, which is in the same class of drugs as albendazole, has some anti-schistosomal effects and it is therefore probable that albendazole synergizes with praziquantel against *S. haematobium* (Al-Waili, 1987; Al-Waili, 1988).

Combined praziquantel-albendazole treatment against STH led to significant cure rate and egg reduction. In isolation, albendazole is known to be highly effective against hookworm and *A. lumbricoides* but is less effective against *T. trichiura* (Appleton *et al.*, 2009; Adams *et al.*, 2004; Watkins *et al.*, 1996; Vercruyse *et al.*, 2011). Combined praziquantel-albendazole treatment against STH in the present study produced similar results and suggests that this combination treatment is equally effective against STH (Olsen, 2007).

A number of studies have been carried out on combined praziquantel-albendazole treatment of children co-infected with schistosomes and STH but the information on how the intensity of individual helminth species may influence the effects of the treatment on other co-infecting helminth species is still lacking (Massa *et al.*, 2009; Kihara *et al.*, 2007; Utzinger *et al.*, 2009; Olsen, 2007; Danso-Appiah *et al.*, 2009). In the present study, the intensity of *S. haematobium* infection did not significantly influence the reduction of hookworm or *A. lumbricoides* following a single treatment with praziquantel and albendazole but there was twice as much reduction of the prevalence of *T. trichiura* in children with heavy *S. haematobium* as in children with light *S. haematobium* infections.

However, no such differences were observed for the reduction in GMI of all three STH species. These results suggest that treatment of heavy *S. haematobium* infections may enhance the effects of albendazole on *T. trichiura*.

In *S. mansoni* infections, there is pronounced pro-inflammatory Th<sub>1</sub> cytokine responses during the acute phase of infections (de Jesus *et al.*, 2002) which shifts to anti-inflammatory Th<sub>2</sub>-type responses during the chronic phase (Araujo *et al.*, 1996; Corrêa-Oliveira *et al.*, 1998). This is also likely to be the case during infections with *S. haematobium*. Such Th<sub>2</sub> immune responses may enhance STH survival in infected individuals (Cooper *et al.*, 2008; Cooper *et al.*, 2000). Treatment of *S. mansoni* infected individuals with praziquantel leads to increased antigen release by the dying schistosomes and infection intensity-dependent changes in cytokine responses (Reimert *et al.*, 2006; Butterworth & Thorne, 1993). Although post treatment immune changes were not assessed in the present study, it can be argued that similar changes may have occurred following combined praziquantel-albendazole treatment of children co-infected with *S. haematobium* and STH. These may then have led to the observed enhanced *T. trichiura* cure rates in children with heavy *S. haematobium* infections but little or no effect on hookworm or *A. lumbricoides*. This may be supported by the fact that although *T. trichiura* inhabits the intestinal lumen, its anterior end is usually embedded within the intestinal epithelium (Ortega *et al.*, 2010) and would therefore be more vulnerable to host immune changes as compared to adult hookworm and *A. lumbricoides* which are restricted to the gut lumen. The GMI of all STH were significantly reduced in children with both light and heavy *S. haematobium* infections following treatment. The lack of significant differences between the two groups with regard to *S. haematobium* infection may be explained as an overall suppression of female STH by albendazole rather than praziquantel (Olsen, 2007).

## 6.5 Conclusion

The findings of the present study suggest that the combined treatment with praziquantel and albendazole is highly effective against *S. haematobium*, hookworm and *A. lumbricoides*; and less effective against *T. trichiura*. These findings also demonstrate that treatment of both *T. trichiura* and heavy *S. haematobium* infections may offer added benefits due to enhanced cure rates of *T. trichiura*.

## **CHAPTER SEVEN: *S. HAEMATOBIIUM* AND STH INFECTIONS: SOCIO-ECONOMIC STATUS AND SPATIAL DISTRIBUTION OF INFECTION IN SCHOOL CHILDREN OF TANA DELTA DISTRICT**

### **7.1 Introduction**

Transmission of schistosomiasis and STH is largely influenced by different behavioral, socio-economic and environmental factors (Bruun & Aagaard-Hansen, 2008; Dumba *et al.*, 2008; Mabaso *et al.*, 2003). These factors may vary from one area to another resulting in differences in transmission patterns. This may have important implications for control efforts. There is, therefore, a need to clearly understand the factors and how they influence transmission in a given area in order to design control programmes that are responsive to local needs.

The behaviour of an individual plays a significant role in transmission of schistosomes and STH. For example, in an area of Zanzibar, swimming in *S. haematobium* intermediate snail-host infested water is one of the most important factors determining infection of children with *S. haematobium* (Rudge *et al.*, 2008). In another area in coastal Kenya, a similar phenomenon which was highly influenced by the household location relative to the local water body was observed (Clennon *et al.*, 2006). For STH infections, unhygienic habits such as not washing hands and eating unwashed fruits and vegetable have been cited as important risk factors for *T. trichiura* and *A. lumbricoides* infections among children (Idowu & Rowland, 2006; Avcioglu *et al.* 2011; O'Lorcain & Holland, 2000). Apart from ingestion of infective eggs, transmission is also influenced by contamination of the environment with eggs. This applies not only for schistosomes but also for STH when infected individuals do not use toilets or latrines (Dumba *et al.*, 2008).

Economic and social factors may also influence human contamination of the environment with urine or stool and contact with intermediate snail-host infested water thus promoting transmission of schistosomes (Bruun & Aagaard-Hansen, 2008). This is more evident in the low socio-economic strata of the endemic communities with poor hygiene standards compounded by intense contact with infective water (Steinmann *et al.*, 2006). In addition, poor housing and low education levels of parents or guardians are reported as important risk factors in a schistosomiasis endemic community in Brazil (Kloos *et al.*, 2008).

Available evidence shows that the physical environment of an area may also significantly influence the spatial distribution of infections with helminths. For example, infections with *S. mansoni* were more prevalent in areas near the lake in the Kenyan Lake Victoria Basin



(Handzel *et al.*, 2003). Similarly, in the southern coastal Kenya and in Zanzibar infections with *S. haematobium* were reported to be clustered around water bodies infested with intermediate snail hosts (Clennon *et al.*, 2004; Clennon *et al.*, 2006; Rudge *et al.*, 2008). The distribution may, however, be different in areas like Tana Delta which apart from having several snail-infested water bodies, they are almost homogeneously flooded during the rainy seasons.

Unlike the distribution of infections with schistosomes, it is the type of soil, moisture and temperatures rather than the presence of water bodies that may significantly influence the spatial distribution of infections with STH (Mabaso *et al.*, 2003; Lilley *et al.*, 1997). This may suggest that infections with STH are more or less homogeneously distributed over wide areas (Howard *et al.*, 2002). In an endemic area of Uganda, however, hookworm was widely distributed whereas *T. trichiura* and *A. lumbricoides* were highly focal (Clements *et al.*, 2010). This suggests that other unknown factors may also influence the spatial distribution of STH infections in an area.

Communities in the Tana Delta District may be socio-culturally different from other communities in schistosomiasis and STH endemic areas of Kenya. For instance, the Giriama people who mainly inhabit the south coast may be socio-culturally different from the Pokomo people who mainly inhabit the north. For example, they are mainly rice farmers where they do a different form of flood irrigation that depends on tidal bore at the mouth of River Tana unlike in the south coast where the Giriama people use flood irrigation from water in the streams during the wet season. The Pokomo people in the Tana Delta also do fishing in the local fresh water swamps whereas in the south coast the Giriama people mainly do their fishing in the Indian Ocean. In addition, the south coast is, to some extent, topographically and climatically different from the north coast as in the Tana Delta flooding is spatially homogenous and does not necessarily depend on rains in the area unlike in the south coast where flooding is rare and almost predictable. As a result, the spatial distribution of *S. haematobium* and STH and factors determining it may differ between the areas. This requires a clear understanding of the factors responsible for the local spatial distribution in order to design effective integrated *S. haematobium* and STH control measures that are suitable given local conditions. The current study was carried out to elucidate effects of selected socio-economic and behavioural factors on *S. haematobium* and STH infections, and the spatial distribution of these infections, in two villages of the Tana Delta District.

## **7.2 Materials and Methods**

### **7.2.1 Study area and study population**

The study area and study population were as previously described in Chapter 2 of this report.

### **7.2.2 Study design**

This was cross-sectional and carried out in two phases. In the first phase children aged between 5 and 12 years from Kau and Ozi primary schools were selected. Three urine and stool samples were collected from each study participant. One urine and one stool sample was collected on each of three different days and examined for *S. haematobium* and STH eggs, respectively. In addition, a questionnaire about risky behaviours relevant to *S. haematobium* and STH transmission was administered to the children. In the second phase, the children were traced to their homes for a study on household location and socio-economic factors relevant to *S. haematobium* and STH transmission. The geographical coordinates of the main house and the presence or absence of a pit latrine were recorded. In addition, one of the parent of each child was interviewed and information on the household socio-economic status relevant to *S. haematobium* and STH transmission obtained using a standard questionnaire.

### **7.2.3 Parasitological examinations**

Urine and stool specimens from the study participants were collected and examined for *S. haematobium* and STH eggs, respectively, as described in Chapter 2.

### **7.2.4 Mapping of households and water bodies**

The households where the children lived were visited, with help of a local guide who was well known and respected in the village. Geographical co-ordinates of the houses were recorded using a hand-held geographical positioning system (GPS) unit (e-trex Venture HC (Garmin®, Canada)). The co-ordinates of the local water contact points were also recorded. The co-ordinates were linked to *S. haematobium* and STH infection data and then exported to ArcGIS programme. These co-ordinates were used to draw spatial distribution maps of household and infection among children in Kau and Ozi.

### **7.2.5 Behavioural and Socio-economic Interviews**

During the baseline survey, the children were interviewed in the schools, through the administration of a questionnaire with close-ended questions, about risk behaviors relevant to *S. haematobium* and STH transmission as described in Chapter 2 (see questionnaire in Appendix II).

Households where the children came from were visited and assessed for some of the socio-economic factors, in the families, that are relevant to *S. haematobium* and STH infections as described in Chapter 2 (see questionnaire in Appendix III). Observations were made on the type of houses the families lived in and the presence or absence of a pit latrine. Houses were ranked based on the materials they were built of as described in Chapter 2 and the questionnaire shown in Appendix III.

Attempts were made to reach and interview parents of all children who participated in the baseline survey. Parents were interviewed, by administering a questionnaire with closed-ended questions, about their education levels and economic activities. They were also asked about the possession of a few selected items and livestock which were ranked based on the minimum market value of such item or animal in Kenya as described in Chapter 2 and the questionnaire shown in Appendix III.

### **7.2.6 Statistical Analysis**

Data were entered in an Excel spread sheet and exported to Stata (Version 10) for statistical analysis. Information collected through questionnaire was first coded and then entered in the Excel spreadsheet. Non-normally distributed continuous variables such as *S. haematobium* and STH eggs were log-transformed, after adding 1 to each value, using the formula:  $\log_{10}(x+1)$ . Geometric means were calculated using the formula:  $\text{antilog}_{10}[(\log_{10}(x+1)/n)]-1$ . Geometric means between the various groups were compared on  $\log_{10}(x+1)$  transformed values using t-test or One-way analysis of variance (ANOVA) as appropriate. Proportions such as prevalences were compared between groups using Pearson  $\chi^2$  test. *p*-values less than 0.05 were considered statistically significant in all tests.

## **7.3 Results**

### **7.3.1 Risky Behaviour of School Children**

#### **7.3.1.1 Study population**

A total of 262 children provided urine and stool samples as described in Chapter 3. Out of these, 211 were interviewed in the school and the data obtained were analysed for behavioural factors related to *S. haematobium* and STH infections. The other 51 children were not interviewed because they could not understand the questions and respond appropriately. A comparison of the 211 children to the initial population of 262 children is shown in Table 7.1. Generally, the two groups of children were very similar with regard to age, sex ratio and prevalence of *S. haematobium* and STH infections.

**Table 7.1:** Comparison between all 262 children who provided stool and urine samples and the 211 whose behavioural data were available.  
G:B ratio = Girls: Boys ratio; S. h. = *Schistosoma haematobium*, T. t.= *Trichuris trichiura*; Hw = hookworm; A. l.= *Ascaris lumbricoides*.

N	G:B ratio	Mean age (yrs)	S. h. prevalence	T. t. prevalence	Hw prevalence	A. l. prevalence
262	1.0	9.7	93.9%	88.2%	80.9%	45.4%
211	1.2	9.7	94.3%	89.6%	81.0%	45.0%

### 7.3.1.2 Children behaviour in Relationship to *S. haematobium* and STH Infections

The types of water bodies where the children went to swim and with which they came into contact while walking in relation to *S. haematobium* infection are shown in Tables 7.2 and 7.3 respectively. From these tables, the prevalence and intensities of *S. haematobium* remained almost the same whether the children went swimming or not. Similarly, it did not matter on the type of water bodies these children went to swim in or came into contact with while walking.

**Table 7.2:** Prevalence and GMI of *S. haematobium* infection among children in Kau and Ozi in relation to type of water body where children went to swim.

Reply	No. of children	<i>S. haematobium</i>	
		Prevalence (%)	GMI (eggs/10 ml urine)
Do not swim	16	93.8	51.8
River	96*	93.8	60.5
Swamp	96*	94.8	58.9
<b>Total</b>	<b>208*</b>	<b>p=0.95</b>	<b>p=0.96; F=0.04</b>

\*) Three children reported swimming in both river and swamps and were not included in the analysis.

**Table 7.3:** Prevalence and GMI of *S. haematobium* infection among children in Kau and Ozi in relation to type of water body with which children came into contact when walking.

Reply	No. of children	<i>S. haematobium</i>	
		Prevalence (%)	GMI (eggs/ 10 ml urine)
None	6	83.3	61.3
Floods	122*	94.3	57.4
Swamps	73*	95.9	68.4
Streams	5*	100.0	36.5
<b>Total</b>	<b>206*</b>	<b>p=0.56</b>	<b>p=0.88, F=0.22</b>

\*) One child reported coming in contact with both flood and swamp water, one reported coming in contact with both swamp and stream water whereas three reported coming in contact with all three types of water. These children were excluded from the analysis.

Among the children interviewed, only 5 reported using latrines at home whereas 206 did not. The prevalence and GMI of *S. haematobium* and STH infections in the two groups of

children are shown in Tables 7.4 and 7.5, respectively. There were no significant differences in the prevalence of *S. haematobium* and hookworm infections between children who used latrines and those who did not. However, children who did not use latrines had significantly higher prevalence of *T. trichiura* and *A. lumbricoides* infections than children who used latrines. Children who did not use latrines had considerably higher GMI of *S. haematobium* and STH infections than children who used latrines, although this difference was only statistically significant for *A. lumbricoides*.

**Table 7.4:** Prevalence of *S. haematobium* and STH infection among children in Kau and Ozi in relation to latrine use at their homes.

S. h. = *Schistosoma haematobium*, T. t.= *Trichuris trichiura*; Hw = hookworm; A. l = *Ascaris lumbricoides*.

Use latrine	No.	Prevalence (%)			
		S. h.	T. t.	Hookworm	A. l.
Yes	5	80.0	60.0	80.0	0.0
No	206	94.7	90.3	81.0	46.1
<b>Total</b>	<b>211</b>	<b>p=0.16</b>	<b>p=0.029</b>	<b>p=0.95</b>	<b>p=0.041</b>

**Table 7.5:** GMI of *S. haematobium* and STH infection among children in Kau and Ozi in relation to their use of latrines at home.

S. h. = *Schistosoma haematobium*, T. t.= *Trichuris trichiura*; Hw = hookworm; A. l.= *Ascaris lumbricoides*.

Use latrine	No.	GMI (eggs/10 ml urine or g stool)			
		S. h.	T. t.	Hw	A. l.
Yes	5	14.5	14.8	24.5	0.0
No	206	62.0	100.0	82.8	9.1
<b>Total</b>	<b>211</b>	<b>p=0.28</b>	<b>p=0.20</b>	<b>p=0.26</b>	<b>p&lt;0.001</b>

Among the children interviewed only 3 reported not eating fruits in the farms whereas the other 208 collected and ate fruits in the farms. When asked whether they washed the fruits before eating them, 178 reported they did not and 33 reported they did. However, there was no significant differences in the prevalences of STH infections.

### 7.3.2 Household Survey

#### 7.3.2.1 Study population and household characteristics

A total of 196 households were visited and their geographical co-ordinates recorded. These households represented 227 children who participated in the school-based baseline data collection. A comparison of the 227 children to the initial population of 262 children generally showed that the two groups of children were similar with regard to age, sex ratio and prevalence of *S. haematobium* and STH infections. However, not all parents

answered all the questions during interviews and therefore the number of children for which interview data are reported is often slightly lower.

The two study villages were very similar in terms of housing structure with three types of houses seen. Thirty-eight per cent of the families lived in houses made of straw walls and grass thatched roofs mainly distributed in farms outside the densely populated central parts of the villages. Another 59% of the families lived in houses made of mud walls and grass thatched roofs mainly distributed in the densely populated central part of the villages. The remaining 3% of the families lived in houses made of mud walls and iron sheet roofs and situated in the densely populated central part of the villages. Houses made of straw wall and thatched roofs were accorded rank 1, those made of mud walls and thatched roofs were accorded rank 2 and those made of mud walls and iron sheet roofs were accorded rank 3.

The prevalence and GMI of *S. haematobium* and STH infections among the children in relation to the rank of family houses is shown in Tables 7.6a and 7.6b, respectively. Analysis by rank and infection prevalences and infection intensity showed no significant difference for all parasitic infections, although there was a general trend of decrease in prevalence and intensity with increasing rank of households.

**Table 7.6a:** Prevalence of *S. haematobium* and STH infection among children in Kau and Ozi in relation to the rank of their family houses.

S. h. = *Schistosoma haematobium*, T. t.= *Trichuris trichiura*; Hw = hookworm; A. l.= *Ascaris lumbricoides*.

House Ranks	No.	Prevalence (%)			
		S. h.	T. t.	Hw	A. l.
Rank 1	91	90.1	87.9	82.7	45.1
Rank 2	128	94.5	88.3	79.7	44.5
Rank 3	8	100.0	75.0	61.3	50.0
<b>Total</b>	<b>227</b>	<b>p=0.33</b>	<b>p=0.54</b>	<b>p=0.70</b>	<b>p=0.96</b>

**Table 7.6b:** GMI of *S. haematobium* and STH infection among children in Kau and Ozi in relation to the rank of their family houses.

S. h. = *Schistosoma haematobium*, T. t.= *Trichuris trichiura*; Hw = hookworm; A. l.= *Ascaris lumbricoides*.

House Ranks	No.	GMI (eggs/10 ml urine or g stool)			
		S. h.	T. t.	Hw	A. l.
Rank 1	91	47.2	100.7	82.7	9.0
Rank 2	128	59.1	87.5	77.0	8.2
Rank 3	8	35.2	39.5	61.3	12.3
<b>Total</b>	<b>227</b>	<b>p=0.63</b> <b>F=0.46</b>	<b>p=0.48</b> <b>F=0.73</b>	<b>p=0.94</b> <b>F=0.06</b>	<b>p=0.93</b> <b>F=0.08</b>

In the two villages, there was no piped water. Thirty-six of the households obtained their water for domestic use from the River Tana whereas 40% of the households obtained it

from open wells. Ten percent of the households, especially those in the farms, obtained their water from the local fresh water swamps. The remaining 13% of the households obtained water from multiple sources. This latter group of children was not included in the analysis.

Comparison of *S. haematobium* and STH infection prevalence and GMI in the examined children in relation to the source of water for the households are shown in Tables 7.7a and 7.7b, respectively. There was no significant difference in the prevalence of any of the infections between the groups of children in relation to the source of water for their households. However, when analysed by infection intensity, there was a significant difference in *S. haematobium* infections between the groups of children in relation to the source of water for their households. Children from households with swamp as the source of water had the highest intensity followed by those with wells.

**Table 7.7a:** Prevalence of *S. haematobium* and STH infection among children in Kau and Ozi in relation to household source of water.

*S. h.* = *Schistosoma haematobium*, *T. t.* = *Trichuris trichiura*; Hw = hookworm; *A. l.* = *Ascaris lumbricoides*.

Water source	No.	Prevalence (%)			
		<i>S. h.</i>	<i>T. t.</i>	Hw	<i>A. l.</i>
River	69	92.8	85.5	84.1	47.8
Well	84	89.3	89.3	82.1	47.6
Swamp	21	100	90.5	90.5	52.4
<b>Total</b>	<b>174</b>	<b><i>p</i>=0.26</b>	<b><i>p</i>=0.72</b>	<b><i>p</i>=0.65</b>	<b><i>p</i>=0.92</b>

**Table 7.7b:** GMI of *S. haematobium* and STH infections among children in Kau and Ozi in relation to household water source.

*S. h.* = *Schistosoma haematobium*, *T. t.* = *Trichuris trichiura*; Hw = hookworm; *A. l.* = *Ascaris lumbricoides*.

Water source	No.	GMI (eggs/10 ml urine or g stool)			
		<i>S. h.</i>	<i>T. t.</i>	Hw	<i>A. l.</i>
River	69	38.2	90.1	68.9	10.9
Well	84	41.3	106.5	108.5	10.4
Swamp	21	176.7	83.9	144.0	13.6
<b>Total</b>	<b>174</b>	<b><i>p</i>=0.009</b> <b>F=4.87</b>	<b><i>p</i>=0.84</b> <b>F=0.17</b>	<b><i>P</i>=0.36</b> <b>F=1.03</b>	<b><i>p</i>=0.94</b> <b>F=0.06</b>

Ten out of 192 children (5.2%) came from households with pit latrines. The prevalences and GMI of *S. haematobium* and STH infections among the children in relation to the presence or absence a pit latrine at home are shown in Tables 7.8a and 7.8b, respectively. The presence or absence of a pit latrine in this population did not affect the prevalences of *S. haematobium*, hookworm and *A. lumbricoides*. However, children from households without pit latrines had a significantly higher prevalence of *T. trichiura* infections than children from households with pit latrines. Similarly for infection intensity,

no significant difference in GMI of *S. haematobium* or STH infections between children from households with and without pit latrines was shown although GMIs of *S. haematobium*, *T. trichiura* and hookworms were markedly lower in households with latrines.

**Table 7.8a:** Prevalence of *S. haematobium* and STH infections among children in Kau and Ozi in relation to presence of pit latrine at home.

*S. h.* = *Schistosoma haematobium*, *T. t.* = *Trichuris trichiura*; Hw = hookworm; *A. l.* = *Ascaris lumbricoides*.

Latrine	No.	Prevalence (%)			
		<i>S. h.</i>	<i>T. t.</i>	Hw	<i>A. l.</i>
Present	10	100.0	60.0	80.0	40.0
Not present	182	95.1	89.0	80.8	45.6
<b>Total</b>	<b>192</b>	<b><i>p</i>=0.47</b>	<b><i>p</i>=0.007</b>	<b><i>p</i>=0.95</b>	<b><i>p</i>=0.73</b>

**Table 7.8b:** GMI of *S. haematobium* and STH infections among children in Kau and Ozi in relation to presence of pit latrine at home.

*S. h.* = *Schistosoma haematobium*, *T. t.* = *Trichuris trichiura*; Hw = hookworm; *A. l.* = *Ascaris lumbricoides*.

Latrine	No.	GMI (eggs/10 ml urine or g stool)			
		<i>S. h.</i>	<i>T. t.</i>	Hw	<i>A. l.</i>
Present	10	43.2	20.9	33.0	14.5
Not present	182	61.6	94.2	82.7	8.8
<b>Total</b>	<b>192</b>	<b><i>p</i>=0.43</b>	<b><i>p</i>=0.15</b>	<b><i>p</i>=0.29</b>	<b><i>p</i>=0.71</b>

### 7.3.2.2 Household interviews

A total of 183 parents, representing 194 children, responded to the question about possession of selected household items. Eight of the parents, representing 11 children, were fathers whereas the rest were mothers. Based on the response given, each household was grouped in three different socio-economic classes, explained in Chapter 2, as follows: Socio-economic class 1 comprising of 135 children from households with selected items worth KES 10,000.00 or less; class 2 comprising of 42 children from households with selected items worth more than KES 10,000.00 but less than KES100,000.00; and class 3 comprising of 17 children from households with selected items worth more than KES 100,000.00.

Comparative analysis of the social class against prevalence and GMI was then done. The prevalence and GMI of *S. haematobium* and STH infections among the children in relation to socio-economic class of their household is shown in Tables 7.9a and 7.9b, respectively. From the analysis, no significant differences in the prevalence and GMIs for *S. haematobium*, *T. trichiura* and *A. lumbricoides* infections in children from the different



socio-economic classes were found. However, for hookworm infection there was a significant difference in the prevalence and GMI in children from the different socio-economic classes. Both the prevalence and GMI decreased with an increase in the social class.

**Table 7.9a:** Prevalence of *S. haematobium* and STH infection status among children in Kau and Ozi in relation to their social class.

n=194, S. h. = *Schistosoma haematobium*, T. t.= *Trichuris trichiura*; Hw = hookworm; A. l.= *Ascaris lumbricoides*.

Social class	No.	Prevalence (%)			
		S. h.	T. t.	Hw	A. l.
Class 1	135	94.1	85.2	87.4	45.9
Class 2	42	90.5	92.9	71.4	42.9
Class 3	17	100.0	88.2	58.8	47.1
<b>Total</b>	<b>194</b>	<b>p=0.77</b>	<b>p=0.43</b>	<b>p=0.003</b>	<b>p=0.93</b>

**Table 7.9b:** GMI of *S. haematobium* and STH infection among children in Kau and Ozi in relation to their social class.

n.=194, S. h. = *Schistosoma haematobium*, T. t.= *Trichuris trichiura*; Hw = hookworm; A. l.= *Ascaris lumbricoides*.

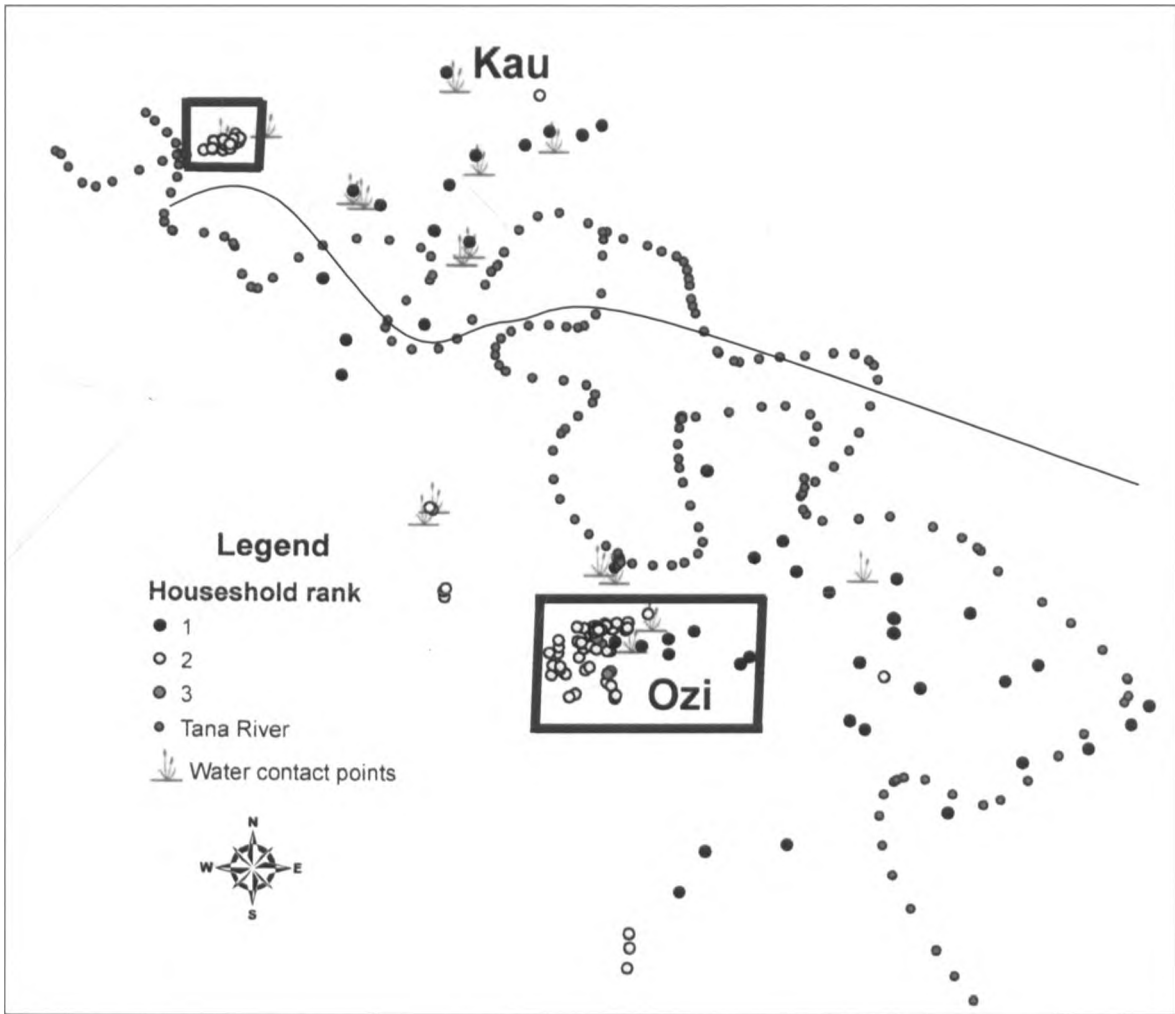
Social class	No.	GMI (eggs/10 ml urine or g stool)			
		S. h.	T. t.	Hw	A. l.
Class 1	135	63.2	79.5	97.4	9.6
Class 2	42	40.4	106.4	49.4	9.1
Class 3	17	59.5	55.6	23.4	14.9
<b>Total</b>	<b>194</b>	<b>p=0.22</b> <b>F=1.54</b>	<b>p=0.54</b> <b>F=0.61</b>	<b>p=0.043</b> <b>F=3.20</b>	<b>p=0.86</b> <b>F=0.16</b>

Economic activities such as farming and small businesses of either parent did not affect the prevalence or intensity of infections among the children. Similarly, the mothers' levels of education did not affect the prevalences and intensity of infection. However, for the father's education, there were significant differences in prevalence and GMI of *S. haematobium* infection between children belonging to fathers with different levels of school education. Children of fathers with primary school education had the highest GMI followed by those with no school education. Children of fathers who had secondary school education had the lowest GMI.

### 7.3.2.3 Spatial distribution of households and infections

The two villages are located on the banks of River Tana with a distance of less than 5 km between the centres of the villages. Most of Kau is located on the northern side of the river, while most of Ozi is located on the southern side. The spatial distribution of

households in the two villages is shown in Figure 7.1. Houses to the north of the black continuous line belong to Kau village and those to the south of the line belong to Ozi village. In general, the two villages appeared rather similar in distribution and structure of the houses. At the centre of each village, most of the houses were made of thatched roof and mud walls. A few houses had roofs made of corrugated iron sheet and mud walls. At the periphery and in the surrounding farms, the houses were mainly made of thatched roofs and walls made of straw. The households of each village were divided into those located in the centre and those located in the periphery, as shown in Figure 7.1.



**Figure 7.1:** Spatial distribution of households in Kau and Ozi (map not drawn to scale). Houses to the north of the black continuous line represent households in Kau village and those to the south of the line represent households in Ozi village. Households within the red boxes were considered to belong to the centre part of the village, while those outside these boxes were considered to belong to the periphery. Household rank 1 = houses made of thatched roofs and straw walls; household rank 2 = houses made of thatched roofs and mud walls and; household rank 3= houses made of corrugated iron sheet roofs and mud walls.

A comparison of household ranks between the central parts and peripheries in the two villages is shown in Table 7.10. There was a significant difference in household ranks between the central part and the periphery in Kau as well as in Ozi.

**Table 7.10:** Comparison of house ranks between the centres and peripheries of Kau and Ozi. P-value represent differences between household at the centre of the village and those at the periphery (t-test).

Village	Village area	No. households*	Arithmetic Mean house rank
Kau	Centre	27	1.96
	Periphery	19	1.16
	<i>p</i> -value	-	<0.001
Ozi	Centre	87	1.98
	Periphery	63	1.17
	<i>p</i> -value	-	<0.001
Kau and Ozi	Centre	114	1.97
	Periphery	82	1.17
	<i>p</i> -value	-	<0.001

\*) The 196 visited and mapped households

A comparison of the prevalences and GMI of *S. haematobium* and STH between the central parts and peripheral parts of Kau and Ozi are shown in Tables 7.11a and 7.11b. There were no significant differences in prevalence and GMI of both *S. haematobium* and STH infections between the central and the peripheral parts in Kau or Ozi.

**Table 7.11a:** Comparison of *S. haematobium* and STH infection prevalences between the centres and peripheries of Kau and Ozi.

*S. h.* = *Schistosoma haematobium*, *T. t.* = *Trichuris trichiura*; Hw = hookworm; *A. l.* = *Ascaris lumbricoides*.

Village	Village area	No. *	<i>S. h.</i> (%)	<i>T. t.</i> (%)	Hw (%)	<i>A. l.</i> (%)
Kau	Centre	31	96.8	87.1	67.7	25.8
	Periphery	25	100.0	80.0	68.0	36.0
	<i>p</i> -value	-	<b>0.37</b>	<b>0.47</b>	<b>0.98</b>	<b>0.41</b>
Ozi	Centre	102	94.1	89.2	84.3	51.0
	Periphery	69	87.0	88.4	87.0	47.8
	<i>p</i> -value	-	<b>0.10</b>	<b>0.87</b>	<b>0.63</b>	<b>0.69</b>
Kau and Ozi	Centre	133	94.7	88.7	80.5	45.1
	Periphery	94	90.4	86.2	81.9	44.7
	<i>p</i> -value	-	<b>0.21</b>	<b>0.57</b>	<b>0.78</b>	<b>0.95</b>

\*227 children whose households were visited and mapped

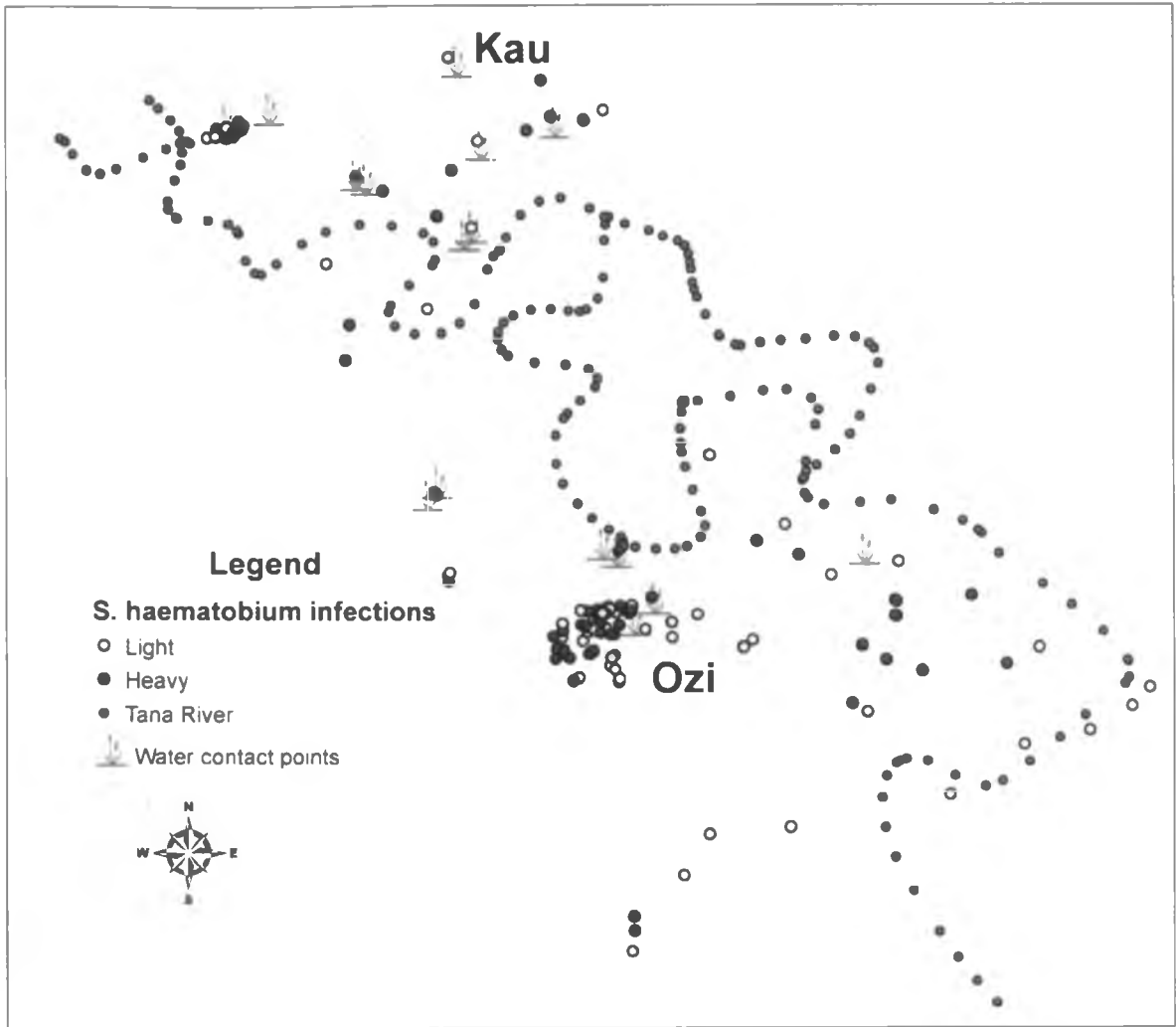
**Table 7.11b:** Comparison of *S. haematobium* and STH infection GMI between the centres and peripheries of Kau and Ozi.

*S. h.* = *Schistosoma haematobium*, *T. t.* = *Trichuris trichiura*; Hw = hookworm; *A. l.* = *Ascaris lumbricoides*.

Village	Village area	No. *	<i>S. h.</i> GMI (egg/10 ml)	<i>T. t.</i> GMI(egg/g)	Hookworm GMI(egg/g)	<i>A. l.</i> GMI (egg/g)
Kau	Centre	31	58.2	46.7	17.8	1.6
	Periphery	25	52.7	43.5	19.9	4.2
	<b>p-value</b>	-	<b>0.85</b>	<b>0.90</b>	<b>0.87</b>	<b>0.27</b>
Ozi	Centre	102	47.1	104.6	129.9	12.9
	Periphery	69	62.2	130.8	115.4	11.6
	<b>p-value</b>	-	<b>0.40</b>	<b>0.51</b>	<b>0.75</b>	<b>0.83</b>
Kau and Ozi	Centre	133	49.5	86.8	82.3	8.4
	Periphery	94	59.5	97.8	72.7	9.0
	<b>p-value</b>	-	<b>0.51</b>	<b>0.68</b>	<b>0.72</b>	<b>0.88</b>

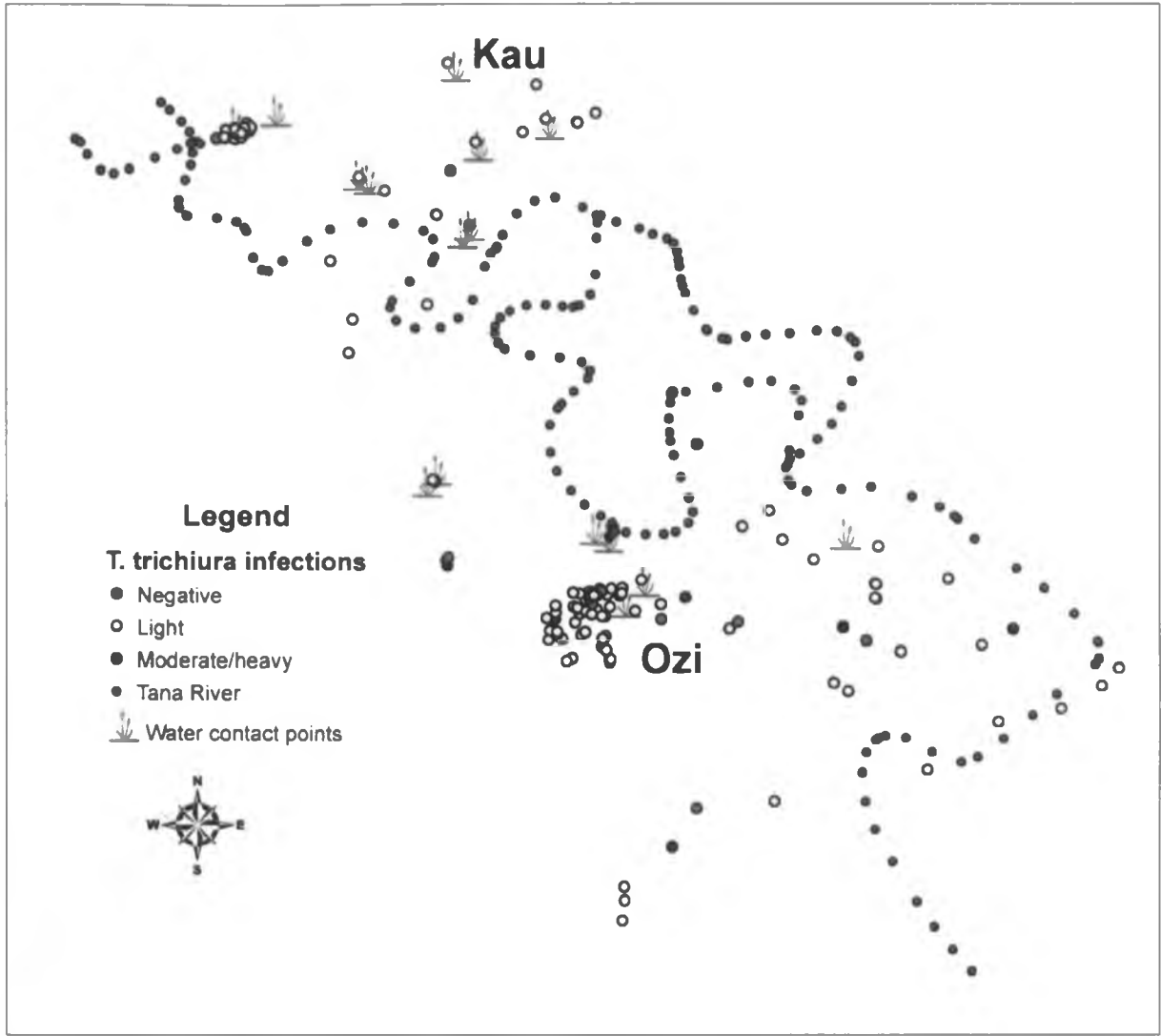
\*227 children whose households were visited and mapped

The spatial distribution of *S. haematobium* infections in Kau and Ozi villages is shown in Figure 7.2. *S. haematobium* infections appeared to be homogenously distributed in the two villages and no obvious clustering was seen. Heavy and light infections were seen both at the centre and in the periphery of each village.



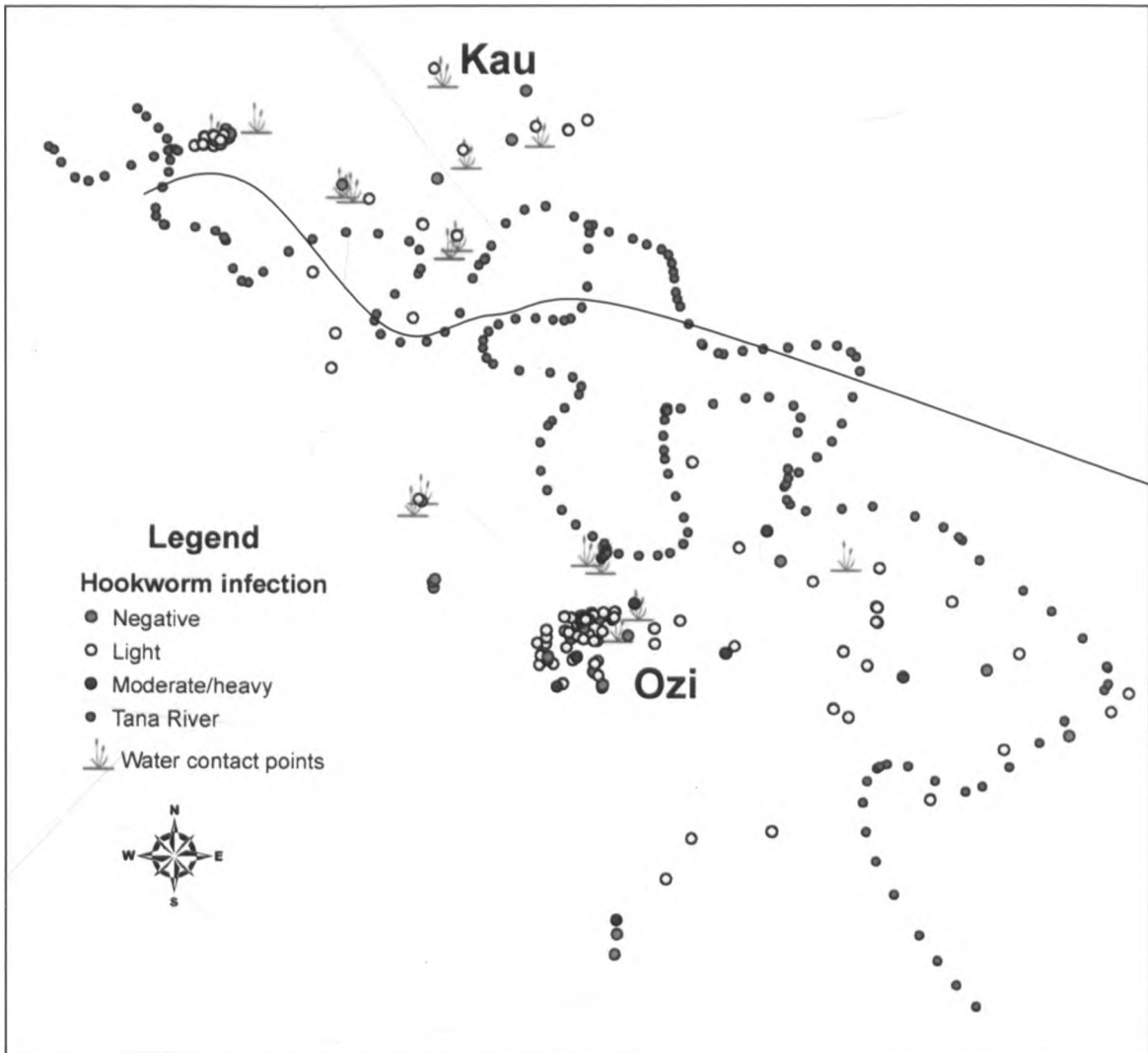
**Figure 7.2:** Spatial distribution of *S. haematobium* infection among children in Kau and Ozi (map not drawn to scale). Houses to the north of the black continuous line represent households in Kau village and those to the south of the line represent households in Ozi village.

The spatial distribution of *T. trichiura* infections in Kau and Ozi villages is shown in Figure 7.3. *T. trichiura* infections also appeared to be homogeneously distributed in the two villages with no obvious clustering.



**Figure 7.3:** Spatial distribution of *T. trichiura* infection among children in Kau and Ozi (map not drawn to scale). Houses to the north of the black continuous line represent households in Kau village and those to the south of the line represent households in Ozi village.

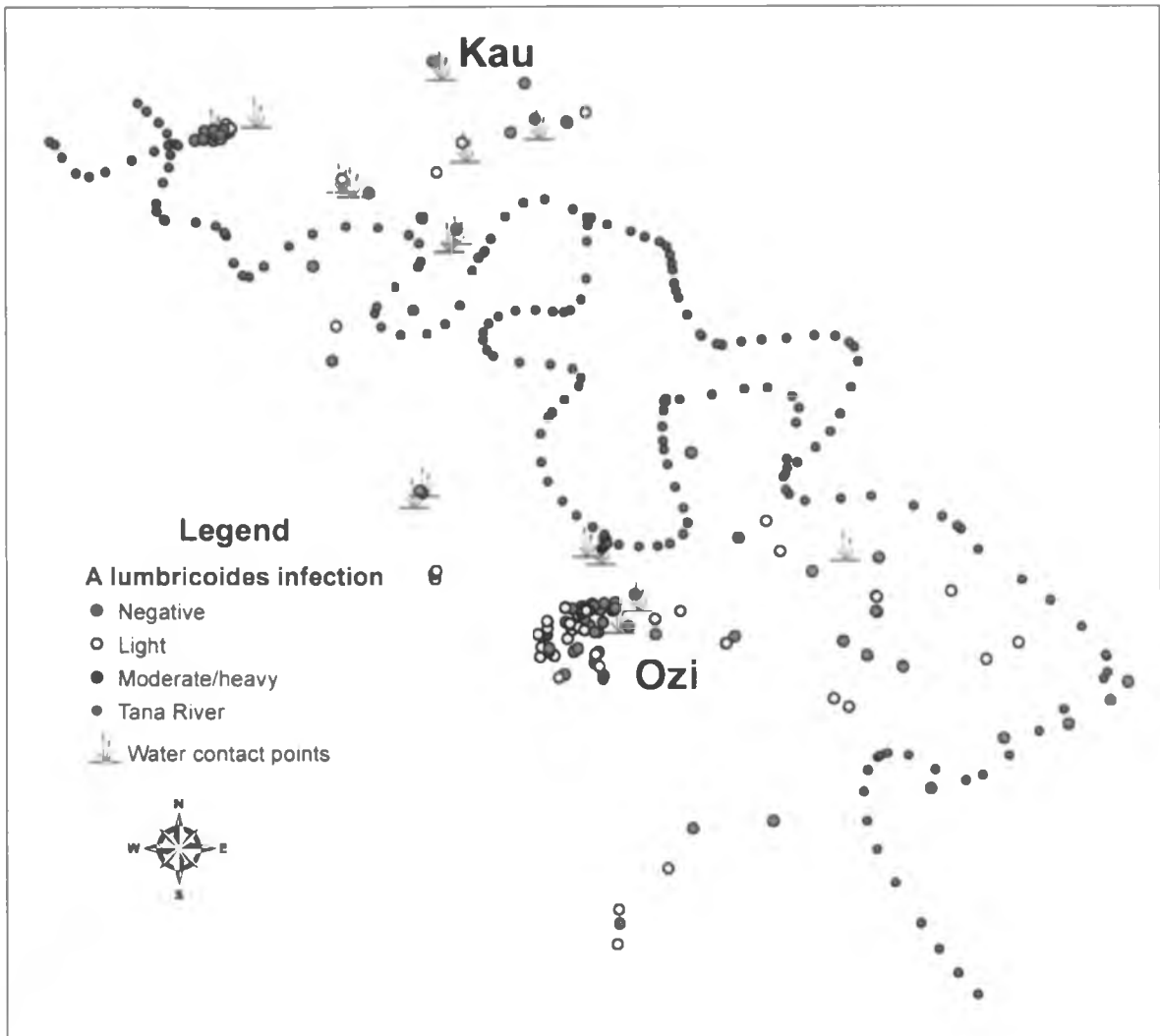
The spatial distribution of hookworm infections in Kau and Ozi villages is shown in Figure 7.4. Both villages had widely distributed hookworm infections and no obvious clustering was observed.



**Figure 7.4:** Spatial distribution of hookworm infection among children in Kau and Ozi (map not drawn to scale).

Houses to the north of the black continuous line represent households in Kau village and those to the south of the line represent households in Ozi village

The spatial distribution of *A. lumbricoides* infections in Kau and Ozi villages is shown in Figures 7.5. Both villages had widely distributed *A. lumbricoides* infections and no obvious clustering was observed.



**Figure 7.5:** Spatial distribution of *A. lumbricoides* infection among children in Kau and Ozi (map not drawn to scale).

Houses to the north of the black continuous line represent households in Kau village and those to the south of the line represent households in Ozi village

#### 7.4 Discussion

Transmission of schistosomes and STH is enabled by a combination of certain behavioural, socio-economic and environmental factors which may vary from one endemic focus to another (Bruun & Aagaard-Hansen, 2008; Kloos *et al.*, 2008). The present study assessed the prevalence and intensity of *S. haematobium* and STH infections among primary school children in relation to selected risky behaviours and socio-economic factors and to the spatial distribution of the households from which they came.

Behaviours that facilitate the seeding of the environment with helminth eggs are, probably, the most important in transmission of *S. haematobium* or STH, and just a few individuals seeding the environment with helminth eggs can spread the infections to an entire village



population (Bruun & Aagaard-Hansen, 2008; Curtis *et al.*, 2000). This mainly occurs when individuals do not use latrines but rather urinate and defecate in the environment. In the present study, only 10 out of the interviewed 211 children (4.7%) had access to latrines at home, and only 5 reported using the latrines. The rest urinated and defecated in the nearby bushes. This means that the environmental contamination with *S. haematobium* and STH eggs in the two villages was high resulting in heavy exposure to infections among the children. As a result, the prevalence and intensity of *S. haematobium* were high (>90%). Similarly, the prevalence of STH, especially *T. trichiura* and hookworm, was high but according to the classification of WHO, the intensities were low (Bergquist *et al.*, 2009). Children who did not use latrines had higher prevalences and intensities of *S. haematobium* and STH infections than children who used them although this was only statistically significant for *T. trichiura* and *A. lumbricoides*. This is similar to reports of other studies that use of latrines is of paramount importance in a reduction of environmental contamination (Olsen *et al.*, 2001; Idowu & Rowland, 2006; Dumba *et al.*, 2008; Curtis *et al.*, 2000). The results also suggest that use of latrines is more effective in controlling *T. trichiura* and *A. lumbricoides* which are transmitted through ingestion of infective eggs than *S. haematobium* and hookworm which are transmitted through percutaneous penetration by larval stages in water and soil, respectively.

Some behaviour may enhance individual infection with helminths. For example, contact with infective water is a major factor predisposing to infections with schistosomes (Aagaard-Hansen *et al.*, 2009). Most of the children reported swimming or wading in river, stream or swamp water and only a few reported not coming in contact with any of these waters. There were, however, no significant differences in *S. haematobium* infections with regard to the type of water the children came in contact with. This implies that the water bodies were equally infective or there was another, yet unidentified, common source of infection for the children. For example, it is noteworthy that although water contact points in the area are more or less defined during the dry season, there are wide-spread floods during the rainy seasons when almost entire villages are submerged in potentially infective water. This can make identification of the temporal role of small sources of infection difficult (Pullan *et al.*, 2011). Infection with hookworm occurs when the larvae penetrate through human skin especially between the toes and can therefore be significantly reduced by wearing shoes (Ortega *et al.*, 2010; Tomono *et al.*, 2003; Midzi *et al.*, 2011). It was observed that children in the two villages did not wear shoes at home or in school. This means they frequently came in contact with soils harbouring infective hookworm larvae due to the prevailing intensive and widespread environmental

contamination. As a result, the prevalence of hookworm infections was high (>80.0%). Eating unwashed raw foodstuffs such as fruits and vegetables is an important risk factor for infection with *T. trichiura* and *A. lumbricoides* (Ilechukwu *et al.*, 2010; Avcioglu *et al.*, 2011; Midzi *et al.*, 2011). Collecting and eating unwashed fruits in the field was common among the children but was not significantly related to *T. trichiura* or *A. lumbricoides* infections. These findings suggest that contamination of fruits was not the main source of infections. There may have existed another unidentified source in the area.

The socio-economic status of a family may be related to helminth infection status. There are several indicators of family socio-economic status which have shown varying associations with helminth infection status (Bruun & Aagaard-Hansen, 2008; Kloos *et al.*, 2008; Pullan *et al.*, 2011; Esrey *et al.*, 1991). The present study assessed the prevalences and intensities of *S. haematobium* and STH infections among primary school children in relation to selected indicators namely; 1) structure of housing, 2) source of water for domestic use, 3) social class as determined by possession of selected valuable items, 4) presence of a latrine in the household and, 5) parents' economic activity and education level.

Unlike in the findings of several other studies (Kloos *et al.*, 2008), no significant relationship was demonstrated between housing structure and *S. haematobium* or STH infections among the children although the prevalence and intensity of *T. trichiura* and hookworm infections tended to decrease with improved housing structure. This suggests that housing structure is a poor predictor of infection with these helminths in the two villages where, despite some differences in the structure of housing, the children spent most of the time together thus probably getting infections from common sources.

The source of water for household use may be an important indicator of the quality and quantity of water available to sustain preventive hygiene standards and may therefore influence infection with *T. trichiura* and *A. lumbricoides* which are transmitted through ingestion of eggs in contaminated hands and foodstuffs (Esrey *et al.*, 1991; Dumba *et al.*, 2008). In the present study there was no significant difference in prevalence or intensity of STH infections between children from households that obtained water from river, wells or swamps which suggests that STH transmission in the study villages is not directly related to the individual water sources or household hygiene standards (Dumba *et al.*, 2008; Kloos *et al.*, 2008). Children may be infected with schistosomes if they come in contact with infective water when they go to fetch water or when they accompany their parents to sources of water for domestic use (Bruun & Aagaard-Hansen, 2008). The present study,

however, did not demonstrate significant differences in the prevalence of *S. haematobium* among the children with regard to sources of water for domestic use but children from households that obtained water from swamps had significantly higher intensity of *S. haematobium* infection. This suggests that apart from there being another common source of infection, swamps acted as additional sources of *S. haematobium* infections, probably during the dry season when children participate in water-related domestic chores such as washing in the swamps (Ndassa *et al.*, 2007; Kloos *et al.*, 1997; Handzel *et al.*, 2003).

There was a significant negative relationship between hookworm infections and the social class of the children's families. These findings agree with those of a study in Brazil (Pullan *et al.*, 2011). There was, however, no significant relationship between *S. haematobium*, *T. trichiura* or *A. lumbricoides* infections and the families' social class. Although this is in agreement with the findings of a study in Kisumu District of Kenya (Olsen *et al.*, 2001), several other studies have shown that negative associations may exist between these infections and social class elsewhere (Bruun & Aagaard-Hansen, 2008; Pullan *et al.*, 2008; Idowu & Rowland, 2006; Brooker *et al.*, 2004; Quihui *et al.*, 2006). This shows that different factors have area-specific effects on helminth infections.

The economic activities and educational levels of parents may relate to the family socio-economic status, which in turn is related to helminth infections (Bruun & Aagaard-Hansen, 2008; Dumba *et al.*, 2008; Quihui *et al.*, 2006). The data indicate that the parents' economic activities were not significantly related to *S. haematobium* or STH infections among the children. Similarly, the mothers' and fathers' education levels were not significantly related to STH infections. However, although there was no significant relationship between the mothers' education levels, children belonging to fathers who had completed primary education had significantly higher prevalence and intensities of *S. haematobium* infections than children whose fathers had no primary education or had finished secondary education. Parents with higher education levels are likely to value health promotion practices more than those with low levels and thus protect their children from helminth infections (Dumba *et al.*, 2008; Quihui *et al.*, 2006). The results of the present study in this aspect did not support this but no immediate and specific explanation could be advanced for this.

Regular deworming of children by parents as a health promoting practice was not well adhered to in the two villages. Only 16.4% of the children came from families where parents dewormed their children with albendazole regularly and 9.9% of the children were from families where parents only dewormed their children if symptomatic. The rest

(73.6%) of the children came from families where parents never dewormed their children. This study did not demonstrate any significant difference in *S. haematobium* or STH infections between these groups of children. No parent reported treating their children with praziquantel. The presence of at least one sick child in the family in the previous three months was used as an indicator of the families' general health in relation to *S. haematobium* and STH infection status among the children. More than 70% of the children whose parents responded to this question came from families that had at least one sick child within this period. These children had significantly higher prevalences and intensities of *A. lumbricoides*, but not *S. haematobium*, *T. trichiura* or hookworm, than children from families that had no sick child in the previous three months.

A number of studies in different areas that have focused on small-scale spatial distribution of schistosomes and STH infections have demonstrated a marked level of heterogeneity in their distribution (Handzel *et al.*, 2003; Brooker *et al.*, 2006; Booth *et al.*, 2004). The present study attempted to elucidate the spatial distribution of *S. haematobium* and STH infections, in two hyper-endemic villages of Tana Delta District in Kenya. No spatial clustering of *S. haematobium* or STH infection prevalence or intensity was observed in the two villages. There were no significant differences between houses located in the central parts and those at the peripheries of the two villages. This disagrees with the findings of many studies carried out elsewhere. For example *S. haematobium* infections in villages in Kenya and Zanzibar were reported to be clustered around water bodies infested with intermediate snail hosts (Clennon *et al.*, 2004; Clennon *et al.*, 2006; Rudge *et al.*, 2008). Similarly, clustering of *S. mansoni* infections around distinct water bodies has been reported in Kenya and elsewhere (Handzel *et al.*, 2003; Brooker *et al.*, 2006; Booth *et al.*, 2004; Pullan *et al.*, 2011). The only explanation for the currently observed homogeneity in spatial distribution of *S. haematobium* infections in the two villages is that, there is a homogeneous transmission in the area that is not dependent on small distinct water bodies but a large source of the infection. This probably happens during the seasonal floods when transmission can take place in vast flood waters.

Transmission of STH does not rely on the presence of water bodies in the environment and therefore may be widely distributed in an area depending on the type of soil, vegetation and ambient temperatures (Mabaso *et al.*, 2003; Lilley *et al.*, 1997). Although the prevalence and intensity of STH was significantly higher in Kau than in Ozi, probably due differences in soil types, there were no significant intra-village differences between the central parts and peripheries. This suggests that low personal hygiene and sanitation, which facilitate transmission, were similar in all parts of the villages as evidenced by

generalised lack of latrines. These findings are different from other reports that STH infections are focal (Handzel *et al.*, 2003; Clements *et al.*, 2010) and suggests that there are other latent factors that influence the spatial distribution of the infections in different areas.

### **7.5 Conclusion**

The findings of the present study suggest that behaviour of the children and the family socio-economic status in Kau and Ozi played minimal roles in transmission of *S. haematobium* and STH among the children with failure to use latrines being a risk factor for infection with *T. trichiura* and *A. lumbricoides*. The fathers' education levels were risk factors for *S. haematobium* infection whereas using swamp as a source of water was a risk factor for heavy infection only. Low social class was a risk factor for hookworm infection. Reports of children deworming, with albendazole, by parents was not a good predictor of STH infections whereas a poor family health status was a predictor of *A. lumbricoides* infection. It is recommended that, in the meantime, all the children in the two villages be targeted for regular treatment against *S. haematobium* and STH (Hotez & Fenwick, 2009).

## CHAPTER 8: GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

The present study assessed and analysed *S. haematobium* and STH infections among school children aged 5-12 years in two adjacent villages in the Tana Delta District of Tana River County, Kenya. A high prevalence of *S. haematobium* infection (>90%) was observed in the children, with no significant differences between the two villages. This agrees with reports from the Ministry of Health indicating that the area is hyperendemic for *S. haematobium* (Brooker *et al.*, 2009). Considering that Kenya is generally regarded as a moderate schistosomiasis transmission country with <50% overall prevalence (Utzinger *et al.*, 2009), the findings suggest that the prevalence of *S. haematobium* infections is over 90% in the study area. Similarly, high prevalences of STH infections were observed among the children, exceeding 80%, 70% and 35% for *T. trichiura*, hookworm and *A. lumbricoides*, respectively. This also agrees with previous reports (reviewed by Brooker *et al.*, 2009) indicating that the area is hyperendemic for STH infections and that control measures tailored towards the needs of the area ought to be put in place.

Most of the observed *S. haematobium* infections were heavy whereas most of the STH infections were light according to WHO classification criteria (Bergquist *et al.*, 2009). No immediate explanation could be given for the apparent discrepancy of low intensity but high prevalence of STH infections. However, high levels of eosinophilia which were significantly associated with *S. haematobium* infection intensity were observed among the children. Since eosinophils have been suggested to be involved in killing of larval worms (Maizels & Balic, 2004; Maizels *et al.*, 2004), it is probable that eosinophil-mediated killing of larval STH was, at least partly, responsible for keeping the STH infections at low intensity. Thus, although *S. haematobium* may not have influenced the prevalence of STH infections, it could have been involved in keeping the intensities at low levels.

*S. haematobium* infections were significantly associated with haematuria and ultrasound-detectable urinary tract pathology. Similar findings, that *S. haematobium* infections are the major cause of urinary tract pathology among school children along the Kenyan coast, have been reported from other studies (Magnussen *et al.*, 1997; King, 2006; Wamachi *et al.*, 2004; Keita *et al.*, 2005). The present study, in addition, showed that development of the pathology started early in life among children in the area.

It is well known that schistosome and STH infections are associated with malnutrition and anaemia (Stephenson *et al.*, 2000 a,b,c; Koski & Scott, 2001; Stoltzfus *et al.*, 1997; Brito *et al.*, 2006; Ezeamana *et al.*, 2008; Friedman *et al.*, 2005), and in the present study anaemia and low BMI were highly prevalent. Anaemia was significantly related to *S. haematobium* and hookworm infections but not to *T. trichiura* or *A. lumbricoides* infections probably because the former two are associated with more blood loss through haematuria and bleeding in the intestines as compared to the latter two (Crompton, 2000). On the other hand, *T. trichiura* and *A. lumbricoides* infections, but not *S. haematobium* or hookworm infections, were significantly associated with low BMI. This was probably because *T. trichiura* and *A. lumbricoides* live in the gut lumen where, apart from competing with the host for nutrients, they also interfere with the gut physiology and thereby reduce nutrients intake (Stephenson *et al.*, 2000a). In addition, it is noteworthy that both anaemia and low BMI may be caused by many other factors, such as lack of proper and adequate diet, which could also have contributed to the observed phenomena (Jardim-Batelho *et al.*, 2008; Koski & Scott, 2001; Lutter, 2008; Beasley *et al.*, 2002; Lartey, 2008).

Some studies have suggested that there could be a biological interaction between schistosomes and STH which increase or decrease the intensity of one or more of the co-infecting helminth species (Cox, 2001; Geiger, 2008). A previous study in coastal Kenya, however, did not detect interaction between *S. haematobium* and STH (Ashford *et al.*, 1992). In the present study, children with light *S. haematobium* infections had significantly heavier *T. trichiura* infections than children with heavy *S. haematobium* infections. This suggests that although *S. haematobium* may not have influenced the prevalence of *T. trichiura* infections, it could have influenced their intensity, perhaps through *S. haematobium*-induced release of eosinophils or cytokines leading to the death of larva stages or the dislodging of adult worms from the intestinal mucosa (Maizels *et al.*, 2004; Cox, 2001; Geiger, 2008). On the other hand, children with heavy *S. haematobium* infections had significantly higher GMI of hookworm infections than those with light *S. haematobium* infections. This may suggest that there was a mechanism which led to increased survival of both *S. haematobium* and hookworms during co-infections and thus higher GMI of both (Cox, 2001). The present study could, however, not identify such a mechanism.

Urinary ECP, *S. haematobium* egg counts, haematuria and ultrasound findings have been used in previous studies to assess level of morbidity due to *S. haematobium* infections (Vennervald *et al.*, 2000; Reimert *et al.*, 2000; Leutscher *et al.*, 2000). In the present

study, there was a general positive relationship between *S. haematobium* infection and these markers of morbidity. Children with heavy *S. haematobium* infections, as determined by egg counts, had significantly higher levels of urinary ECP than children with light *S. haematobium* infections, indicating that they had active *S. haematobium*-related urinary tract inflammation (Vennervald *et al.*, 2000). This was supported by the finding that children with haematuria, an indication of bleeding lesions in the urinary tract, also had significantly higher levels of urinary ECP. On the other hand, levels of urinary ECP were not significantly influenced by concurrent infections with STH, indicating that urinary ECP was a reliable marker of *S. haematobium*-related urinary tract inflammation even in those co-infected with STH. Children with ultrasound-detectable urinary tract morbidity had significantly higher levels of urinary ECP as well as *S. haematobium* egg counts than children without ultrasound-detectable urinary tract morbidity. Since ultrasound mainly detects old lesions in the urinary tract (Vennervald *et al.*, 2000), the presence of ultrasound-detectable pathology indicated that most of the children had chronic *S. haematobium* infections with a long period of pathological development.

ECP and EPX excreted in stool are useful markers of intestinal inflammation due to *S. mansoni* infections (Reimert *et al.*, 2008; Wagner *et al.*, 2008). The relationship between faecal ECP or EPX and STH infections, however, is not well documented. In the present study, there were high levels of faecal ECP and EPX but no clear-cut relationship between these markers and STH infections, except for hookworm infection which was significantly associated with faecal EPX. It is known that STH secrete immunomodulatory molecules that may reduce intestinal inflammation, but hookworm and *T. trichiura* may also cause considerable intestinal inflammation especially in heavy infections (Johnston *et al.*, 2009; Maizels *et al.*, 2004; Loukas *et al.*, 2005; Hsieh *et al.*, 2004; Khuroo *et al.*, 2010; Tuan Sharif *et al.*, 2010). Perhaps *T. trichiura* and *A. lumbricoides* were present in so low intensities that they did not cause significant intestinal inflammation whereas hookworm intensities were high enough to cause significant inflammation. This is an indication that faecal ECP and EPX could be poor markers of STH-related intestinal inflammation, especially in low intensity infections.

Previous studies in patients with urinary tract *E. coli* infection or chronic hepatitis C-related cryoglobulinaemic glomerulonephritis showed a lack of correlation between serum and urinary IL-6 (Otto *et al.*, 1999; Korotchaeva *et al.*, 2011). The findings of the present study, that there was no correlation between serum and urinary levels of IL-6, IFN- $\gamma$ , TNF- $\alpha$  or IL-10 in the children, corroborate these previous reports. This suggests that serum and



urinary cytokines have different origins, with urinary cytokines being secreted locally from the urinary tract mucosa.

*Ex vivo* studies have reported both positive and negative relationships between cytokine production by peripheral blood cells and schistosome-related morbidity (Abath *et al.*, 2006; Friedman *et al.*, 2005). In the present study there was no relationship between serum levels of IFN- $\gamma$ , TNF- $\alpha$  or IL-10 and markers of *S. haematobium*-related morbidity. Levels of serum IL-6 were positively related to ultrasound-detectable morbidity but not to any of the other markers of morbidity. The difference between the findings of the present study and those of previous *ex vivo* studies could be due to the different conditions under which the two studies were conducted (Moore *et al.*, 2001). For instance, the previous studies mainly assessed *ex vivo* cytokine production whereas the present study assessed *in vivo* cytokine production by blood cells and there could be important differences between *ex vivo* and *in vivo* conditions.

The present study for the first time reports on the relationship between urinary cytokines and *S. haematobium* infections. There were variations in the relationships between levels of urinary cytokines and markers of *S. haematobium*-related urinary tract morbidity, egg counts, urinary ECP, microhaematuria and ultrasound-detectable morbidity. Urinary levels of TNF- $\alpha$  and IFN- $\gamma$  were not well correlated to these markers of morbidity. This suggests that apart from the urinary tract, urinary TNF- $\alpha$  and IFN- $\gamma$  may have been induced by other unidentified factors and thus do not necessarily reflect *S. haematobium*-related urinary tract morbidity. There were significant positive relationships between levels of urinary IL-6 and all the studied markers of *S. haematobium*-related urinary tract morbidity. On the other hand, there were significant negative relationships between levels of urinary IL-10 and these markers. In addition, levels of urinary IL-6 and IL-10 seemed not to be amenable to co-infections with STH. These results suggest that urinary IL-6 and IL-10 have a local origin in the urinary tract mucosa and thus accurately reflect *S. haematobium*-related urinary tract morbidity.

Many countries started implementing large mass chemotherapy programmes using praziquantel and albendazole/ mebendazole to control schistosomiasis- and STH-related morbidity when important information regarding the combination of these drugs was still lacking (WHO, 2002). The findings of the present study suggest that combined praziquantel-albendazole treatment against *S. haematobium* infections is highly effective. It is probable that albendazole has some effects against *S. haematobium* and the two drugs act in synergy against this worm. This is supported by the findings that, when

administered alone, mebendazole, which is related to albendazole, has some effects against *S. haematobium* (Al-Waili, 1987; Al-Waili, 1988; Danso-Appiah *et al.*, 2009).

Combined praziquantel-albendazole treatment led to significant hookworm and *A. lumbricoides* cure rates and egg reduction rates but was less effective against *T. trichiura*. This is in agreement with the reports of other studies and indicates that *T. trichiura* is less amenable to chemotherapy as opposed to other STHs (Olsen, 2007; Appleton *et al.*, 2009; Adams *et al.*, 2004; Watkins *et al.*, 1996; Vercruyse *et al.*, 2011). The treatment resulted in significantly higher reduction of *T. trichiura* in children with heavy *S. haematobium* infections than in those with light *S. haematobium* infections. This suggests that praziquantel-albendazole combined treatment of heavy *S. haematobium* infections in co-infected individuals may lead to enhanced effects of albendazole against *T. trichiura* probably due to schistosome-induced cytokine changes and subsequent expulsion of *T. trichiura* from the gut (Geiger, 2008). This argument may draw support from reports indicating that following praziquantel treatment against schistosomes, there are infection intensity-dependent changes in cytokine responses (Reimert *et al.*, 2006; Butterworth & Thorne, 1993).

The combination of socio-economic, environmental and behavioural factors that enable transmission of schistosomes and STH may vary from one endemic focus to another (Bruun & Aagaard-Hansen, 2008; Kloos *et al.*, 2008). The present study assessed how selected risk behaviour, structure of housing, source of water for domestic use, social class (as determined by possession of selected valuable items), presence and use of latrines in the households, and parents' economic activity and education level were related to *S. haematobium* and STH infections among the examined primary school children. In addition, the study also assessed the household-related spatial distribution of the infections among the children.

The relationship between most of the studied factors and *S. haematobium* or STH infections among the children was not clearly elucidated. Use of latrines by the children, however, was significantly negatively related to *S. haematobium* and STH infections and social class was significantly negatively related to hookworm infection. Other studies have shown that lack of latrines in households is an important risk factor for the infections among children as it results in environmental contamination with helminth eggs, while social class is significantly negatively related to hookworm infection (Stephenson *et al.*, 2000a; Olsen *et al.*, 2001; Idowu & Rowland, 2006; Dumba *et al.*, 2008; Curtis *et al.*, 2000; Pullan *et al.*, 2011). There was no significant relationship between sources of water

for domestic use and *S. haematobium* infections among the children although the results indicated that swamps acted as one of the important sources of the infections, probably during the dry season when children participate in water-related domestic chores such as washing in the swamps (Ndassa *et al.*, 2007; Kloos *et al.*, 1997; Handzel *et al.*, 2003).

One general explanation for the observed lack of clear relationships between *S. haematobium* or STH infections and socio-economic status or predisposing behaviours is that environmental contamination with the helminth eggs and, as a result, exposure to the infections was high in the two villages. This is supported by the general lack of latrines and means that almost all the children had high chances of being infected regardless of their differences in socio-economic status or predisposing behaviours. A similar explanation can be given for the lack of clear spatial clustering of the infections as generalised lack of latrines would lead to almost all parts of the villages being contaminated with *S. haematobium* and STH eggs. This would in turn result in children from all parts of the villages being exposed to the infections. In addition, although small local water bodies such as swamps could be important sources of infections with *S. haematobium*, the villages experience widespread flood during the wet seasons meaning that children from all parts of the villages are exposed to *S. haematobium* infections.

### 8.1 Summary of the Main Findings

1. The two study villages were hyperendemic for both *S. haematobium* and STH causing important morbidity in primary school-age children,
2. Anaemia and low BMI were highly prevalent among the children. *S. haematobium* and hookworm were the main parasitic causes of anaemia and *T. trichiura* and *A. lumbricoides* were the main parasitic causes of low BMI,
3. Intensities of *S. haematobium* infections were negatively related to *T. trichiura* infection intensities and positively related to hookworm infection intensities among the children,
4. Urinary ECP levels reflected *S. haematobium*-related urinary tract pathology, and were not influenced by co-infection with STH. They were therefore good markers of early urinary tract morbidity due to *S. haematobium* infection in the children,
5. Faecal ECP and EPX were poor markers of intestinal pathology due to STH, except hookworm, infections in the children,
6. There was no correlation between serum levels and urinary levels of IL-6, IFN- $\gamma$ , TNF- $\alpha$  or IL-10 in the children,
7. Urinary levels of IL-6 were positively related whereas urinary levels of IL-10 were negatively related to *S. haematobium*-related urinary tract pathology and the two

were not influenced by co-infection with STH thus making them good markers of urinary tract morbidity due to *S. haematobium* infection in the children,

8. Combined praziquantel-albendazole treatment was highly effective against *S. haematobium* in the children
9. Combined praziquantel-albendazole treatment of children co-infected with *T. trichiura* and *S. haematobium* lead to an *S. haematobium* intensity dependent enhancement of the drug effect against *T. trichiura*, and
10. Lack of toilets and prevailing unhygienic conditions in the study villages led to widespread environmental contamination with *S. haematobium* and STH eggs thus obviating the role of other socio-economic and environmental factors in transmission.

## 8.2 Recommendations

Based on the findings of the present study, the following recommendations can be made:

1. The Ministry of Public Health and Sanitation should facilitate and advocate building and use of latrines to control the infections in the area
2. The Ministry of Public Health and Sanitation should facilitate sustained mass administration of praziquantel and albendazole in the area to control *S. haematobium*- and STH-related morbidity
3. Urinary IL-6 and IL-10 be adopted as markers of *S. haematobium*-related urinary tract pathology

## 8.3 Further studies

Based on the findings of the present study, further studies are needed on:

1. How co-infection with *S. haematobium* and STH influence development of morbidity to these infections and the response to treatment,
2. The interaction between *S. haematobium* and STH infections particularly the mechanisms by which *S. haematobium* limits the intensity of *T. trichiura* infections,
3. The effects of praziquantel treatment on urinary IL-6 and IL-10 levels and their potential as tools for monitoring the performance of *S. haematobium*-related morbidity control programmes
4. The potential of faecal EPX as a marker of hookworm-related intestinal inflammation
5. The interaction between praziquantel and albendazole and the possible synergy between the drugs against *S. haematobium*, particularly how *S. haematobium* influences the effects of albendazole against *T. trichiura*, and

6. Socio-economic and environmental risk factors for *S. haematobium* and STH infection including identification of local hot spots for intermediate host snail infections with *S. haematobium* and areas of intense soil contamination with STH eggs in the study areas.

#### 8.4 Study Limitations

The study was faced with a number of drawbacks which should also be considered in future studies. Some of these drawbacks were such as:

1. Very high prevalences of infections and thus lack of sufficient numbers of children without *S. haematobium* infections for comparison between those infected and those not infected,
2. Limitation of resources which prevented the conduct of follow-up studies in experiments such as on cytokine and haemoglobin levels among the children after treatment, and
3. Lack of the children's birth registration documents which means that some of the given ages of the study participants were only estimates by their parents, teachers or the children themselves.
4. During mapping, some of the households had more than one child whose data were simultaneously exported to ArcGIS in which case such households had more than one sets of data.
5. Some households were also very close to one another and thus masked by others therefore making geospatial maps clouded.
6. There was a lack of information regarding the actual environmental sources of *S. haematobium* and STH infections and did not detect any clear spatial clustering of these infections in the study communities.

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## APPENDICES

### Appendix I: Sample size calculation

The main focus of the study was morbidity due to *S. haematobium* infections. According to previous prevalence studies in the study area, about 98% of the children between 5 and 12 years old are infected with *S. haematobium*. It was Assumed about 28% of them have heavy infections (excreting over 1000 eggs/10 ml urine) and about 72% of the children have light *S. haematobium* infections (excreting between 2 – 100 eggs/10 ml urine). Let  $\pi_1 = 0.72$ ;  $\pi_2 = 0.28$ ;  $N$  = minimum number of children in each group;  $\mu$  = one-sided percentage point of the normal distribution corresponding to 100% less the power (90%) in this case 1.28 and;  $v$  = percentage point of the normal distribution corresponding to the significance level of 5% (i.e. 1.96).  $N$  was calculated using the formula given by Kirkwood and Sterne (2003) as:

$$N = \frac{\left[ u\sqrt{\pi_1(1-\pi_1) + \pi_2(1-\pi_2)} + v\sqrt{(\pi_1 + \pi_2)\left(1 - \frac{(\pi_1 + \pi_2)}{2}\right)} \right]^2}{(\pi_1 - \pi_2)^2}$$

This resulted in  $N = 100$  children meaning that a minimum of 200 children were required for the study.

## Appendix II: Questionnaire for child

ID. No.: \_\_\_\_\_ Class: \_\_\_\_\_ Name: \_\_\_\_\_

School: \_\_\_\_\_

Sex: \_\_\_\_\_ Village: \_\_\_\_\_ GPS: \_\_\_\_\_

Age: \_\_\_\_\_ Weight: \_\_\_\_\_ Height: \_\_\_\_\_

1. Have you felt sick within the last one month? (Tick **ONLY ONE** box)

No  Yes

If Yes how many times? \_\_\_\_\_

2. What were the symptoms?

Fever  Pain when urinating  Stomach-ache  Diarrhoea

Other: \_\_\_\_\_

3. Have you ever seen blood in your urine?

No  Yes

4. Did you go to any hospital/ dispensary? (Tick **ONLY ONE** box)

No  Yes

5. If yes to Q4 above which hospital/ dispensary? \_\_\_\_\_ Km \_\_\_\_\_

6. Which of the following specimens did you give at the hospital/ dispensary?

None  Capillary blood  Venous blood  Urine  Stool

7. Were you given medication at the hospital/dispensary? Show praziquantel tablet

No  Yes

8. Do you go swimming and if yes, where?

No  Yes  River  Swamp

9. Do you wade in water? if yes enquire and Tick appropriate box(es)

Flood water  Swamp  Stream

10. Do you use a pit latrine at home?

No  Yes

11. Do you collect and eat fruits (mangoes) in the farm/ bushes?

No  Yes

Do you wash them before eating? \_\_\_\_\_



### Appendix III: Questionnaire for parent

Date \_\_\_\_\_ Names of Subject/ Children \_\_\_\_\_

Name of Parent/Guardian \_\_\_\_\_

Main House No. \_\_\_\_\_

GPS Co-ordinates \_\_\_\_\_

1. Type of house:

Type of walls: Mud  Wooden  Iron sheets  Stones  Straws

Roofing: Iron sheet  Thatch

Is a pit latrine in use? No  Yes

Do the children use it? No  Yes

2. What is the education level of the **mother**?

None  Primary dropout  Secondary dropout  College dropout

Finished Primary  Finished Secondary  Finished College

3. What is the education level of the **father**?

None  Primary dropout  Secondary dropout  College dropout

Finished Primary  Finished Secondary  Finished College

4. What is the main economic activity of the **father**?

Farming  Livestock keeping  Casual employment

Formal employment  Fishing

5. How many animals do you have?

Cattle \_\_\_\_\_ Goats \_\_\_\_\_ Sheep \_\_\_\_\_ Donkeys \_\_\_\_\_

6. Does your family own the following at home?

Bicycle  Television  Radio  Cell phone  Canoe

7. Which of the following does your family eat at least once a week?

Meat  Milk  Vegetable  Maize  Beans

8. What is the main economic activity of the **mother**?

Farming  Business  Casual employment  Self employed

9. Do your children normally eat the following:

Break fast: No  Yes

Lunch: No  Yes

Supper: No  Yes

10. Do you deworm your children? No  Yes

If yes, how often per year? \_\_\_\_\_

11. Has your child/ subject fallen sick in the last three months? No  Yes

12. If yes to 11 above, what were the symptoms? \_\_\_\_\_

13. Did he or she get treatment from any clinic/ hospital? No  Yes

14. Was the child given artemisinin-combined therapy (ACT)? Show a pack of Coartem®.  
No  Yes

15. Where do you fetch water for bathing and domestic use?

River  Well  Swamp

Other \_\_\_\_\_

***Thank you for your responses.***