Prevalence of Malaria, Schistosmiasis and Soil-Transmitted Helminthiasis in School Children of Nyamatongo in Sengerema District, Northwest Tanzania

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DECLARATION

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

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DEDICATION

This work is dedicated to my mother, Stella Wilbert Turuka whose love for me cannot be imitated by any known loquacious creatures ever existed in this universe.

Romans 11: 33-36

Oh, the depth of the riches both of the wisdom and knowledge of GOD! How unsearchable are His ways past finding out!

"For who has known the mind of the LORD? Or who has become His counselor? "Or who has first given to Him and it shall be repaid to him?" For of Himthrough Him and to Him are all things, to whom be glory forever. Amen.

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LIST OF ABBREVIATIONS

ALU	Artemisinin Lumefantrine
BMC	Bugando Medical Center
BS	Blood smear
EPG	Eggs per gram of feaces
G/dL	Gram per deciliter
Hb	Haemoglobin
IPT	Intermittent Presumptive Treatment
IRS	Insecticides Residual Sprays
ITNs	Insecticides Treated Nets
ITROMID	Institute of Tropical Medicine and Infectious Diseases
MAL	Mid Axillary Line
MCL	Mid Clavicular Line
MDA	Mass Drug Administration
MEB	Mebendazole
MOH	Ministry of Health-Tanzania
MSL	Mid Sternal Line
NIMR	National Institute for Medical Research
SP	Sulphadoxine Pyremethrine
SPSS	Statistical Packages for Social Sciences
STH	Soil-Transmitted Helminth
WBUCHS	Weill-Bugando University College of Health Sciences
WHO	World Health Organization

ABSTRACT

Infections with malaria parasites, schistosomes and soil-transmitted helminths are widespread in sub-Saharan Africa and pose an enormous challenge on the socio-economic development of the region. Polyparasitism involving malaria and helminth infections is common and often associated with severe morbidities and mortalities. Co-infections of malaria, schistosomiasis and soil-transmitted helminth exist in parts of Tanzania, however, very little is known about their prevalence. A cross-sectional survey was conducted in the Nyamatongo ward of northwest Tanzania near Lake Victoria and a total of 400 primary school children from four schools were examined for *Plasmodium falciparum,* the malaria parasite, *Schistosoma mansoni,* the bilharzia parasite and soil-transmitted helminths. Stool specimens obtained from the children were processed on microscope glass slides using the Kato-Katz method and were examined for helminth eggs under a compound microscope. Giemsa-stained thick and thin blood smears were also prepared from finger prick blood samples and examined under a microscope for malaria parasites. Hemoglobin levels were determined from the finger prick blood samples using the Hemocue system (HemoCue AB, Ängelholm, Sweden) and organomegaly (liver and spleen enlargement) was determined by clinical examination of the study subjects.

Prevalence of *P. falciparum* was 13.5% (95%CI, 10.2-16.8) while *S. mansoni* was 64.3% (95%CI, 59.6-68.9). The soil-transmitted helminths present in the study children were hookworms with a prevalence of 38% (95%CI, 33.2-42.8). Apparently, *A. lumbricoides* and *T. trichiura* were absent. About 26.5% (95%CI, 21.9-30.6) of the children examined harbored two (2) parasite species with *S. mansoni* and hookworm co-infections being the

most common at 69% (95%CI, 60.2-77.8) followed by *S. mansoni -P. falciparum* co-infections at 22.6% (95%CI, 14.7-30.5), and hookworm - *P. falciparum* co-infections at 5.7% (95%CI, 1.3-10.1). The prevalence of co-infection of *P. falciparum, S. mansoni* and hookworm was 2.8% (95%CI, 1.15-4.4). The overall prevalence of co-infections with these parasites was 29.3%.

Prevalence of anaemia (<11g/dL) was 20% (95%CI, 15.9-23.7) and about 61% (95%CI, 50.2-71.8) of the anaemic children were infected with one or more of the parasites in the present study. Prevalence of hepatomegaly, splenomegaly or hepatosplenomegaly were 22% (95%CI, 17.9-26.1), 11.5% (8.4-14.6) and 7.5% (95%CI, 4.9-10.1) respectively. About 41% (95%CI, 31.6-50.4) of the children with organomegaly were infected with one or more parasite species in the study.

In conclusion, the findings of this study suggest that *S. mansoni*, hookworm and *P. falciparum* are prevalent among the school children in the study area and co-infections are common. The findings support the need for initiatives to implement a new framework for an integrated approach in disease management. Longitudinal studies which will clearly identify the associations between parasites and associated morbidities are required.

CHAPTER ONE

BACKGROUND

1.1. Parasitic infections in Africa and their public health significance

Plasmodium falciparum malaria and intestinal helminth infections are among the most common of all infections in the tropics (de Silva *et al*., 2003; Snow *et al*., 2005), and they share the same spatial distribution. The high prevalence of both infections among individuals living in Africa means that co-infections of *P. falciparum* and helminth infections are extremely common (Petney and Andrews, 1998).

The public health impact of *P. falciparum* and helminth infections include undernutrition, anaemia, poor cognitive and motor performance, stunting and impaired growth in children living in endemic areas (Walker *et al.*, 2006). Schistosome infections are also associated with malnutrition and stunting in children, particularly in nutritionally vulnerable populations (King *et al.*, 2005). Childhood infection with *S. mansoni* is commonly associated with hepatomegaly and splenomegaly as a result of eosinophilic inflammatory and granulomatous reactions against parasite eggs trapped in host tissues (Gryseels *et al.*, 2006). Chronic *P. falciparum* malarial infection is also an important cause of hepatomegaly and splenomegaly in the tropics (Grobusch and Kremsner, 2005).

Despite many efforts to control and eliminate parasitic infections in Africa, these infections continue to be prevalent in many parts of the continent. The main challenges facing control programs are: - (i) Poor population coverage and population compliance to intervention (ii) Inadequacies of resources (both funding and human) (iii) Lack of

political commitment to support the control programs (iv) Poor organization of mass drug administration (MDA) campaign (v) Political unrest or civil war in endemic countries (vi) Extreme poverty of endemic community (vii) Lack of access to safe, potable water and absence of proper excreta disposal systems (viii) Population increase, immigration and population movement (ix) Drugs and other control tools e.g. insecticides, ITNs are expensive and endemic communities cannot afford (Kyelem *et al*., 2008).

1.2. Malaria

Malaria is a parasitic disease of man transmitted from an infected to a non-infected individual by a female anophelene mosquito. The disease occurs in tropical regions of the world, mainly in sub- Saharan Africa, Asia and Latin America (WHO, 2000a). In these regions of the world, malaria continues to present an immense public health challenge with the World Health Organization (WHO) estimating that about 40% of the world's population is at risk of the disease (WHO, 2000a). The disease accounts for about half a billion clinical attacks each year and kills more than one million people per year, mainly children under five years, 90% of whom are in sub-Saharan Africa (WHO, 2000a; Lopes *et al*., 2006).

1.3. Schistosomiasis

Human schistosomiasis is a complex parasitic infection caused by digenetic trematodes commonly referred to as schistosomes or blood flukes in the genus *Schistosoma* (Rollinson and Southgate, 1987). Five species are recognized as human parasites, namely *S. mansoni*, *S. japonicum*, *S. mekongi*, *S. intercalatum* and *S. haematobium*

(Rollinson and Southgate, 1987) while several other schistosome species infect mostly animals. The disease ranks second only to malaria in terms of social-economic and public health importance in the tropical and subtropical regions of the world primarily Africa and neighboring regions, south east Asia, China, the Caribbean regions and parts of South America (WHO, 2004). It afflicts populations in 76 countries worldwide (Chitsulo *et al*., 2000), causing cumulative damage to the bodies of those afflicted by attacking their urinary bladder or gastrointestinal system (WHO, 1993). About 60% of the affected countries worldwide are in Africa (Chitsulo *et al*., 2000) and it is estimated that 200 million people are infected, with 85% of these being concentrated in Africa (Chitsulo *et al*., 2000; Engels *et al*., 2002).

1.4. Intestinal helminthiasis

The soil-transmitted helminths are a group of parasitic nematodes that cause human intestinal helminthiasis through ingestion of infective eggs of the parasites or penetration of intact skin by infective larval forms of the parasite (Crompton, 1999; WHO, 2005). Eggs and larval forms thrive well in the warm and moist tropical and subtropical countries (Crompton, 1999; WHO, 2005). Soil-transmitted helminths of public health importance worldwide include roundworms (*Ascaris lumbricoides*), whipworms (*Trichuris trichiura*) and hookworms (*Necator americanus* and *Ancylostoma duodenale)* (Bethony *et al*., 2006). These helminths commonly occur as co-infections and tend to run chronic courses, especially in children living in endemic developing countries (Bethony *et al*., 2006). Consequences of infections with intestinal worms include malnutrition, stunted growth, intellectual retardation, cognitive and

education deficits (Stoltzfus *et al*., 1996; Bethony *et al*., 2006). It is estimated that more than one billion people worldwide are infected by the soil-transmitted helminths (WHO, 2005; WHO, 2006). Intestinal worm infections cause significant morbidity in human populations worldwide, with 39 million disability adjusted life years (DALY) lost each year, more than 36 million from malaria, with hookworm alone causing the loss of 22 million DALYs annually (WHO, 2006).

1.5. Potential impact of polyparasitism on human health

Malaria, schistosomiasis and intestinal helminthiasis are the important parasitic infections from public health point of view in developing countries, afflicting in particular the poorest segments of populations (Buck *et al*., 1978; Petney and Andrews, 1998; Drake and Bundy, 2001). It also follows that multiple parasite infections are wide spread across diverse ecosystems in the tropics and subtropics (Brooker *et al.*, 2000a; Raso *et al*., 2004). Individuals with multiple parasite infections are often at an elevated risk of morbidity (Howard *et al*., 2002), hence appraisal of the extent of polyparasitism in a population is a key measure of disease burden, and an important guide for planning sound control strategies.

1.6. Problem statement

Falciparum malaria, schistosomiasis mansoni and soil-transmitted helminthiasis are important parasitic infections in Tanzania (Mboera *et al*., 2007). The distribution of malaria and helminth infections is largely determined by climate which dictates survival of the malaria mosquito, snail hosts of schistosomes and free-living larval forms of soil-transmitted helminths. Malaria, schistosomiasis and intestinal helminthiasis often occur

and co-exist under relatively similar climatic conditions and this partly explains why these infections are common in populations around Lake Victoria. In particular, prevalence of co-infections of the three (3) infections needs to be fully understood, given their importance in terms of morbidity and disease burden.

There is evidence that co-infections with multiple parasites may alter the immune responses in ways that are not fully understood (Nacher *et al*., 2001). For example, interactions of malaria and helminth infections increase the severity of anaemia and organomegaly observed in school children (Mwangi *et al*., 2007), thus may create a great challenge for disease control programs in the tropics.

Although falciparum malaria, schistosomiasis and intestinal helminthiasis have recently been reported to co-exist in parts of Tanzania (Mboera *et al*., 2007), the prevalence of co-infections remains unclear and requires further investigation.

1.7. Study justification

The localities around Lake Victoria in Tanzania are known to be endemic for falciparum malaria, schistosomiasis mansoni and soil-transmitted helminth infections (Ajanga *et al*., 2006), whereas co-infections of *S. mansoni*, *S. haematobium* and soil transmitted helminths in human populations have been reported around L.Victoria (Lwambo *et al*., 1999). Prevalence of co-infections of malaria and helminth infections is unknown. Furthermore local variations in the prevalence of the various parasitic infections and especially prevalence of co-infections remain unknown. The co-existence of these parasitic infections in an area or locality provides an opportunity for individuals to acquire infections with multiple parasite species. Such infections may have severe

consequences on the health of the affected populations leading to more severe clinical symptoms and pathology than for the infections with single parasite species.

Although the significance of co-infections of malaria and helminthiasis on the health of affected individual remains unclear (Nacher *et al*., 2000), its implication on disease control and disease burden reduction efforts could be significant.

The goal of the present study was to determine the prevalence of malaria, schistosomiasis mansoni and soil-transmitted helminthiasis in schoolchildren in Nyamatongo in Sengerema district, northwest Tanzania, with a focus on co-infections. Until now, very little information is available on these infections in this locality.

1.8. Research questions

1. What is the prevalence and intensity of *P. falciparum, S. mansoni* and soil-transmitted helminths in schoolchildren in Nyamatongo ward?
2. What is the prevalence of co-infection of *P. falciparum* with *S. mansoni, T. trichiura, A. lumbricoides* or hookworms among school children in Nyamatongo ward?
3. What is the prevalence of anaemia, splenomegaly, hepatomegaly and hepatosplenomegaly in relations to parasitic co-infections in school children in Nyamatongo ward?

1.9. Hypotheses

1.9.1. Null hypothesis

Co-infection of malaria and helminth infections is not common in school children living in Nyamatongo ward.

1.9.2. Alternative hypothesis

Co-infection of malaria and helminth infections is common in school children living in Nyamatongo ward.

1.10. Objectives

1.10.1. General objective

The broad objective was to determine the prevalence of malaria, schistosomiasis and soil-transmitted helminthiasis in school children of Nyamatongo in Sengerema District, Northwest Tanzania.

1.10.2. Specific objectives

1. To determine the prevalence and intensity of falciparum malaria, schistosomiasis mansoni and intestinal helminthiasis in school children of Nyamatongo, northwest Tanzania.
2. To determine the prevalence of co-infections of falciparum malaria, schistosomiasis mansoni and intestinal helminthiasis infection in school children of Nyamatongo ward.

3. To determine the prevalence of anaemia, hepatomegaly, splenomegaly and hepatosplenomegaly in children with single infections or co-infections.

CHAPTER TWO

LITERATURE REVIEW

2.1. Malaria

2.1.1. Plasmodium and malaria

The protozoan parasites of the genus *Plasmodium* are of public health concern of which four species in this genus are responsible for causing human malaria (Sinden and Gilles, 2003). About 120 or so species of *Plasmodium* parasitize mammals, reptiles and birds, and these are recognized taxonomically by the presence of two types of asexual division: schizogony, occurring in the vertebrate host and sporogony, taking place in the insect vector (Sinden and Gilles, 2003). The parasites belong to the family Plasmodiidae and the order Coccidae (Sinden and Gilles, 2003). A more detailed taxonomic classification is provided in Table 2.1. Human malaria parasites belong to two subgenera, *Laverania* and *Plasmodium* (Sinden and Gilles, 2003). The subgenus *Laverania* includes *P. falciparum*, the most pathogenic species and accounting for the majority of malaria infections (Chen *et al*., 2000). The subgenus *Plasmodium* includes *P. malariae, P. ovale* and *P. vivax* (Chen *et al.*, 2000).

2.1.2. Biology of malaria parasites

The life cycle of malaria parasites is complex and requires two hosts for completion; the human as the intermediate host (in which asexual development occurs) and a female anopheline mosquito as the vector or the definitive host (in which sexual development occurs) (Sinden *et al*., 2003).

Table 2.1.Taxonomic classification of malaria parasites

Kingdom	Chromalveolata
Superphylum	Alveolata
Phylum	Apicomplexa
Class	Aconoidasida
Order	Haemosporidia
Family	Plasmodiidae
Genus	*Plasmodium*
Species	*Plasmodium falciparum*

In the human host, *Plasmodium* species are in the haploid state and reproduce asexually in the hepatocytes (liver cells) and then in red blood cells (Aikawa *et al*., 1978; Chitnis, 2001). In the female anopheline mosquito, the parasites reproduce sexually, whereby the gametes join to produce the diploid oocysts (Garnham, 1988). The oocysts undergo several stages of development in the mosquito, resulting in the formation of sporozoites, found in the salivary glands of the mosquito (Garnham, 1988). To initiate an infection in the human, sporozoites are inoculated into the human blood stream when the mosquito bites. A diagrammatic representation of the Plasmodium life cycle of human malaria parasites is provided in Figure 2.1.

The sporozoites then invade hepatocytes (the liver cells) where they propagate into schizonts, in which the merozoites are formed (Garnham, 1988). Within 1-2 weeks, the merozoites are released from the liver and invade red blood cells (Chen *et al*., 2000). The merozoites in red blood cells then develop into the ring stages, eventually into trophozoites and then into schizonts which may either develop into male or female gametocytes or they may form merozoites (Sinden *et al*., 2003). The host erythrocyte

ruptures to release the merozoites which then invade new erythrocytes and initiate another round of schizogony. The blood-stage parasites usually undergo a synchronous schizogony. As an alternative to schizogony some of the parasites may undergo asexual cycle and terminally differentiate into either micro- or macrogametocytes. Gametocytes do not cause pathology in the human host but can be taken up by a female *Anopheles* mosquito for continuation of the sexual reproduction (Garnham, 1988; Heddin, 2001).

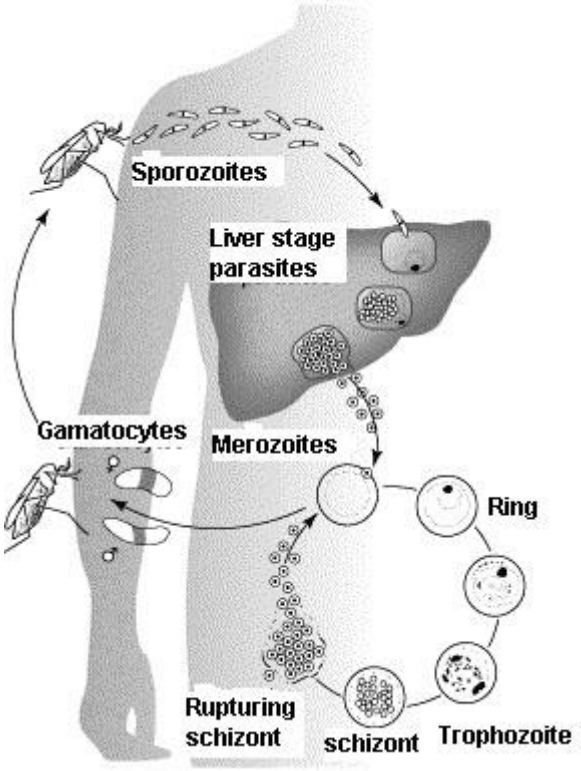


Figure 2.1. A diagrammatic representation of the life cycle of human malaria parasites (Source: Miller *et al*., 2002)

Sporozoites are inoculated by the female Anopheles invide the liver, multiply and mature into schizonts which rupture to release merozoites. Merozoites infect and multiply in the erythrocytes. Mature merozoites differentiate in male and female gametocytes which are ingested by female Anopheles. Inoculation of the sporozoites into a new human host perpetuates the malaria life cycle.

2.1.3. The world epidemiology of malaria

Malaria is found throughout the tropics, in Africa, *P. falciparum* predominates in causing infections while *P.vivax* has the widest geographical range, occurring in the temperate regions, subtropics and in the tropics (Snow and Gilles, 2003). *P. vivax* is particularly common in Central America, parts of South America, North Africa, the Middle East and on the Indian subcontinent (White, 2003).

In parts of South America, East Asia and Oceania, the prevalence of both *P. falciparum* and *P. vivax* is about 50:50 (White, 2003). However *P. vivax* is rare in sub-Saharan Africa and *P. ovale* is relatively uncommon outside West Africa (Marsh and Snow, 1999). *Plasmodium malariae* is found in most parts of Africa but is less common outside Africa (Marsh and Snow 1999). More than 200 million people live in areas where malaria is endemic with children and pregnant women being the most affected (WHO, 2002a; Snow *et al*., 2005). In Africa alone, it is estimated that 300-500 million clinical cases of malaria occur annually resulting in one to two million deaths with *P. falciparum* accounting for 95% of the malaria cases every year on the continent (WHO, 2002a; Snow *et al*., 2005). Host- related factors, human behaviour, socio-economic, environmental and climatic factors play an important role in the epidemiology of malaria (Cattani *et al*., 1986).

Human reservoirs of viable gametocytes facilitate parasite transmission (Molyneux *et al.,* 1988). In areas of stable transmission, infants and young children are more susceptible to malaria infection than the more immune older children and adults (Molyneux *et al.,* 1988). Parasite densities are higher in children than in adults and gametocytaemia is detected more frequently in children (Bousema *et al*., 2004).

Protection against severe malaria is acquired in early childhood (Gupta *et al*., 1999). Although immunoprotective mechanisms may clear the infection in peripheral blood, some parasites may persist in circulation leading to asymptomatic parasitaemia (Miller *et al.*, 1994). Also a high proportion of individuals living in malaria- endemic areas remain asymptomatic despite of harboring large numbers of malaria parasites (Smith *et al.*, 1994). In fact asymptomatic *P. falciparum* infection seems to be fairly common in the endemic areas (Bousema *et al.*, 2004; Kimbi *et al*., 2005; Dal-Bianco *et al.*, 2007).

Human behaviour and social economic factors are thought to increase human-vector contact within a given ecological environment (Carme *et al*., 1994; Koram *et al*., 1995; Luckner *et al*., 1998). Social-economic factors influencing human-vector contact include lack of bed nets, mosquito repellents, and most likely poorly constructed houses which give easy access to mosquitoes (Worrall *et al*., 2003). Low education levels have also been associated with poor malaria prevention outcomes and poor access to effective anti-malarial (Carme *et al*., 1994; Varandas *et al*., 2000).

The large natural water bodies and irrigation schemes provide excellent opportunities for mosquito vector breeding and consequently an increase in the burden of malaria in sub-Saharan Africa. Dams and irrigation schemes transform ecosystems and lead to environmental changes that increase the risk of malaria (Service, 1991). Such ecological changes result in the creation of new mosquito breeding sites (Service, 1991). Rice fields and flooded paddy fields provide ideal breeding sites for *Anopheles gambiae* complex, the principal vectors of malaria in Africa (White, 1974; Carnevale and Robert, 1987).

2.1.4. Clinical manifestation and pathology of malaria

Malaria is an acute febrile illness whose severity and course is influenced by the species, strains of the infecting parasite and its geographical origin. Also patient age, genetic constitution, immunity status, general health, and nutritional status as well as the effects of chemoprophylaxis or chemotherapy administered to the patient influence the outcome of malaria (Warrell, 2003). Malaria causes severe morbidity and death through two main mechanisms; (1) severe anaemia leading to profound hypoxia and congestive cardiac failure; and (2) cerebral malaria (Philips and Pasvol, 1992). Falciparum malaria is responsible for almost all of the two million or more attributed malaria cases each year worldwide (Warrell, 2003).

Malaria illness starts with headache, which may be severe and accompanied by dizziness, pains in the neck, back, limbs or joints, malaise, anorexia, nausea, vague abdominal pain, vomiting or mild diarrhea and a feeling of chill (Warrell, 2003). Other symptoms include, jaundice, tender enlargement of the liver and hyperparasitaemia (Clark and Schofied, 2000; Menendez *et al*., 2000; Warrell, 2003). The syndrome is caused by *P. falciparum* and is typically characterized by fever, metabolic acidosis, hypoglycaemia, seizures, anaemia, coma and cerebral oedema particularly in children (Warrell, 2003). The coma exhibited by severe cases of falciparum malaria results from mechanical blockage of blood vessels by sequestered parasitized red blood cells, causing local cerebral hypoxia (Clark and Schofied, 2000). Those who live in endemic areas and have been frequently infected acquire some immunity so that they can tolerate *P. falciparum* parasitaemia with trivial or no symptoms (Bousema *et al*., 2004).

2.1.5. Control of malaria

Major malaria intervention strategies in Africa include case management, preventive measures, environmental management, community participation, malaria intermittent treatment in pregnant mother, malaria epidemics prevention, health education and awareness (Mboera *et al*., 2007).

Preventive measures: These includes, use insecticides treated bed nets (ITNs), curtains on doors and windows, and eaves strips impregnated with permethrin, repellants, insecticides and insecticides residual sprays (IRS) (Beales and Gilles, 2003). For personal protection, application of repellants on skin, clothing or use of mosquito coils or joss sticks containing pyrethrum and wearing of long sleeved shirts and long trousers are used (Beales and Gilles, 2003).

Chemotherapy or case management is another preventive measure. This involves case identification through diagnosis and timely treatment using combination therapy, intermittent presumptive treatment in pregnancy (IPTP) using sulphadoxine-pyrimethamine (SP) (Shulman *et al*., 1999). Mosquito Control is undoubtedly the best method for protecting a community against malaria. Mosquito vector control approaches includes, (a) use of chemical insecticides targeting adult mosquitoes, larval and pupal stages (b) biological control of mosquito vectors involving the introduction into the environment of pathogens or predators of the insect vectors. The use of larvivorous fish has been the most successful and most widely used (Beales and Gilles, 2003). The most important and best known biological controls are the top-feeding minnow *Gambusia affinis* (Beales and Gilles, 2003).

Environmental management: Vegetation clearance around homesteads helps to destroy mosquito resting places and reduces number of malaria vectors (Service, 1989). Removal of clogged and blocked drainages which accumulate water and form pools and empty containers help to reduce breeding sites and resting places for mosquitoes (Service, 1989). Other measures include, filling in breeding sites and draining standing water (Beales and Giles, 2003).

Community participation: The community's effort can be put into promotion of insecticide treated bed nets usage, provision of public health education, use of recommended antimalarial drugs, environmental management, and in the training of mosquito control personnel in an area (WHO, 1992).

2.1.6. Malaria in Tanzania

In Tanzania, over 95% of the 38.7 million people are at risk of contracting malaria (MOH, 2006). Malaria contributes about 39.4% and 48% of all of outpatient cases in the under 5 years and in the 5 year olds and above respectively (MOH, 2006; Mboera *et al*., 2007). Like in most of sub-Saharan Africa, falciparum malaria is the most common in Tanzania, with an estimated 16 million episodes and 100,000 to 125,000 deaths reported annually (MOH, 2002a; Makundi *et al*., 2006). Malaria is endemic in most parts of Tanzania (MOH, 2002a) (Figure 2.2).

In terms of hospital admissions, malaria accounts for 33.4% of the cases in children under the age of 5 years and 42.1% in children above 5 years old. It has been estimated that on average there are about 0.7 and 0.6 episodes of clinical malaria and severe anaemia respectively per child per year in children under five years of age (Menendez *et*

al., 1997). Official statistics are likely to grossly underestimate the number of malaria cases and deaths, especially in rural and remote areas of Tanzania (Menendez *et al*., 1997).

The endemicity of malaria in Tanzania is not homogenous (Figure 2.2). The variations in endemicity are conveniently classified as unstable seasonal malaria, stable malaria with seasonal variations, and stable perennial malaria (Mboera *et al*., 2007)

Unstable seasonal malaria occurs with a transmission period of not more than three months a year. In such situations, malaria may occur in epidemics with increased transmission, morbidity and mortality (Mboera and Kitua, 2001; Mboera, 2004). Areas with unstable malaria transmission include highlands with altitudes of up to 2000m. Up to 25% of the Tanzanian population live in areas prone to malaria and generally, this population has very little immunity to malaria (Mboera, 2004).

Stable malaria with seasonal variations occurs where there is seasonal intense transmission for 3-6 months in a year. It occurs in high altitude plains and about 33% of the population in Tanzania live in such areas (MOH, 2002a). Stable perennial malaria occurs in coastal Tanzania and areas around the Lakes Nyasa and Victoria. About 42% of the Tanzanian population live in these areas (MOH, 2002a).

In Tanzania, malaria is mainly transmitted by *Anopheles gambiae*, *An. arabiensis* and *An. fenestus* (White, 1974; Mboera, 2000). *An. merus* is also an important vector in some localities in coastal Tanzania (Mnzava, 1991; Kigadye, 2006). Vector distribution in Tanzania follows the distribution of malaria endemicity. In the humid coastal and

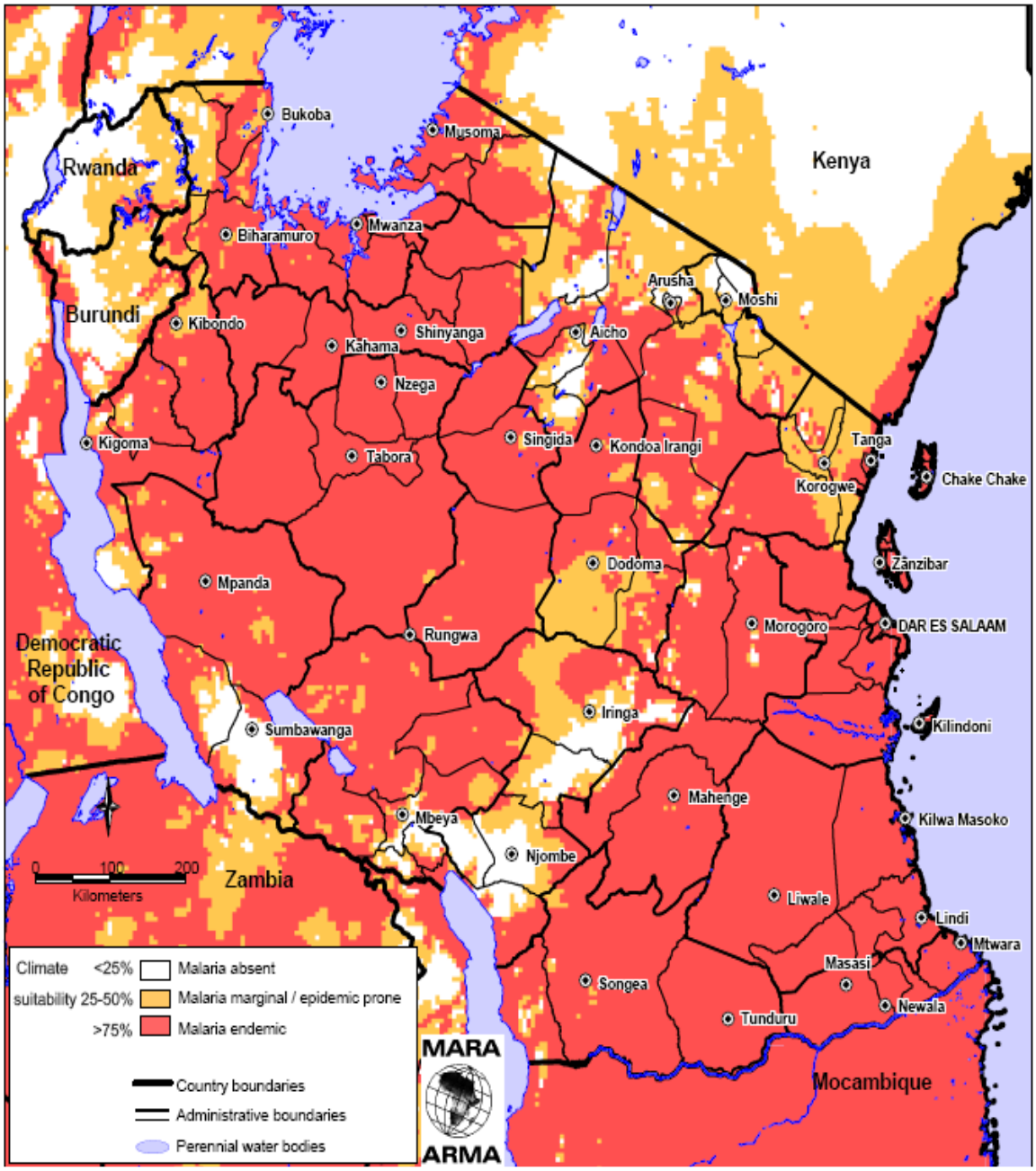


Figure 2.2. Malaria distribution in Tanzania (Source: www.mara.org.za)

humid lacustrine areas where malaria is holoendemic, the predominant species are *Anopheles gambiae* and *An. funestus*. In the dry and semi-arid areas, where malaria may occur in epidemics or may have hyperendemic status, *Anopheles arabiensis* is the main vector (Mnzava and Kilama, 1986).

2.2. Schistosomiasis

2.2.1. Schistosomes and schistosomiasis

Schistosomiasis is a parasitic disease caused by digenetic trematodes (Rollinson and Southgate, 1987) of the genus *Schistosoma* and transmitted to man and livestock by aquatic or amphibious freshwater snails (Brown, 1980). Of the 18 *Schistosoma* species currently recognized, only 5 infect man (Rollinson and Southgate, 1987), these are *Schistosoma haematobium, S. japonicum, S. mansoni, S. intercalatum and S. mekongi* (Rollinson and Southgate, 1987). Schistosomes are blood dwelling parasites and differ from other digenean trematodes in that they are dioecious (separate male and female) Two forms of the human disease exist: the intestinal disease attributed to *Schistosoma mansoni, S. japonicum, S. intercalatum* and *S. mekongi* and the urinary form which is caused by *S. haematobium* (Rollinson and Southgate, 1987).

2.2.2. Schistosome biology

In general, schistosomes have a complex life cycle which alternates between the human, (the definitive host) and a snail (intermediate host). In the definitive host adult schistosomes live in the blood stream and are usually paired (Figure 2.3). The female worm lays eggs intravascularly in the peripheral branches of the capillary venules.

Immature eggs pass through the vessel wall, aided by the spine on the parasite eggs and cytolytic secretions (Mitchell, 1990) into the lumen of the genitourinary tract (in the case of *S. haematobium)* or the intestine (in the case of *S. japonicum, S. intercalatum* and S. *mansoni)*. The parasite eggs are excreted with urine or faeces, depending on species into the environment. In the presence of fresh water the eggs hatch releasing a larval stage known as the miracidium (Jordan and Webbe, 1982). Within 8-12 hrs, the miracidium finds the snail host (Jordan and Webbe, 1982) and it penetrates the soft tissue of the snail where it transforms into a mother sporocyst which in turn produces a daughter sporocyst. The daughter sporocysts migrate to the digestive glands of the snail and develop into the larval forms known as cercariae, which are infective to the definitive host (Jordan and Webbe, 1982). After incubation period of 4-6 weeks in the snail, free swimming, fork-tailed cercariae are released. Cercariae enter into a susceptible host by penetration of intact skin, and transform into schistosomula (Jordan and Webbe, 1982). These enter the blood circulatory system and migrate to the liver and eventually to the bladder or mesenteries, where pairing of male and female schistosomes take place, followed by migration to the preferred sites of egg deposition (Sturrock, 1993).

Schistosoma haematobium inhabits the terminal venules in the wall of the urinary bladder and the pelvic plexus causing urinary schistosomiasis, whereas *S. japonicum, S. mekongi, S. intercalatum* and *S. mansoni* inhabit the pericolonic venules within the distribution of the portal venous system.

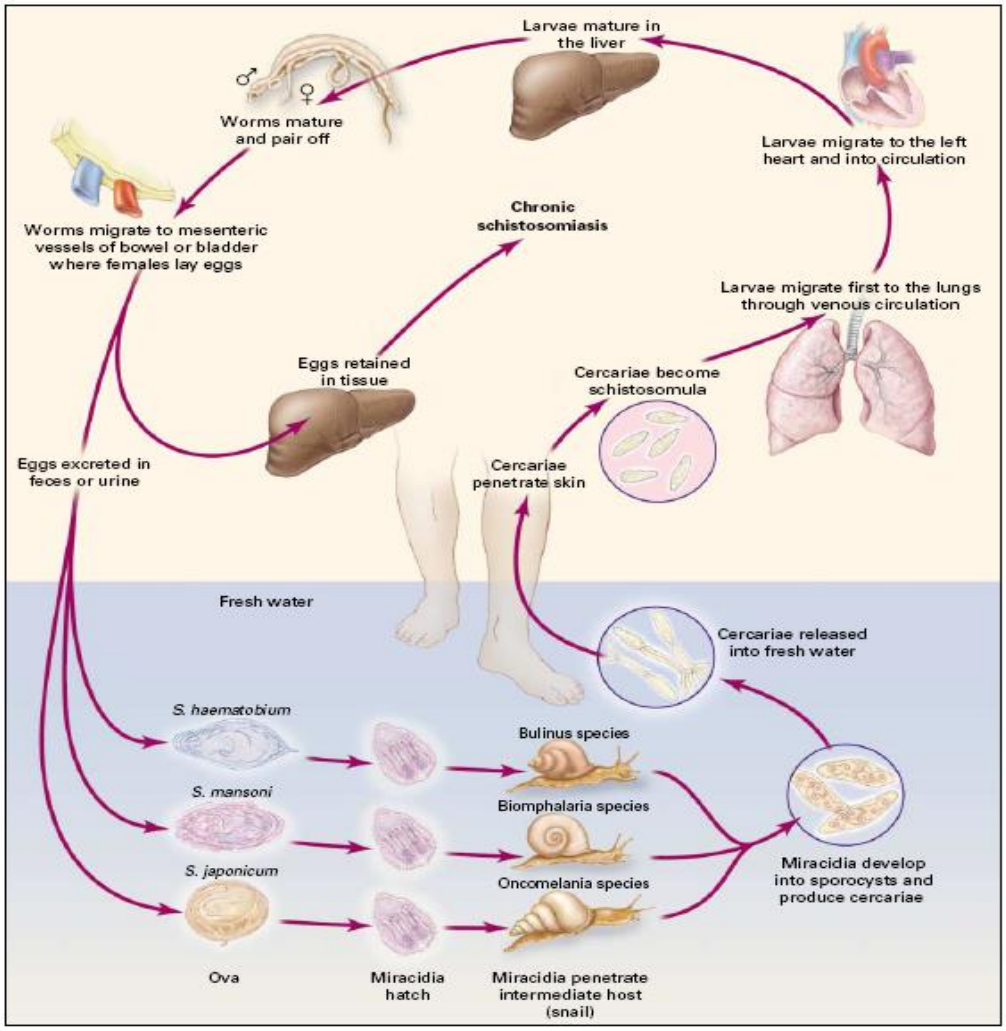


Figure 2.3. Diagramatic representation of life cycle of human schistosomes (Source: Ross *et al*., 2002).

Eggs are eliminated with feaces or urine and hatch to release miracidia, which swim and penetrate specific snail intermediate gosts. The infective cercariae are released from the snail and penetrate the human host and become schistosomulae. Adults worms in hosts reside in the mesenteric venules in various systems. The female lay eggs in the small venules of the portal and perivesical systems of the urinary bladder (*S. haematobium*) and intestines (*S. mansoni* and *S. japonicum*) are eliminated with feces or urine.

2.2.3. The world epidemiology of schistosomiasis

Schistosoma species are widely distributed in the tropical and subtropical areas of the world. *Schistosoma mansoni* occurs in Africa, the Middle East, the Caribbean region, in parts of South America whereas *S. haematobium* is endemic in Africa and the Middle East (Chitsulo *et al*., 2000) (Figure 2.4). *Schistosoma japonicum* is confined to the Far East, especially in China and the Philippines, while *S. intercalatum* occurs in Central and West Africa specifically the Democratic Republic of Congo, Congo Brazzaville, Central African Republic, Gabon, Cameroon, Chad and Burkina Faso) (Chitsulo *et al*., 2000). *Schistosoma mekongi* is endemic in the Mekong Valley of the Yunman Province in China, in Vietnam and surrounding countries (Chitsulo *et al*., 2000) (Figure 2.4). *Schistosoma mansoni*, the causal agent of human intestinal schistosomiasis, is the most widespread and probably the most important in terms of the number of people afflicted worldwide and the morbidity it inflicts (Bundy, 1997).

Transmission of schistosomiasis is influenced by a number of factors, the major ones being, the distribution, biology and population dynamics of the snail intermediate hosts (Barreto, 1991; Huang and Manderson, 1992), the patterns and extent of environmental contamination with human excreta (Huang and Manderson, 1992; Marcal *et al*., 1993), the socio-economic and hygienic status of the affected (Fulford *et al*., 1996) host-parasite relationships (in both the snail and human host) and human host protective mechanisms (Modha *et al.*,1998). Prevalence and intensity of schistosomiasis is highest in children, adolescent and young adults (Woolhouse, 1998). In endemic areas, children are particularly important as reservoirs of the infection because of their indiscriminate excretory habits, their unrivalled opportunities for water contact in hot climate and also

because they suffer most the brunt of the infection (Huang *et al*., 1992; Jordan and Sturrock, 1993; Lwambo *et al.*, 1999).

The distribution of schistosomiasis is influenced by the distribution of its snail intermediate hosts. In Africa, the freshwater pulmonate snails in the family Planorbidae serve as intermediate hosts of mammalian schistosomes (Rollinson and Southgate, 1987). The planorbid snails in the genus *Biomphalaria* serve as intermediate hosts of *S. mansoni* whereas members of the genus *Bulinus* are hosts of *S. haematobium* and *S. intercalatum*. Various *Biomphalaria* species are involved in the transmission of *S. mansoni* worldwide; *B. glabrata* in the Neotropical region, *B. alexandrin* in the Nile Delta, *B. pfeifferi* in much of sub-Saharan Africa, *B. sudanica* in the Great Lake region of equatorial Africa, *B. choanomphala* in Lake Victoria and *B. arabica* in the Arabian Peninsula (Rollinson and Southgate, 1987).

Bulinus species are important in transmitting *S. haematobium* with *B. senegalensis* in Cameroon and Senegal (Greer *et al*., 1990), *B. globosus, B. nasutus* and *B. africanus* in much of sub-Saharan Africa and adjacent islands of the Indian Ocean (Mauritius and Malagasy) and in the Middle East (Rollinson and Southgate, 1987).

Environmental changes that result from the development of water resources for electricity production, irrigations, and population growth and migration have also facilitated the spread of schistosomiasis (Patz *et al*., 2000).

The construction of Diama Dam on the Senegal River for instance, led to the introduction of *S. mansoni* into Mauritania and Senegal (Patz *et al*., 2000). Human population displacement and movement has also resulted in the introduction of *S. mansoni* into Somalia and Djibouti (Patz *et al*., 2000). Also, construction of the Aswan

Dam in Egypt led to the virtual elimination of *S. haematobium* from Nile Delta but has resulted in the establishment of *S. mansoni* in Upper Egypt (Patz *et al*., 2000).

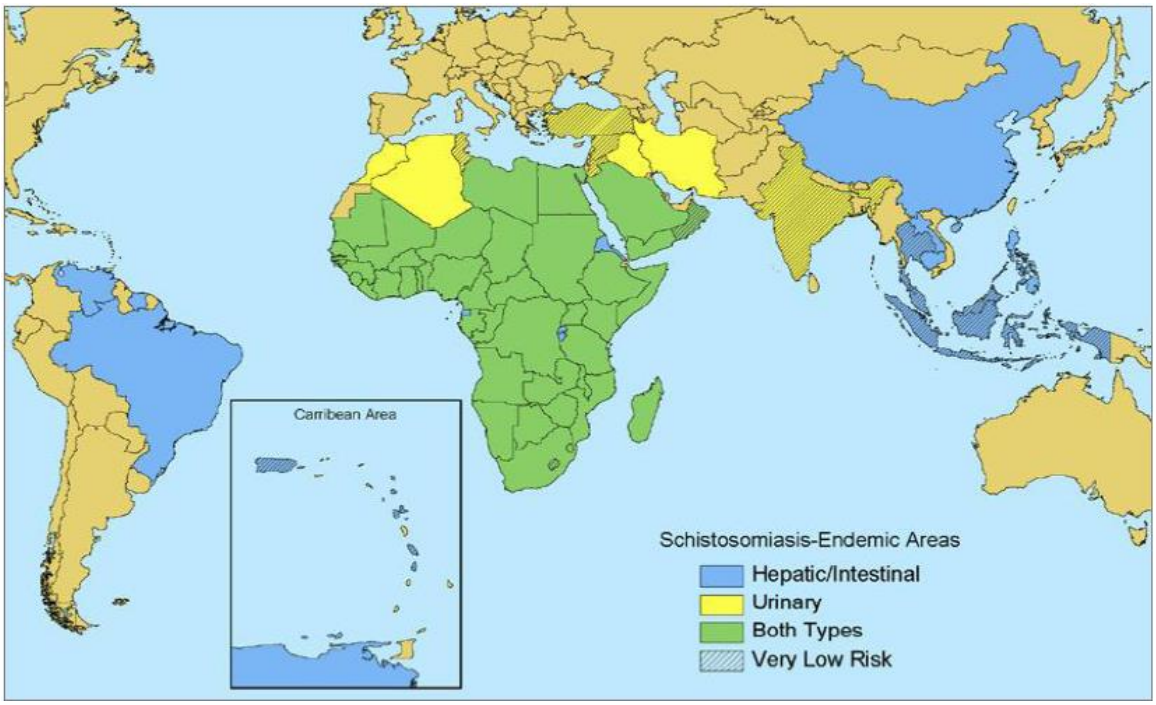


Figure 2.4. Global distribution of schistosomiasis (Source: CDC, 2007)

2.2.4. Human schistosomiasis in Tanzania

Schistosomiasis is endemic in Tanzania, and is considered a major public health problem. Both *S. haematobium* and *S. mansoni* are endemic in many parts of Tanzania primarily in (Webbe, 1962; Rugemalila, 1979; Kitinya *et al.*, 1986; Lwambo *et al.*, 1999) the areas surrounding Lake Victoria in northwest Tanzania, in the Kilimanjaro and Tanga regions in northern Tanzania; and in coastal Tanzania (Mallya, 1988). In 1988, the prevalence of schistosomiasis in Tanzania was estimated at 38% (Mallya, 1988) and in 2002; it ranged betwen 12.7% to 87.6% (MOH, 2002b). *Schistosoma*

mansoni is the most common and probably the most important from a public health view point. It occurs commonly around Lake Victoria and lowlands areas of Kilimanjaro and Tanga regions. *Schistosoma haematobium* tends to be focal in distribution and occurs in localities further away from the shore of the Lake Victoria and in localities in coastal Tanzania (Mugashe, *et al.*, 1994). *Both S. haematobium* and *S. mansoni* are common in schoolchildren in and around L. Victoria (Tanzania-Bureau of statistics, 1992; Lwambo *et al.*, 1999; Guyatt *et al.*, 1999). For instance, in Magu district alone, the prevalence of *S. mansoni* and *S. haematobium* infections in 1992 was 30% and 40% respectively (Bureau of statistics, 1992) and in the late 1990's it was about 11% and 56% respectively in schoolchildren (Lwambo *et al*., 1999).

In and around L. Victoria, both *Biomphalaria sudanica* and *B. choanomphala* are the main intermediate hosts of *S. mansoni* whereas *B. pfeifferi* is responsible for seasonal transmission in permanent ponds away from the lake (Lwambo *et al.*, 1999). *Bulinus* snail species in the africanus group are responsible for the transmission of *S. haematobium* in Tanzania where *B. nasutus, B. africanus* and *B. globosus* are the most important. The most abundant and widespread species is *Bulinus nasutus*, which is adapted to temporary freshwater habitats (Lwambo *et al*., 1999).

2.2.5. Morbidity in schistosomiasis

Human schistosomiasis affects the urinary and intestinal systems. The pathogenesis and clinical manifestations of schistosomiasis are essentially the same for all the four species of schistosomes infecting man, but differences arise in response to differences

in location and in egg-laying capacity of the worms (Mitchell, 1990). Severe disease in schistosomiasis begins with the deposition of eggs in the tissues (Mitchell, 1990).

The exposure to schistosome cercarie may provoke a rash on the skin of an exposed person known as “swimmers itch” or schistosome demartitis (Amer, 1982). The development of the schistosomula into adults may cause marked eosinophilia and a feverish syndrome known as Katayama fever (Mitchell, 1990). This fever develops within 3-9 weeks after exposure to cercariae (Boros, 1989) but is common in individuals who are exposed for the first time to a heavy infection (WHO, 1998). *Schistosoma mansoni* worms live in the terminal venules of the mesenteric system which facilitate passage of the laid eggs to the lumen of the intestines. However, about 50% of the eggs never reach the environment, but are trapped in the tissues where they are responsible for most of the pathological consequences of the disease (Mitchell, 1990), *Schistosoma haematobium* inhabits the terminal venules of the urinary bladder wall and the pelvic plexus and causes urinary schistosomiasis. The eggs of *S. haematobium* are trapped in the tissue of the urinary bladder wall causing massive inflammatory reaction that is associated with much of the pathology (Mitchell, 1990).

The eggs lodged in the host tissue contain the broad forms, miracidia and this release a complex mixture of antigens through microscopic pores within the eggshell (Mitchell, 1990). These antigens then induce cellular reactions leading to the development of granulomatous lesion and scars in the affected organs where they are trapped. In mild infections, the granulomatous reactions and subsequent fibrotic changes are limited. In *S. mansoni* infections, symptoms may include diarrhea, bloody stool, abdominal pain, nausea, fatigue and drowsiness (Mitchell, 1990). In heavier *S. mansoni* infections,

extensive fibrosis of the liver may lead to hepatomegaly, splenomegaly and ascites (Mitchell, 1990). Subsequently, decompensated portal hypertension may develop with oesophageal and gastric varices which can rupture, causing fatal massive bleeding (Gryseels, 1990). In *S. haematobium* infections, eggs deposited in the bladder tissue give rise to granulomatous lesions, formed in the bladder mucosa which develop into tumours, and are referred as pseudopapillomas (Farid *et al*., 1993). When egg granulomas form near the ureteric orifices or in the ureters themselves, the ureters may become obstructed and this is often the cause of early obstructive uropathy (Farid *et al*., 1993). Other urinary tract symptoms may include recurrent painless haematuria, anaemia, stunted growth, burning on micturition and suprapubic discomfort or pain and egg excretion in the urine with proteinuria (Farid *et al*., 1993).

2.2.6. Schistosomiasis control

The public health significance of human schistosomiasis in endemic countries, calls for feasible and sustainable control measures. For many years, control of schistosomiasis aimed at breaking the cycle of transmission by controlling the intermediate host snails and by reducing human exposure to cecariae-infested water and prevention of water contamination with schistosome eggs (Gryseels, 1990). Snail control was achieved through modification of ecological conditions of the snail habitats to make then unsuitable for survival and breeding of the snail hosts.

Later, focus shifted from transmission control to eliminate transmission to containment of morbidity through regular treatment of infected individuals with the antischistosomal drug, praziquantel (WHO, 1998). This approach has proved efficient in reducing the

worm load as assessed by egg excretion (Frenzel *et al*., 1999). It dramatically reduces disease burden, thus preventing the chronic sequelae (WHO, 1993).

Nevertheless, an integrated approach to the control of schistosomiasis remains valid and practical as chemotherapy does not prevent re-infections (WHO, 1993). Provision of safe water, use of sanitation facilities, and health education are prerequisites for long-term and sustainable control of schistosomiasis (WHO, 1993). However, with respect to snail control experience has shown that the cost of implementing chemical snail control far outweighs the benefits it offers and overall impact on disease burden and therefore, this option should only be used after a thorough analysis of the desired benefits (WHO, 1998).

2.3. Human soil-transmitted helminthiasis

2.3.1. Background

The term soil-transmitted helminth (STH) applies to a group of helminth parasites whose life cycles depend on a period of development outside the human host, typically in the soil (Bethony *et al*., 2006). They can be further subclassified according to their mode of transmission as direct, modified direct or penetration of the skin (Bethony *et al*., 2006). Direct transmission occurs when eggs are transmitted from anus to mouth, and ingested without ever reaching the soil, for example in the case of whipworm (*Trichuris trichiura*) infection. Modified direct transmission occurs when eggs are passed in the faeces and become infectious after a period of development in the soil. e.g. the roundworms (*Ascaris lumbricoides*) (Awasti *et al*., 2003).

Penetration through intact skin on the other hand is the mode of transmission for hookworms, *Ancylostoma duodenale* and *Necator americanus*. Under favourable environmental conditions, hookworm larvae hatch out from eggs deposited in the soil. The larvae lie in wait in the surface layers of the soil until they come into contact with the skin of their unsuspecting host (WHO, 2000b; Awasti *et al*., 2003). Over 1 billion people are infected with STH and the greatest burden of disease occurs among children, particularly in areas of poor hygiene and sanitation, and has a significant effect on physical and intellectual development (WHO, 2000b; Bethony *et al*., 2006).

2.3.2. Hookworm

Human hookworm infections are caused by the helminth nematode parasites, *Necator americanus* and *Ancylostoma duodenale*, and are transmitted through contact with contaminated soil. It is one of the most common and chronic infections, with an estimated 740 million cases in the poor of rural areas in the tropics and subtropics (de Silva *et al*., 2003). The greatest numbers of hookworm cases occur in Asia, followed by sub-Saharan Africa (de Siva *et al*., 2003). *Necator americanus* is the most common hookworm worldwide, whereas *A. duodenale* is more geographically restricted e.g West Africa and China (de Silva *et al*., 2003). Highest rates of hookworm transmission occur in the world's coastal regions, where infective third-stage larvae can migrate freely in sandy soil and where temperatures and moisture are optimal for viability of larval hookworms (Mabaso *et al*., 2003). Hookworms are slender tube like worms, measuring about 1 cm long. They have a mouth and oesophagus at the anterior end, connected by the gut to the anus at the rear end.

The teeth in *A. duodenale* and the cutting plates in *N. americanus* are used to pierce the intestinal mucosa whereas the mouth and pharynx are used to attach the worms to the mucosa by suction (Bethony *et al*., 2006). Hookworm eggs are passed in the faeces and hatch in warm moist conditions liberating rhabditiform larvae. These develop into filariform larvae which inhabit the surface layer of soil (Hawadon and Hotez, 1996). While in the soil, third-stage larvae are in a state of developmental arrest. Development resumes after the larvae enter the human host (Hawadon and Hotez, 1996). In humans, entry through the skin is followed within ten days by larval migration to the lungs. Here they enter the alveoli, migrate to the pharynx and then to the small intestine where they mature into adults (Maxwell *et al*., 1987). Adult hookworms attach themselves to the upper half of the small intestine and feed on blood (Maxwell *et al*., 1987). Figure 2.5. illustrates the life cycle of hookworms.

2.3.3. *Ascaris lumbricoides*

Ascaris lumbricoides, (roundworm) is a remarkably infectious and persistent parasite that infects a quarter of the world population (Crompton, 1994). It is widely recognized that ascariasis plays a major role in the aetiology of childhood malnutrition (Crompton, 1992). The worms infect 1.221 billion people with peak prevalence and intensity of infection being children aged 3 - 8 years (Bethony *et al*., 2006).

Ascaris eggs, contaminating vegetables, soil or dust are swallowed when accidentally ingested, and in the gut they hatch to release larval forms as they pass through the stomach and small intestine (Faust and Russell, 1964). The larvae penetrate the

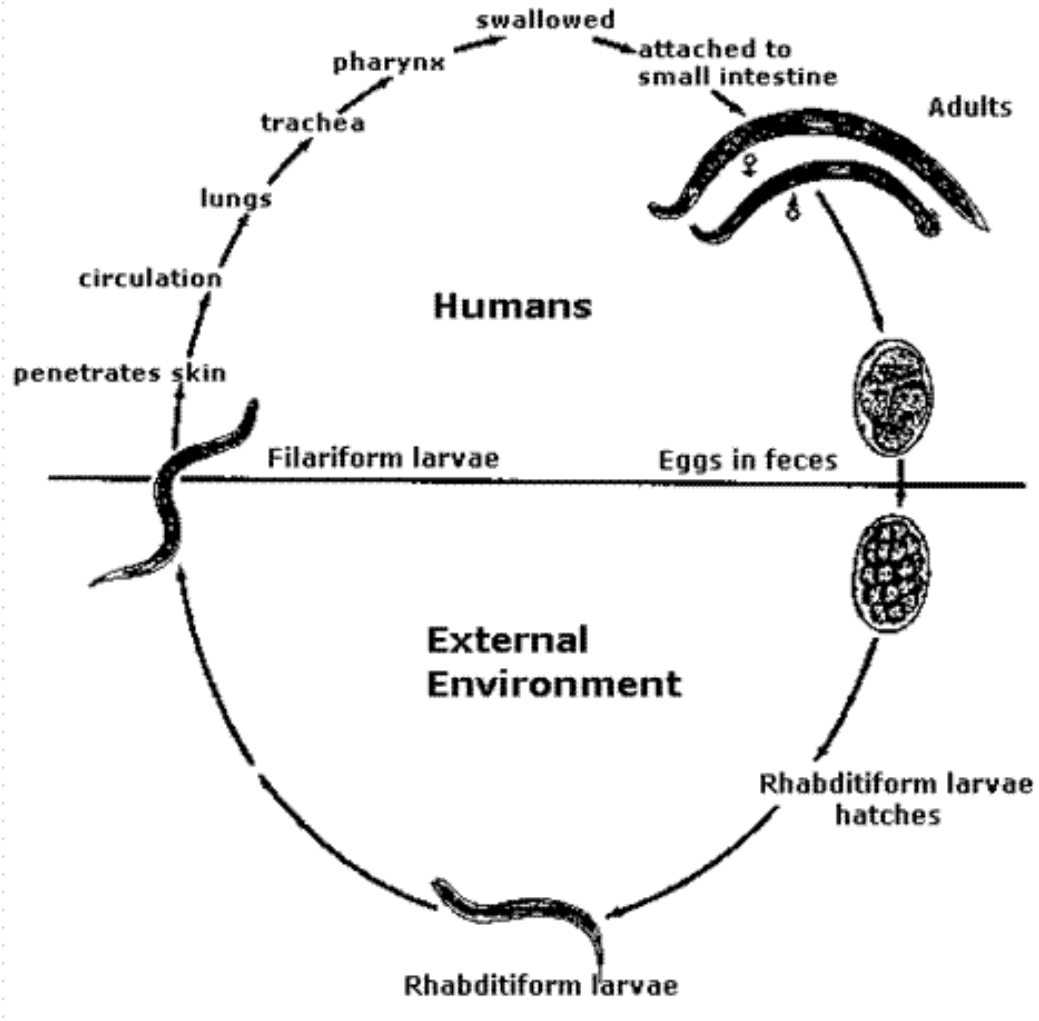


Figure 2. 5. A diagrammatic representation of the life cycle of hookworms

(Hotez *et al*., 2005).

Female in human host produce eggs which are passed out in the feaces. In soil, eggs hatch into rhabditiform larvae which molts into infective filariform larvae which penetrates the skin of human host. They are carried in blood circulation to the heart and then to the lungs and finaly swallowed. The larvae reach the small intestine and mature into adults.

intestinal mucosa, enters the bloodstream and lymphatics, and reaches the lungs 4-16 days after exposure. The larvae penetrate the alveoli, moult and migrate via the respiratory tract to the oesophagus and on to the small intestine, where they develop into adult worms; they mate and start producing eggs 6 - 8 weeks after infection (Faust and Russell, 1964).

The adult worms are large cream coloured worms with males measuring 15-30 cm long, females 20-40 cm. The parasites live in the small intestine and obtain nourishment from the intestinal contents.

Female worms produce up to 200,000 eggs per day and these are excreted in faeces into the environment and the ova mature worms in soil into infective embryos within 1- 4 weeks remain viable in soil for years (Faust and Russell, 1964). The life cycle of *A. lumbricoides* is illustrated in Figure 2.6.

2.3.4. *Trichuris trichiura*

The other soil-transmitted helminth commonly infecting humans in sub-Saharan Africa is *T. trichiura,* the whipworm, most common in the warm and moist, tropical and subtropical regions, also occurs in temperate climates. Children are most commonly infected with prevalence reaching >90%. An estimated 795 million persons globally harbor *Trichuris* infections (de Silva *et al*., 2003) and these numbers include 114 million preschool-age children and 233 million school-age children 5-14 years (Chan, 1997). At least 27 million school-age children in sub-Saharan Africa are estimated to be infected (Michael *et al*., 1997). Humans become infected directly by ingesting the

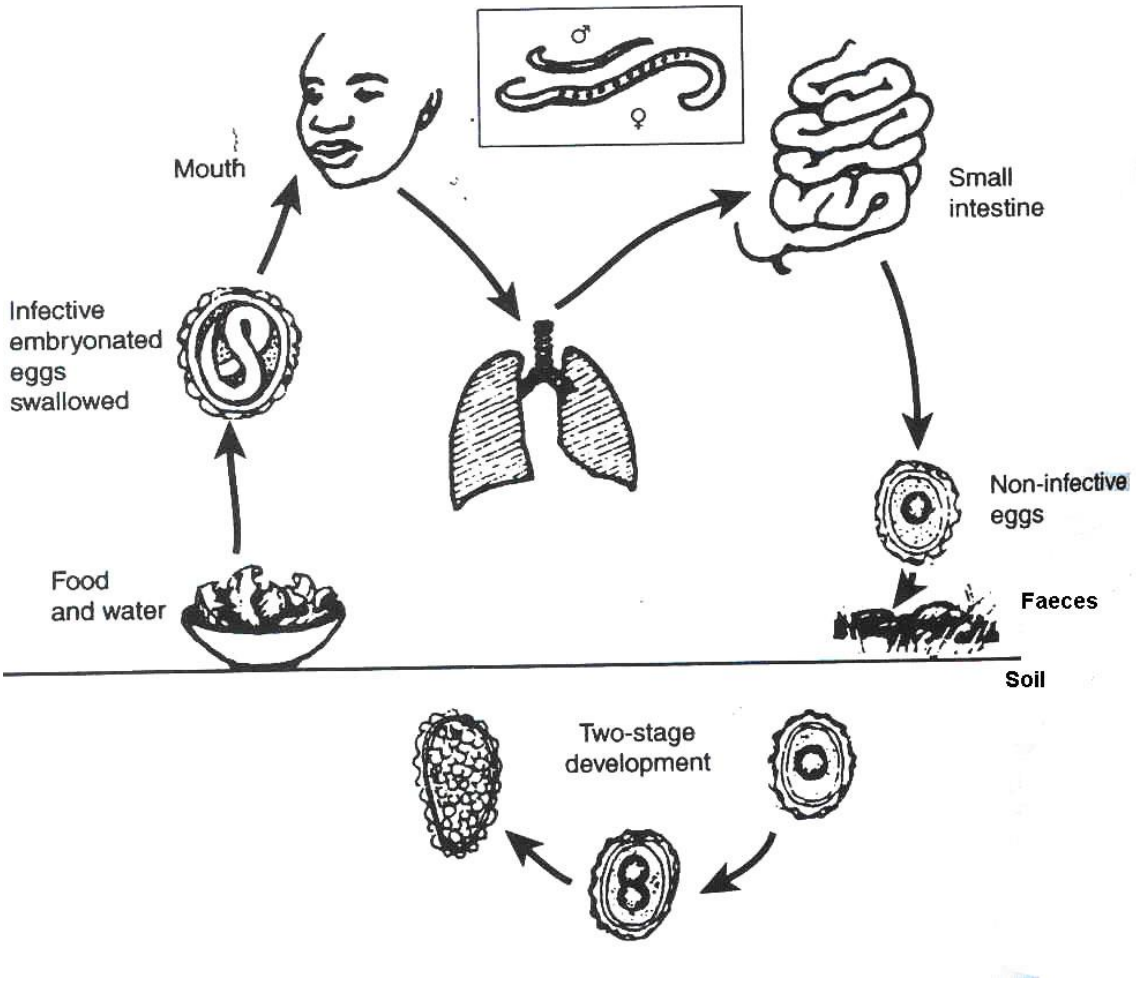


Figure 2.6. A diagram illustrating the life cycle of *Ascaris lumbricoides* (Melvin *et al*., 2001)

Adult worms live in the lumen of the small intestine. A female produces which are passed out with the feces and fertile eggs embryonate in soil. Infective eggs are swallowed, the larvae hatch, invade the intestinal mucosa, and are carried via the portal, then systemic circulation to the lungs. The larvae mature further in the lungs, penetrate the alveolar walls, ascend the bronchial tree to the throat, and are swallowed. They develop into adult worms in small intestine (Melvin *et al*., 2001).

embryonated eggs from contaminated hands, food, soil or water (Figure 2.7). *Trichuris trichiura* larvae are liberated in the caecum, penetrate the crypts of Lieberkühn and migrate within the mucosa (Faust and Russell, 1964).

Mature adult worms are 2-5cm long; the thinner anterior half of the body being normally partly buried in the mucosa of the large bowel of the host (caecum, colon, and rectum). Female worms release several thousands of eggs per day. After two week's development in warm moist soil, the eggs are embryonated and become infective (Faust *et al*., 1964). The life cycle of *T. trichiura* is presented in Figure 2.7.

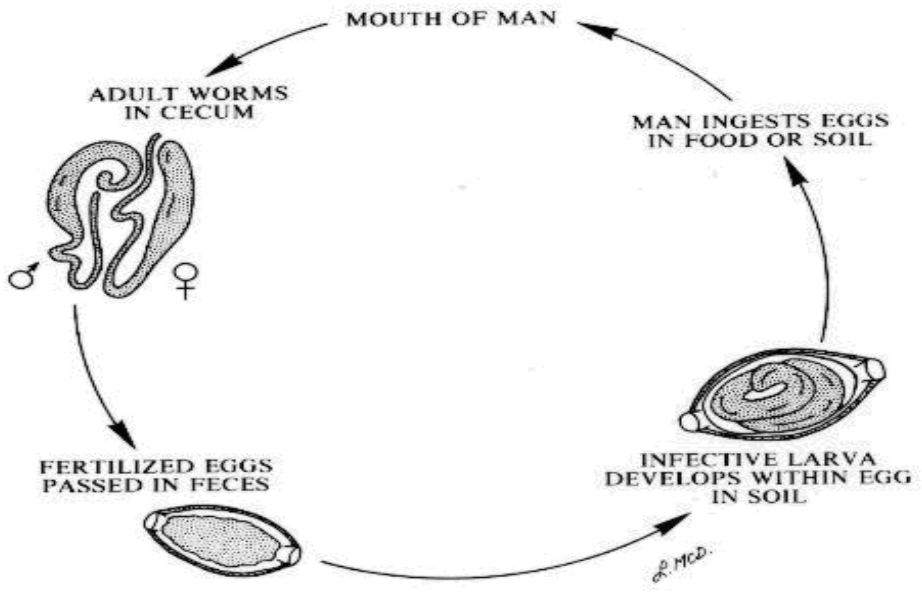


Figure 2.7. Life cycle of *Trichuris trichiura* (Melvin *et al*., 2001)

The unembryonated eggs are passed with the stool and in the soil, the eggs develop and then they embryonate. After ingestion the eggs hatch in the small intestine, and release larvae that mature and establish themselves as adults in the colon. The adult worms live in the cecum and ascending colon.

2.3.5. The world epidemiology of soil-transmitted helminthiasis

Soil-transmitted helminth infections are widely distributed throughout the tropics and subtropics. Climate is an important determinant of transmission of these infections adequate moisture and warm temperatures (22-32^{0}C) are essential for larval development in the soil (Brooker and Michael, 2006b). Infection is prevalent in areas with high population densities in tropical and subtropical countries. Equally important determinants are poverty, inadequate water supplies and sanitation (de Silva *et al*., 2003). In such conditions, soil-transmitted helminth species are commonly co-endemic.

There is evidence that individuals living in the tropics have heavier infections with soil-transmitted helminths (Raso *et al*., 2004). Intensity of infection is the main epidemiological index used to describe soil-transmitted helminth infection (Brooker *et al*., 2006).

Hookworms are the most widespread species of soil-transmitted helminth in sub-Saharan Africa (Brooker and Michael, 2000b), where iron stores are low. Although, hookworm also occurs in childhood, the frequency and intensity commonly remains high in school age children, adulthood, or elderly people because of the high ability of the hookworm larvae to survive in high temperature of the tropics (Bethony *et al*., 2002).

2.3.6. Morbidity of soil-transmitted helminths infections

Generally, only soil-transmitted helminth infections of moderate to high intensity produce clinical manifestations, with the highest-intensity of infections occurring in children (Chan *et al*., 1994). Infection in school-age children is intense and debilitating,

resulting in malnutrition, physical and intellectual growth retardation and cognitive and educational deficits (Stephenson *et al.*, 1989; Koroma *et al*., 1996). Each of the major soil-transmitted helminths produces characteristic disease syndrome. The major hookworm-related injuries in humans occur when the adult parasites cause intestinal blood loss (Hotez *et al*., 1995; Stoltzfus *et al*., 1997a; Albanico *et al*., 1998).

Blood loss occurs when the worms use their cutting apparatus to attach themselves to the intestinal mucosa and submucosa. Capillaries and arterioles are ruptured through the action of hydrolytic enzymes (Hotez *et al*., 1995) and the worm releases anticlotting agents to ensure flow of blood (Stanssens *et al*., 1996).

The major clinical manifestations of hookworm disease are the consequences of chronic intestinal blood loss. The hookworm disease occurs when the blood loss exceeds the nutritional reserves of the host, thus resulting in iron-deficiency anaemia (Stoltzfus *et al*., 1997b; Hotez *et al*., 2004). The presence of more than 40 adult worms in the small intestine is estimated to be sufficient to reduce host haemoglobin concentration to below 11g/dl (Lwambo *et al*., 1992). *Ancylostoma duodenale* causes more blood loss than *N. americanus* and the degree of iron-deficiency anaemia induced by hookworms depends on the species (Albanico *et al*., 1998). The chronic protein loss from heavy hookworm infection can result in hypoproteinaemia. Anasarca from extensive plasma hypoproteinemia is associated with eodema of the face and lower limbs and with potbelly (Hotez *et al*., 2004). Due to the fact that school-age children have reduced iron reserves, they are in particular at risk of hookworm disease.

The presence of large numbers of adult Ascaris worms in the small intestine can cause abdominal distension and pain. Ascaris can also cause lactose intolerance and

malabsorption of vitamin A and possibly other nutrients (Taren *et al*., 1987) which might partly cause the nutritional and growth failure. In young school-age children, the worms can aggregate in the ileum and cause partial obstruction (Khuroo *et al*., 1990; Villamizar *et al*., 1996). Various grave consequences can ensue including intussusceptions, volvulus and complete obstruction (Khuroo *et al*., 1990) leading to bowel infarction and intestinal perforation.

Adult whipworms are the causal agent of trichuriasis, they live preferentially in the caecum, although in heavy infections, whipworm can be seen throughout the colon and rectum. The adult parasite leads both an intracellular and an extracellular existence, with the anterior end being embedded in epithelial tunnels within the intestinal mucosa. Inflammation at the site of attachment by the large numbers of whipworms results in colitis. Longstanding colitis may cause chronic abdominal pain and diarrhea and its long term effect may be sequelae of impaired growth, anaemia and finger clubbing (Bundy and Blumenthal, 1989). Trichuris dysentery syndrome is an even more serious manifestation of heavy whipworm infection, resulting in chronic dysentery and rectal prolapse (Bundy and Blumenthal, 1989).

2.3.7. Control of soil-transmitted helminth infections

Soil-transmitted helminth infections in humans remain a worldwide public-health threat in the developing world. The United Nations agencies have now recognized the health and educational effect of these infections in children (WHO, 2002b). Thus, since early 1990s the World Health Organization has focused on the control of soil-transmitted helminth infection on global deworming of school-aged children (WHO, 2002b). World

Health Organization recommends that all school-age children at risk of infection within endemic areas should receive regular deworming drugs (WHO, 2002b). The drugs of choice for the elimination of adult worms from intestines are either a single dose of albendazole (400mg) or mebendazole (400mg) (WHO, 2002b). Regular deworming with these drugs in school-age children reduces and maintains the worm burden below the threshold associated with disease (Savioli *et al*., 2002).

The benefits of regular deworming in this age group include improvement in iron stores, growth and physical fitness (Stephenson *et al*., 1989), cognitive performance and improved school attendances (Miguel and Kremer, 2003). When deworming is undertaken along side other control measures such as sanitation and provision of public health education to increase awareness in the population, disease control is sustainable. A major drawback in the control of soil-transmitted helminthiasis is reinfection which often occurs a few months after deworming (Albanico *et al*., 1995). Poverty, lack of funds to maintain control programs and environment contamination and other factors hinder helminthiasis control program in Africa (Albanico *et al*., 1995). Therefore, socioeconomic reforms in endemic countries will facilitate reduction of disease burden due to soil-transmitted helminth infections with the support of WHO.

2.3.8. Soil-transmitted helminths in Tanzania

In Tanzania, information on the prevalence and intensity of soil-transmitted helminth infections is not consistent, although published data suggests that STH infections occur in all the regions. It is believed that infection prevalence could reach 100% in certain ecological settings (MOH, 2002c). Soil-transmitted helminth infections typically occur

sympatrically with schistosomiasis (Kihamia, 1981) and hookworm infection is primarily due to *N. americanus* (Lwambo *et al*., 1999). A cross-sectional study around Lake Victoria, reported that 37% of the school children aged 7-20 years were infected with hookworm, and 76% were infected with either *A. lumbricoides* or *T. trichiura* (Lwambo *et al*., 1999). In the same study, anaemia and stunted growth was also common in the studied subjects. Figure 2.8 show the point prevalence of hookworm infections in selected parts of northwest Tanzania.

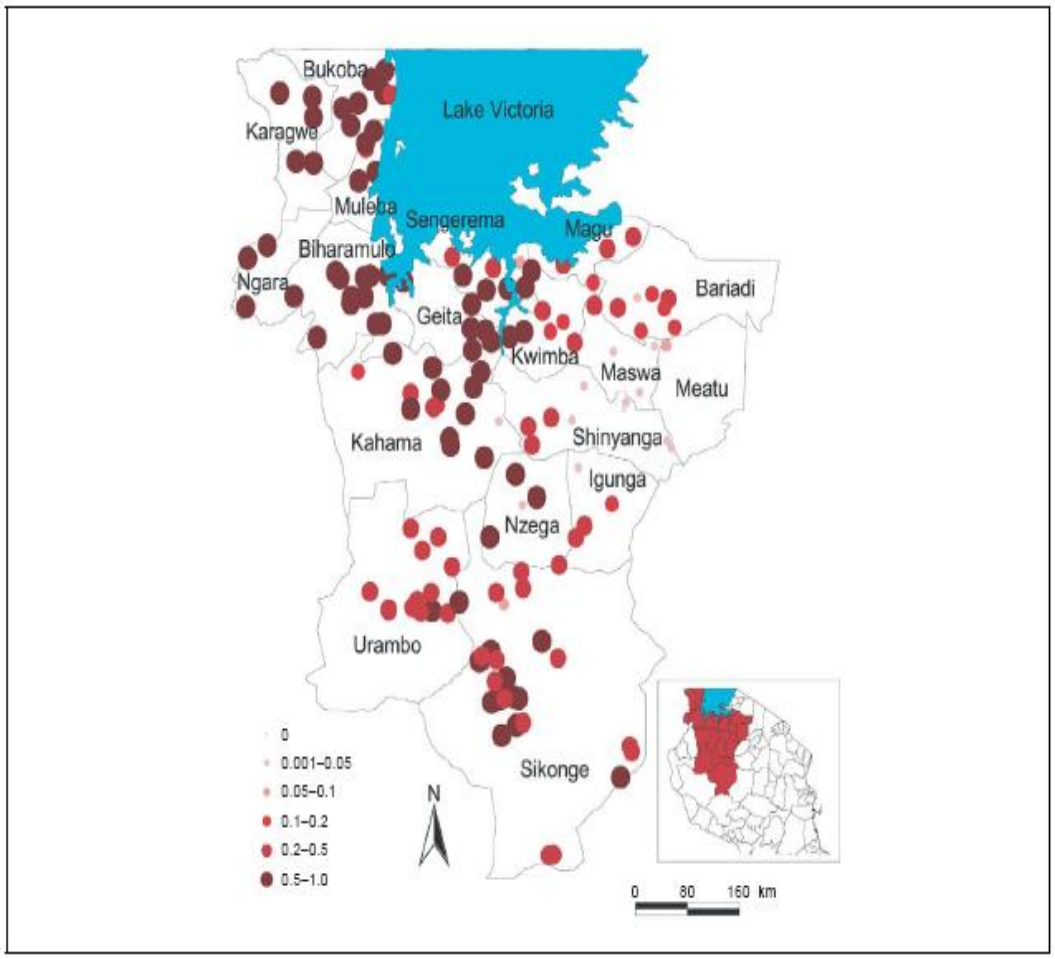


Figure 2.8. A map of the Lake and Western Zones of mainland Tanzania, showing the point prevalences of hookworm infection across six regions, ranging from 0 to 100% (0–1.0) (Kabeteraine *et al*., 2006).

2.4. Polyparasitism/ parasites co-infections and their clinical consequences

Malaria and intestinal helminths are among the most common chronic infections in populations living in sub-Saharan Africa (Mwangi *et al*., 2007). These two parasitic diseases have similar geographical distribution and co-infections are common (Mwangi *et al*., 2007). It is estimated that over a third of the world's population, in the tropical and sub-tropical regions are infected with helminth infections or malaria (de Silva *et al*., 2003; Snow *et al*., 2005). Crompton and Tulley (1987) have listed 47 protozoan and helminth species often found in association with *A. lumbricoides* infections, 24 of which are common. Co-existence of parasites in a particular population provides opportunity for co-infections to occur (Brooker *et al*., 2006c). A survey conducted in coastal Kenya in the mid-1980 showed that, the prevalence of hookworm, *N. americanus* was 50%, *P. falciparum* 47.5%, *S. haematobium* and soil-transmitted helminths species approximately 13%-37% (Bush and Holmes, 1986). Multiple parasite species infections involved malaria parasites and soil-transmitted nematodes, with a prevalence of >60% (Bush and Holmes, 1986).

The large-scale geographical distributions of malaria and helminth infections are determined largely by climate, that in turn influences survival of the mosquito vector and helminth free-living stage (Brooker and Michael, 2000b; Hay *et al*., 2000). Thus, it is probable that the geographic congruence of malaria, soil-transmitted helminths and schistosomes, reflect common climatic drivers of parasite geographic ranges (Mwangi *et al*., 2007). Among the soil-transmitted species, hookworm appears to have a wider thermal tolerance than *A. lumbricoides* or *T. trichiura* occurring throughout most of

sub-Saharan Africa, congruently with malaria (Mwangi *et al*., 2007). Other factors thought to be involved in determining distribution include socio-economic status (SES), human behaviour (Mwangi *et al*., 2007) and host-specific factors e.g. host physiology, host immunological status, population dynamics and genetic factor (Petney and Andrews, 1998).

It has been speculated that helminth infections may alter host susceptibility to clinical malaria (Nacher *et al*., 2001; Druilhe and Tall, 2005). However, malaria may also exacerbate the consequences of helminth infections (Mwangi *et al*., 2007). An important consequence of both malaria and helminth infection in particular hookworm is anaemia, an important public health problem in the tropics (Brooker *et al*., 2006c). It is well recognized that malaria contributes significantly to the development of anaemia in young children while hookworm infection is an acknowledged significant cause of anaemia as a result of intestinal blood loss (Hotez *et al*., 2004). It has recently been demonstrated that hookworm and malaria infections are additive in their effect in reducing haemoglobin concentrations among East African children (Brooker *et al*., 2006c).

In school-age children infection with *S. mansoni* is commonly associated with hepatomegaly and splenomegaly as a result of eosinophilic inflammatory and granulomatous reactions against parasite eggs trapped in host tissues (Gryseels *et al*., 2006). Aberrant immune responses to repeated or chronic *P. falciparum* malarial infection are also an important cause of hepatomegaly and splenomegaly in the tropics (Grobusch and Kremsner, 2005). In addition to serving as an antecedent of severe disease in young and middle-aged adults with long-standing intense schistosome

infection (Gryseels *et al*., 2006), splenomegaly is associated with anaemia, possibly because sequestration of red blood cells in the spleen reduces the effective circulating mass of red blood cells (Fulford *et al*., 1991). It is possible therefore that co-infections with schistosomes and malaria parasites may exacerbates organ pathology. Indeed it has been suggested that malaria co-infection exacerbate schistosomiasis-associated hepatosplenomegaly (Gryseels *et al*., 2006). Evidence from epidemiological studies suggests that chronic exposure to malaria and infection with schistosome may interact in childhood hepatosplenomegaly (Fulford *et al*., 1991).

Undernutrition, whether characterized in terms of growth impairment or micronutrient malnutrition is a pervasive problem in the developing world, contributing substantially to both child mortality and morbidity (WHO, 2002b), and to poor cognitive and motor performance (Walker *et al.,* 2006). As important contribution to undernutrition, *A. lumbricoides*, *T. trichiura* and malaria co-infection impact upon host nutrition through a number of mechanism including anorexia, chronic blood loss and malabsorption. Multiple species infections may have an additive or multiplicative impact on nutrition, especially in school-age children.

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study area

Nyamatongo ward located in Sengerema District in the Mwanza Region (Figure 3.9), northwest Tanzania is divided into 5 administrative localities (sub-villages), namely, Kamanga, Karumo, Nyamatongo, Irunda and Nyalwambu, and is one of the 25 administrative wards in the district. The ward is located on the eastern side of Sengerema district and borders Lake Victoria to the east. According to Tanzania Meteorogical Agency, the annual rainfall ranges between 930 - 1,200mm with temperature in the range of 25^0C – 28^0C with maximum temperatures being experienced from September to December. The area is cool and dry from June to August when it experiences low temperatures in the range of 11^0C- 20^0C. According to the 2002 Tanzania National Census (Tanzania National Bureau of Statistics, 2002) the population of Nyamatongo ward was 21,262 (females = 10,648 and males= 10,614) representing about 4% of the population of Sengerema district. The soils are red to yellow-red, gritty sandy and clay loams which are widely cultivated with most of the population living on the sandy soils (Tanzania National Bureau of Statistics, 2002). The economic activities in Nyamatongo are predominantly farming, livestock keeping, small scale business and fishing. Major crops in the area are maize, bananas, sweet potatoes, cassava, rice and beans. Cash crop production is dominated by cotton and groundnuts. There are 3,800 schoolchildren (in 6 primary schools) in the 5 study sub-villages. One of the challenges in the area is lack of piped water supply and community obtains water for domestic

purposes directly from the Lake, small streams or open man-made wells. The majority of the inhabitants are Sukuma tribe and other ethnic groups represented are Zinza and Kerewe who are the minority. The ward has 3 dispensaries, which are operated by the gorvement.

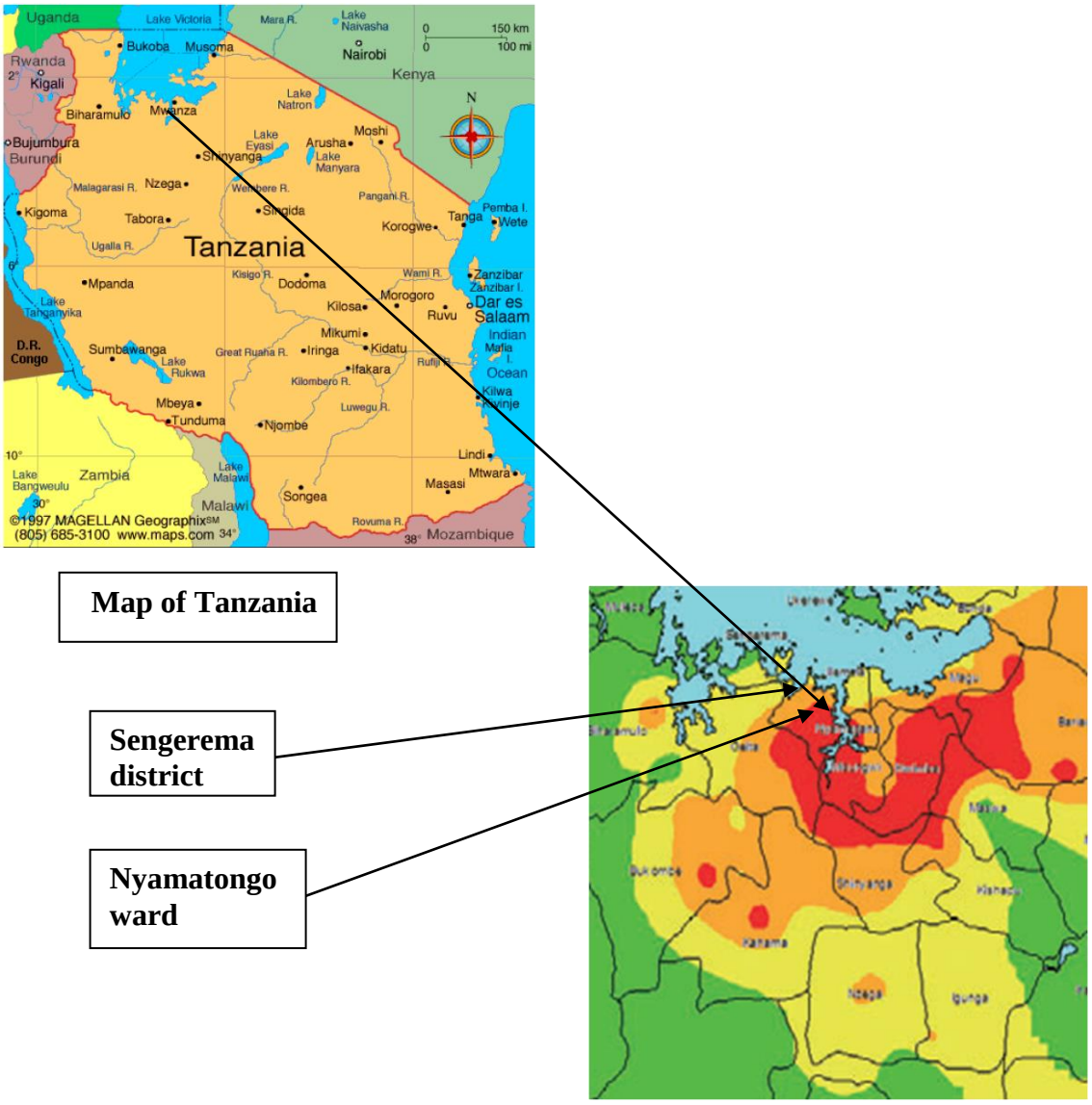


Figure 3.9. Map of Sengerema district showing the location of Nyamatongo ward (Source: Clements *et al*., 2006)

3.2. Study design

This was a cross-sectional study undertaken during the period of December 2008 and May 2009 in 4 primary schools (Karumo, Irunda, Nyalwambu and Kamanga) located in Nyamatongo ward, Sengerema district, in northwestern Tanzania, in the Lake Victoria basin.

3.3. Study population

The study population comprised primary school children who live and attend schools located in Nyamatongo ward and this particular study targeted school children that live and attend primary schools located near the Lake Victoria shore and in the age range 8-16 years. Study schools were Irunda, Karumo, Nyalwambu and Kamanga who consented to participate in the study. The children who participated in the study were those whose parents/guardians had given consent.

3.4. Inclusion criteria

Children were enrolled into the study if:-

1. Their age ranged between 8 – 16 years.
2. They provided a written assent and their parents/guardians provided written consent
3. They were generally considered by a qualified physician to be medically fit to participate in the study.
4. They lived in the study area.
5. They have not received antihelmintics for the past one year

3.5. Exclusion criteria

Children, on the other hand were excluded if:-

1. Their parent/guardian refused to give consent.
2. They were less than 6 years or more than 16 years of age.
3. They refused to provide a written assent.
4. They didn't live within the study site.
5. The physician adviced that they were unfit to participate in the study.
6. They have received antihelmintics for the past one year.

3.6. Sample size determination

The sample size was calculated using the Kish and Leslie formula (Fisher, 1960) based on the national prevalence of malaria and helminth infections, which was reported to be between 38% - 40% (MOH, 2002c).

Sample size $N = \frac{Z^2 P (1 - P)}{d^2}$

Where: - P = prevalence of infection (Malaria and schistosomiasis 38% - 40%)

Z = Score of confidence interval (CI= 1.96)

d = Tolerable error (Absolute precision) 5%

Sample size $N = \frac{1.96 \times 1.96 \times 0.38 (1 - 0.38)}{0.05 \times 0.05} = 362$

A minimum of 362 school children were expected to be recruited to participate in the study, but **400** school children joined the study voluntarily and were allowed to drop out at any time they wished without any prejudice.

3.7. Sampling procedures

A simple random sampling method was used to select children to participate in the study. Using the attendance register, each child was given a number. The selection procedure of the children to participate in the study was achieved by random assortment using tables. A topographical map of the Nyamatongo ward was divided into 10 squares equal in size and schools were selected from the squares. For the purpose of the present study, four randomly selected schools located near the lake shore were selected.

3.8. Stool sample collection and laboratory examination for helminth infections

Demographic information and parasitological samples were collected from school children enrolled in the study (Figure 3.11). A single stool sample was obtained from each child once using labeled container. The samples were processed within an hour after collection by experienced technicians at a field laboratory (Figure 3.12). Duplicate Kato-Katz cellophane thick feacal smears were prepared on a glass microscope slide according to described procedures by Katz *et al*., (1972) and WHO (1991). The smears were prepared using 41.7g of the feacal sample and then examined for hookworm ova under a compound microscope within 30-60 minutes after preparation. Slides were then left to clear for 24 hrs at room temperature and re-examined for ova of *S. mansoni, A. lumbricoides, T. trichiura* or any intestinal helminths ova. Average parasite egg counts for the duplicate smears were then multiplied by 24 (a factor used for templates which

takes 41.7g of feaces) to obtain the number of eggs per gram (epg) of faeces. As a quality control measure, 10% randomly selected smears were re-examined by an experienced parasitologist who was blinded to the results of the first reader. Helminth infection intensities for each species were categorized as shown in table 3.2.

Table 3.2. Classification of infection intensities of intestinal schistosomiasis and soil-transmitted helminths

Parasite species	**Infection intensity categories (epg)**				**Reference**
	Light	Moderate	Heavy	Very heavy	WHO, 1994
S. mansoni	1 – 100	101 – 399	400-1000	1000+	
Hookworms	1 – 999	1000-3999	≥ 4000		WHO, 1999
A. lumbricoides	1 – 999	1000-4999	≥ 5000		
T. trichiura	1 - 999	1000-9999	≥ 10,000		

3.9. Blood sample collection for malaria parasites examination

A finger prick blood sample was collected from each child enrolled in the study using a sterile lancet after swabbing the finger with methylated spirit sterilized cotton wool. Thick and thin blood films were prepared, fixed in absolute ethanol and stained in 10% Giemsa (Sigma). The slides were examined under a microscope using objective X 100 under oil-immersion (WHO, 1991; Cheesbrough, 1998). The thick film served to confirm the presence or absence of the *Plasmodium* parasite, whereas the thin film was used to identify the *Plasmodium* species. A slide was considered negative for malaria

parasite if no parasites were seen in at least 100 oil-immersion fields on the thick film (Muller *et al.*, 2003). Parasite density was determined per 200 or 500 leukocytes and then expressed using the following formula (WHO, 1991):

$$\frac{\text{Number of asexual parasites} \times 8000 \text{ leukocytes}}{200 \text{ leukocytes}} = \text{number of parasite/µl blood}$$

All blood smears that were positive for malaria and a 10% sample of slides that were considered negative were subsequently re-examined by an independent technician at Bugando Medical Center (BMC), Mwanza, Tanzania as blind slides. Malaria parasitaemia was categorized as follows: - 0, 1-50/µL, 51-500/µL, 501-5000 and > 5,000/µL which were used in data analysis (Raso *et al*., 2004).

3.10. Determination of anaemia

Finger prick blood samples obtained for malaria diagnosis were used for determination of anaemia. Heamoglobin (Hb) levels were measured using the HemoCueâ system (HemoCue AB, Ängelholm, Sweden). A drop of blood was placed on a special microcuvette which contained a hemolyzing agent (sodium desoxycholate, sodium nitrite, and sodium-azide). The erythrocyte membranes are disintegrated by sodium desoxycholate, releasing the haemoglobin. Sodium nitrite that converts the haemoglobin iron from the ferrous to the ferric state to form methaemoglobin, which then combines with azide to form azidemethaemoglobin. The Hb level was estimated in the microcuvette at the wave length of 540nm range at the HemoCueâ system. The method has been found to be reliable, simple and accurate for measuring haemoglobin (von Schenck *et al*. 1986).

Anaemia levels of analysed blood samples were classified according to the following criteria provided by World Health Organization (WHO, 1989) in Table 3.3.

Table 3.3. Classification of anaemia based on measurement of haemoglobin (Hb) levels in blood (WHO, 1989).

Classifications	**Hb levels**
Normal	≥ 11g/dL
Mild anaemia	10.0 – 10.9g/dL
Moderate anaemia	7.0 – 9.9g/dL
Severe anemia	4.0 – 6.9g/dL
Very severe anaemia	≤ 3.99g/dL

3.11. Clinical examination for organomegaly

Organomegaly was used to measure morbidity associated with schistomiasis and/or malaria in the study children. A combination of oral interview and physical examination by a qualified physician were used to determine organomegaly. Morbidity due to *S. mansoni* and *P. falciparum* malaria was defined by enlargement of the liver or spleen (Booth *et al*., 2004). The children underwent a general physical examination to determine whether or not they had anaemia, jaundice, oedema, fever or lymphadenopathy. They were also examined for cardiovascular, respiratory and central nervous systems problems. Each child was examined clinically by palpation

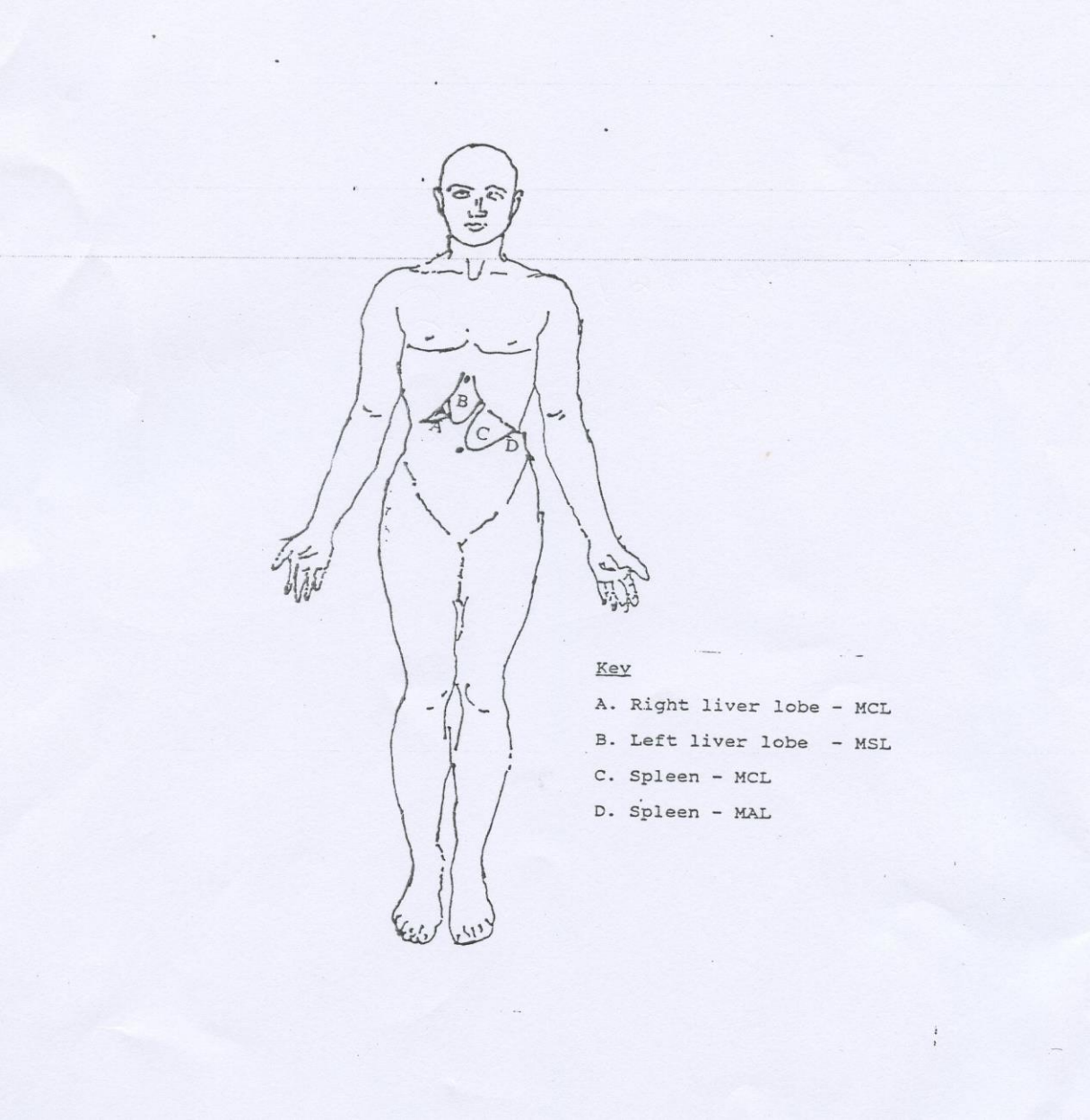


Figure 3.10. Organ enlargement measurements (**A.** right liver lobe **B.** Left liver lobe), (**C** and **D s**pleen). Measurement of liver and spleen enlargement (organomegaly) to detect irregularities and extension of liver lobe and spleen beneath the sternum and rib cages. The extension of liver and spleen are measured in the mid-sternal line (MSL), mid-clavicular line (MCL) and mid-axillary line (MAL) in both left and right side of the subject (Vennervald *et al*., 2004a).

for enlarged liver and spleen in the supine position on a bench or table as described by Vennervald *et al*., (2004a).

An organ was considered enlarged if it was palpable more than 2cm measured below the costal line (Figure 3.10 and 3.13). The liver enlargement was measured in reference to the right subcostal margin while spleen was measured in reference to the left subcostal margin to the organ edge in order to assess extent of enlargement (Figure 3.10 and 3.13). Proper care was taken to distinguish mid-axillary enlargement of spleen from that of the left kidney. The findings were categorized as follows: absence/presence of organomegally, firm/hard splenomegaly, firm/hard hepatomegaly or firm/hard hepatosplenomegaly according to the criteria provided by Vennervald *et al*., (2004a).

3.12. Study approval and ethical considerations

Ethical approval to collect feacal and blood samples from school children was obtained from Weill-Bugando University College of Health Sciences Research and Publications Committee, Mwanza, Tanzania (No. BREC/001/03/2009) and also from the Scientific Steering and Ethical Review Committees of the Kenya Medical Research Institute (KEMRI). This project was referred as KEMRI SSC 1595 (Appendix V).

Prior to the beginning of the study, district health authorities and district education authorities as well as village leaders were informed about the study and gave their approval in writing (Appendix II).

Information meetings were held with the parents, children and village leaders in each school to explain the purpose of the study, the procedures involved and the benefits and risks of taking part in the study. The benefits were that school children received free

laboratory investigations and free treatment for, anaemia, schistosomiasis mansoni, STH and malaria. The risks of taking part in the study were mainly due to short period mild pain during finger prick, bruising around the needle sites, little heamorrhage, mild and transient adverse drug reactions. Written consent was sought from parents whose children were involved in the study. Oral and written assent from school children was also sought before specimens were collected.

All the information collected from study participants remained confidential and only codes were used for analysis and presentation of the study results. To minimize risk of microbial infections at the site of injection and oral-feacal helminth egg contamination, the needle sites were sterilized using cotton wools soaked in methylated spirit before blood collection and sterile lancet blades were used for pricking. After collection of feacal samples children washed their hands using medicated soaps and disinfectants.

3.13. Data management and statistical analysis

Data entry was done using Microsoft Excel (Microsoft Corp., Redmond, WA, USA). Data analysis was done using SPSS version 11.5 for window (SPSS Inc., Chicago, IL, USA). Description of the study population was done by sex or age. Prevalence of infection was determined at 95% confidence interval and compared according to age or sex using the Chi-square test (χ^2). To calculate the statistical significance for differences between frequencies, the Pearson χ^2 test was used. For each individual, the arithmetic mean egg count of *S. mansoni*, hookworms, *A. lumbricoides* and *T. trichura* was calculated from the Kato-Katz smear average readings for two slides. In the present study, a multiple parasite (polyparasitism) was defined as co-infection with two or more

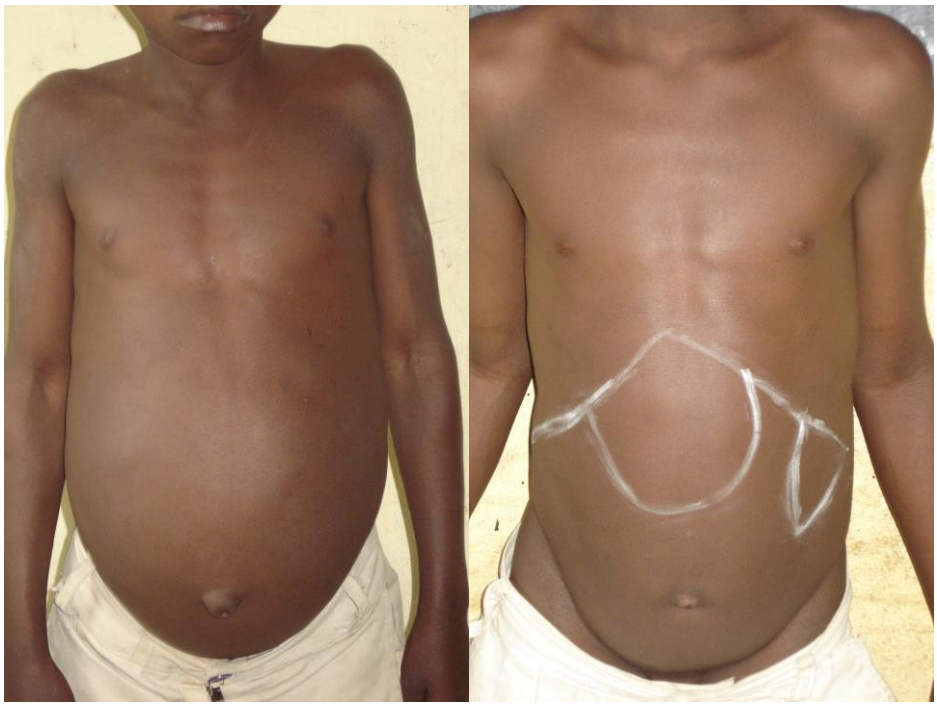
parasite species. Statistical significance for all analyses was determined at 5% alpha level.

3.14. Treatment of malaria, schistosomiasis and soil-transmitted helminthiasis

At the end of the study, children infected with *S.mansoni* or STH were treated with praziquantel at a single oral dose of 40mg/kg (Shelys Pharmaceuticals Ltd, Tanzania) and a single 500mg mebendazole tablet (Remedica Ltd, Cyprus) (WHO, 1993). Among other remaining children, those who had no infection with soil transmitted helminths or schistosomiasis mansoni but had hemoglobin level <11g/dl received ferrous sulphate tablets (hematinics), (Shelys Pharmaceuticals Ltd, Tanzania). Children positive for *P. falciparum* with an axillary temperature above 37^0C were treated with a combination therapy of Lumefantrine and Artemether in collaboration with the nearby health centers according to local malaria case-management guidelines. Children received porridge and sweet potatoes before the tablets were given in order to reduce the nauseating effect of praziquantel.

Figure 3.11. Organizing **s**chool children for faecal specimen collection at Irunda primary school.

Figure 3.12. Kato-Katz thick- smears preparation, laboratory set up for processing faecal samples for detection of *S. mansoni* and soil-transmitted helminth eggs at Karumo primary school.



A **B**

Figure 3.13. Two primary school boys suffering from hepatosplenomegaly at Nyalwambu **(A)** and Kamanga **(B)** primary schools.

CHAPTER FOUR

RESULTS

4.1. Demographic characteristics of the study population

A total of 400 school children aged 8-16 years old from 4 schools in Nyamatongo, Sengerema district of northwest Tanzania, whose parents had given consent for participation were examined for schistosomiasis, intestinal helminthiasis and malaria. They were also examined for organomegaly and anaemia. Mean age of the children was 12.2 ± 0.034 years and the male: female ration was roughly 1:1. The demographic characteristics of the study population are shown in Table 4.4.

Table 4.4. Age categories and sex of school children from Nyamatongo ward who were enrolled in the study

Age (years)	Males	%	Females	%	Total
≤10	33	16.8	50	24.5	83
11 - 13	104	53.1	106	52	210
14 – 16	59	30.1	48	23.5	107
Total	196	100	204	100	400

4.3. Prevalence of single parasitic infection

A total of 218 out of the 400 school children examined, 54.5% (95%CI, 49.6-59.4) harboured at least one parasite species. The prevalence of *S. mansoni* was 64.3% (257/400, 95%CI, 59.6-68.9)**,** hookworm 38.5% (152/400, 33.7-42.8) and that of *P. falciparum* malaria was 13.5% (54/400, 10.2-16.9) (Table 4.5). *Ascaris* and *Trichuris* infections were not detected in the feacal samples.

Table 4.5. The prevalence of parasites detected in the children from Nyamatongo ward

Parasites	**Prevalence of infection**	
	Overall (%)	(95% CI)
Schistosomes		
Schistosoma mansoni	64.3	59.9-68.8
Soil-transmitted helminth		
Hookworm	38.5	33.7-42.8
Trichuris trichiura	-	-
Ascaris lumbricoides	-	-
Plasmodia		
Plasmodium falciparum	13.5	10.2-16.9
Plasmodium ovale	0.8	-9.3-10.9

4.3. Prevalence and intensity of malaria by age and sex

Malaria was present in 57 (14.25 %, 95%CI, 10.8-17.7) of the 400 children tested for malaria and two *Plasmodium* species were detected, namely *P. falciparum* and *P. ovale*. Prevalence of *P. falciparum* was 13.5% (95%CI, 10.2-16.9) and that of *P. ovale* was 0.8 % (Table 4.5). *P. vivax* and *P. malariae* on the other hand were not detected. Female

had the highest prevalence of asymptomatic malaria (16.6%) and children aged 11-13 years old had the highest prevalence of asymptomatic malaria at 15.3% (n=30), (95%CI, 11.7-18.7). Table 4.6. shows number of children infected by *P. falciparum* classified in different infection intensities by age and sex.

Table 4.6. Number of children (%) with different infections intensities of *P. falciparum*, classified by sex and age (n= 397, children infected with *P. ovale* were excluded).

Variable	**Infection intensity of *P. falciparum* (parasite/µl blood)**					*P*-value
	0	1-50	51-500	501-5000	> 5000	
Sex						
Male	175 (44.1)	0	5 (1.25)	13 (3.3)	2 (0.5)	
Female	166 (41.8)	1 (0.25)	10 (2.5)	22 (5.5)	1(0.25)	< 0.262
Age (years)						
≤ 10	72 (18.1)	0	2 (0.5)	7 (1.8)	0	
11 - 13	177 (44.5)	1 (0.25)	7 (1.8)	22 (5.5)	2 (0.5)	
14 - 16	95 (23.9)	0	6 (1.5)	6 (1.5)	1 (0.25)	< 0.729
Total	347 (86.5)	1 (0.25)	15 (3.8)	35 (8.8)	3 (0.75)	

4.4. Prevalence and intensity of schistosomiasis infection by sex and age

Of the 400 stool specimens examined for *S. mansoni* eggs, 257 (64.3%), 95%CI, 59.6-68.9) were positive and the overall prevalence was 64.3% (Table 4.2) but prevalence in males and females were similar (P<0.313). Figure 4.14 shows the prevalence of *S. mansoni* in different infection intensities according to sex. About 60% of female had light infection intensities (1-100 epg) and in general, majority of the infected children, (n= 91/257, 35.4%) had light infection (1-100 epg). The prevalence and intensity of *S. mansoni* infections were observed to decrease with increase in ages of the study subject (Figure 4.15) and there was a significant differences in the infection intensity between age groups (χ^2=11.183, P<0.004).

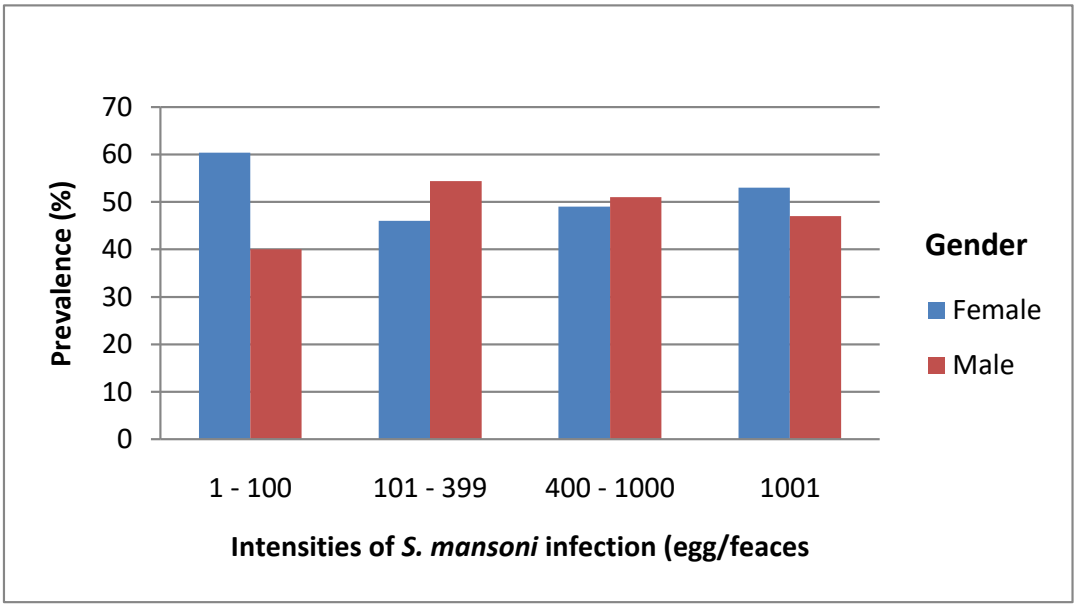


Figure 4.14. Prevalence *S. mansoni* in different infection intensities according to sex among 400 school children in Nyamatomango ward**.**

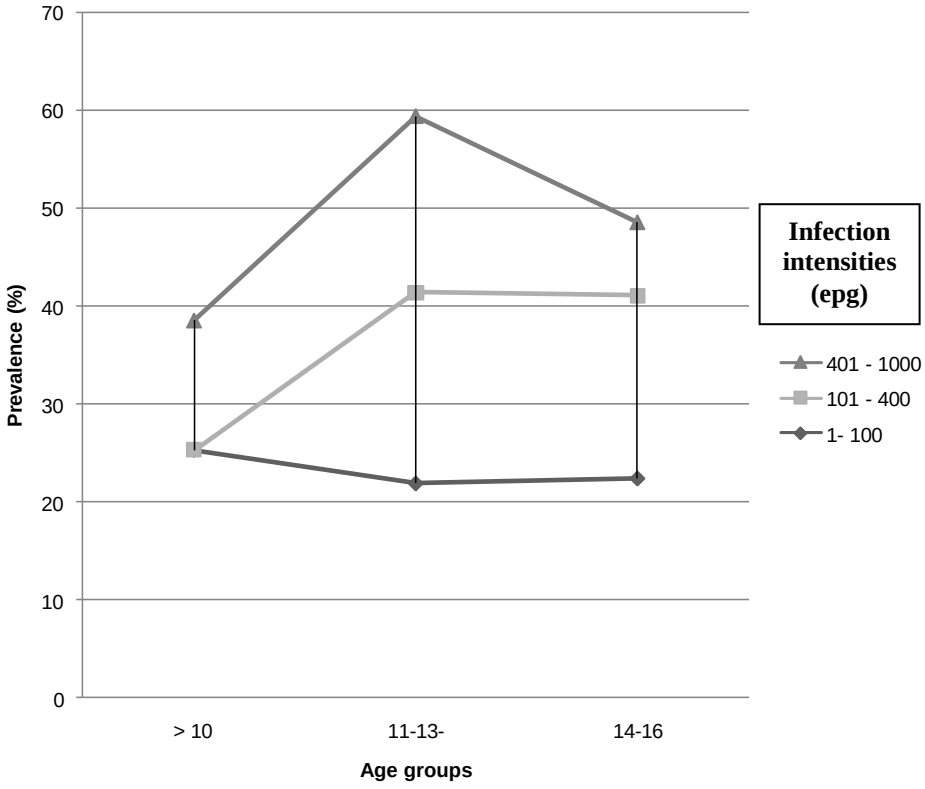


Figure 4.15. Prevalence and intensity of *S. mansoni* infection according to age among 400 school children in Nyamatongo ward.

4.5. Prevalence and intensity of hookworm infection by sex and age

Of the 400 school children who provided stool samples for the diagnosis of intestinal helminth infections, 152 (38%), 95%CI 33.7-42.8) were found to harbour hookworms as determined by the presence of characteristics of parasite eggs (Table 4.2). No attempt was made to differentiate between the hookworm species. Hookworm prevalence in males and females was similar (38%). The prevalence of hookworm in different infection intensities among male and female children are presented in Figure 4.16. Majority of the children (32%, 95%CI, 27.2-36.3) had light hookworm infections (1-999epg) and only < 5% of them harboured moderate to heavy infections (<4,000epg) (Figure 4.16). The prevalence of hookworm infections was observed to increase with

increase in age reaching a maximum at 44.8% (n= 48/107, 95%CI, 40-49.8) in the 14 - 16 years age groups (Figure 4.17). No significant association was observed between age and infection intensity (χ^2=5.885, *P*< 0.436).

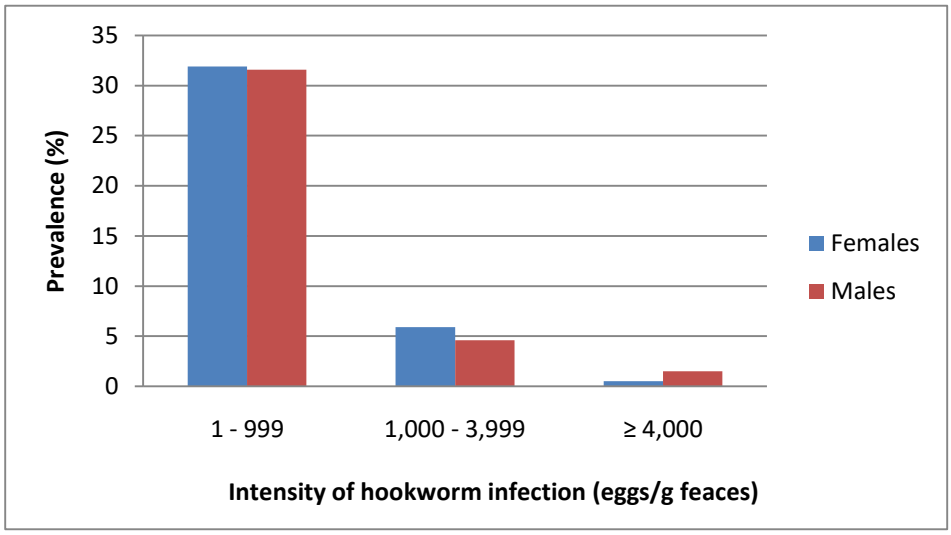


Figure 4.16. Prevalence of hookworm in different infection intensities among male and female school children in Nyamatongo ward.

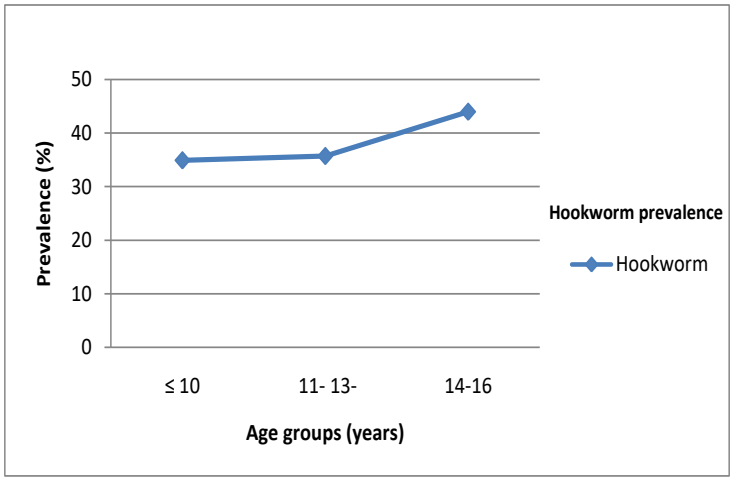


Figure 4.17. Prevalence of hookworm infection according to age among 400 school children in Nyamatongo ward.

4.6. Multiple parasitic infections

At least 26.3% (n=105/400), 95%CI, 22.2-30.8) of the children harboured 2 parasite species and about 69%, (95%CI, 64.4-73.4) of these had co-infections of hookworms and *S. mansoni*. Other dual infections observed were between *S. mansoni* and *P. falciparum*, 22.6%, (95%CI, 14.7-30.5), hookworm and *P. falciparum* and 5.7%, (95%CI, 1.3-10.1). Figure 4.18 shows the prevalence of double and triple parasitic infections according to sex. Only about 3% had 3 parasite species and these were for *S. mansoni* + hookworm + *P. falciparum*. Triple parasites species infections were most common in children in the aged range 11-13 years, and accounted for 72.7% (n= 8/11) of the cases.

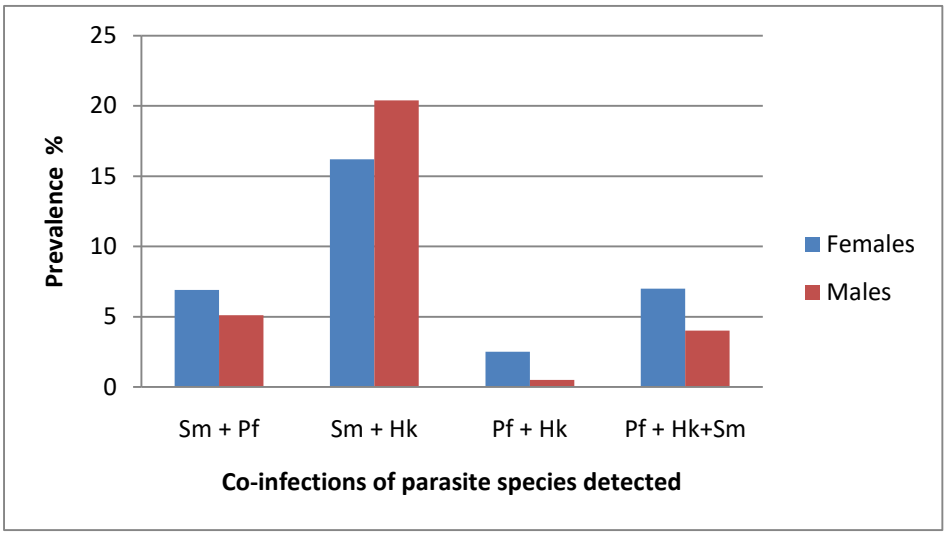


Figure 4.18. Prevalence of double and triple parasitic infections according to sex among 400 schoolchildren in Nyamatongo ward. (Keys: sm= *Schistosoma mansoni*, Pf= *Plasmodium falciparum*, Hk=hookworm)

4.7. Prevalence of anaemia

Haemoglobin (Hb) levels were used to measure anaemia. Mean haemoglobin level was 12.1g/dL (7.1 – 16.9g/dl). The mean Hb in males and females was 12.1 ± 0.543g/dl and

12.9 ± 0.624g/dL respectively and the difference was significant (χ^2= 14.485, *P*< 0.006) (Figure 4.19). About 20% (n= 79, 95%CI, 15.9-23.7) of the school children were anaemic (<11g/dL) (Figure 4.19) and the highest prevalence of anaemia was observed in age group 11-13 years, 19% (n= 40, 95%CI, 15.2-22.8) (Table 4.7).

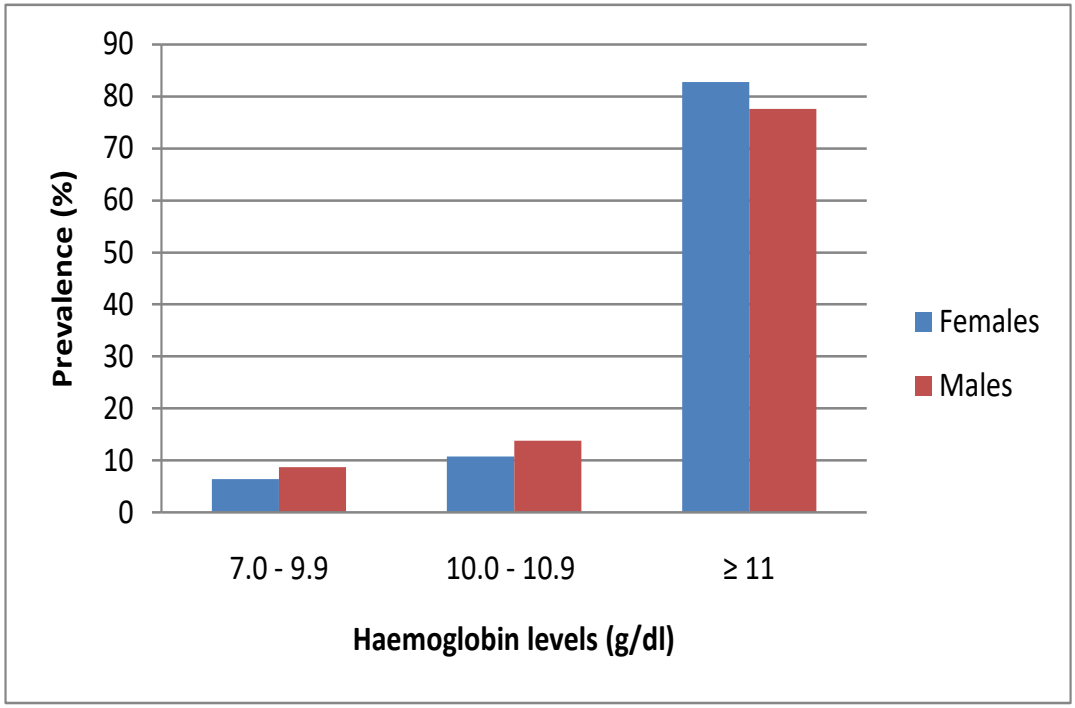


Figure 4.19. Prevalence of anaemia among school children according to sex among 400 school children in the Nyamatongo ward.

Table 4.7. Prevalence of anaemia among school children according to age groups (in both male and female) in the Nyamatongo ward.

Haemoglobin level (g/dl)				
Variable		Moderate 7.0 – 9.9g/dL	Mild 10.0 – 10.9g/dL	Normal ≥ 11g/dL
Age (years)	**N**			
≤ 10	83	8(9.6%)	14 (16.9%)	61(73.5%)
11 - 13	210	16(7.6%)	24(11.4%)	170(80.9%)
14 - 17	107	6 (5.6%)	11(10.3%)	90(84.1%)

4.7.1. Haemoglobin levels and parasite infections

4.7.1.1. Haemoglobin levels in malaria infections

Of all the 54 children diagnosed to have malaria (*P. falciparum*), only 3.7% (95%CI, 1.3- 8.7) were considered anaemic (<11g/dl) and one of the anaemic children had a parasitaemia of < 5,000 parasites/μL. The majority of the children, 85% (n=46) had normal Hb levels (≥11g/dl) and 2 children among these had parasitaemia of <5,000 parasites/μL. No relationship was observed between the intensity of *P. falciparum* infection and haemoglobin levels (χ^2= 5.834, $P \leq 0.67$).

4.7.1.2. Haemoglobin levels in *S. mansoni* infections

Of the 257 children diagnosed to have *S. mansoni* infection, 12.5% (n=32/257), 95%CI, 8.5-16.5) had Hb levels of <11g/dl and therefore, were considered to be aneamic. The majority of the anaemic individuals had light to moderate *S. mansoni* egg intensities. Although 87.5% (n=203/257) of the children had *S. mansoni* infection, their Hb levels were within the normal ranges (≥ 11g/dl).

4.7.1.3. Haemoglobin levels in hookworm infections

Approximately, 9.2% (14/152), 95%CI, 4.6-13.8) of the children diagnosed to have hookworm infections had Hb levels ≤ 10.9g/dl and therefore were considered to be anaemic. Of these, only 2 individuals had heavy intensities (≥ 4,000 eggs/g feaces). Majority of the children (77%) had light to moderate hookworm infection intensities and had normal Hb values (>11g/dl).

Hb levels were observed to generally decrease with increase in the intensity of hookworm eggs, children with ≥ 4,000epg had a mean Hb of <11g/dl (Figure 4.20) however infection with hookworm was not significantly associated with anaemia (χ^2= 1.405, $P\leq$ 0.704).

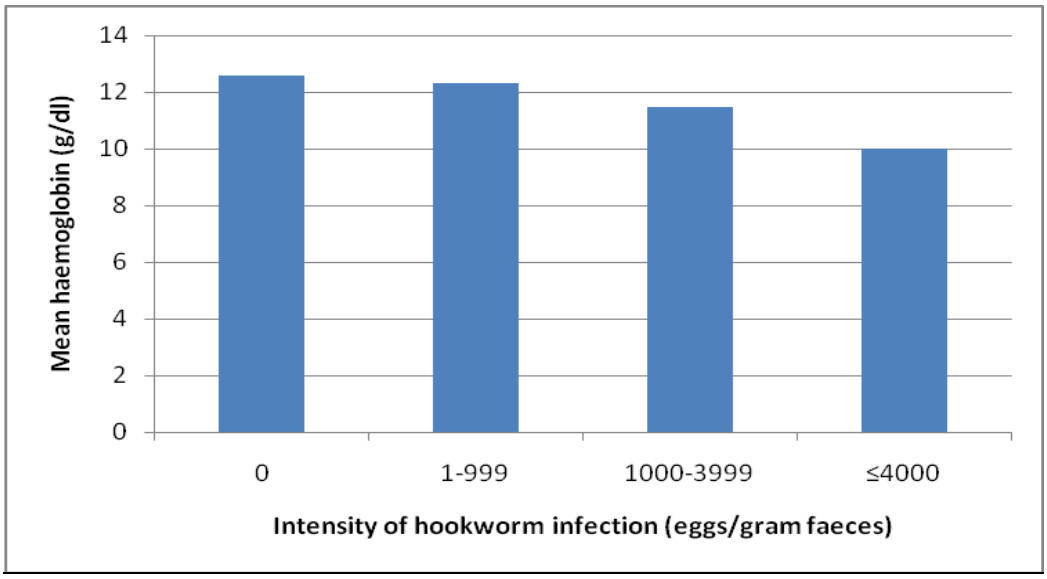


Figure 4.20. Relationship between mean haemoglobin (Hb) levels and intensity of hookwoms infection.

4.7.1.4. Haemoglobin levels and multiple parasitic infections

Of the 400 children examined for malaria or helminth infections, 26.5% (n=106/400) had dual parasite infections, and 20% (21/106) of these had ≤ 10.9g/dl Hb levels, and

were considered to be anaemic. About 14.2% were co-infected with *S. mansoni* + hookworm, 4.7% with *S.mansoni* + *P. falciparum* and 0.9% with hookworm + *P. falciparum*. About 77% (n=82/106) of the children who had dual parasite infections and had normal Hb levels (>11g/dl). However, about 18% (n = 2/11) of the children who harboured three parasites species (*S. mansoni* + hookworm + *P. falciparum*) had Hb levels of ≤ 10.9g/dl and therefore were anaemic.

4.8. Prevalence of organomegaly

Hepatomegaly was the most common condition, with overall prevalence of 22% and prevalence of splenomegaly was 11.5%. Only 7.5% of the total study population had hepatosplenomegaly (Figure 4.21).

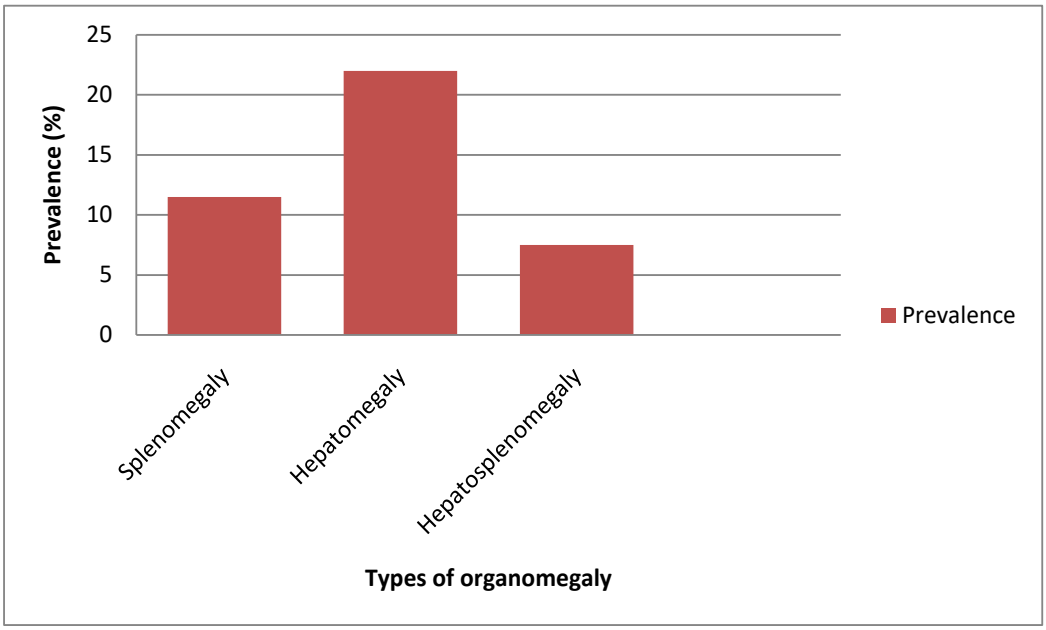


Figure 4.21. Pevalence of organomegaly among 400 school children of Nyamatongo ward

4.8.1. Prevalence of organomegaly according to sex and age

Figure 4.22 shows the prevalence of splenomegaly, hepatomegaly and hepatosplenomegaly in males and females. The overall, prevalence of splenomegaly, hepatomegaly and hepatosplenomegaly in both sex was 35.7% (n=46, 95%CI, 31-40.4), 62.2% (n=88, 95%CI, 57.5-66.9) and 22% (n=30, 95%CI, 17.9-26.1) respectively. The prevalence of hepatomegaly and splenomegaly was higher in male than in females (P<0.04) (Figure 4.22) and the sex-related differences were significant (*P*< 0.008). Prevalence of hepatosplenomegaly was significantly higher in the males than in the females, 11.2% vs 3.9% (χ^2=7.685, *P*< 0.006). Overall, children in the age group 11-13 years old had a higher prevalence of splenomegaly, hepatomegaly and hepatosplenomegaly and prevalence rates were 46%, 59% and 53.3% respectively (Figure 4.23).

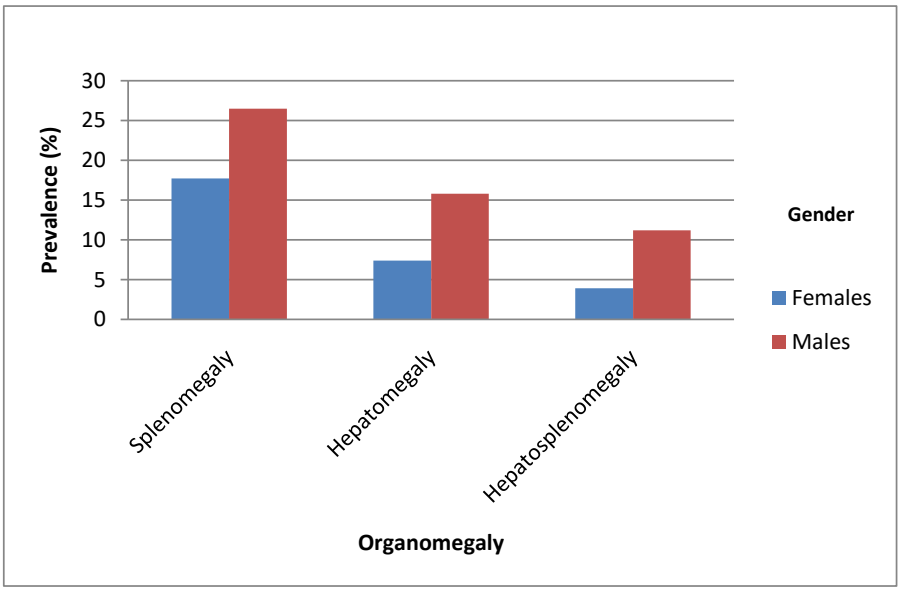


Figure 4.22. Frequency of organomegaly in the clinical palpation of school children in Nyamatongo ward categorized according to sex

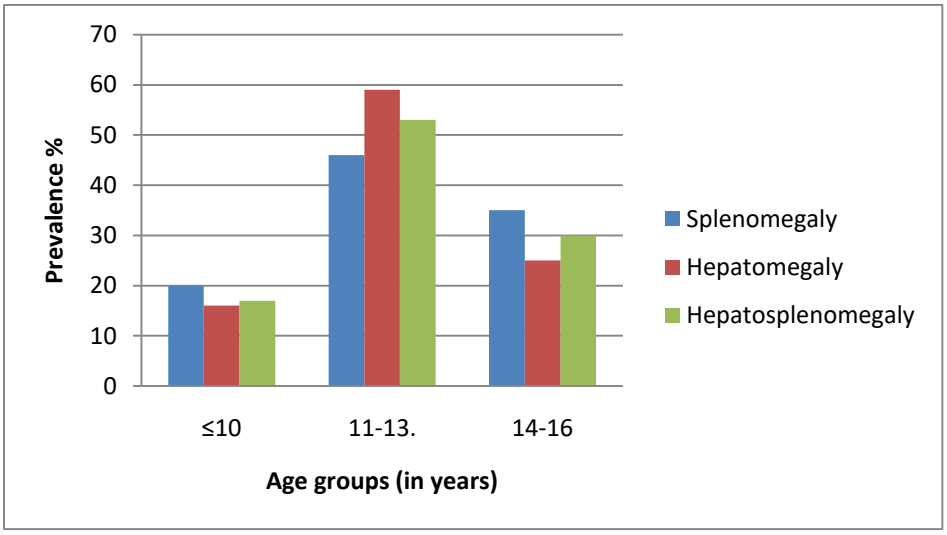


Figure 4.23. Prevalence of splenomegaly, hepatomegaly and hepatosplenomegaly in the clinical palpation among 400 school children in Nyamatongo ward according to age of both male and female.

4.8.2. Organomegaly and the individuals parasitic infections investigated

Of the 54 children with *P. falciparum* infections, 7.4% (4/54), 16.6% (9/54), and 5.6% (3/54) had splenomegaly, hepatomegaly and hepatosplenomegaly respectively. Intensities of *P. falciparum* infections was not associated with organomegaly (χ^2= 4.85, P<0.73), but hepatomegaly was the most common condition in malaria (Figure 4.24).

Of the 257 children diagnosed with *S. mansoni* infections by feacal examination for parasites eggs, 9.3% (25/257), 23% (59/257) and 6.2% (16/257) had splenomegaly, hepatomegaly and hepatosplenomegaly, respectively. No significant association was however observed between intensity of *S. mansoni* infections and organomegaly (χ^2=4.05, *P*<0.39). Hepatomegaly was the most common condition in the intestinal schistosomiasis (Figure 4.24).

Similarly, of the 400 school children provided stool samples, 152 (38%) were infected with hookworm and 13.8% (21/152), 23.7% (36/152) and 11% (11/152) had

splenomegaly, hepatomegaly and hepatosplenomegaly. No significant association was observed between intensity of hookworm infections and organomegaly (χ^2=2.5, P<0.48).

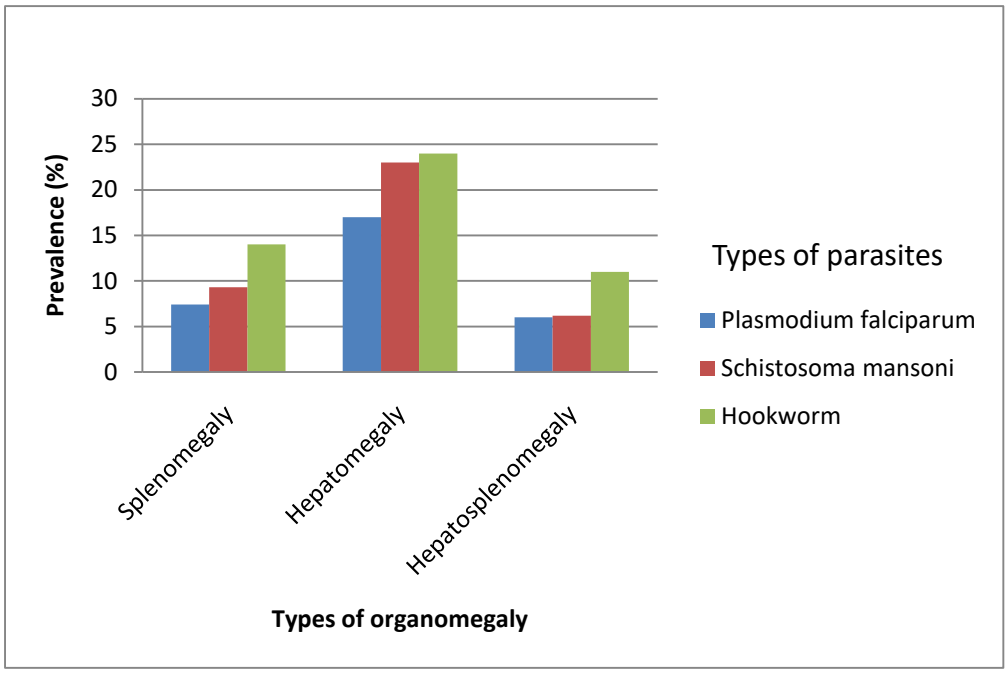


Figure 4.24. Prevalence of organomegaly in relation to single parasitic infection

4.8.3. Parasite combinations and organomegaly

About 27% (106/400) and 2.8% (11/400) of the school children who provided blood and stool samples were infected with 2 and 3 parasites respectively. Table 4.8 shows the prevalence of organomegaly in children co-infected with 2 or 3 parasites. Children co-infected with *S. mansoni* and hookworm had significantly higher prevalences of hepatomegaly (16%, n= 17/106) than children co-infected with *S. mansoni* and *P. falciparum* (3.8%, n= 4/106) (*P*<0.03). Only 18% (n= 2/11) children co-infected with 3 parasites (*S. mansoni* + *P. falciparum* + Hookworm) had hepatomegaly and the rest (8 children) had hepatosplenomegaly or splenomegaly. Hepatosplenomegaly and splenomegaly were most common in children co-infected with *S. mansoni* and

hookworm (Table 4.8). No significant associations were observed between parasites co-infections and organomegaly (χ^2= 0.07, P< 0.8).

Table 4. 8. Prevalence (%) of organomegaly among 400 schoolchildren co-infected with 2 or 3 parasites in Nyamatongo ward. (Key: Sm = *Schistosoma mansoni*, Hk= Hookworm, Pf= *Plasmodium falciparum*)

Organomegaly	**Parasite co-infections**							
	Sm + Hk		Sm + Pf		Hk + Pf		Sm + Pf + Hk	
		(%)		(%)		(%)		(%)
Splenomegaly	10	(9.4)	1	(0.9)	1	(0.9)	1	(9.1)
Hepatomegaly	17	(16)	4	(3.8)	1	(0.9)	2	(18)
Hepatosplenomegaly	4	(3.8)	1	(0.9)	0	(0)	1	(9.1)
Total	31	(29.2)	6	(5.6)	2	(1.8)	4	(36.4)

4.8.4. Prevalence of anaemia and organomegaly in relation to parasitic infections

Of all 400 school children who provided blood samples for haemoglobin level examination, about 20% (n=79/400) were anaemic (Hb level <11g/dl) but 39.2% (31/79) of these were not infected with any of the parasites investigated in the present study. About 41% (32/79), 18% (14/79) and 2.5% (2/79) of the anaemic children were infected with *S. mansoni*, hookworm and *P. falciparum* respectively. About 20% (15/79) were co-infected with *S. mansoni* and hookworm, 5.1% (4/79) with *S. mansoni* + *P. falciparum* while 1.3% (1/79) had hookworm and *P. falciparum* respectively. Only 1 child among the anaemic children had triple infection of *S. mansoni* + hookworm + *P. falciparum* (Figure 4.25).

On the other hand, about 19% (6/32), 3.1% (1/32) and 13% (4/32) of the anaemic children infected with *S. mansoni* had hepatomegaly, splenomegaly, and hepatosplenomegaly. Of the anaemic children infected with hookworm, 21% (3/14), 7.1% (1/14) and 7.1% (1/14) had hepatomegaly, splenomegaly and hepato-splenomegaly. None of the anaemic children infected with *P. falciparum* had organomegaly.

Of the children co-infected with *S. mansoni* + hookworm, 40% (6/15), 13.3% (2/15) and 6.7% (1/15) had hepatomegaly, splenomegaly and hepatosplenomegaly. Only 1 child out of those co-infected with *S. mansoni* and *P. falciparum* (1/4) had hepatomegaly. Among the anaemic children, only 1 child had splenomegaly with none of the parasitic infection diagnosed in the study.

Out of the 15 anaemic children diagnosed to have *S. mansoni* + hookworm co-infection, 40% (6/15) had no form of organomegaly. Of those co-infected with *S. mansoni* + *P. falciparum*, 75% (3/4) had no organomegaly and those co-infected with hookworm + *P. falciparum*, none had organomegaly. No statisitical significant association was observed between parasitic infections and anaemia observed among the 400 school children investigated (Figure 4.25).

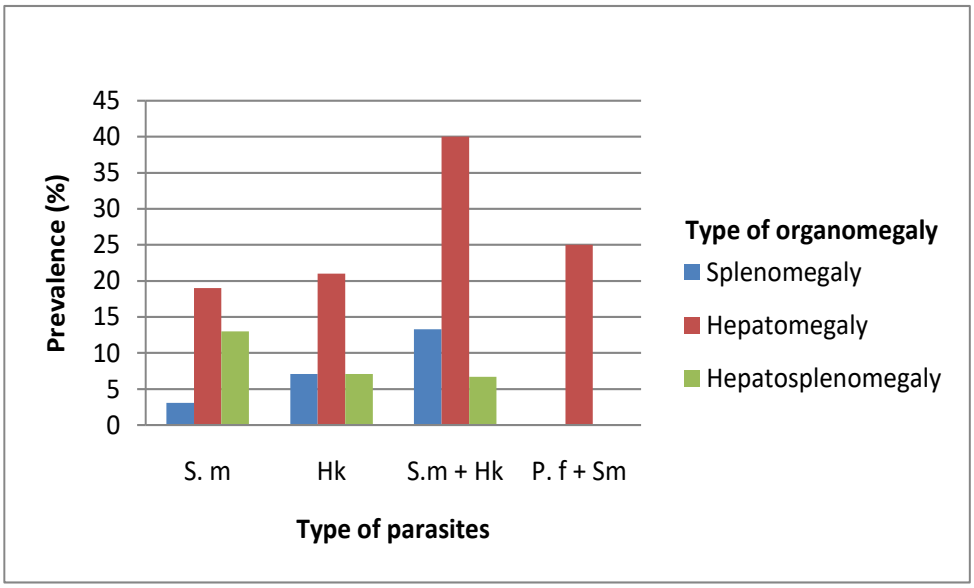


Figure 4.25. The prevalence of organomegaly among anaemic children infected with single and double parasitic infection (S.m. =*Schistosoma mansoni*, Hk=hookworm, P.f = *Plasmodium falciparum*)

CHAPTER FIVE

DISCUSSION

5.1. Prevalences and intensities of *S. mansoni*, hookworm and *P. falciparum* infections

This study confirms earlier observations that *S. mansoni* and hookworm are endemic in the Lake Victoria basin (Lwambo *et al*., 1999; Handzel *et al*., 2003; Ajanga *et al*., 2006). In the case of *S. mansoni*, a prevalence of 64.3% was recorded in the present study, which was 6 folds greater than that recorded previously in other localities within the area (Lwambo *et al*., 1999) or elsewhere within the Lake Victoria basin (Handzel *et al*.,2003; Ajanga *et al*., 2006). However, prevalence of *S. mansoni* may vary from locality possibly due to micro-geographical variations in exposure to *S. mansoni* cercaria (Handzel *et al*., 2003: Booth *et al*., 2004) or it is possible that ecological changes within the lake basin have caused an increase in schistosomiasis transmission.

Majority of the children had light to moderate *S. mansoni* infections and only 7.5% of the children had heavy infections. These findings are not different from what is expected in an endemic community where it has previously been observed that only few individuals excrete large numbers of eggs (Butterworth *et al*., 1991). Similarly, children of 11-13 years old had the highest prevalence of *S. mansoni* infection and this has been observed in other studies of schistosomes elsewhere in sub-Saharan Africa (Kabatereine *et al*., 2000; Keiser *et al*., 2002). One explanation is that children in this age group contact water more frequently than the other age groups and hence are more likely to contaminate the environment (Handzel *et al*., 2003) compared to any other age groups.

In the present study, it was observed that *S. mansoni* prevalence was higher in the female children than in male children. A similar observation was made by Kabatereine *et al*., (2000) at Piida village in Uganda. Several other studies, have however observed higher prevalence of *S. mansoni* infection in males than in females (Bundy and Blumenthal, 1990; Lwambo *et al*., 1999). Differences between frequency of exposure to water contact between male and female children seems to vary from place to place (Lwambo *et al*., 1999; Handzel *et al*., 2003) and this may account for such differences in the prevalence of *S. mansoni*.

On the other hand, the prevalence of hookworm infection observed in Nyamatongo was similar to that made in the neighbouring Magu district within northwest Tanzania (Lwambo *et al*., 1999) and was within the range observed in western Kenya (Handzel *et al*., 2003), and Brazil, in south America (Fleming *et al*., 2006). It appears that hookworm prevalence does not vary significantly over large geographical area. Nevertheless, the present study confirms that hookworm infection is a major public health problem in the Lake Victoria basin (Lwambo *et al*., 1999; Handzel *et al*., 2003), and is probably the most widespread of the 3 most common soil-transmitted helminth infections, namely *A. lumbricoides* and *T. trichiura* which occur throughout much of the sub-Saharan African (Brooker *et al*., 2006b) but were not observed in the current study. Apparently, the intensity of hookworm infections in the present study increased with increase in host age, reaching the highest intensity in children in the age range of 14-16 years old. A similar observation was made in a locality in rural Côte d'Ivoire by Keiser *et al*., (2002). Suprisingly, but not totally unexpected neither *A. lumbricoides* nor *T. trichiura* were present in the study children investigated in Nyamatongo ward.

Indeed, earlier studies in the nearby Magu district, reported a prevalence of <1% (Lwambo *et al*., 1999). On the other hand, report by Handzel *et al*., (2003) reported a prevalence of 22.9% and 17.9% for *A. lumbricoides* and *T. trichuris* in Asembo, Nyanza province in Kenya, within the lake basin. One reasons for the local differences within the lake basin could be variability in endemicity of the infections, environmental factors such as temperature, soil types (sand soil) or inability of the helminth eggs to withstand the variable local high temperatures that may be experienced within the lake region.

Malaria prevalence in Nyamatongo ward was 13.5%, even though this locality is within a malaria holoendemic area according to Tanzanian's Ministry of Health reports (Ministry of Health, Tanzania, 2002a unpublished report; Mboera *et al*., 2007). *Plasmodium falciparum* was the most predominant species in Nyamatongo as well as many parts of Tanzania (Mboera *et al*., 2008). It is not clear why malaria prevalence was low in the current study area, probably; the freely distributed ITNs by the Tanzanian government have reached most of the children in the study area and hence protect them against mosquito bites.

5.2. Prevalence of co-infection of *P. falciparum*, *S. mansoni* and hookworm

In the present study, *S. mansoni* and hookworm co-infections were the most common combination. This observation is similar to that reported from Brazil (Fleming *et al*., 2006) and Côte d'Ivoire (Keiser *et al*., 2002). These co-infections could be attributed to the co-endemicity of the two species in this area, a phenomenon that seems to be common in other areas (Keiser *et al*., 2002; Raso *et al*., 2004). Lwambo *et al*., (1999)

also reported a high prevalence of co-infections of *S. haematobium* and hookworm in school children in Magu district, within the region. In the present study, *S. haematobium* was not investigated.

The co-infections of *S. mansoni* and *P. falciparum* observed in the present study, have also been observed in other studies undertaken for instance in Senegal (Sokhana *et al*., 2004) and Zimbabwe (Midzi *et al*., 2008), and prevalence of *P. falciparum* among school children was high in those infected with *S. mansoni*. One explanation for co-infections of *P. falciparum* and *S. mansoni* being common due to the fact that schistosomes snail hosts (*Biomphalaria*) and malaria vectors (*Anopheles* mosquitoes) share common breeding sites.

In the present study, hookworm and *P. falciparum* co-infections were also common and the prevalence observed was within the range (<1-41%) reported in other studies in sub-Saharan Africa (Olsen *et al*.,1998; Nkuo-Akenji *et al*., 2006; Midzi *et al*., 2008). Differences in geographical variations in exposure and endemicity could be attributed to the wide variations in prevalence of *P. falciparum* and hookworm co-infections. Triple infections of *P. falciparum*, hookworm and *S. mansoni* were also observed in the present study but their prevalence was generally very low (3%). Triple co-infections have been reported in studies conducted in Côte d'Ivore, Cameroon and Zimbabwe (Raso *et al*., 2004; Nkuo-Akenji *et al*., 2006; Midzi *et al*., 2008). One study has reported occurence of up to six parasites species within a single individual (Raso *et al*., 2004). The present study and others conducted elsewhere in sub-Saharan Africa suggest that most parasitic infections do not occur singly but rather as co-infections (Brooker *et al*., 2006). Although both malaria and helminths have distinct modes and patterns of

transmission, environmental and host factors may influence their epidemiology and geographical distributions (Mwangi *et al*., 2007).

5.3. Prevalence of anaemia, hepatomegaly, splenomegaly and hepatosplenomegaly in relation to single or co-infections

The present study demonstrated that anaemia is still a problem in school children; this is consistent with reports of previous studies (Partnership for Child Development, 1998; Lwambo *et al*., 2000). In this study, majority of the children were observed to have mild to moderate anaemia (≤ 10.9g/dl) and the overall percentage of 19.5% of anaemia which was observed, was not so high as reported in Magu district and Tanga region in Tanzania (Lwambo *et al*., 2000; Tatala *et al*., 2008) and western Kenya (Sturrock *et al*., 1996). One explanation for this large difference in prevalence of anaemia between populations in Nyamatongo ward and the other locality could be attributed to variations in nutrition, nature of parasitic infections and other environmental related factors within the population.

Among the parasitic infections, hookworm, schistosomiasis and malaria are the major causes of anaemia, with hookworm infections accounting for between 35-73% of the iron-deficiency anaemia in Africa (Stoltzfus *et al*., 1997a). In the hookworm infections, blood loss occurs when worms use their cutting apparatus to attach themselves to the intestinal mucosa, submucosa and suck blood (Stanssens *et al*., 1996). The worms release anticoagulant that causes chronic intestinal blood loss (Stanssens *et al*., 1996). In the present study, a slightly and insignificant decrease in Hb levels was observed with increase in hookworm egg intensity, children with heavy hookworm intensity

(≥4,000) had Hb levels of ≤10.9g/dl. Olsen *et al*., (1998) made similar observations in western Kenya. In the current study, no significant association was observed between hookworm infection and anaemia, which contrasts sharply with the results of other studies that showed that hookworm intensity, is strongly associated with anaemia (Olsen *et al*., 1998). Lack of association with anaemia was probably due to the fact that relatively low intensity of hookworm infection was observed in the study population at Nyamatongo ward in which only 1% had hookworm egg counts ≥1000 epg.

In the present study, 41% of the anaemic children were infected with *S. mansoni*. However, no relationship was observed between Hb levels and the intensity of *S. mansoni* infection which has been observed in an earlier study in Zimbabwe (Ndamba *et al*., 1991). It is believed that *S. mansoni* causes anaemia through gastrointestinal blood loss via eggs passage through the intestinal wall (Haidar, 2001), and the influence, therefore depends on the intensities. The infection intensities in the present study were probably too low to have any impact on Hb levels.

In this study, only 2.5% of the anaemic children were infected with *P. falciparum* malaria. Malaria infection contributes to low haemoglobin levels due to a number of mechanisms, principally through the destruction of parasitized red blood cells, phagocytosis of parasitized and unparasitized red blood cells and autoimmune haemolysis (Philips and Pasvol, 1992). In advanced stages of malaria, erythroid hypoplasia, dyserythropoiesis and imbalances of cytokines are likely to be the main causes of anaemia (Philips and Pasvol, 1992). In the present study, however, no associations were observed between the mean haemoglobin and *P. falciparum* parasitaemia. A similar observation has been reported in Zanzibarian school children

(Stoltzfus *et al*., 1997a), where it was suggested that asymptomatic malarial infection did not influence Hb. Alternative explanation for this could be that in endemic areas, children suffer from several malaria attacks which may decrease their Hb to low levels, but the time interval between one malaria episode to another is sufficient to restore Hb values, thus resulting in a lack of fluctuation in response to parasitaemia.

Co-infections of *P. falciparum, S. mansoni* and hookworm are known to enhance the severity of anaemia due to undelying mechanisms (Mwangi *et al*., 2007). However, in the present study multiple parasitic infections (double/triple) were not associated with anaemia. Prevalence of multiple co-infections was 2.8% and in any case anaemia levels were mild to moderate.

In the present study, the prevalence of hepatomegaly and splenomegaly were higher in males than in females. A similar finding was observed in Uganda (Kabatereine, 2000). This could be partly explained by difference in exposure between the two sexes. In endemic populations, boys spend much time swimming and fishing in contaminated water as compared to girls (Huang and Manderson, 1992). Hepatomegaly was common in children aged 11-13 years old (Gryseels, 1990). A similar observation has been reported from Makueni district, Kenya (Vennervald and Dunne, 2004b). In endemic areas, the infections with *S. mansoni* starts at early ages and peaks at the age of 11-13 years and at this age organomegaly and immunity against schistosomes antigens starts to build up (Vennervald and Dunne, 2004b). Among the parasitic infections, schistosomiasis mansoni and malaria are the major causes of organomegaly in African children. *S. mansoni* causes hepatomegaly, splenomegaly or hepatosplenomegaly as a result of eosinophilic inflammatory and granulomatous reactions against parasite eggs

trapped in host tissues (Vennervald and Dunne, 2004b), whereas aberrant immune response to chronic *P. falciparum* malarial infection is also an important cause of hepatomegaly and splenomegaly in the tropics (Grobusch and Kremsner, 2005). Thus, it is possible that co-infections with *Schistosoma* and *Plasmodium* species may have synergistic effects on organ pathology. However, in the present study, no direct associations were observed between organomegaly (hepatomegaly, splenomegaly or hepatosplenomegaly) with infection intensities in the case of *S. mansoni* and *P. falciparum* or their co-infections. Similar observations were made in Kenya, (Wilson *et al*., 2007) and in Uganda (Kabatereine, 2000). However, the findings of Fulford *et al*., (1991) showed that hepatomegaly correlated well with *S. mansoni* infection intensities. Also, Vennervald *et al*., (2004b) showed a significant correlation between *S. mansoni* infections intensities and splenomegaly, both in terms of prevalence and extent after adjusting for spleen length.

In the present study, anaemic children infected with *S. mansoni*, hookworm or *P. falciparum* were observed to have hepatomegaly, splenomegaly or hepatosplenomegaly. Splenomegaly is known to be associated with anaemia, possibly because of sequestration of red blood cells in the spleen which reduces the effective circulating mass of red blood cells (Kloos *et al.*, 1997). However, in the present study, no correlation was observed between splenomegaly and anaemia.

In the current study organomegaly was also detected in children with hookworm or without eggs of *S. mansoni* or *P. falciparum* infections, an argument for a non schistosome or malaria-related aetiology such as viral hepatitis B or C, and other viruses (Mbugua, 1995). Also, anaemia was detected in children with no parasitic

infections investigated in the study, suggesting that, there may be other causes of anaemia such as *S. haematobium* and malnutritions in the tropics of Africa (Sturrock *et al*., 1996; Olsen *et al*., 1998).

5.4. Conclusion, limitations and recommendations

5.4.1. Conclusions

The following conclusions can be drawn from the present study:-

- Hookworm and *S. mansoni* are common parasitic infections in the area and they do co-exist with *P. falciparum*. Thus, co-infections of two or three parasite species in a single individual is common among schoolchildren in Nyamatongo area.
- The study further confirmed that helminths and malaria associatted morbidities are higher in the area but no correlations could be observed between infections intensity and morbidity. Thus, the intensity of infections alone could not explain the difference in the morbidity between uninfected and infected individuals.
- Majority of the children had light to moderate infections of *S. mansoni* and hookworm eggs, thus suggesting that infections of *S. mansoni* and hookworm start at early age in endemic areas.

5.4.2. Limitations of the study

- The study was cross-sectional in design, thus could not demonstrate the temporal associations between the co-infections and morbidity.

- The study used only clinical palpations method to diagnose organomegaly which easily detected individuals with obvious organomegaly but most likely missed those with mild organomegaly. Thus, the use of ultrasonography is more important and crucial in identifying organomegaly.
- Due to time and funds limitations, the study could not identify the risk factors associated with co-infections among the study subjects.

5.4.3. Recommendations

From the observations and conclusions outlined above,the following is reccomended:-

5.4.3.1. Recommendations for further research

- A large longitudinal or cohort study is required to identify a clear association between co-infections and morbidity.

5.4.3.2. Recommendations for policy makers

- There is a need to implement a framework by the WHO and Ministry of health in Tanzania for intergrated approach for the management and control of parasitic diseases in Nyamatongo that targets all parasitic infections present rather than a single parasitic infection. In endemic areas, parasitic infections occur as multiple infections rather than single parasite.
- It is important from the public view point to consider the joint impact of multiple-species infections when addressing the health of schoolchildren.
- To reduce the impact of co-infections, it is important to incorporate malaria control in programmes that focus on the control of helminthiasis.

- The results of the present study, can be used by the Ministry of Health and Social Walfare of the Tanzania government to implement control intenvetions such as mass drug administrion for the school children in the study area as per recommendation of the WHO.

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APPENDICES:

APPENDEX I: Clinical examination

Study subject identification number…… Date……

School/village name:…….....

Name:…….....Sex:……Age:……

Clinical diagnosis

After interrogating the participant about coughing, vomiting, abdominal pain, swelling scars, fever for the past three days and any other health problem. The clinician will continue with general and systemic examination on the study subjects to rule out other diseases other than malaria and schistosomiasis mansoni. Then, abdominal examination will be carried out to detect masses on the liver and spleen which will be measured in centimeter.

1: Symptoms:

Symptoms	Tick	Symptoms	Tick
Coughing		Fever	
Vomiting		Vomiting blood	
Abdominal pain		Blood in feaces (melena/fresh blood	
Swelling scars			

2: Liver

A)Not palpable B) Palpable and soft C) Palpable and firm D) Palpable and hard

The liver enlargement measured in reference to the right subcostal margin:……cm

3: Spleen

A) Not palpable B) Palpable and soft C) Palpable and firm D) Palpable and hard

The spleen enlargement measured in reference to the left subcostal margin:……cm

APPENDEX II

APPENDEX II: Consent /assent form

Consent Seeking Document: English Version

2: CONSENT /ASSENT FORM

Consent Seeking Document: English Version

Title: The prevalence of co-infections of malaria, schistosomiasis mansoni and soil-transmitted helminthiasis in school children in Nyamatongo ward in Sengerema district, northwest Tanzania

INTRODUCTION

You are asked to allow your child/children to participate in a research study conducted at Nyamatongo ward in Sengerema district by the following investigators:-

1: Humphrey D. Mazigo ITROMID- JKUAT, P.O. Box 62000-00200, Narobi, Kenya
Bugando University., P.O. Box 1464, Mwanza, Tanzania
2. Dr Gerald Mkoji CBRD- KEMRI, P.O. Box 54840-00200, Nairobi, Kenya
3. Dr. Nicholas Lwambo NIMR, Mwanza Center, P.O. Box 1462, Mwanza, Tanzania
4. Dr. Rebecca Waihenya JKUAT, P.O. Box 62000-00200, Nairobi, Kenya.

PARTICIPATION

Participation of your child/children in this study is voluntary. You should read all the information below, and ask questions about anything you do not understand, before deciding whether to allow your child/children or not to allow them to participate.

CONSENT EXPLANATION

Parasitic disease, especially malaria and helminths are important diseases in many part of Tanzania. These diseases are most common in school-age children. Various studies have demonstrated the health effects of these diseases in school-age children, ranging from malnutrition, impaired growth, impaired cognitive functions and clinical diseases which make children absent from school. Also, chronic infections of these diseases lead to hepatomegaly and splenomegaly. To achieve a maximum control measure of the

diseases is important to understand the level at which they occur together in children living in endemic area.

CONTINUATION OF APPENDEX II

PURPOSE OF THE STUDY

- The purpose of this research is to determine the prevalence of malaria, schistosomiasis mansoni and soil-transmitted helminthiasis in school children
- To determine the prevalence of co-infections of malaria, schistosomiasis mansoni and soil-transmitted helminthiasis in school children.
- The determine prevalence of anaemia and hepatomegaly or splenomegaly or both,

PROCEDURES

If you allow your children to volunteer to participate in this study, we will ask your child/children to provide an early morning stool sample in a container for examination of intestinal helminth and a finger prick blood sample for malaria parasites examination and anaemia determination. Stool sample obtained will be processed using Kato Katz technique for examination of intestinal helminth ova. Two thick and thin blood smears will be prepared from finger prick blood samples and stained with Giemsa for examination of malaria parasites under the microscopy. Haemoglobin level will be estimated using Hemocue system from the finger prick blood samples.

Children will also be examined clinically for the presence of any irregularities of liver and spleen at supine position on the table.

POTENTIAL RISKS AND DISCOMFORTS

We do not anticipate any serious risk for your child/children to participate in this study. Mild pain of short period, bruising around the needle sites may occur. To minimize these pains, all the procedures will be carried out by qualified personnel. To minimize risk of microbial infections and oral-feacal contamination, the needle site will be sterilize and children will be provide with disinfectants or medicated soaps to wash their hands after stool sample collection.

ANTICIPATED BENEFITS TO SUBJECTS

Your child/children will receive free laboratory diagnosis and in case your children will be found infected with any parasite under the study, he/she will receive treatment free of charge according to national treatment guidelines.

CONTINUATION OF APPENDEX II

CONFIDENTIALITY

When the results of the research are published or discussed in conferences, no information will be included that would reveal the identity of your child/children. If photographs, videos, or audio-tape recordings of your child/children will be used for educational purposes, the identity of the child/children will be protected. During data analysis, only codes instead of names will be used and all the information will be kept in a computer protected with a password.

PARTICIPATION AND WITHDRAWAL

The participation of your child/children in this research is voluntary. If you choose not to allow your child/children to participate, that will not affect your relationship with (health centers in Sengerema district) or your right or right of the child/children to health care or other services to which your child/children or yourself are otherwise entitled. If you decide for your child/children to participate, you are free to withdraw your consent and discontinue your child/children from participation at any time without prejudice.

IDENTIFICATION OF INVESTIGATORS

In the event of a research related injury or if your child/children experience an adverse reaction, please immediately contact Dr. Humphrey Mazigo, Weil-Bugando University College of Health Sciences, P.O. Box 1464, Mwanza, Tanzania. Mobile +255 786 060067 and e-mail- *humphreymazigo@gmail*.com. If you have any questions about the research, please feel free to contact us using the above address.

RIGHTS OF RESEARCH SUBJECTS

You may withdraw your consent for your child/children at any time and discontinue them from participation without penalty. You are not waiving any legal claims, rights or remedies because of allowing your child/children to participate in this research study. If you have questions regarding your rights or the rights of your child/children as a research subject, you may contact Dr. Humphrey Mazigo, Weil-Bugando University College of Health Sciences, P.O. Box 1464, Mwanza, Tanzania. Mobile +255 786 060067 and e-mail- *humphreymazigo@gmail.com.*

CONTINUATION OF APPENDEX II

SIGNATURE OF THE PARENT/GUARDIAN/TEACHERS

I have read the information provided above. I have been given an opportunity to ask questions and all of my questions have been answered to my satisfaction. I have been given a copy of this form.

I……..(Name of a parent or guardian) being 18 years or

older and a guardian or parent having fully capacity to consent. I agree my child or children to participate in the study.

Signature……Date……

….

Thumb

print……..date…….

Witness: I certify that, the participant have agreed to participate in the study without being forced.

Signature:…….Date……

CONTINUATION OF APPENDEX II

ASSENT FORM FOR CHILDREN (under 18 years old)

Assent Seeking Document: English Version

Title: The prevalence of co-infections of malaria, schistosomiasis mansoni and soil-transmitted helminthiasis in school children in Nyamatongo ward in Sengerema district, northwest Tanzania

Dear children, you are asked to participate in a research study conducted at Nyamatongo ward in Sengerema district by the following investigators:-

1: Humphrey D. Mazigo ITROMID- JKUAT, P.O. Box 62000-00200, Narobi, Kenya
Bugando University., P.O. Box 1464, Mwanza, Tanzania
2. Dr Gerald Mkoji CBRD- KEMRI, P.O. Box 54840-00200, Nairobi, Kenya
3. Dr. Nicholas Lwambo NIMR, Mwanza Center, P.O. Box 1462, Mwanza, Tanzania
4. Dr. Rebecca Waihenya JKUAT, P.O. Box 62000-00200, Nairobi, Kenya.

PARTICIPATION

We are asking you to participate in this research study because most of the diseases we wish to investigate, affect more children of 6-17 years of age. Research is a way to test new ideas or to investigate the cause of diseases in children or other members of the community. Research helps us learn new things and to identify people with various health problems. You can say Yes or No. Whatever you decide is OK. We will still take good care of you. We ask you to read all the information below, and ask questions about anything you do not understand, before deciding whether to participate or not participate.

PURPOSE OF THE STUDY (Why are we doing in this study?)

We are doing a research study about parasitic diseases which infect children and other members of the community. In this study, we will diagnose children for malaria parasites, schistosomiasis and other intestinal worms. Then, children will be examined for haemoglobin levels and clinically for any irregularities of the liver and spleen.

PROCEDURES (If I am in the study what will happen to me?)

If you volunteer to participate in this study, we will ask you to provide an early morning stool sample in a container which will be provided to you by a research team for

CONTINUATION OF APPENDEX II

examination of intestinal helminth and a finger prick blood sample for malaria parasites examination and anaemia determination will be obtained from you. Stool sample obtained will be processed using laboratory procedures and examined of intestinal helminth eggs. Also, a finger prick blood samples will be prepared using laboratory procedures, stained and examined of malaria parasites under the microscopy. Haemoglobin level will be estimated from the finger prick blood samples using another laboratory procedure.

Volunteers will also be examined clinically for the presence of any irregularities of liver and spleen caused by these parasitic infections at supine position on the table.

POTENTIAL RISKS AND DISCOMFORTS (Will I be hurt if I am in the study?)

We do not anticipate any serious risk for you to participate in this study. Mild pain of short period, bruising around the needle sites may occur. To minimize these pains, all the procedures will be carried out by qualified personnel. To minimize risk of microbial infections and oral-feacal contamination, the needle site will be sterilize and you will be provide with disinfectants or medicated soaps to wash your hands after stool sample collection.

ANTICIPATED BENEFITS TO SUBJECTS (Will the study help me?)

If you volunteer to participate, you will receive free laboratory diagnosis and in case you will be found infected with any parasite under the study, you will receive treatment free of charge according to national treatment guidelines.

CONFIDENTIALITY (What happens after the study?)

When the results of the research are published or discussed in conferences or used in any form, no information will be included that would reveal your identity. If photographs, videos, or audio-tape recordings of your child/children will be used for educational purposes, your identity will be protected.

PARTICIPATION AND WITHDRAWAL (Do I have to be in this study?)

You do not have to be in this study, if you do not want to be. The participation in this research is voluntary. If you do not want to be in this study, we will agree with your decision. If you decide that you don’t want to be in the study after we begin, that’s OK

CONTINUATION OF APPENDEX II

too. Nobody will be angry or upset. If you choose not participate that will not affect your relationship with the school or teachers or get any punishment.

IDENTIFICATION OF INVESTIGATORS (What if I have questions?

You can ask questions if you do not understand any part of the study. If you have questions later that you don't think of now, you can talk to me again or call Dr. Humphrey Mazigo, Weil-Bugando University College of Health Sciences, P.O. Box 1464, Mwanza, Tanzania. Mobile +255 786060067 and e-mail- humphreymazigo@gmail.com .

RIGHTS OF RESEARCH SUBJECTS (What are my rights as a volunteer?)

You may withdraw yourself from the study at any time and without punishment or penalty. If you have questions regarding your rights as a research volunteer, you may contact Dr. Humphrey Mazigo, Weil-Bugando University College of Health Sciences, P.O. Box 1464, Mwanza, Tanzania. Mobile +255 786 060067 and e-mail- *humphreymazigo@gmail.com.*

Assent:

If you decide you want to be in this study, please write your name and signature. If you decide that you don't want to be in the study, even if you have started in the study, then all you have to do is tell us:

I,…… (your name) would like to be in this research study.

…….. (Date of assent)

…… (Name of person who obtained assent)

…… (Signature of person who obtained assent and Date)

Witness: I certify that, the participant have agreed to participate in the study without being forced.

Signature:…….Date……

CONTINUATION OF APPENDEX II

Fomu ya kukubali au kukataa kushiriki katika utafiti

(Wazazi/Walezi/wasimamizi)

Tafsiri ya Kiswahili

Kichwa cha/jina la utafiti: Kiwango cha maambukizi ya malaria, minyoo ya kichocho cha tumbo na minyoo ya tumbo kwa watoto wa shule katika kata ya Nyamatongo iliyopo wilaya ya Sengerema, kasikazinimagharibi mwa Tanzania.

Unaombwa kuruhusu mwanao/wanao kushiriki katika utafiti huu unafanywa katika kata ya Nyamatongo na watafiti wafuatao:

1: Humphrey D. Mazigo ITROMID- JKUAT, S.L.P. 62000-00200, Narobi, Kenya
Chuo Kikuu Bugando, S.L.P. 1464, Mwanza, Tanzania

2. Dr Gerald Mkoji CBRD- KEMRI, S.L.P. 54840-00200, Nairobi, Kenya

3. Dr. Nicholas Lwambo NIMR, Mwanza Center, S.L.P. 1462, Mwanza, Tanzania

4. Dr. Rebecca Waihenya JKUAT, S.L.P. 62000-00200, Nairobi, Kenya.

Ushiriki

Ushiriki wa mwanao/wanao katika utafiti huu ni suala la hiari. Soma taarifa zote katika fomu hii na kuwa huru kuuliza swali lolote ambalo uelewi, kabla ya kuamua mwanao/wanao kushiriki au kutokushiriki katika utafiti huu.

Madhumuni ya utafiti

Kuangalia kiwango cha maambukizi ya vijidudu vya malaria, minyoo ya kichocho cha tumbo na minyoo mingine ya tumbo. Pia tutaangalia kitalaamu kama watoto wana matatizo ya kuvimba kwa ini au bandama kutokana na madhara ya maambukizi ya vijidudu vya malaria na minyoo ya kichocho cha tumbo. Watoto watachnguzwa pia kama wana matitizo ya upungufu wa damu.

Taratibu za ushiriki

Kama utakubali mwanao/wanao kushiriki katika utafiti huu, watoto wataombwa kufanya yafuatayo:-

- Mtoto/watoto wako watachukuliwa damu kutoka katika kidole na kuweka kwenye kioo cha darubini kwaajili ya kuangalia vijidudu vinavyosababisha malaria
- Mtoto/watoto wataombwa kutoa haja kubwa ya asubuhi kwa ajili ya kufanya uchunguzi wa kimaabara kuangalia mayai ya minyoo ya tumbo.
- Pia mtoto watapimwa kuangalia kama wana upungufu wa damu kwa kutumia vipimo vya kimaabra.

CONTINUATION OF APPENDEX II

Madhara

Hatutarajii madhara yeyote kutokea kwa watoto iwapo watashiriki katika utafiti huu. Mtoto/watoto watapata maumivu kidogo na ya muda mfupi wakati wa kutoa damu kwenye kidole na wakati mwingine kidole kubadilika rangi. Hili kupunguza maumivu, kazi hii itafanywa na mtalaamu wa maabara. Pia, kuzuia maambukizi katika eneo lililochomwa sindano dhidi ya vijidudu vingine vinavyosababisha magonjwa mengine, dawa maalumu ya kuuwa wadudu itatumika kusafisha eneo hilo la kidole kabla ya kuchoma sindano. Pia watoto watanawa mikono yao baada ya kumaliza kuchukua haja kubwa hili kuzuia maambukizi ya minyoo kupitia mikono yao.

Faida ya kushiriki katika utafiti

Mtoto/watoto wako watapata huduma ya bure ya kupimwa kama wana ugonjwa wa malaria na minyoo ya tumbo. Pia watoto watapata dawa zinazotibu ugonjwa wa malaria, Kichocho na minyoo mingine bure (pasipo malipo yeyote). Pia , utafiti huu utaisaidia serikali kufanya maamuzi juu ya kutoa dawa za minyoo bure kwa wanafunzi wote katika eneo hili.

Usiri wa taarifa

Wakati majibu ya utafiti huu yatakapokuwa tayari kwa kuchapishwa or kuongelewa katika makungamano, hakuna taarifa inayo mhusu/wahusu mtoto/watoto itatolewa hadharani kwa majina yao. Washiriki wote watatambuliwa kwa namba zao na si kwa majina yao

Haki ya kushiriki au kujitoa katika utafiti

Ushiriki wa mtoto/ watoto wako katika utafiti huu ni uamuzi wako mwenyewe. Kama utaamua mtoto/ watoto wako wasishiriki katika utafiti huu, haitakuzuia kupata huduma za afya au huduma zinginezo zitolewazo katika wilaya ya Sengerema. Kutoruhusu mwanao/wanao kushiriki au kujitoa katika utafiti hakutasababisha kupigwa faini au kuchukuliwa hatua zozote za kisheria.

Utambuzi wa watafiti

Kwa suala lolote linahusiana na utafiti huu or iwapo mtoto atapata madhara makubwa kutokana na kutumia dawa au maumivu yeyote makali kutokana na utafiti huu, wasiliana moja kwa moja na Dr. Humphrey Mazigo, Chuo Kikuu cha sayansi ya tiba ya binadamu

CONTINUATION OF APPENDEX II

Well-Bugando S.L.P.1464, Mwanza, Tanzania. Simu ya mkononi +255 786 060067 and barua pepe- *humphreymazigo@gmail*.com.

Haki ya mshiriki katika utafiti

Unaweza kuondoa fomu ya mtoto/watoto wako katika utafiti huu wakati wowote na kuwaondoa katika utafiti huu pasipo kutozwa faini yeyote. Hauvunji sheria yeyote kwa kuwaondoa kushiriki katika utafiti huu. Kama una swali kuhusiana na haki za mtoto/watoto wako katika utafiti huu, wasiliana Dr. Humphrey Mazigo, Chuo kikuu cha sayansi ya tiba ya binadamu, Weill-Bugando, S.L.P 1464, Mwanza- Tanzania**.** Simu namba +255 786060067

Sahihi ya mzazi/msimamizi/mwalimu

Nimeisoma fomu hii na kuielewa vizuri. Nimepewa nafasi ya kuuliza maswali na maswali niliyouliza yamejibiwa vizuri. Nimepewa durufi ya fomu hii.

Jina la mzazi/msimamizi sahihi Tarehe

……. ……. …….

Sahihi ya shahidi

Sahihi yangu kama shahidi kwamba mshiriki amekubali na kuweka sahihi yake mbele yangu bila kulazimishwa.

Sahihi:…… Tarehe:-…….

Sahihi ya mtafiti:…….

CONTINUATION OF APPENDEX II

Assent form

Kichwa cha/jina la utafiti: Kiwango cha maambukizi ya malaria, minyoo ya kichocho cha tumbo na minyoo ya tumbo kwa watoto wa shule katika kata ya Nyamatongo iliyopo wilaya ya Sengerema, kasikazinimagharibi mwa Tanzania.

Wapendwa watoto, mnaombwa kushiriki katika utafiti huu unafanywa katika kata ya Nyamatongo na watafiti wafuatao:

1: Humphrey D. Mazigo ITROMID- JKUAT, S.L.P. 62000-00200, Narobi, Kenya
Chuo Kikuu Bugando, S.L.P. 1464, Mwanza, Tanzania
2. Dr Gerald Mkoji CBRD- KEMRI, S.L.P. 54840-00200, Nairobi, Kenya
3. Dr. Nicholas Lwambo NIMR, Mwanza Center, S.L.P. 1462, Mwanza, Tanzania
4. Dr. Rebecca Waihenya JKUAT, S.L.P. 62000-00200, Nairobi, Kenya.

Ushiriki

Tunawaomba mshiriki katika utafiti huu kwasababu magonjwa tunayoyafanyia utafiti yanashambulia sana watoto wa umri kati ya miaka 6-17. Utafiti ni njia mojawapo ya kugundua vitu vipya au kutafiti magonjwa yanayoshambulia watoto na watu wengine katika jamii zetu. Utafiti unatusaidia sote kujifunza vitu vipya and pia kugundua watu wenye matatizo mbalimbali ya afya. Unaweza kusema ndiyo au hapana kushiriki katika utafiti huu. Vyovyote utakavyo amua ni sahihi na tutaendelea kukupa huduma za afya wakati wowote.. Tunakuomba usome fomu hii kwa uangalifu na umakini mkubwa kabla hujaamua kushiriki au kutokushiriki katika utafiti huu. Sehemu yeyote ambayo hautaielewa vizuri, usisite kuuliza swali lolote bila woga.

Madhumuni ya utafiti (nini malengo ya utafiti huu?)

Kuangalia kiwango cha maambukizi ya vijidudu vya malaria, minyoo ya kichocho cha tumbo na minyoo mingine ya tumbo kwa watoto. Pia tutaangalia kitalaamu kama watoto wana matatizo ya kuvimba kwa ini au bandama kutokana na madhara ya maambukizi ya vijidudu vya malaria na minyoo ya kichocho cha tumbo. Watoto watachnguzwa pia kama wana matitizo ya upungufu wa damu.

Taratibu za ushiriki (Nini napaswa kufanya endapo nitaamua kushiriki?)

Kama utakubali mwana/wanao kushiriki katika utafiti huu, watoto wataombwa kufanya yafuatayo:-

CONTINUATION OF APPENDEX II

- Kukubali kuchukuliwa damu kutoka katika kidole na kuweka kwenye kioo cha darubini kwaajili ya kuangalia vijidudu vinavyosababisha malaria
- Utaombwa kutoa haja kubwa ya asubuhi kwa ajili ya kufanya uchunguzi wa kimaabara kuangalia mayai ya minyoo ya tumbo.
- Pia utapimwa kuangalia kama una upungufu wa damu kwa kutumia vipimo vya kimaabara.

Madhara (Je, nitapata madhara yeyote nikishiriki katika utafiti huu?)

Hatutarajii madhara yeyote kutokea kwako iwapo utashiriki katika utafiti huu. Utapata maumivu kidogo na ya muda mfupi wakati wa kutoa damu kwenye kidole na wakati mwingine kidole kubadilika rangi. Hili kupunguza maumivu, kazi hii itafanywa na mtalaamu wa maabara. Pia, kuzuia maambukizi katika eneo la lililochomwa sindano dhidi ya vijidudu vingine, dawa maalumu ya kuuwa wadudu itatumika kusafisha eneo hilo la kidole. Pia utanawa mikono yako baada ya kumaliza kuchukua haja kubwa hili kuzuia maambukizi ya minyoo kupitia mikono yako.

Faida ya kushiriki katika utafiti (Je, nitapata faida yeyote kwa kushiriki?)

Ndiyo, utapata huduma ya bure ya kupimwa kama una ugonjwa wa malaria na minyoo ya tumbo. Pia utapata dawa zinazotibu ugonjwa wa malaria, Kichocho na minyoo mingine bure (pasipo malipo yeyote).

Usiri wa taarifa (Je, kuna usiri wowote kuhusu taarifa nitakzotoa kuhusu afya yangu?)

Wakati majibu ya utafiti huu yatakapokuwa tayari kwa kuchapishwa or kuongelewa katika makungamano, hakuna taarifa inayo kuhusu itatolewa hadharani kwa jina lako. Washiriki wote watatambuliwa kwa namba zao na si kwa majina yao

Haki ya kushiriki au kujitoa katika utafiti (Je, haki zangu ni zipi nikiamua kushiriki?)

Ushiriki wako katika utafiti huu ni uamuzi wako mwenyewe. Kama utaamua kutokushiriki katika utafiti huu, haitakuzuia kupata huduma za afya au kupewa adhabu shuleni au na wazazi.

CONTINUATION OF APPENDEX II

Utambuzi wa watafiti (Je, nitawatambuaje washiriki?)

Kwa suala lolote linahusiana na utafiti huu au iwapo utapata madhara makubwa kutokana na kutumia dawa au maumivu yeyote makali kutokana na utafiti huu, wasiliana moja kwa moja na Dr. Humphrey Mazigo, Chuo Kikuu cha sayansi ya tiba ya binadamu Well-Bugando S.L.P.1464, Mwanza, Tanzania. Simu ya mkononi +255 786 060067 and barua pepe- *humphreymazigo@gmail*.com.

Haki ya mshiriki katika utafiti (Je, mshiriki ana haki zipi katika utafiti huu?)

Unaweza kujiondoa katika utafiti wakati wowote pasipo kupewa adhabu yeyote au wazazi wako kukupa au kupewa adhabu yeyote. Kama una swali kuhusiana na haki za mtoto/watoto wako katika utafiti huu, wasiliana Humphrey Mazigo, Chuo kikuu cha science ya tiba ya binadamui, Weill-Bugando, S.L.P 1464, Mwanza- Tanzania**.** Simu namba +255 786060067

Sahihi ya mtoto/mzazi/msimamizi

Nimeisoma fomu hii na kuielewa vizuri. Nimepewa nafasi ya kuuliza maswali na maswali niliyouliza yamejibiwa vizuri. Nimepewa durufi ya fomu hii.

Jina la motto sahihi Tarehe

……. ……. ……

Sahihi ya mzazi/msimamizi

Sahihi:…… . Tarehe:-……

Sahihi ya mtafiti:……

APPENDEX III: Protocol for diagnosis of intestinal helminths (*S.mansoni* and soil-transmitted helminths)

Kato Katz thick smears technique

Materials

- Cellophane cover slips
- Glass microscope slides
- Screen of metal or nylon mesh (mesh size 250μm)
- Wooden spatula
- Template (with well volume of 50mg)
- Disposable paper
- Compound microscope with 50 – 100X magnification.
- Glycerine-Malachite green solution

Procedures

- Coverslips made from cellophane strips (20X30mm, 40-50μm thick wettable) are soaked in a 50% glycerine-malachite green solution for at least 24hours before use.
- A smooth faecal sample is needed. Therefore, to remove fibrous material, a small amount of the specimen is forced through a screen of metal or nylon mesh (mesh size 250μm).
- A template (perforated plate containing exactly 50 mg feacal materials in the well) is placed on a microscope slide. By using a wooden spatula, fill completely the hole in the surface of template.
- Remove the template carefully, leaving all the faecal material on the slide and none sticking to the template.
- Now cover the 50mg specimen on the slide with a soaked cellophane coverslip. If an excess of glycerine solution is present on the upper surface of the cellophane, wipe off the excess with a small piece of absorbent paper.

CONTINUATION OF APPENDEX III

- Invert the slide against a smooth surface and spread the feacal specimen evenly under the cellophane by pressing it against the surface.

- Leave the specimen for 30 to 60 minutes, then examine for the eggs of hookworm (*A.doudenale, Necator americanus), Ascaris lumbricoides* and *T.trachiura*.
- Leave the specimen at 24hours for clearing before examine the schistome eggs. The specimen should be examined with 50 – 100X magnification. To obtain the number of eggs per gram of the patients faeces, the number of schistosome eggs counted in the specimen is multiplied by 20 (Kato, *et al*., 1972; WHO,1991)

Glycerine-malachite green

Materials

- Malachite green powder
- Glycerine
- Water

Procedures

- Make 3% aqueous solution of methyline blue by dissolving 3 grams of malachite green in 100ml of water.
- Prepare 50% methyline blue solution by mixing 100ml glycerine + 100ml water + 1ml of 3% aqueous methyline blue solution. ((Kato, *et al.*,1972;WHO,1991)

APPENDEX IV: Staining of malaria parasites

Giemsa stain

Material

- Giemsa powder 3.8g
- Glycerol (glycerine) 250ml
- Methanol (methyl alcohol) 250ml
- Microscopic slides
- Lancet prickers and Buffered water (pH 7.1 – 7.2)

Method

Staining the blood films

Regular method, for 20 or more slides

Equipment

- Stock of Giemsa stain
- Methanol*
- Absorbent cotton wool
- Staining troughs (to hold 20 slides)
- Distilled/deionized water, buffered to pH 7.2
- Measuring cylinder, capacity 100-500 ml (depending on the number of slides to be stained)
- Measuring cylinder, capacity 10-25 ml (depending on the amount of stock stain to be measured)
- Flask or beaker (capacity will depend on the amount of stain to be made up) Timing clock
- Slide-drying rack

CONTINUATION OF APPENDEX IV

* Methanol (methyl alcohol) is highly toxic and can cause blindness or death if swallowed. It should be stored in a lockable cupboard.

Method

Step 1 Fix each thin blood film by dabbing it gently with a pledget (small piece) of cotton wool dampened with methanol or by dipping it in a container of methanol for a few seconds. Avoid methanol, or its fumes, coming into contact with the thick film, otherwise fixation may take place and will prevent proper staining.

Step 2 Place the slides, back to back, in a staining trough, making sure that all thick films are at one end of the trough.

Step 3 Prepare a 3% solution of Giemsa stain by adding 3 ml of Giemsa stock solution to 97 ml of buffered water.

Step 4 Pour the stain gently into the trough until the slides are totally covered. Avoid pouring the stain directly on to the thick films.

Step 5 Leave the slides in the stain for 30-45 minutes. Experience will indicate the correct time for each batch of slides.

Step 6 Pour clean water gently into the trough to float off the iridescent “scum” on the surface of the stain. The water should be poured into the end of the trough where the thin films are, to avoid undue disturbance of the thick films.

Alternatively, gently immerse the whole trough in a bowl or basin filled with clean water.

Step 7 Gently pour off the remaining stain and rinse again in clear water for a few seconds. Then pour off the water.

CONTINUATION OF APPENDEX IV

Step 8 Remove the slides one by one and place them, film side downwards, in a drying rack to drain and dry, making sure that the thick film does not touch the edge of the rack.

This is a practical method of reasonable and acceptable accuracy. The number of parasites per microlitre of blood in a thick film is counted in relation to a standard number of leukocytes (8000). Although there are variations in the number of leukocytes between healthy individuals and even greater variations between individuals in ill health, this standard allows for reasonable comparisons.

You will need two tally counters, one to count parasites and the other to count leukocytes.

(a) If, after 200 leukocytes have been counted, 10 or more parasites have been identified and counted, record the results on the record form in terms of the number of parasites per 200 leukocytes.

(b) If, after 200 leukocytes have been counted, 9 or fewer parasites have been counted, continue counting until you reach 500 leukocytes on your tally counter; then record the number of parasites per 500 leukocytes.

In each case, the number of parasites relative to the leukocyte count can be converted to parasites per microlitre of blood by the simple mathematical formula:

$$\frac{\text{number of parasites} \times 8000}{\text{number or leukocytes}} = \text{parasites per microlitre}$$

In effect, this means that if 200 leukocytes are counted, the number of parasites is multiplied by 40 and if 500 leukocytes are counted the number of parasites is multiplied by 16.

APPENDEX IV: Protocol for anaemia determination using Hemocue system

Sample collection: The preferred site for collection of a capillary blood sample is from the middle or ring finger of children or adults. Blood obtained by finger stick (lancet) must be free flowing and not forced; do not "milk" the finger to get sufficient blood. Wipe away the first and second drop with clean, dry gauze or lint-free tissues. Collect the third drop of blood for analysis.

Procedures:-

1. Select an appropriate puncture site. For best results, use the middle finger or the ring finger for sampling. Avoid fingers with rings for sampling.
2. If cold, warm client's fingers with warm water. The client's fingers should be straight but not tense, to avoid stasis.
3. Remove a microcuvette from the vial and recap the vial immediately.
4. Clean site for blood collection with alcohol-soaked gauze or a newly-opened alcohol pledget.
5. Using your thumb, lightly press the finger from the distal knuckle (phalanx) to the tip to stimulate flow of blood to the sampling point. For the best blood flow and the least pain, sample on the palmar surface of the distal phalanx away from the tip and midline of the surface of the finger.
6. Position the lancet device so that the puncture will be made across the whorls (lines) of the fingerprint. Press the lancet firmly off-center on the fingertip prior to activating the lancet to aid in obtaining a good sample.
7. Activate the lancet to puncture the finger. Discard the lancet in an approved sharps container.
8. Wipe away the first two large drops of blood. This stimulates the blood flow and lessens the likelihood of a dilutional effect by

CONTINUATION OF APPENDEX IV

interstitial fluid. If necessary, apply light pressure again, until another drop of blood appears. Avoid "milking of the finger".

9. Make sure the third drop of blood is big enough to fill the microcuvette completely. Hold the microcuvette at the "wing" end and touch the tip into the middle of the drop of blood from above the finger. Avoid touching the microcuvette to the skin. Keep the microcuvette in contact with the blood and fill in one continuous process. Do not refill a partially filled microcuvette.
10. Wipe any residual control material from the sides of the microcuvette with a piece of gauze, as if wiping excess butter from a knife. Do not touch the opened end of the microcuvette with the gauze since this will draw blood out of the microcuvette.
11. Visually inspect for air bubbles in the center of the cuvette eye. If bubbles are present in the cuvette eye, discard the microcuvette and obtain another specimen.
12. The filled (test) microcuvette should be analyzed within three (3) minutes after being filled. Filled (test) microcuvettes are to be kept in the horizontal position. Place the filled (test) microcuvette into the cuvette holder and gently slide the holder into the measuring position.
13. The Hemoglobin (hgb) value will be displayed in grams/dL after approximately 30-50 seconds.
14. Record the result before removing the microcuvette from the instrument.
15. Dispose of the microcuvette in the biohazardous waste container.
16. If an "ERROR" code is displayed, refers to the manufacturer's "Troubleshooting Guide" found in the Hemocue operating manual.

APPENDEX V: Ethical clearance certificates

BUCHS/BMC Research Ethical Committee (BREC)

Research Clearance Certificate No. **BREC/001/03/2009**

Ethical clearance is hereby granted to the following Principal Investigator/Researcher.

DR. HUMPHREY MAZIGO OF THE DEPARTMENT OF PARASITOLOGY AND ENTOMOLOGY OF WEILL BUGANDO UNIVERSITY COLLEGE OF HEALTH SCINCES, MWANZA TANZANIA

On this day of: **08th March 2009** to conduct health research

Involving human subjects titled: **PREVALENCE OF CO-INFECTION OF MALARIA, SCHISTOSOMIASIS MANSONI AND SOIL – TRANSMITTED HELMINTHIASIS IN SCHOOL CHILDREN IN NYAMATONGO WARD IN SENGEREMA DISTRIC.**

The research period is from **March 2009** to **March 2010.**

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CONTINUATION OF APPENDEX V

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26/03/2009

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MWANZA.

Yah: KURUHUSIWA KUFANYA UTAFITI WA UAMBUKIZO WA UGONJWA WA KICHOCHO NA MINYOO WILAYANI MWETU:

Kichwa cha barua hapo juu chahusika.

Mtajwa hapo juu ameruhusiwa kufanya utafiti wa uambukizo wa magonjwa ya kichocho na minyoo wilayani. Hii itakuwa pia ni faida ya wilaya kujua kiwango cha Maambukizi.

Tafadhali mpeni ushirikiano atakaohitaji.

.....
Dr. B. Ndaki
Kaimu Mganga Mkuu (W),
SENGEREMA.
DISTRICT MEDICAL OFFICER
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CONTINUATION OF APPENDEX V

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k.k. Mratibu Elimu Kata,
NYAMATONGO

YAH: IDHINI YA KUFANYA UTAFITI DR. HUMPHREY MAZIGO:

Mtajwa hapo juu ni mwanafunzi wa Chuo Kikuu cha Afya Bugando.

Tumemruhusu afanye utafiti katika Shule za Kata yenu.

Tafadhali mpeni msaada afanye Utafiti wake.

L. Samike
Kny: AFISA ELIMU (W)
SENGEREMA

AFISA ELIMU WA WILAYA
SENGEREMA