THE EFFECT OF ONE VERSUS TWO PRAZIQUANTEL TREATMENTS ON *SCHISTOSOMA MANSONI* MORBIDITY AND RE-INFECTION ALONG LAKE VICTORIA IN UGANDA

BY

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Declaration

This study is original, except when stated by reference, and has not been published and/or submitted for any other degree award to any other University before.

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List of abbreviations

BMI	Body mass index
DALYs	Disability-adjusted life years
DANIDA	Danish International Development Agency
DBL	Danish Bilharziasis Laboratory
DRC	Democratic Republic of Congo
epg	Eggs per gram
ERR	Egg reduction rate
GMI	Geometric mean intensity
GPS	Global Positioning system
HAZ	Height for age z-scores
IL	Interleukin
MAL	Mid axillary line
MCL	Mid clavicular line
MSL	Mid sternal line
NTD	Neglected Tropical Diseases
PPF	Periportal fibrosis
PSL	Parasternal line
PVD	Portal vein diameter
Th2	T helper cells
VCD	Vector Control Division
USAID	United States Agency for International Aid
WAZ	Weight for age z-scores
WBC	White blood cells
WHO	World Health Organisation
μL	Micro litre

Abstract

Schistosomiasis is a debilitating disease and the most widespread water-borne parasitic disease. It affects approximately 4 million people in Uganda and 13% of the population are at risk of infection. Schistosomiasis control target is to reduce morbidity. In Uganda, a national control program for Schistosomiasis and other intestinal worms was launched in 2003. Since then, affected communities have been undergoing mass treatment using a single standard dose (40mg/kg body weight) of praziquantel taken once a year. However, schistosomiasis is still prevalent along large water bodies. More information is required to find out whether different chemotherapeutic regimens of praziquantel would combat the levels of schistosomiasis infection. To fill this information gap, a longitudinal randomised intervention study was carried out to compare the effect of two doses versus one dose of praziquantel on schistosomiasis infection and related morbidity. The aim of this study was to enhance our knowledge about the use of different praziquantel treatment regimens in the control of schistosomiasis.

The study was conducted in Musoli village along Lake Victoria, Eastern Uganda. A cohort of 446 people was followed over a period of 2 years. Pre-treatment S. mansoni infection and related morbidity data, and water contact patterns were obtained. All participants were given a single dose of praziquantel after which they were randomised to two groups. Two weeks later, one of the groups received a second dose of praziquantel while the other group was not given any other treatment. Follow up surveys were performed nine weeks, eight and 24 months after treatment. Analysis was performed using ANOVA, student`s t test, paired t test, pairwise correlation and chi-square test using Stata for windows (Intercooled Stata 10.1, Stata Corporation, USA). A P value <0.05 was used to determine statistical significance.

The *S. mansoni* infection prevalence and the geometric mean intensity (GMI) were 88.6% (95% confindence interval [95% CI]: 85.6 – 91.5) and 236.2 (95% CI: 198.5 – 460.9) eggs per gram of faeces respectively. The prevalence of splenomegaly, hepatomegaly and hepatosplenomegaly were 4.9%, 24.2% and 30.3% respectively. Periportal fibrosis was minimal. Cure rates of single and double dose treatment groups were 47.9% and 69.7% respectively, significantly different (Relative risk = 1.7, 95% CI = 1.3 – 2.2). The egg

reduction rate of a single dose (92.3%) was not significantly different from that of two doses (93.9%). Occurrence of re-infection was not significantly different between the two treatment groups. There was no significant difference in growth parameters and prevalence of anaemia between the two treatment groups. The prevalence of splenomegaly and hepatomegaly were not significantly different between the two treatment groups. However, 24 months after treatment, prevalence of hepatosplenomegaly showed a significant difference between the two treatment groups (95% CI: 14.1 – 24.5 for single dose and 95% CI: 4.6 – 13.0 for two doses).

In conclusion, Musoli village is highly endemic for schistosomiasis. Two doses of praziquantel are not superior to a single dose with regard to reducing schistosomiasis re-infection and related morbidity. This study therefore supports the current treatment strategy of a single annual dose of praziquantel in the control of schistosomiasis infection. Nonetheless, schistosome parasites can develop resistance to praziquantel especially in control programmes. Therefore there should be mechanisms to monitor parasite tolerance to praziquantel and control of schistosomiasis should be integrated with other preventive measures.

Chapter 1: Introduction

Schistosomiasis is the most widespread water-borne parasitic disease and remains a public health problem where it occurs. Five species of schistosomes infect man but in Africa the most common ones are *Schistosoma mansoni* and *S. haematobium* that cause intestinal and urinary schistosomiasis respectively. Global estimates indicate that 600 million are at risk of schistosome infection and 200 million people from 76 countries are infected with schistosomiasis, out of which 85% are from sub-Saharan Africa (Engels *et al*., 2002; WHO, 2002). Schistosomiasis is a debilitating disease that mainly affects but not limited to the poor rural communities, where health care, sanitation, clean and safe water supply are minimal. Approximately 120 million of the infected people are asymptomatic (van der Werf *et al*., 2003; Savioli *et al*., 1997), while 20 million have severe clinical symptoms. Mortality rate due to schistosomiasis is about 200,000 deaths per year worldwide (Conlon, 2005) but predictions based on standardised data from sub-Saharan Africa show that mortality rates due to urinary and intestinal schistosomiasis are 150,000 and 130,000 persons per year respectively (van der Werf *et al*., 2003). The disability-adjusted life years (DALYs) lost due to schistosomiasis are estimated at 4.5 million (WHO, 2002) but several authors have noted that this is underestimated since it excludes some morbidity parameters (van der Werf *et al*., 2003; King, 2005). In Uganda, the most prevalent schistosome species is *S. mansoni* (Kabatereine *et al*., 2003) and schistosomiasis affects approximately 4 million people and 13% of the population live in endemic areas (Kabatereine *et al*., 2004; 2006), hence at risk of being infected.

1.2 Schistosome biology

1.2.1 Species and their hosts

Schistosomiasis is a parasitic disease caused by infection with platyhelminth, trematode blood fluke worms of the genus *Schistosoma*. In addition to infecting man, the parasite infects a range of animals and birds. In human beings the disease is caused by mainly five species: *S. haematobium* in Africa and Middle East; *S. mansoni* in Africa and South America; *S. japonicum* in South East Asia, China and Philippines; *S. mekongi* in South East Asia and *S. intercalatum* in Africa (Rollinson & Southgate, 1987). Specific parasites have

different snail intermediate hosts, egg morphology and final location in the definitive hosts. *S. haematobium* and *S. intercalatum* develop in *Bulinus* species of snails; *S. mansoni*, in genus *Biomphalaria*; *S. japonicum* in genus *Onchomelania* and *S. mekongi* in *Tricula* species (Rollinson & Southgate, 1987). The most common parasites affecting man in Africa are *S. mansoni* and *S. haematobium*, whose adult worms reside in the blood vessels of the intestines and urinary tract respectively.

1.2.2 Life cycle

Schistosomes are digenetic trematodes of the family *Schistosoma*tidae. The adult worms are dioecious with separate sexes. Schistosome life cycle involves an intermediate host, usually a fresh water snail and a definitive host that can be humans, various animals or birds (figure1.1). The life cycle is the same for all species but the focus in this study report is on *S. mansoni*. It begins with schistosome eggs deposited in the environment through stool by an infected person (Jordan & Webbe, 1993). The eggs are non-operculate with an asexual embryo called a miracidium. *S. mansoni* eggs have a lateral spine, which together with host blood pressure and secretion of proteolytic enzymes help the eggs to penetrate and pass through the venule walls into the lumen of intestines and then be excreted in faeces (Jourdane & Theron, 1987; Gryseels *et al*., 2006). When the eggs get into fresh water they hatch into free-living miracidia. Guided by light and chemical stimuli, the miracidia swim and penetrate a compatible snail intermediate host. Over a period of 3-8 weeks within the snail, the miracidia undergo a series of asexual multiplications, from primary (mother) sporocysts through secondary (daughter) sporocysts until they mature into cercariae. Cercariae have small forked tails and are motile. In response to light, free-swimming cercariae are shed from snails into water and cercariae can live for about 24 hours swimming in search of a definitive host (man). When the cercariae penetrate the host skin, they immediately loose their tails and undergo a series of structural changes and transform into schistosomulae. The schistosomulae are carried through the lymphatics and veins to the heart and then to the lungs. From the lungs they migrate to the hepatic sinusoids of the liver, where they mature and female worms pair up with male worms 4-6 weeks after the infection (Jourdane & Theron, 1987). The male worm has a groove called a gynaecophoric canal, in which it clutches the cylindrical female and they live together the

rest of their life (Sturrock, 2001). The paired worms of *S. mansoni* migrate from the liver to settle in the mesenteric veins, where egg-laying takes place. Each female worm lays 100-300 eggs per day. Approximately 50% of the eggs are excreted in faeces to begin the cycle while others are trapped and retained in the host tissue (Gryseels & Nkulikyinka, 1988).

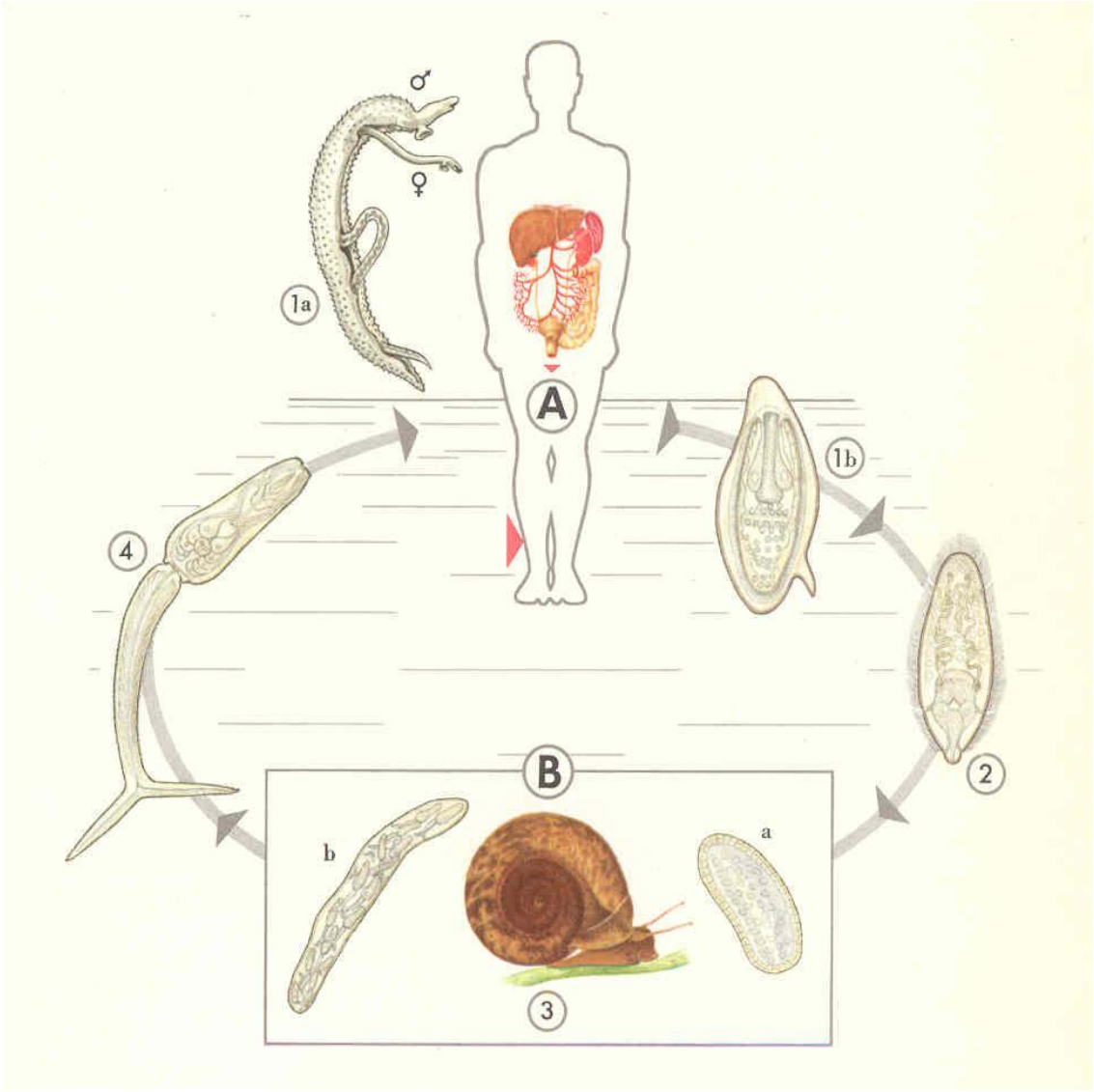

Figure 1. 1: Life cycle of *Schistosoma mansoni* (from Piekarski, 1962)

A: Final host with worms in the mesenteric venules; 1a: A pair of adult worms; 1b: Mature egg of *S. mansoni*; 2: Miracidia.

B: Intermediate host (*Biomphalaria* snail spp); 3a: Mother sporocyst; 3b: Daughter sporocyst

4: Cercaria (infective stage).

1.3 Clinical manifestations of schistosomiasis *mansoni*

Eggs trapped in the host tissues are the major cause of pathology. Some infected individuals remain asymptomatic (Utzinger *et al*., 2001), while others may incur severe irreversible morbidity depending on immunological factors, age, gender and duration of exposure (Doehring-Schwerdtfeger *et al*., 1992; Booth *et al*., 2004). In general, clinical symptoms occur in about 20% of the *S. mansoni* infected people and the level of symptomatology (Van der Werf *et al*., 2003; Booth *et al*., 2004; Conlon, 2005), is associated with infection intensity levels (Boisier, 1995; Ouma *et al*., 2001; Kabatereine *et al*., 2003). However, in certain communities the prevalence of symptoms may be very high (Kabatereine *et al*., 2004: Vennervald *et al*., 2004). Clinical disease can be divided into three categories, as described in the following section, depending on the schistosome stage of infection.

1.3.1 Cercarial dermatitis

When the cercaria penetrates the skin, an immediate reaction to cercarial antigens occurs that leads to cercarial dermatitis, commonly called 'swimmer's itch' and the patient scratches. The dermatitis presents as erythymatous lesions and after approximately 10-15 hours they appear as a maculo papular rash that peaks 2-3 days after cercarial penetration. The lesions disappear spontaneously after 5-7 days (Gryseels *et al*., 2006; Leutscher & Magnussen, 2006). Due to scratching, a secondary infection may occur and result into vesicles or pustules (Leutscher & Magnussen, 2006). Cercarial dermatitis is rare in individuals living in endemic areas and may go unnoticed.

1.3.2 Acute schistosomiasis

Acute schistosomiasis is a systemic hypersensitivity reaction against migrating schistosomulae and starts 3 to 9 weeks after cercarial penetration (Akhiani, 1996). Acute schistosomiasis can occur in both light and heavy infections. The schistosomulae grow into adult worms that lay eggs and the eggs pass through the intestinal walls but approximately 50% of them are trapped in the walls and mediate an immune reaction that cause mucosal granulomatous inflammation and formation of pseudopolyps and scars around the trapped eggs (Vennervald & Dunne, 2004; Gryseels *et al*., 2006) leading to an inflammatory

reaction and the response to this reaction causes a sudden feverish syndrome with severe systemic illness referred to as Katayama fever (Gryseels *et al*., 2006). Individuals with acute schistosomiasis may have enlarged tender livers and on rare occasions a slightly enlarged spleen. Other symptoms associated with acute schistosomiasis include; intermittent abdominal pains, fever, high eosinophilia, headache, malaise, localised oedema, unproductive irritating cough, loss of appetite, weight loss, nausea, vomiting, bloody diarrhoea and muscular pains (Cheever *et al*., 2000; Gryseels *et al*., 2006). The acute stage lasts 14-21 days. As with Swimmer's itch, Katayama fever mainly affects those from non-endemic areas who are exposed to the parasite for the first time, like tourists and immigrants.

1.3.3 Early chronic schistosomiasis

This is an early reaction triggered by some of the retained eggs that are carried to the liver via the portal circulation where they get trapped in the presinusoidal periportal spaces in the liver. The eggs contain a developing miracidium, which releases proteolytic enzymes through minute pores in the egg shell. The released enzymes give rise to typical eosinophilic inflammatory and granulomatous reactions that result in granuloma formation (Mitchell, 1990; Gryseels *et al*., 2006). When the miracidium in the egg dies, usually after about 6-8 weeks, the antigen load decreases and the granuloma sometimes shrinks. Healing of the affected area leads to formation of fibrotic lesions around the portal venules. The granulomatous reactions and the fibrotic lesions may result in hepatosplenic schistosomiasis, which may be accompanied by increased portal pressure. The severity of these symptoms is associated with intensity of infection and related to the type of immune responses generated by the infected individual (Vennervald *et al*., 2004; Gryseels *et al*., 2006). Hepatosplenic schistosomiasis is more common in children and adolescents than in adults (Gryseels & Polderman, 1991; Vennervald *et al*., 2004).

1.3.4 Late chronic schistosomiasis

After several years of infection, the granulomas and fibrotic lesions around the portal venules block the hepatic portal system leading to reduced portal blood flow and portal hypertension develops. This results in Symmer's periportal fibrosis (Gryseels *et al*., 2006).

Portal hypertension may give rise to a hard but not necessarily enlarged liver, splenomegaly and formation of intestinal and oesophageal varices, which when they burst may cause fatal haematemesis (Vennervald & Dunne, 2004; Conlon, 2005). During late chronic stage, some individuals may not excrete eggs but will exhibit irreversible fibrotic streaks and portal vein dilatation (Gryseels & Polderman, 1987; Homeida *et al*., 1988; Gryseels *et al*., 2006).

1.4 Control

Schistosomiasis control measures include regular chemotherapy, health education; good sanitation, avoiding contact with contaminated water and control of the vector snails (Gryseels, 1990; WHO, 2002). Integrating various measures is expected to yield better results. However, snail control is in many instances less applicable because it involves use of molluscicides that kill other aquatic biota. Another option of snail control is modification of snail habitat but this is expensive and not feasible in large water bodies like Lake Victoria. Considering all these limitations, the World Health Organisation changed the schistosomiasis control policy from interrupting the transmission by snail control to morbidity control by treating endemic populations with praziquantel once a year (Engels *et al*., 2002; WHO, 2002; Magnussen, 2003; Richter, 2003).

Praziquantel is still the only drug used for treatment of all schistosome species in Sub-Saharan Africa (Doenhoff *et al*., 2002; Utzinger & Keiser, 2004). It is administered as a single standard dose of 40 mg/kg body weight and treatment now includes pregnant and lactating women (Olds, 2003). Praziquantel is anticipated to give cure rates of 60-90% and egg reduction rates of 90-95% six to twelve weeks after treatment (Ismail *et al*., 1994; Utzinger *et al*., 2000; Danso-Appiah & De Vlas, 2002; WHO, 2002). In the past most of the affected countries could not afford the cost of praziquantel but it is now available at a much cheaper price; treatment per person is estimated to be US $ 0.175 (Cioli, 2000; Fenwick *et al*., 2003; Conlon, 2005). With this reduced price, N'goran *et al*., (2003) anticipated that the control programmes will continue using praziquantel for several years. Furthermore, it can be administered by non-medical personnel and has tolerable mild adverse events (Boisier *et al*., 1994; Jaoko *et al*., 1996; Raso *et al*., 2004; Gryseels *et al*., 2006). However, Stelma *et al*., (1995) observed that some individuals with high pre-treatment schistosomiasis

infection intensity may experience serious adverse events. Praziquantel reduces the number of eggs in the host body by killing the adult worms (Fenwick *et al*., 2003; Gryseels *et al*., 2006) and is also toxic to mature *S. mansoni* eggs (Giboda & Smith, 1994; Richards *et al*., 1989). This will eventually lead to a reduction in morbidity. Many countries have used it in their control programmes (N'Goran *et al*., 2003), among which Egypt treated 20 million people between 1997 and 1999 (Cioli, 2000; Doenhoff *et al*., 2000). Magnussen (2003), in his review of 10 year's experience with schistosomiasis control in different African, Asian and American endemic settings, proposed various treatment targets for *S. mansoni* with regard to prevalence of infection. With a prevalence ≥ 50%, the school-age children should be mass treated once every year; with a prevalence in the range of 20 – 49.9%, all school-age children should be treated once in every 2 years and at a prevalence < 20%, only the infected individuals should be treated or enrolled school children should be treated twice in their school time, i.e. at the beginning and completion of primary school level.

In Uganda, The Bill and Melinda Gates Foundation through the Schistosomiasis Control Initiative supported and launched a Programme for the Control of Schistosomiasis and intestinal worms in 2003, and this support lasted for five years. Currently control of schistosomiasis is integrated within the Neglected Tropical Disease (NTD) Control Programme supported by USAID through Research Triangle Initiative. The major target is school-age children and the strategy used is annual mass treatment of mainly school-age children and also adults from highly endemic areas, using a single dose of 40 mg/kg body weight of praziquantel.

1.5 Statement of the problem

Despite control programmes in a number of countries, schistosomiasis still remains a significant public health problem in Sub-Saharan Africa. In Uganda, schistosomiasis affects approximately 4 million people (Kabatereine *et al*., 2004, 2006) and transmission takes place along all large water bodies, irrigation schemes and big ponds (Bukenya *et al*., 1994; Kabatereine *et al*., 1996). The control strategy aims at reducing morbidity in the endemic areas using chemotherapy (Cioli *et al*., 1995; Engels *et al*., 2002; Olds, 2003). So far praziquantel is the only drug used in Africa for mass administration as a single annual dose

(WHO, 2002; Doenhoff *et al*., 2002; Engels *et al*., 2002; Fenwick *et al*., 2003; Sayed *et al*., 2008).

It is documented that an infected person can excrete eggs immediately after treatment and continue excreting eggs ten weeks after treatment (Cioli, 1998; Botros *et al*., 2005; Gryseels *et al*., 2006) and viable eggs may be present in the host tissue several months after treatment if the infection is not radically cured (Nibbeling *et al.,* 1998). This means that the eggs will sustain egg-stimulated inflammatory granulomatous response which may slow down the process of repair and hamper morbidity regression. Praziquantel works partly by paralysing the worms and causing damage to their tegument and this happens within one hour of ingestion of the tablets. However, the effect of praziquantel is stage-specific mainly killing adult worms and mature eggs, and the half-life of the active component in plasma is approximately 2 hours (Utzinger *et al*., 2000). This implies that with a single dose of praziquantel in a high transmission area, pre-treatment juvenile worms may survive, grow to maturity and start laying eggs some weeks later. It has been observed that a second dose given few weeks apart can kill 90-95% out of the 5-10% previous survivors (Cioli, 2000; Tchuem Tchuente *et al*., 2001; Kabatereine *et al*., 2003).

Studies have been carried out to assess the effect of praziquantel on schistosomiasis prevalence and infection intensity and most of them reported very low cure rates with a single dose of praziquantel. Stelma *et al*., (1995) administered 40 mg/kg body weight and obtained 18-36% cure rate and 77-88% egg reduction rate in Senegal. In another study, Gryseels *et al*., (2001) realised cure rate ranging from 31-36%. In a clinical trial, where one group was treated with praziquantel (40mg/kg body weight) and another one with two doses of 30 mg/kg body weight given 6 hours apart, cure rates were 34% and 44% respectively (Guisse *et al*., 1997). Kabatereine *et al*., (2003) reported cure rates of 41.9% and 69.1% for single dose and double dose given six weeks apart respectively in Uganda.

However, these studies mainly report the effect of repeated treatment on cure rates and egg reduction rates. Considering the aim of our study which is to assess and compare the effect of one versus two doses of treatment on re-infection and morbidity among others; there is

still a gap in knowledge about the effect of different treatment regimens on schistosomiasis infection indicators. Knowledge on comparison of one and two standard doses of praziquantel on morbidity over a long period of time is vital but still lacking. Therefore, in addition to cure rates and egg reduction rates, our study also compared the effect of one versus two treatment doses of praziquantel on morbidity regression and incidence of re-infection.

1.6 Conceptual framework

Schistosomiasis is a debilitating parasitic disease affecting about 200 million people globally (Engels *et al*., 2002). Mortality due to schistosomiasis is estimated at 200,000 deaths per year (Conlon, 2005) with 4.5 million DALYs (WHO, 2002). Morbidity related to schistosomiasis infection ranges from acute to chronic manifestations (Gryseels, 2006). In children, it leads to growth retardation and reduced mental development (Assis *et al*., 1998).

Factors influencing the occurrence of schistosomiasis range from socio-economic to environmental factors. However, literacy levels of a community may influence their economic status. Lack of adequate formal education may lead to poverty, which in turn greatly influences schistosomiasis infection. The poor are always marginalized in that the diseases affecting them are never prioritized, accessibility to health services is poor, they do not get social services like safe water supplies, health education, vector control, all of which enhance schistosomiasis infection. The poor usually engage in activities such as fishing, water development projects involving dams and agricultural irrigation schemes, where sanitation is inadequate and they get exposed to schistosomiasis infection. Dams and rice paddies provide suitable breeding grounds for schistosome snail vectors (Mouchet & Carnevale, 1997). It is also common for people with such occupations to migrate from place to place hence spreading schistosomiasis to other new areas or missing services like mass treatment for schistosomiasis. It is very common for schistosomiasis endemic communities to receive inadequate health services such as poor accessibility to health facilities, lack of disease diagnosis equipment and drugs. Such people end up suffering from chronic inflammatory process due to long term schistosomiasis leading to high

morbidity, mortality, poor work productivity and school performance (Audibert &Leshem *et al*., 2008).

Until recently, control of schistosomiasis was geared towards transmission interruption (Bradley *et al*., 1967; Gryseels, 1990). The current strategy of schistosomiasis control aims at morbidity reduction using chemotherapy. There are a number of anti-schistosomal drugs such as: oxamniquine, a safe but expensive drug that is only effective on *S. mansoni* species; metrifonate which is only effective on *S. haematobium*; and artemisinin derivatives that are effective against young stages of almost all schistosome species but since they are used as anti-malarials, their use in the control of schistosomiasis is limited so as avoid malaria parasite drug resistance. The current anti-schistosomal drug used is a single annual dose of praziquantel, a cheap and broad spectrum drug that kills adult worms of all schistosome species. However, in high transmission areas, it is likely that there are many juvenile worms that would not be affected by one dose of praziquantel. Thus a second dose given two weeks later would kill the previous juvenile worms that survived the first dose. Besides, treatment with praziquantel is reported to enhance resistance to re-infection (Joseph *et al*., 2004). With increased cure rate and reduced incidence of re-infection, schistosomiasis related morbidity is expected to reduce.

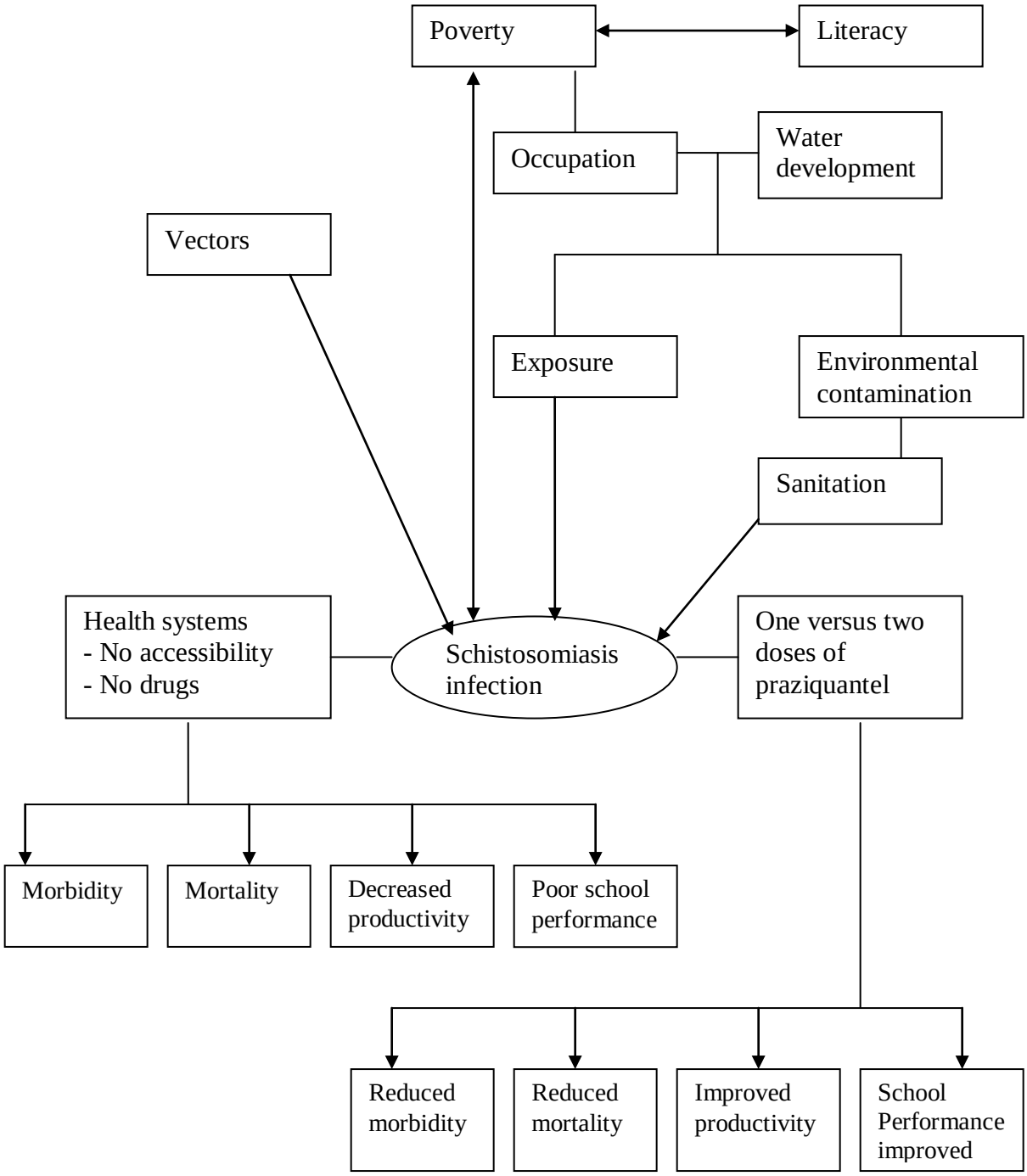

Figure 1.2: Conceptual framework to show factors affecting schistosomiasis infection

1.7 Hypothesis

General hypothesis

The cure rate for *S. mansoni* infection, reduction of re-infection and morbidity regression would be similar after treatment with either two doses of praziquantel given two weeks apart or a single dose.

Specific hypotheses

1. The efficacy of one dose of praziquantel is not different from that of two doses given two weeks apart.
2. Incidence of re-infection after a single dose is not different from the incidence of re-infection after two doses given two weeks apart.
3. Two doses of praziquantel given two weeks apart are not superior to a single dose with regard to morbidity regression.

1.8 Objectives

The major objective was to enhance our knowledge about the use of different praziquantel treatment regimens in the control of schistosomiasis *mansoni*.

The specific objectives were:

1. To describe the epidemiology and morbidity of *S. mansoni* in Musoli village.
2. To describe peoples' exposure to schistosome-contaminated water.
3. To compare the efficacy of one and two doses of praziquantel.
4. To compare the effect of one and two doses of praziquantel on schistosomiasis *mansoni* morbidity, re-infection and children's growth.

1.9 Justification

A post treatment assessment of the Schistosomiasis/Worm Control Programme in Uganda revealed far less egg reduction rates (49%) than the expected of >80% (Balen *et al*., 2006). This implies that many worms were still alive and most likely the released eggs were viable, which may result in poor or no morbidity reduction. It has been observed that infection can be more radically cured by treating twice using standard doses of praziquantel

given a few weeks in between (Guisse *et al*., 1997; Renganathan & Cioli, 1998; Giboda & Smith, 1994; Picquet *et al*., 1998; N'Goran *et al*., 2003).

In Uganda, studies carried out along Lake Albert by Kabatereine *et al*., (2003) where treatment with 40 mg/kg body weight of praziquantel was repeated at an interval of 6 weeks revealed low cure rates. No further intervention studies have been conducted to assess the effect of two doses of praziquantel given at a closer interval and look into its impact on schistosomiasis *mansoni* related morbidity and re-infection. Other studies evaluating the effectiveness of praziquantel have used an interval between the first treatment and second treatment of four or more weeks (Utzinger *et al*., 2000; Gryseels *et al*., 2001; Kabatereine *et al*., 2003; N'Goran *et al*., 2003) whereas our study gave the second dose two weeks after the first dose. This may raise questions as to why we used a shorter interval than that of earlier studies. Despite the fact that the previous studies (Guisse *et al*., 1997; Kabatereine *et al*., 2003) obtained cure rates below the recommended rate of 60 – 90% (WHO, 2002), we considered the life cycle of *S. mansoni*, which takes 4-6 weeks to mature into adult worms after penetration. Basing on the interval of two weeks between the two doses, during which the parasites are expected to mature and the stage specificity of praziquantel action, it was speculated that the second dose given two weeks later would kill the previous juvenile worms which would now be mature and susceptible to praziquantel and also kill some mature viable eggs in the host tissues.

It is appropriate to try and identify different chemotherapeutic strategies for possible adaptation in continuously schistosomiasis exposed communities. Thus there is need to carry out research to find out whether repeating the dose given at a short interval would increase praziquantel efficacy, reduce the incidence and intensity of re-infection and have more effect on morbidity regression and children's growth than a single dose. In order to bridge this gap, a study was carried out to compare the effect of two doses versus one dose of praziquantel (40 mg/kg body weight) on cure rate, egg reduction, morbidity regression, re-infection with *S. mansoni* and children's growth. This study will give an insight contribution to when and how to implement mass chemotherapy and to monitor praziquantel efficacy (Engels *et al*., 2002). The knowledge obtained from this study will be

applied in policy-making to improve on the existing intervention strategies for the control of schistosomiasis.

Schistosoma mansoni in Uganda

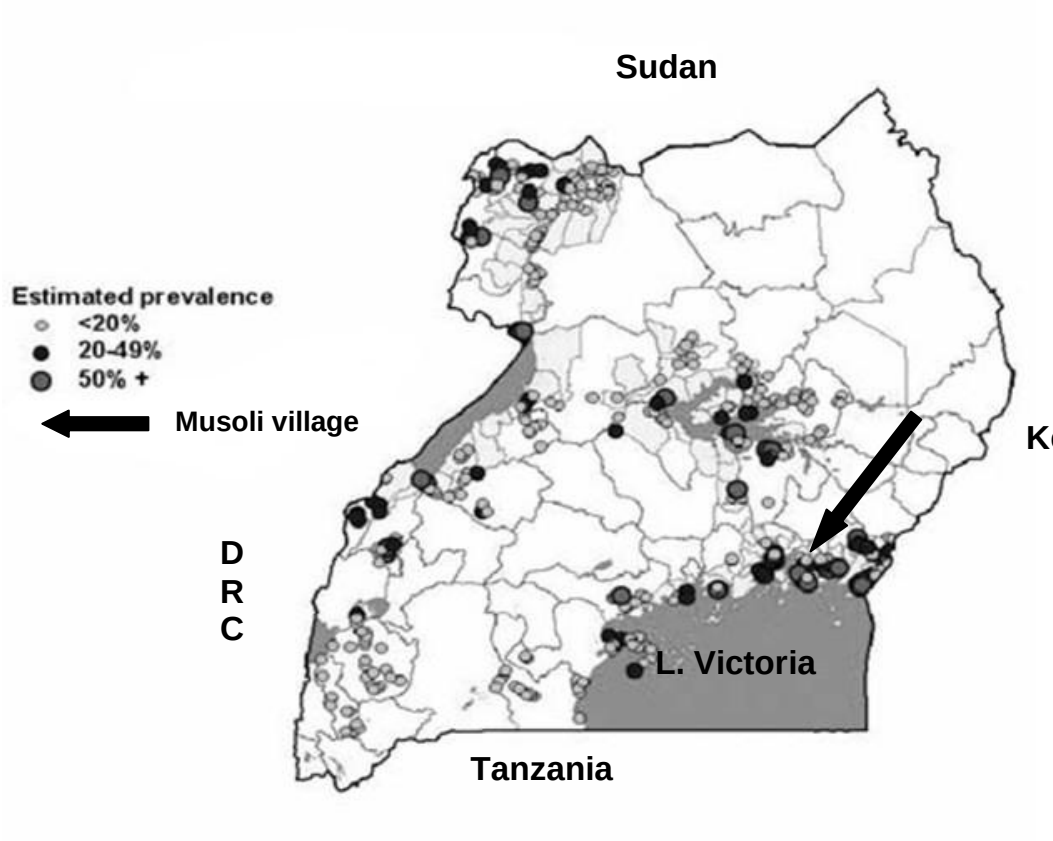

Figure 1. 3: The distribution of *S. mansoni* in Uganda. Map derived from a rapid mapping approach (Brooker *et al*., 2005)

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Chapter 2: Literature review

2.0 Epidemiology

The most common species of schistosomes responsible for schistosomiasis in sub-Saharan Africa are *Schistosoma mansoni* and *S. haematobium* which cause intestinal and urinary schistosomiasis respectively (Chitsulo *et al*., 2000). Schistosomiasis is common in poor communities where sanitation and clean water supply are inadequate. Its distribution has been escalated by environmental changes, water development projects and migrations of people from endemic to non-endemic areas (Savioli, 1997) and it has spread to urban areas in developing countries (Prentice, 1972; Firmo *et al*., 1996, Kabatereine *et al*., 1996). School children in the capital city of Uganda, Kampala, were found to be infected with schistosomiasis and transmission was speculated to have been local (Kabatereine *et al*., 1996).

In Uganda, *S. mansoni* is the species that cause intestinal schistosomiasis (Wright, 1973; Kabatereine, 2000) and infection is prevalent along large water bodies, rivers, swamps, man-made lakes and big ponds, Lake Victoria region inclusive (Prentice *et al*., 1970; Frenzel *et al*., 1999; Kabatereine *et al*., 2004a; Odogwu *et al*, 2006; Karanja, 2002). *S. mansoni* infection along the northern shores of Lake Victoria was reported as early as 1959 (Prentice *et al*., 1970; Prentice, 1972) but with low prevalence (<20%) of infection. It was predicted that with more people settling near the lake shores, schistosomiasis would rise over time (Prentice *et al*., 1970). Thereafter, hyperendemicity of *S. mansoni* in communities living along L. Albert and the Albert Nile has been reported in various studies. Prentice, (1972) recorded prevalence up to 100% in the communities along Albert Nile. Studies along Lake Albert reported a prevalence of 90.7% in Kibale district while in Masindi district a prevalence of 72% was reported (Kabatereine *et al*., 1996; Kabatereine *et al*., 2004b). Schistosomiasis also occurs in water development projects in Uganda as reported by Bukenya *et al*., (1994) who obtained 20% infection rate of *S. mansoni* from workers of Kibimba Rice Scheme.

In the past, the urinary type of schistosomiasis was reported in northern parts of Lake Kyoga (Bradley *et al*., 1967; Wright, 1973) but it was not as widespread as the intestinal

type (Prentice, 1972). However, parasitological and vector sampling surveys conducted between 2003 - 2008 by Vector Control Division (VCD) – Ministry of Health have not mapped any region with urinary schistosomiasis and there is no recent documentation on its occurrence in the country. It appears to have naturally cleared from the environment.

Schistosomiasis affects people of all age groups including children below 5 years (Kabatereine, 2000; Kabatereine *et al*., 2004b). In their study of infants less than 3 years of age living along Lake Victoria in Uganda Odogwu *et al*., (2006) reported a prevalence of 7%, but the overall intensity of infection was below 100 eggs per gram of faeces. However, schistosomiasis has an age distribution pattern of infection prevalence and intensity that peaks between ages 10-19 years and decrease as one grows older (Boisier *et al*., 1995; Kabatereine, 2000; Barakat *et al*., 2000; Kabatereine *et al*., 2004a; Conlon, 2005). The common age pattern of infection levels can be influenced by age-related immune responses (Hagan, 1992; Butterworth, 1998; Woolhouse, 1998; King, 2001) in that IgE antibodies, associated with resistance to infection, have been reported to increase with age (Naus *et al*., 1999; Mutapi *et al*., 2002). Although some authors have attributed higher infection levels in children than in adults to children's higher degree of exposure to infested water (Kabatereine *et al*., 2003; Scott *et al*., 2003), other studies have noted a similar age related intensity pattern in communities where adults are more exposed than children due to their occupation like fishing (Kabatereine *et al*., 1999; Booth *et al*., 2004a). Another study carried out in a community that had migrated from a schistosomiasis free area to a schistosomiasis endemic area in Kenya at 12-18 months after migration, where both children and adults were equally exposed, revealed age pattern of infection typical of an endemic area in that infections were higher in children and declined with age (Ouma *et al*., 1998). Other factors that could affect age-related pattern of infection have been described. During puberty, growth hormone levels increase and induce physiological changes in the young individuals, for example skin thickness increases and more fat is deposited, all of which may inhibit cercarial penetration (Fulford *et al*., 1998), hence reduce infection.

Many studies have observed that males are more heavily infected than females (Bundy, 1988; Bukenya *et al*., 1994; Nakazawa *et al*., 1997; Barakat *et al*., 2000; Kabatereine *et al*.,

2004b) and they have mainly attributed this to males' prolonged occupational exposure to infested water. In situations where exposure time is the same, the sex difference in adults could be due to the nature, time and extent of water contact activities (King, 2001), in that men have more of their bodies immersed in water than females. In most endemic areas, prevalence and intensity of infection are usually high (Utzinger *et al*., 2000; Gryseels *et al*., 2001; N'Goran *et al*., 2003). However, a small proportion of the infected population are reported to carry heavy infections (Utzinger *et al*., 2001; Conlon, 2005), but this may not always be the case as reported by Kabatereine *et al*., (2004b) along Lake Albert where more than 33% of the infected people had >1000 eggs per gram of faeces.

2.1 Cure rates and egg reduction rate

Praziquantel, a broad spectrum antiparasitic drug known to be effective against all species of schistosomes and other trematodes has a cure rate of 60 – 90% and reduce egg load by 90 – 95% using the recommended standard single dose of 40 mg/kg body weight (Raso *et al*., 2004; WHO, 1998, 2002). However, praziquantel is not effective against immature worms (Cioli, 1998) and this may result in poor cure rates especially in high transmission areas where infection levels are high. Studies from a newly infested *S. mansoni* endemic focus in Senegal carried out 12 weeks after treatment with a standard dose of praziquantel recorded very low cure rates and moderate egg reduction rates of 18-36 % and 77-88% respectively (Stelma *et al*., (1995). In Cote d'Ivoire, Tchuem Tchuente *et al*., (2001) found cure rates of 58.1% after a single dose with praziquantel. Gryseels *et al*., (2001) recorded a cure rate of 18% and an egg reduction rate of 86% after treating a Senegalese community whose pre-treatment prevalence was 91% and intensity of infection was >1000 eggs per gram of faeces. In Zaire, Polderman *et al*., (1988) reported cure rates of 47% for children aged 6-20 years and 69% for those above 20 years. Guisse *et al*., (1997) treated one group with 40 mg/kg and another group was given a double dose of 30mg/kg at 6 hours interval. Six weeks later cure rates were 34% and 44% for single and double doses respectively while egg reduction rates were 99% in both groups. Kabatereine *et al*., (2003) recorded cure rates of 41.9% with a single dose and 69.1% with two doses of 40 mg/kg of praziquantel given six weeks apart in a fishing community living in a high transmission area along Lake Albert in Uganda.

It has been suggested that the low cure rates recorded in a number of studies could be due to development of resistance to praziquantel. *S. mansoni* isolates less susceptible to praziquantel have been described in a situation where 2.4 % of infected people living in the Nile delta region in Egypt remained positive for *S. mansoni* after treatment with 40 mg/kg body weight followed by 60 mg/kg body weight of praziquantel (Ismail *et al*., 1994). Studies were carried out to monitor the efficacy of praziquantel and it was reported that the poor cure rates in Senegal could have been due to a very high transmission level in an area not previously endemic to *S. mansoni* (Cioli, 1998; Gryseels *et al*., 2006) in that people had not yet developed an adequate level of acquired immunity to schistosomiasis (Cioli, 1998, 2000; Danso-Appiah & De Vlas, 2002; Gryseels *et al*., 2006; Doenhoff *et al*., 2002). Another school of thought attributed the poor cure rates to high pre-treatment intensity of infection with large numbers of pre-patent infections and immature worms that are not susceptible to praziquantel (Cioli *et al*., 1995; Picquet *et al*., 1998; Berhe *et al*., 1999; Van Lieshout *et al*., 1999; Utzinger *et al*., 2000; N'Goran *et al*., 2001; Danso-Appiah & De Vlas, 2002; Gryseels *et al*., 2006).

More support to high infection intensity yielding low cure rates was by Gryseels *et al*., (2001) who reported findings from school children who had low intensity of infection in a boarding school within a non-transmission area, and were treated with a single dose of 40 mg/kg. Three weeks after treatment, cure rate and egg reduction rate were 93% and 90% respectively. It should be noted that none of the Senegalese studies report findings from egg viability tests, hence it is possible that after treatment some of the excreted eggs were dead. It is common for non-viable eggs to be retained in the tissue up to 10 weeks after treatment (Cioli, 1998; Botros *et al*., 2005). Evidence has shown that when the uncured people are given an extra standard dose or higher dose the cure rates increase (Ismail *et al*., 1994; Gryseels *et al*., 2006). Picquet *et al*., (1998) reported a cure rate of 42.5% after first treatment with 40 mg/kg and egg reduction rate of 70.7%. When the uncured were treated again 40 days later, they yielded cure rate of 76.1% and egg reduction rate of 88.1%. The fact that the rates increased after the second dose indicates that the uncured might not have harboured resistant parasites but probably had pre-patent infections and or immature worms

at the time of the first treatment. Nonetheless, praziquantel being more or less the only drug available for treatment of schistosomiasis caused by all species of schistosomes, the possibility of resistance building up is a substantial issue and needs to be monitored.

2.2 Morbidity

S. mansoni related morbidity is mainly caused by inflammatory and immunological reactions induced by eggs lodged in the host's tissue (Vennervald & Dunne, 2004). Morbidity ranges from acute to chronic manifestations with signs such as persistent abdominal pain, general weakness, fever, high eosinophilia, headache, weight loss, nausea, vomiting, diarrhoea, cough and muscular pains, blood in stool, hepatosplenomegaly, portal hypertension and liver fibrosis (Utzinger *et al*., 1998; van der Werf *et al*., 2003; Gryseels *et al*., 2006). Not all infected people experience morbidity (Utzinger *et al*., 2001) and the level of schistosomiasis related morbidity differs among affected communities and endemic areas (Gryseels, 1992; Boisier *et al*., 2001). Hepatosplenic schistosomiasis, a chronic manifestation of *S. mansoni* can present as early inflammatory hepatic schistosomiasis or late hepatosplenic schistosomiasis with periportal fibrosis. Inflammatory hepatic schistosomiasis usually presents with an enlarged but smooth and firm left liver lobe, and an enlarged firm and hard spleen (Prata, 1982; Gryseels *et al*., 2006). Inflammatory hepatic schistosomiasis is common in children and adolescents and may affect up to 80% of the infected individuals (Gryseels & Polderman, 1991; Gryseels *et al*., 2006; Vennervald *et al*., 2004) and its severity and frequency may be influenced by the intensity of infection (Kabatereine *et al*., 2004b).

Late hepatosplenic schistosomiasis with periportal fibrosis affects young and middle aged adults that have been exposed to infection for at least 5 years (Homeida *et al*., 1988; Booth *et al*., 2004a; Gryseels *et al*., 2006). Periportal fibrosis occurs when collagen is deposited in the periportal space and this may lead to obstruction of the portal veins, which will in turn result into portal hypertension, splenomegaly, dilatation of the portal vein, and development of collateral veins and oesophageal varices. The liver becomes hard and may or may not be enlarged (Gryseels *et al*., 2006). Oesophageal varices may burst due to severe periportal pressure and result in haematemesis that will either be fatal or recur and

cause anaemia. Portal hypertension can also result into ascites (Chen & Mott, 1988; Gryseels, 1990; Kabatereine, 2000).

With *S. mansoni*, it may take 5-15 years for fibrosis to be eminent and by this time, the individual may no longer be excreting eggs (Gryseels & Polderman, 1987; Homeida *et al*., 1988). Studies have shown that periportal fibrosis increases with duration of exposure but not age and this could be the reason why children rarely present with periportal fibrosis (Kardorff *et al*., 1996; Dessein *et al*., 1999; Booth *et al*., 2004a). Nevertheless, children can also develop hepatosplenic schistosomiasis and portal hypertension, but without fibrosis (Gryseels & Polderman, 1987; Boisier *et al*., 2001; Vennervald *et al*., 2004; Gryseels *et al*., 2006 ; Malenganisho *et al*., 2008) and it is influenced by intensity of infection (Arap-Siongok, 1976; Gryseels & Polderman, 1987; Fulford *et al*., 1991; Vennervald *et al*., 2004).

Earlier studies in Uganda reported morbidity due to *S. mansoni* in the West Nile region (Nelson, 1958). Ongom and Bradley (1972) observed a similar occurrence of high morbidity within the same region in that bloody diarrhoea, hepatosplenomegaly and eosinophilia were highly prevalent. It was noted that cases of hepatosplenic disease in the hospital records of West Nile region were related to *S. mansoni* (Ongom *et al*., 1972). Thereafter, more studies have reported on *S. mansoni* related morbidity in most endemic areas. Frenzel *et al*., (1999) reported a prevalence of >46% of periportal thickening in West Nile districts of Uganda. In fishing communities along L. Albert, Booth *et al*., (2004a) reported 31.5% and 6.1% of peri-portal fibrosis in Booma and Bugoigo villages respectively. They attributed this difference in prevalence of fibrosis in the two adjacent villages to duration of exposure to contaminated water, whereby communities in Booma village had been exposed for longer time than communities of Bugoigo village.

Chemotherapy using praziquantel reduces morbidity even in high transmission areas where re-infection levels are high (Frenzel *et al*., 1999). Treatment lowers the patient's worm burden (Fenwick *et al*., 2003) thereby reducing the number and size of hepatic granulomas and provide an opportunity for the liver to resolve the fibrous lesions (Frenzel *et al*., 1999). A number of studies have assessed the effect of treatment on *S. mansoni* related morbidity

and shown that morbidity can regress after treatment but may take several years (Doehring-Schwerdtfeger *et al*., 1992). Doehring-Schwerdtfeger *et al*., (1992) found a higher reduction of periportal fibrosis 23 months after treatment than 7 months after treatment. Kabatereine, (2000) reported a significant decrease from 53.4% to 35.6% in prevalence of hepatosplenomegaly one year after treatment with a standard dose of praziquantel (40 mg/kg body weight).

Richter, (2003) noted that repeated treatment delays the development of severe morbidity. In a study of school-aged children that were treated annually over three years where transmission was interrupted, the prevalence of splenomegaly and hard spleens decreased independently of age (Vennervald *et al*., 2005). Frenzel *et al*., (1999) observed an age-influenced reversibility of periportal thickening after two annual treatments, in that adolescents and young adults had the highest reversibility and those above 30 years had the lowest. In this study, reversibility was higher in males than in females despite similar exposure pattern. In Madagascar, a cohort treated annually for three consecutive years showed a decrease in prevalence of hepatic fibrosis from 28% to 10.3% two years after the first treatment and the prevalence of splenomegaly decreased three years after treatment (Boisier *et al*., 1998). However, the repeated treatment reported in these studies are annual treatments as applied in mass drug administration programmes and not treatments repeated within a short interval of time. The current study assessed whether repeated treatment with praziquantel within an interval of 14 days would have an additional impact on the regression of morbidity over a period of two years.

2.3 Re-infection

Most studies have shown that populations in endemic areas get re-infected. Although *S. mansoni* re-infection intensity is usually lower than that before treatment, it follows a pattern similar to what is seen before treatment, i.e. peaking between 10-14 years and thereafter declining with age (Bensted-Smith *et al*., 1987; Butterworth *et al*., 1988, 1991; Kabatereine *et al*., 1999; Barakat *et al*., 2000; Conlon, 2005; Vereecken *et al*., 2007). With this pattern, adults are less re-infected and it is postulated that this is because adults exhibit less water contact than children (Bensted-Smith *et al*., 1987; Butterworth *et al*., 1991;

Karanja *et al*., 2002; Conlon, 2005). Lack of age-dependent innate resistance to re-infection or duration of infection-dependent acquired immunity (Gryseels, 1994; Fitzsimmons *et al*., 2004; Dunne *et al*., 2006) can also explain the high re-infection levels in children. Age-dependant immunity is further supported by Kabatereine *et al*., (1999) report where adults spent more time in water than children but adults were less re-infected than children.

It is known that schistosome infection triggers the release of IgE antibodies in response to schistosome worm antigens (Webster *et al*., 1998; Naus *et al*., 1999; Mutapi, 2002; Fitzsimmons *et al*., 2004). It is also documented that IgE antibodies against parasite antigens are responsible for resistance to (re)infection (Dunne *et al*., 1992; Fitzsimmons *et al*., 2004; Joseph *et al*., 2004) and the antibody release increases with age (Fitzsimmons *et al*., 2004). Farghaly, (1993) reported that treatment with praziquantel enhances short-lived resistance to re-infection by mediating changes in parasite specific humoral and cellular immune responses. Praziquantel enhances the production of IL-4 and IL-5, Th2 cytokines in response to adult worm antigen and this may result in increased IgE levels (Rihet *et al*., 1991; Dunne *et al*., 1992; Mutapi, 2001; Fitzsimmons *et al*., 2004; Joseph *et al*., 2004; Secor, 2005). However, post-treatment increase in IL-4 and IL-5 is rarely evident in children less than 15 years of age, implying that young children exhibit lower IgE levels after treatment (Rihet *et al*., 1991; Dunne *et al*., 1992; Corrêa-Oliveira, 2000; Secor, 2005) hence low resistance to re-infection. Elsewhere, observations have also shown that worm antigen mediated responses after praziquantel treatment 'vaccinates' people against re-infection (Fallon *et al*., 1992; Dunne *et al*., 1992, 2006; Colley & Secor, 2004; Karanja *et al*., 2002; Vereecken *et al*., 2007). Repeated chemotherapy is expected to enhance this effect and have an impact on re-infection intensities (Richter, 2003).

2.4 Schistosomiasis and haemoglobin levels

It is documented that schistosomiasis leads to reduced haemoglobin levels and cause anaemia (Koukounari *et al*., 2006; Gryseels and Polderman, 1987; Friedman *et al*., 2005). *S. mansoni* may lead to anaemia through the following mechanisms: (i) schistosome eggs rupture the blood vessels around the intestines and blood is lost through stool; (ii) splenic sequestration where the spleen rapidly and prematurely destroys blood cells; (iii)

autoimmune hemolysis and (iv) reduced production of red blood cells by pro-inflammatory cytokines (Friedman *et al*., 2005). It is also reported that the host's red blood cells form a major part of the diet for schistosome adult worms (Dalton *et al*., 1996; McGarvey *et al*., 1996; Gryseels *et al*., 2006), which might enhance the development of anaemia. A study by Sturrock *et al*., (1996) revealed that levels of haemoglobin decreased with increasing intensity of infection. It is evident that schistosomiasis infected children have lower haemoglobin levels than uninfected children (Olds *et al*., 1999; Koukounari *et al*., 2006). On the other hand, some studies failed to show any association between haemoglobin levels and intensity of schistosomiasis infection (Olsen *et al*., 1998; Leenstra *et al*., 2006). These discrepancies might be attributed to confounding factors such as malaria and hookworm infections (Friedman *et al*., 2005; Sturrock *et al*., 1996).

2.5 Effect of schistosomiasis on growth

Schistosomiasis affects children's mental development, physical fitness and growth while in adults it hampers physical fitness and working capacity. Studies on school children reported that schistosomiasis infected ones were more retarded in growth than the uninfected ones (Corbett *et al*., 1992; Parraga *et al*., 1996; Assis *et al*., 1998). Befidi-Mengue *et al*., (1992) in their study of school children in Cameroun observed that height and weight corrected for age were higher in uninfected children than in infected ones. In a study by Parraga *et al.,* (1996), more boys were growth retarded than girls even when intensity of infection was corrected for. This difference was probably due to normal hormone-related growth where girls suddenly grow and reach adolescent stage earlier than boys. It should be noted that in developing countries, alongside infectious diseases, malnutrition in children can be influenced by other factors like poor food quality or inadequate amount of food intake. It is also documented that low haemoglobin levels reduce appetite thereby decreasing dietary intake hence retarding growth mainly in children (Latham *et al*., 1990).

Chemotherapy targeted to kill adult worms is therefore expected to have a positive impact on children's growth (Richter, 2003). However information on the effect of treatment of schistosomiasis with praziquantel on children's growth is limited.

2.6 Exposure to infection

Duration and nature of exposure to infested water influences the levels of (re)infection (Scott *et al*., 2003; Kabatereine *et al*., 2003; Booth *et al*., 2004a) and varies among individuals. Children who commonly go to contaminated water for leisure expose larger parts of their bodies to schistosome cercariae penetration and get more infected than adults (Butterworth *et al*., 1988; Ximenes *et al*., 2001). An association between age patterns of exposure and age patterns of infection prevalence and intensity has been observed (Scott *et al*., 2003). This age pattern of infection and exposure is contrary to some studies where children were more (re)infected than adults and yet adults were more exposed to infested water than children (Kabatereine *et al*., 1999; Chandiwana & Woolhouse, 1991). Elsewhere, water contact observations reported women having more frequent water contact than men but women were less infected than males (Bundy, 1988; Huang & Manderson, 1992; Bukenya *et al*., 1994; Kabatereine *et al*., 1996, 1999, 2004b). In another study, Gazzinelli *et al*., (2001) observed that women 30-39 years of age had more frequent water contacts but no significant difference in prevalence and intensity of *S. mansoni* was realised between females and males. The most pertinent issue here is not frequency of exposure but duration since males spend longer time in contact with water than females (Scott *et al*., 2003; Booth *et al*., 2004a).

Duration of exposure to schistosome infected water has been shown to affect the level of morbidity in a population. Booth *et al*., (2004a) observed an association between the prevalence of liver fibrosis and duration of exposure among communities living along the shores of Lake Albert in Uganda. There was 12-fold increased risk of fibrosis in populations who had lived in the area for more than 22 years as compared to those who had lived there for less than 15 years.

2.7 Co-infection of *S. mansoni* with other parasitic infections

In most parts of sub-Saharan Africa, schistosomiasis is co-endemic with other parasitic diseases such as malaria and hookworms. Similar to schistosomiasis infection, malaria affects the liver and spleen (Sowunmi, 1996; Clarke *et al*., 2002) and may act synergistically in the development of hepatosplenomegaly (Ongom & Bradley, 1972; Booth

et al., 2004b; Wilson *et al*., 2007). Ongom and Bradley, (1972) reported hepatosplenomegaly prevalence above 40% in a schistosomiasis endemic community and noted that the causative effect of hepatosplenomegaly by *S. mansoni* was confounded by malaria infection. An association between hepatosplenomegaly and *Plasmodium falciparum* schizont antigen (Pfs-IgG3) levels was observed in Kenyan school children (Mwatha *et al*., 2003). Comparable prevalence of hepatosplenomegaly was obtained in children with and those without *S. mansoni* but who were all exposed to malaria infection (Wilson *et al*., 2007). Booth *et al*., (2004b) in their study of Kenyan school children found that both *S. mansoni* and malaria are aetiological agents of chronic hepatosplenomegaly. In the same study, three years after treatment with praziquantel, children whose *S. mansoni* intensities of infection had markedly reduced did not exhibit a regression in hepatomegaly and it was postulated that hepatomegaly was maintained by malaria infection (Booth *et al*., 2004b).

Another schistosomiasis-related morbidity symptom influenced by malaria is anaemia (Stephenson, 1993; Sturrock *et al*., 1996; Friedman *et al*., 2005; Koukounari *et al*., 2008). Malaria causes anaemia through: (i) Destruction of the red blood cells by mature asexual intraerythrocytic parasites; (ii) Suppression of erythropoiesis by malaria parasite-induced cytokines (iii) peripheral destruction of red blood cells by the spleen (Krogstad, 1999; Menendez *et al*., 2000).

Another parasitic disease that commonly co-exists with schistosomiasis and influences schistosomiasis related morbidity is hookworm infection (Flemming *et al*., 2006; Raso *et al*., 2006; Hotez *et al*., 2008). The epidemiology and pathology of schistosomiasis and hookworm infection are almost similar. Both parasites feed on the host's blood and render the victim anaemic (Stoltzfus *et al*., 1997; Friedman *et al*., 2005; King *et al*., 2005). Schistosomiasis and hookworm co-infection exacerbates anaemia levels (Ezeamama *et al*., 2005; Brito *et al*., 2006). Olsen *et al*., (1998) in their study in Western Kenya observed a negative relationship between hookworm intensity and haemoglobin levels. This is further supported by a study where children infected with schistosomiasis and geohelminths experienced an increase in haemoglobin levels after treatment with standard doses of

albendazole and praziquantel (Sturrock *et al*., 1996; Olds *et al*., 1999; Bhargava *et al*., 2003; Friis *et al*., 2003; Koukounari *et al*., 2006).

Schistosoma mansoni is endemic in communities living along large water bodies in Uganda and it affects all age groups including children <5 years. Some of the schistosomiasis infected persons remain asymptomatic while others develop morbidity. Schistosomiasis infection may also lead to children's growth retardation. Praziquantel is the drug of choice for the control of schistosomiasis related morbidity. However several studies have reported lower cure rates than the WHO expected ones of 60 – 90% using a single standard dose (40 mg/kg body weight) of praziquantel. It is also reported that after treatment, a number of people living in endemic areas get re-infected but with low *S. mansoni* infection intensities. Morbidity related to schistosomiasis is reported to regress after treatment but it may re-occur approximately 2 years after treatment. In this study, we assessed and compared the effect of two doses of praziquantel given two weeks apart versus a single dose on cure rate, morbidity and re-infection rate. Before intervention, socio-demographic characteristics, infection levels and morbidity indicators were similar in the two treatment groups.

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Chapter 3: Material and methods

3.1 Study area

The study was conducted in Musoli village community on the shores of Lake Victoria, Mayuge district in South East Uganda (Figure 3.2). Mayuge district, with an estimate population of 406,600 people, borders Lake Victoria to the south, Bugiri district to the east, Jinja district to the west and Iganga district to the north. The study area is in the North Western part of Mayuge district (Figure 3.3) and is 15 km from Jinja town, the second largest town in Uganda. There is one major fish-landing site in the study area called Musoli beach, where the majority of human water contact takes place, and other small water contact points along the shores where people access the lake to fetch water for domestic use. There is one primary school and two small trading centres within Musoli village. The nearest Health Centre (level II) is 2 km away in an adjacent village of Busuyi. The study area is near Kakira Sugar Factory and there are many individual-owned sugar plantations in the village. The village lies at an altitude of 1,070 – 1,161 metres above sea level and the vegetation is mainly savannah grassland with scattered tropical forests. There are two peak rainy seasons with 1,250- 2,200 mm of annual rainfall in March - May and September – December. The weather is hot, wet and humid with temperatures ranging from 19 – 27^0 C. Lake Victoria is the only source of water in this village and like other lakes in Uganda (Kabatereine *et al*., 2003), transmission of schistosomiasis is stable and high throughout the year. Musoli village is 6 km on a murram road that connects to the main road going to Jinja.

Mayuge district – the study area

Figure 3. 1: Map of Uganda showing the districts

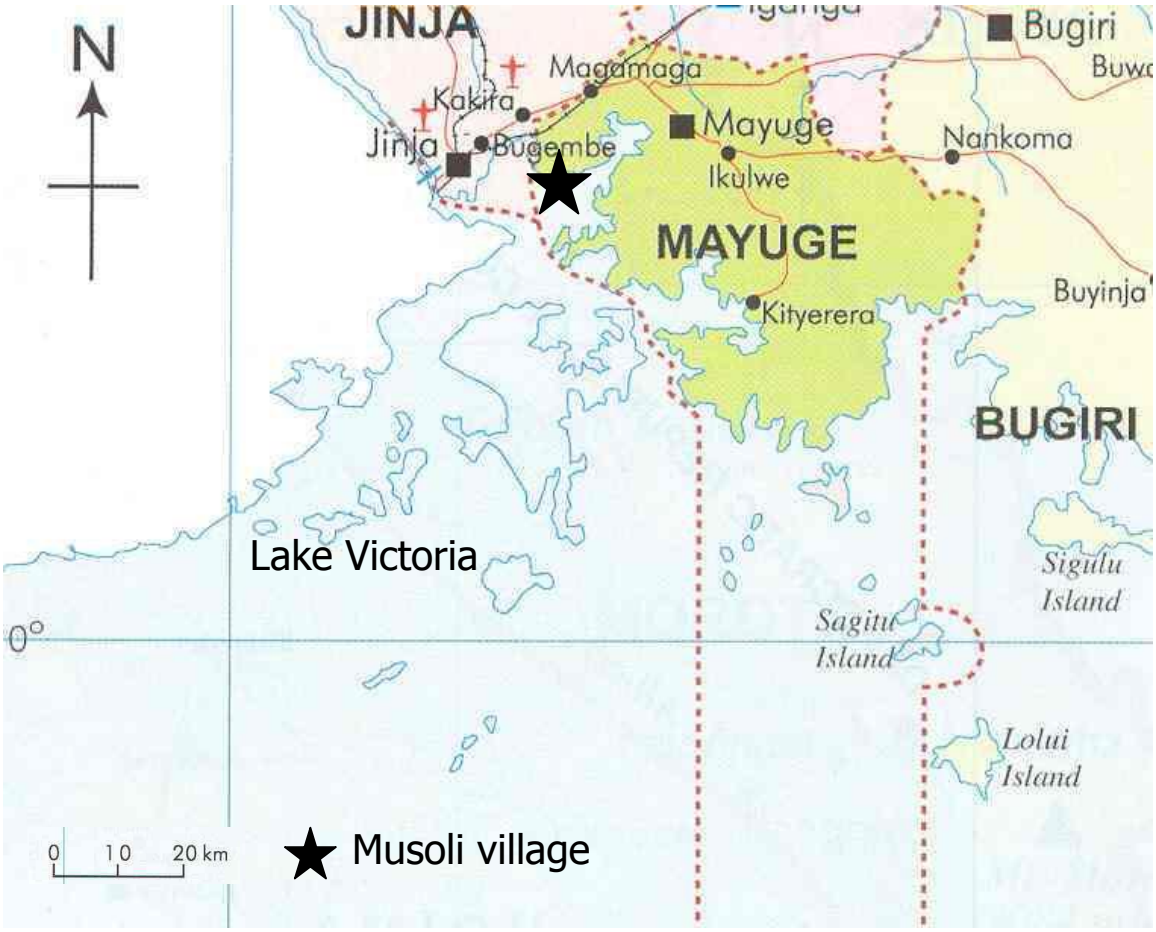

Figure 3. 2: Map of Mayuge district showing the location of the study area

3.2 Study population

From the demographic survey, the village is inhabited by approximately 2,000 people, most of whom were born in the village. The inhabitants belong to 5 major tribes of which Basoga are the predominant. Other tribes are Japadhola, Samia, Atesot and Bagwere. The ethnic heterogeneity is due to the fact that the village is located close to Jinja town, which was in the past 2 decades the most industrialised town in the country. When people from all over the nation came to work in the factories, they settled in the neighbourhood including Musoli village. Being close to a big town, their level of literacy is higher than that of other rural fishing communities and most children go to school. Unlike other fishing communities' settlement pattern where people are clustered at landing sites, people in Musoli village live in relatively scattered homes on individual owned land. They grow a variety of food crops like maize, cassava, sweet potatoes, beans, vegetables and these crops coupled with fish puts their dietary in-take at fairly balanced levels. Fishing is the major economic activity alongside subsistence farming and the village being near a sugar factory, people grow sugar cane as a commercial crop as well as vanilla. Although most people are permanent residents, like any other fishing community, they migrate temporary in search of better fishing grounds. Lake Victoria is the only source of their domestic and recreational needs and this exposes them to schistosomiasis infection.

3.3 Study design

The study consisted of a cross sectional baseline survey followed by longitudinal randomised intervention follow-up surveys (Figure 3.4). At baseline participants were recruited and a cross sectional study was carried out to collect pre-treatment information about the level of *S. mansoni* infection and related morbidity, anthropometric measurements, haemoglobin levels, malaria parasitemia and water contact patterns of the study cohort after which, all participants were treated with a single dose of praziquantel (40 mg/kg body weight). After treatment, participants were randomised to two groups and two weeks later one group received a second dose of praziquantel while the other one was not given any treatment. Nine weeks after the first dose, stool was examined to determine efficacy of praziquantel. Eight and 24 months after treatment, stool was examined to assess *S. mansoni* re-infection levels, anthropometric measurements were taken to assess change

in children's growth, clinical and ultrasound examinations were also performed to assess changes in morbidity.

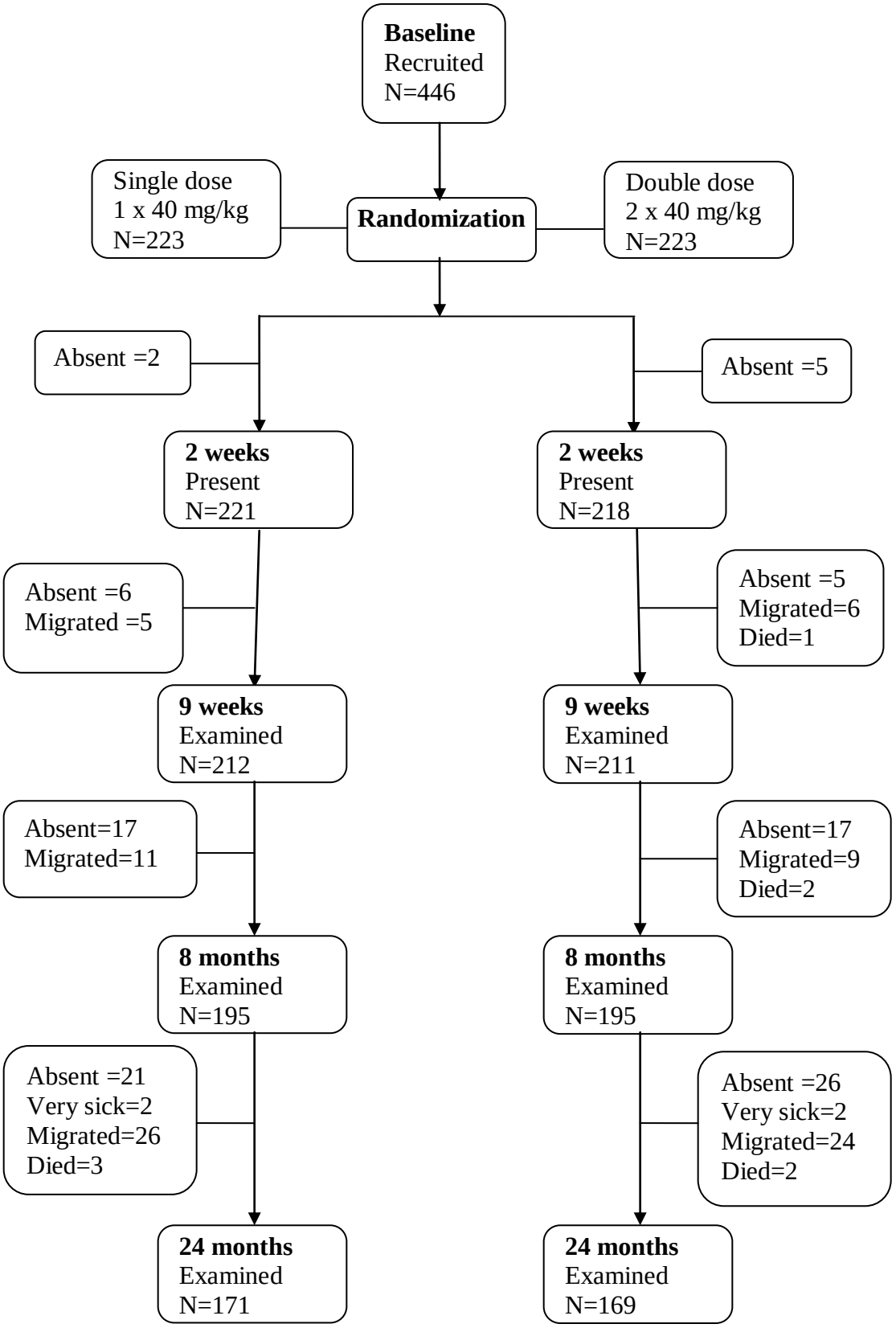

Figure 3.3: Trial profile

3.4 Sample size determination

The primary aim of the study was to compare the effect of one versus two doses of praziquantel on cure rate, re-infection and morbidity regression. The sample size calculation for the cohort was based on the expected difference in cure rate between the two study groups as well as the expected loss to follow-up. Sample size was calculated using Kirkwood's 1988 modified formula for comparing two rates (Hardon *et al.*, 1994). Basing the calculations on cure rates reported in communities living on Lake Albert (Kabatereine *et al.*, 2003) of 41.9% and 69.1% in the first treatment and second treatment respectively, the sample size at 95% significance level and power of 90% was calculated as follows:

$$n = \frac{(u + v)^2 (r_1 + r_2)}{(r_1 - r_2)^2},$$

Where r = cure rate; u = two-sided percentage point of the normal distribution corresponding to the power of 90%; v = percentage point of the normal distribution corresponding to the significance of 95%.

The sample size per group: $\frac{(1.64 + 1.96)^2 (0.42 + 0.69)}{(0.42 - 0.69)^2} = 197$

In order to allow for a 20% annual loss to follow-up (total loss 40% in two years) 79 people were added and each group had 276 participants, giving a total sample size of 552 people to be recruited in the study.

3.4.1 Sampling procedure

Village meetings were held to explain the purpose, activities and implications of the study. Trained and experienced demographers conducted a comprehensive census of the village population, and each household was given a unique identification number that all household members used, followed by an individual number. The inhabitants in each household were registered, their year of birth, gender, occupation, duration of residency in the village and tribal membership were all recorded. Particulars of people living in the household but absent at the time of demography were also recorded. Individuals were asked whether they had ever been treated with praziquantel and if so, when they received the treatment. Coordinates of each household were mapped using a portable Global Positioning

System (GPS) tool. The shoreline, schools, roads and other important features were also mapped. Demography data was entered in the computer and computer generated random numbers were used to select a representative cohort stratified for age and sex. A replacement list was also randomly drawn for substitution of those absent at the time of recruitment into the study. Only individuals > 6 years of age were enrolled because this is the age from which morbidity becomes eminent. After the baseline data collection, a list of all the examined participants was drawn and the cohort stratified according to sex. Again using computer generated random numbers, odd numbers in each stratum were located to single dose group while even numbers were located to double dose group. Randomisation was perfomed by a Scientist, independent of treatment and laboratory testing.

3.5 Data collection methods and procedures

3.5.1 Baseline interview

The questionnaire (appendix I), containing information about schistosomiasis symptoms, sanitary habits and water contact exposure patterns, was developed and translated into Lusoga language, the most commonly used language in the village, by an independent researcher. The questionnaire was back translated to English and corrections of the Lusoga version made accordingly. Thirty questionnaires were administered to a community living in another village with similar socio-demographic characteristics. The questionnaire was edited and interviewing instructions were developed. Interviewers underwent a training session, during which the objectives and methods of the study were explained in detail. Thereafter, they conducted trial interviews and the data was discussed before the actual interview of the study subjects. All the adult participants were interviewed whereas parents or guardians provided the required information for children who could not respond to the questions on their own.

3.5.2 Clinical examination

Each individual that provided three stool samples was clinically palpated by three experienced examiners, i.e. one physician and two nurses, for consistency at baseline, eight and 24 months after treatment. The examiners were all blinded to which treatment group the subjects belonged. The subjects lied on an examination table, with knees bent so as to

relax the abdominal muscles and the abdomen was palpated as previously described (Vennervald *et al.*, 2004). Using a tape measure, the following measurements were taken: the extension of the left liver lobe beneath the sternum was measured in the mid sternal line (MSL); the extension of the right liver lobe beneath the rib cage was measured in the right mid clavicular line (MCL); the extension of the spleen below the rib cage was measured both in the left MCL and left mid axillary line (MAL). The liver and spleen data were translated into a clinical score reflecting the degree of organomegaly (Table 3.1), as described by Vennervald *et al*., (2004) and shown in the clinical form (Appendix II). Each subject was palpated by all the examiners independently and the order of examination was randomised such that each examiner had a chance of being the first. When all the three finished the examination, the obtained measurements were discussed and a final measurement agreed upon and recorded in the clinical form. If the measurements of the three examiners greatly varied, all the examiners repeated the examination and reached a consensus about the final measurements. Eight and 24 months after treatment, all participants were clinically examined again using the same protocol.

Table 3. 1: Clinical scores describing the degree of organomegaly

Score	**Description of organomegaly**
0	No organomegaly
1	Hepatomegaly, splenomegaly or both but with soft consistency. Liver may be tender.
2	Spleen enlarged with firm or hard consistency. No hepatomegaly
3	Liver enlarged, especially along the MSL. May be tender, firm or hard. No splenomegaly.
4	Firm or hard splenomegaly plus firm or hard hepatomegaly, may be irregular.
5	Massive hepatosplenomegaly, with or without ascites and collaterals.

3.5.3 Ultrasound examination

Ultrasonography examination was performed on each study participant using a portable ultrasound machine (SSD 500 Aloka with 3.5 MHz curvlinear - 60% probe). The examination was conducted by two experienced ultrasonographers working alternatively and they were both blinded to the subject's treatment group. Each study subject was examined by one ultrasonographer, who would consult the other ultrasonographer in case of unclear liver texture pattern. The subjects were examined in a supine position lying with their legs stretched on an examination table. The following were measured: the spleen length, splenic vein diameter, liver size measured in the parasternal and mid-clavicular longitudinal lines, segmental left portal branch walls and portal vein diameter measured at the point of entrance into porta hepatis at the ventral lower end of the caudate lobe as described by Wahab and Esmat (1992). The measurements were recorded in an ultrasound form (appendix III). Liver texture patterns were graded as shown in table 3.2 and according to WHO guidelines (Richter *et al*., 2000). Using the same protocol ultrasonography was repeated eight and twenty four months after the second treatment to assess morbidity changes.

Table 3. 2: Liver texture patterns

Pattern	Description of pattern
A	Normal structure
B (B1, B2)	Diffuse echogenic foci ('feather streaks', 'flying saucers', 'spider thickening')
Patterns related to schistosomiasis *mansoni* infection	
C (C1, C2)	Highly echogenic 'ring echoes', which correspond to the 'pipe stems' seen in a scan perpendicular to the one where rings are seen.
D (Dc)	Highly echogenic 'ruff' around portal bifurcation and main stem
E (Ec)	Highly echogenic 'patches' expanding from the main portal vein and branches into the parenchyma.
F (Fc)	Highly echogenic 'bands' and 'streaks' extending from the main portal vein and its bifurcation to the liver surface, where they retract the organ surface.
Patterns indicating pathology different from periportal fibrosis	
X	Diffusely coarse liver texture, irregular liver surface, distorted hepatic veins, rounded caudal liver edge
Y	Diffusely increased liver echogenicity, loss of highly reflective edges of peripheral portal branches, possibly distal sound extinction, rounded caudal liver edge.
Z	Any other liver abnormalities.

3.5.4 Anthropometric measurements

A single set of anthropometric measurements was taken at all time points. Standing in an upright position and without shoes, each individual's height and weight measurements were taken. The height was measured to the nearest 0.1 centimetres using a portable stadiometer and weight measured to the nearest 0.1 kg using a Seca portable digital scale. A waxed paper insertion tape and a calliper were used to measure mid upper arm circumference (nearest to 0.1 cm) and triceps skinfold thickness (nearest to 0.1mm) respectively. These

were taken between the bony points of the shoulder and elbow of the left arm hanging relaxed. Anthropometry assessment was repeated eight and 24 months after treatment. The person measuring was blinded to the participant's treatment group allocation.

3.5.5 Laboratory examinations

3.5.5.1 Stool

In order to improve diagnostic sensitivity, three early morning stool specimens were taken from each participant on three consecutive days (Cheever *et al*., 1994; Utzinger *et al*., 2001; Booth *et al*., 2003). From each specimen; two slides were prepared using the modified Kato Katz thick smear technique (Katz *et al*., 1972) with a 50 mg template, giving rise to six slides per participant. Two experienced technicians examined the slides under the microscope (10x) within one hour of slide preparation so as to assess presence of hookworm eggs.

In order to minimise intra-observer variation, both technicians read slides from the same sample in such a way that each technician read one of the duplicate slides. The numbers of *S. mansoni* eggs observed per slide were recorded. The technicians were blinded to the participant's treatment group. Other intestinal helminths were not quantified; instead they were reported as positive or negative. Stool examination was repeated nine weeks after treatment to determine those who were stool egg negative and again at eight and 24 months to determine the re-infection levels.

3.5.5.2 Blood sampling for malaria and haemoglobin

A finger prick blood sample was taken from each subject and a thick blood smear prepared on a microscope slide for diagnosis of malaria parasites. The slides were stained with Giemsa for 20 minutes, thereafter washed and dried. The slides were read on a microscope under an oil-immersion objective magnification 40x and the number of malaria parasites per 200 white blood cells recorded. Another drop of blood was absorbed into a micro-cuvette, inserted into a portable photometer (HemoCue Hb 201^{+} Analyser manufactured by Quest Diagnostics Company, Norrköping - Sweden) and haemoglobin readings taken

directly from the machine. Blood sampling was repeated eight and 24 months after the second treatment to assess malaria infection and haemoglobin change.

3.5.6 Treatment

After all baseline examinations were completed, each study participant from both groups was treated with a single dose of praziquantel (40 mg/kg body weight) and one tablet of albendazole 400 mg to clear other intestinal helminths. The brands of albendazole and praziquantel used were Alzental® 400mg and Distocide® 600 mg respectively all manufactured by Shin Poong Pharmaceuticals, Seoul Republic of Korea. Two weeks later, one of the groups received another dose of praziquantel while the other did not receive any treatment. Treatment was performed by and under direct observation of an experienced nurse. A piece of bread and a cold soft drink were given to each participant before swallowing the drug so as to minimise occurrence or magnitude of any adverse events. Participants were kept at the field research station for two hours after treatment to observe and manage any possible adverse events.

3.6 Data management and analysis

3.6.1 Quality control

Results from different observers were not compared, instead for stool, quality control was the responsibility of an independent experienced microscopist reading a random 10% of slides, after which the results were compared and where there was controversy, all slides of the same individual were read by different experienced technicians. Clinical and ultrasound examinations were performed by more than one person and results compared. Data was double-entered by two experienced data entry clerks using excel computer programme and the two files were cleaned, compared and a master file compiled, which was used for all analyses.

3.6.2 Data management

Data were imported from Excel computer programme to Stata for windows (Intercooled Stata 10.1, Stata Corporation, USA) for analysis. From baseline interview, data were entered in the computer as recorded in the questionnaire, after which similar responses were

grouped and each group assigned one code. The codes were used during data analysis. Frequency of water contact was defined as number of days per week one went into the lake. The arithmetic mean of the individual *S. mansoni* egg counts was calculated from all the six slide results and multiplied by 20 to obtain each individual's eggs per gram (epg) faeces. Histograms, with normal curves, of continuous dependent variables were generated before analysis to check whether data were normally distributed and where it did not follow a normal distribution, the data was normalised using appropriate transformation. The individual egg counts were logarithmically transformed and the intensity of infection was obtained as the geometric mean of the egg counts per gram of faeces of positive ones only. The egg reduction rate (ERR) was calculated as:

$$\frac{1 - \text{Geometric mean intensity after treatment}}{\text{Geometric mean intensity before treatment}} \times 100$$

Intensity of infection was categorised based on World Health Organisation criteria as: 1-99, 100-399, ≥400 defined as low, moderate and heavy intensities of infection respectively (WHO, 2002). The cure rate was calculated as the proportion of treated persons who were egg-positive at baseline but became negative nine weeks after treatment. Re-infection was defined as persons cured at nine weeks but who became positive again eight or 24 months after treatment.

Malaria parasites were counted against 200 white blood cells (WBC). Using an estimate of a normal WBC count at 8000 cells/μL of blood, the number of parasites counted on a slide was multiplied by 40 (8000/200) to obtain malaria parasite density per microlitre of blood (Cheesbrough, 2005). Anaemia was based on haemoglobin (Hb) levels according to age and sex and was defined as: Hb < 115 g/L for children 5 – 11 years; Hb <120 g/L for children 12 – 14 years; Hb <120 g/L for non-pregnant women ≥ 15 years; Hb <110 g/L for pregnant women; Hb <130 g/L for men ≥ 15 years (WHO, 2001). For growth assessment, only children ≤ 15 years of age were considered. Z-scores of height-for-age (HAZ); weight-for-age (WAZ) were calculated using Nutritional Index Calculator, Epi Info, Version 6.04 (Centers for Disease Control and Prevention, USA). Body Mass Index (BMI) was calculated as weight in kilograms divided by the square of height in meters (kg/m^2).

For assessment of ultrasonographically measured spleen length and portal vein diameter (PVD), height categories were created as follows: 110-129, 130-149, 150-169, ≥170 cm according to normograms suggested by Richter *et al*., (2000) of heights from Senegalese non-infected population (Yazdanpanah *et al*., 1997). Ultrasound parameters were standardised upon these height categories and scores were calculated for each ultrasound parameter within each height category. Individual ultrasound values were expressed as standard deviations (SD) away from the mean of the height-category. PVD and splenic length values below or equal to the mean + 2 SD were classified as normal; if they were > mean + 2 SD but ≤ mean + 4 SD they were classified as moderately abnormal and they were considered markedly abnormal if they were > mean + 4 SD.

3.6.3 Data analysis

Baseline analysis included data from all the recruited participants while follow up analysis was performed for only those who were infected with *S. mansoni* before treatment. Pair-wise correlation was used to determine the pre-treatment relationships of *S. mansoni* infection against liver and spleen sizes while correcting for malaria infection as a confounder. Student`s t test and ANOVA were applied to compare means of height, weight, intensity of *S. mansoni* infection and haemoglobin levels between various parameters before treatment. They were also used to compare children's height and weight among the two treatment groups. The effect of the two dose regimens on mean intensity of re-infection, organ sizes and haemoglobin levels was tested using Student's t test. Also used was the 95% confidence interval (95% CI) of GMI to compare the effect of the two treatment regimens on mean intensity of re-infection. Paired t tests were performed to compare the mean differences of height and weight between different time points.

Odds ratios and 95% CI were applied to compare proportions of organ consistency, organomegaly, anaemia, water contact activities, ultrasound measured splenic length and portal vein diameter morbidity indicators with regard to *S. mansoni* infection before treatment. Cure rates of the two dose groups were compared using risk ratios (RR) and their corresponding 95% CI. Chi square tests were used to compare proportions of growth parameters, organ consistency, organomegaly and anaemia between the two dose groups.

Proportions and their 95% CI were used to compare incidence of re-infection between the two treatment groups. A *P* value <0.05 was used to determine statistical significance in all analyses.

3.7 Ethical considerations

Ethical approval was obtained from the Higher Degrees Research and Ethics Committee of the School of Public Health, Makerere University. Ethical clearance was obtained from the Uganda National Council of Science and Technology. This study was supported by DANIDA, thus clearance was also sought from the Danish National Committee on Biomedical Research Ethics in Denmark. Consent forms were developed in the local language. Although most of the potential study participants could read the consent forms themselves, the purpose and contents of the study were explained in detail to the community in the local language. They were informed that the decision to participate in the survey was voluntary and any one who wished to withdraw was free without any reprimand. Informed consent was obtained from individual adult participants but for children; the parents or guardians consented on their behalf. Thereafter, each individual signed a consent form before commencement of any activity. A sterile and less paining sharp lancet was used to prick the participants. All information obtained from participants was kept confidential.

To minimise occurrence of known side effects of praziquantel when taken on an empty stomach, it is advisable to take the tablets after at least a light meal. Thus, a snack and a soft drink were given before treatment. A trained nurse was available during treatment to attend to any adverse event. Other minor ailments like laboratory diagnosed malaria, anaemia, diarrhoea and others were treated according to the national guidelines. Adhering to the National Schistosomiasis and Worm Control Programme strategy of mass treatment in endemic areas, the rest of the community was treated after the second treatment of the cohort.

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Chapter 4: Epidemiology and morbidity of *Schistosoma mansoni* in Musoli village along Lake Victoria, Uganda

Summary

A cross-sectional survey was carried out to assess the epidemiology and morbidity of *Schistosoma mansoni* in the community of Musoli village, along Lake Victoria in Uganda. Water contact interviews, anthropometric measurements, parasitological, clinical and ultrasonographical examinations of 446 people, comprising of 217 females and 229 males, with mean age of 26 years (SD ± 16, range 7-76 years) were performed. The prevalence of *S. mansoni* was very high (88.6%, 95% Confidence interval [95% CI]: 85.6 – 91.5). The geometric mean intensity (GMI) of *S. mansoni* was 236.2 (95% CI: 198.5 – 460.9) eggs per gram (epg) faeces. Males had significantly higher GMI (370.2 epg) than females (132.6 epg) ($t = 0$, $P<0.001$). Age significantly affected GMI ($t = 169$, $P<0.001$). Occupation influenced GMI in that fishermen had the highest GMI ($F = 26.8$, $P<0.001$). People who were engaged in all the reported activities in the lake had the highest GMI than those who had fewer activities ($P<0.001$).

Splenomegaly, hepatomegaly and hepatosplenomegaly were found in 4.9%, 24.2% and 30.3% of the study population respectively. There was no significant difference in occurrence of organomegaly between *S. mansoni* infected and non-infected people. Liver image patterns C and D indicative of fibrosis were found in 2.2% and 0.2% of the cohort respectively. Moderately and marked dilated portal vein diameters were found in 10.3% and 0.2% respectively. Prevalence of moderately enlarged spleens was 47% while that of severely enlarged spleens was 21.8%. *S. mansoni* intensity of infection was associated with portal vein dilatation (coefficient = 0.139, $P = 0.006$) and abnormal spleen length (coefficient = 0.149, $P = 0.003$). Anaemia was observed in 36.4% of the participants but it was not associated to *S. mansoni* intensity of infection. Intensity of *S. mansoni* significantly affected the level of stunting ($t = -2.86$, $P = 0.005$) but there was no evidence of the effect of intensity on wasting and underweight. The major water contact activities reported were fishing, swimming or bathing and washing clothes or utensils. Those who reported to go to the lake and perform all of the mentioned activities had significantly higher intensity of *S.*

mansoni infection (F = 18.76, *P*<0.001). We conclude that *S. mansoni* and hepatosplenomegaly are prevalent in Musoli village, however periportal fibrosis is rare.

4.1 Introduction

Schistosoma mansoni infection affects over 200 million people worldwide (Engels *et al*., 2002). It causes hepatomegaly and splenomegaly in 8.5 and 6.3 million people respectively in sub-Saharan Africa (van der Werf *et al*., 2003). In Uganda, *S. mansoni* is the major cause of schistosomiasis (Wright, 1973; Frenzel *et al*., 1999; Kabatereine, 2000). It occurs mainly in rural areas along large water bodies and affects more than 10% of the population (Kabatereine *et al*., 2004a, 2006). It has also been reported in urban populations (Kabatereine *et al*., 1996a), along small streams (Odongo-Aginya *et al*., 1994) and in irrigation canals (Bukenya *et al*., 1994). Schistosomiasis related morbidity has been reported in various studies (Frenzel *et al*., 1999; Kardorff *et al*., 1996; Dessein *et al*., 1999; Kabatereine, 2000; Booth *et al*., 2004a; Vennervald *et al*., 2004; Malenganisho *et al*., 2008). Schistosome eggs trapped in the liver cause granulomatous reactions and lead to formation of periportal fibrosis which may in turn cause hepatic portal hypertension (Gryseels *et al*., 2006).

The results presented here are from a cross-sectional study that was carried out to describe the epidemiology of *S. mansoni* infection and its related morbidity among communities living along Lake Victoria. Malaria infection, which is known to aggravate schistosomiasis *mansoni*-related hepatosplenomegaly (Ongom & Bradley, 1972; Mwatha *et al*., 2003; Booth *et al*., 2004b; Wilson *et al*., 2007) and hookworm infection, which affects haemoglobin levels (Olsen *et al*., 1998; Muller, 2002; Friedman *et al*., 2005; King *et al*., 2005) were also assessed.

4.2 Material and methods

4.2.1 Study area and population

The study was conducted in Musoli village community on the shores of Lake Victoria, Mayuge district in South East Uganda. Mayuge has an estimate population of 406,600 people and it borders Lake Victoria to the south, Bugiri district to the east, Jinja district to the west and Iganga district to the north. The weather in this area is hot, wet and humid with temperatures ranging from 19 – 27^0 C. Like the rest of other lakes in Uganda,

transmission of schistosomiasis in Lake Victoria is stable and high throughout the year (Kabatereine *et al*., 2003).

From the demographic survey, Musoli village is inhabited by approximately 2,000 people, most of who were born in the village. The major tribes are Basoga, Japadhola, Samia, Atesot and Bagwere. The level of literacy is high as compared to other fishing communities and most children go to school. Unlike other fishing communities where people are clustered at landing sites, people in Musoli village live in relatively scattered homes on individual owned land. Other than subsistence farming, fishing is the major economic activity. Lake Victoria is the only source of their domestic and recreational needs and this exposes them to schistosomiasis infection.

4.2.2 Study design

This chapter reports baseline results of a longitudinal randomised intervention study. The baseline survey assessed the level and intensity of *S. mansoni* infection, morbidity, children's anthropometric trends, water contact patterns, haemoglobin levels, hookworm infection and malaria parasitemia of the study cohort.

4.2.3 Sample size determination and sampling procedure

The primary aim of the major study was to compare the effect of one versus repeated doses of praziquantel on *S. mansoni* cure rate, re-infection and morbidity regression. Sample size was calculated using Kirkwood's 1988 modified formula for comparing two rates (Hardon *et al*., 1994). Basing the calculations on cure rates reported from a study of communities living along the shores of Lake Albert (Kabatereine *et al*., 2003) of 41.9% and 69.1% with a single dose and two doses respectively, the sample size at 95% significance level and power of 90% was calculated as follows:

$$n = \frac{(u + v)^2 (r_1 + r_2)}{(r_1 - r_2)^2},$$

Where r = cure rate; u = two-sided percentage point of the normal distribution corresponding to the power of 90%; v = percentage point of the normal distribution corresponding to the significance of 95%.

The sample size per group: $\frac{(1.64 + 1.96)^2 (0.42 + 0.69)}{(0.42 - 0.69)^2} = 197$

The sample size per group was 197 and in order to allow for a 20% annual loss to follow-up (total loss 40% in two years) 79 people were added and each group had 276 participants, giving a total sample size of 552 people to be recruited in the study. However, a sample size required for this part of the study to detect a true difference, was calculated using Statcalc calculator, Epi Info, version 6.04 (Centers for Disease Control and Prevention, USA). Considering sex as one of the risk factor and a probability of 95% with power of 80%, a sample size of 85 was obtained for each group. Since this sample size is smaller than the calculated one for the cohort study, the used cohort sample size is adequate enough to detect a difference in *S. mansoni* levels of infection between the risk factors.

All households in the village were given a unique identification number that all household members used, followed by an individual number. Data was entered in the computer and computer generated random numbers were used to select a representative cohort stratified for age and sex. A replacement list was also randomly drawn for substitution of those absent at the time of recruitment into the study. Only individuals > 6 years of age were enrolled because this is the age from which morbidity becomes eminent.

4.2.4 Data collection methods

4.2.4.1 Baseline interview

The questionnaire (appendix I), containing information about schistosomiasis symptoms, sanitary habits and water contact exposure patterns, was developed and translated into Lusoga language, the most commonly used language in the village, by an independent researcher. The questionnaire was back translated to English and corrections of the Lusoga version made accordingly. Thirty questionnaires were administered to a community living in another village with similar socio-demographic characteristics. The questionnaire was edited and interviewing instructions were developed. Interviewers underwent a training session, during which the objectives and methods of the study were explained in detail. Thereafter, they conducted trial interviews and the data was discussed before the actual interview of the study subjects. All the adult participants were interviewed whereas parents

or guardians provided the required information for children who could not respond to the questions on their own.

4.2.4.2 Anthropometric measurements

A laboratory technician was trained in anthropometry, after which he performed the anthropometric measurements. A single set of anthropometric measurements was taken. Standing in an upright position and without shoes, each individual's height and weight measurements were taken. The height was measured to the nearest 0.1 centimetres using a portable stadiometer and weight measured to the nearest 0.1 kg using a Seca portable digital scale.

4.2.4.3 Stool

In order to improve diagnostic sensitivity, three early morning stool specimens were taken from each participant on three consecutive days (Cheever *et al*., 1994; Utzinger *et al*., 2001; Booth *et al*., 2003). From each specimen; two slides were prepared using the modified Kato Katz thick smear technique (Katz *et al*., 1972) with a 50 mg template, giving rise to six slides per participant. Two experienced technicians examined the slides under the microscope (10x) within one hour of slide preparation so as to assess presence of other helminth eggs. In order to minimise intra-observer variation, both technicians read slides from the same sample in such a way that each technician read one of the duplicate slides. The number of *S. mansoni* eggs observed per slide was recorded. Hookworm infection was not quantified but reported as positive or negative.

4.2.4.4 Blood sampling for malaria parasitaemia and haemoglobin

A finger prick blood sample was taken from each subject and a thick blood smear prepared on a microscope slide for diagnosis of malaria parasites. The slides were stained with Giemsa for 20 minutes, thereafter washed and dried. The slides were read on a microscope under an oil-immersion objective 40x and the number of malaria parasites per 200 white blood cells was recorded. Another drop of blood was absorbed into a micro-cuvette, inserted into a portable photometer (HemoCue Hb 201^{+} Analyser manufactured by Quest

Diagnostics Company, Norrköping - Sweden) and haemoglobin readings taken directly from the machine.

4.2.4.5 Clinical examination

Each individual that provided three stool samples was clinically examined by three experienced examiners, i.e. one physician and two nurses, for consistency. This is likely not to have created any bias since the status of *Schistosoma mansoni* infection was the focal parameter and this applied to measurement of all the other parameters. The subjects lied on an examination table, with knees bent so as to relax the abdominal muscles and the abdomen was palpated as previously described (Vennervald *et al.,* 2004). Using a tape measure, the following measurements were taken: the extension of the left liver lobe beneath the sternum was measured in the mid sternal line (MSL); the extension of the right liver lobe beneath the rib cage was measured in the right mid clavicular line (MCL); the extension of the spleen below the rib cage was measured both in the left MCL and left mid axillary line (MAL). The liver and spleen data were translated into a clinical score reflecting the degree of organomegaly as described by Vennervald *et al*., (2004) and shown in the clinical form (Appendix II). Each subject was palpated by all the examiners independently and the order of examination was randomised such that each examiner had a chance of being the first. When all the three finished the examination, the obtained measurements were discussed and a final measurement agreed upon and recorded in the clinical form. If the measurements of the three examiners greatly varied, all the examiners repeated the examination.

4.2.4.6 Ultrasound examination

Ultrasonography was performed using a portable ultrasound machine (SSD 500 Aloka with 3.5 MHz curvlinear - 60% probe). The examination was conducted by two experienced ultrasonographers working alternatively and they were both blinded to the subject's treatment group. Each study subject was examined by one ultrasonographer, who would consult the other ultrasonographer in case of unclear liver texture pattern.. The subjects were examined in a supine position lying with their legs stretched on an examination table. The following were measured: the spleen length, splenic vein diameter, liver size measured

in the parasternal and mid-clavicular longitudinal lines, segmental left portal branch walls and portal vein diameter measured at the porta hepatis at the ventral lower end of the caudate lobe as described by Wahab and Esmat (1992). The measurements were recorded in an ultrasound form (appendix III). Liver texture patterns were graded according to WHO guidelines (Richter *et al.*, 2000).

4.2.5 Data management and analysis

4.2.5.1 Quality control

Results from different observers were not compared, instead an independent experienced microscopist read a random 10% of the stool slides, after which the results were compared and where there was controversy, all the six slides of the same individual were read by two different experienced technicians and the counts were harmonised. Data was double-entered by two experienced data entry clerks using excel computer programme and the two files were cleaned, compared and a master file compiled, which was used for all analyses.

4.2.5.2 Data management

Data were entered into Excel computer programme and later exported to Stata for windows (Intercooled Stata 10.1, Stata Corporation, USA) for analysis. Two age categories, for children (<15 years) and adults (15 years and above) were created and these were used in analysis. Histograms of continuous dependent variables with normal curves were generated before analysis to check whether data were normally distributed and where it did not follow a normal distribution, the data was normalised using appropriate transformation. The arithmetic mean of the individual *S. mansoni* egg counts was calculated from all the six slide results and multiplied by 20 to obtain each individual's eggs per gram (epg) faeces i.e. *S. mansoni* infection intensity. *S. mansoni* infection intensity was found to be skewed, thus individual egg counts were normalised using base 10 logarithmic transformations. The geometric mean intensity (GMI) of infection was obtained as the antilog of the mean of the transformed egg counts of positive ones only and is reported as eggs per gram (epg) of faeces. Intensity of infection was categorised based on World Health Organisation criteria as: 1-99 epg, 100-399 epg, ≥400 epg defined as low, moderate and heavy intensities of infection respectively (WHO, 2002). Malaria parasites were counted against 200 white

blood cells (WBC). Estimating a normal WBC count at 8000 cells/μL of blood (Cheesbrough, 2005), to obtain malaria parasite density per microlitre of blood, the individual number of malaria parasites were multiplied by 40 (8000/200).

Height categories, matched to those of the Senegalese non-infected population (Yazdanpanah *et al*., 1997), were created as follows: 110-129.9, 130-149.9, 150-169.9, ≥170 cm. The spleen length and portal vein diameter measured by ultrasound were standardised upon these height categories and normal scores were calculated for each parameter within each height category using normograms of the Senegalese population (Richter *et al*., 2000). Individual ultrasound values were expressed as standard deviations (SD) away from the mean of the height-category. Portal vein diameter and spleen length values below or equal to the mean + 2 SD were classified as normal; if they were > mean + 2 SD but ≤ mean + 4 SD they were classified as moderately abnormal and they were considered severely abnormal if they were > mean + 4 SD of the values for the corresponding height groups from a Senegalese non-infected population (Yazdanpanah *et al*., 1997) as suggested by Richter *et al*., (2000).

Anaemia was based on haemoglobin levels according to age and sex and was defined as: Hb < 115 g/L for children 5 – 11 years; Hb <120 g/L for children 12 – 14 years; Hb <120 g/L for non-pregnant women ≥ 15 years; Hb <110 g/L for pregnant women; Hb <130 g/L for men ≥ 15 years, (WHO, 2001). Height and weight of children below 16 years were compared with those of World Health Organisation recommended reference curve for international use to derive z-scores. Growth was analysed for only children below 15 years of age because this is the growth stage that is more susceptible to debilitating effects of schistosomiasis infection than the older age (Stephenson, 1993; Parraga *et al*., 1996; Assis *et al*., 1998; King *et al*., 2005; Corbett *et al*., 1992; Zhou *et al*., 2005; Leenstra *et al*., 2006). Growth reference curve from American children collected by the National Centre for Health Statistics was used. Z-scores of height-for-age (HAZ); weight-for-age (WAZ), obtained as the difference between the value for an individual and median value of the reference for the same age and sex, divided by the standard deviation of the reference population (Kirkwood & Sterne, 2003), were calculated using Nutritional Index Calculator,

Epi Info, Version 6.04 (Centers for Disease Control and Prevention, USA). The nutritional index calculator flags any implausible z-scores and WHO recommends such scores to be treated as missing values. We used ¯2 Standard deviations (SD) from the reference mean because 95% of the measured variable lies within 2 SD of a standard normal distribution curve, and since African children are expected to be less developed than American ones; using 2 SD would cover most of the African children. Thus, HAZ and WAZ values less than ¯2 SD were considered as stunting and wasting respectively as described by WHO (1995). Body Mass Index (BMI) of each child was calculated as weight in kilograms divided by the square of height in meters (kg/m^2). In our study, like Sacko (2006), BMI <15 kg/m^2 was considered as underweight, otherwise BMI beyond 15 kg/m^2 would have rendered all the children in our study to be underweight.

4.2.5.3 Data analysis

Student`s t test and ANOVA were applied to compare means of height, weight, intensity of *S. mansoni* infection and haemoglobin levels between various parameters. Odds ratios and 95% confidence intervals were used to compare proportions of organ consistency, organomegaly, anaemia, water contact activities, ultrasound measured splenic length, portal vein diameter and morbidity indicators with regard to *S. mansoni* infection. Pair-wise correlation was used to determine the relationships of *S. mansoni* infection against liver and spleen sizes while correcting for malaria infection as a confounder. *P* value <0.05 was used to determine statistical significance in all analyses.

4.2.6 Ethical consideration

Ethical approval was obtained from the Higher Degrees Research and Ethics Committee of the School of Public Health, Makerere University. Ethical clearance was obtained from the Uganda National Council of Science and Technology. This study was supported by DANIDA, thus clearance was also sought from the Danish National Committee on Biomedical Research Ethics in Denmark. Informed consent was obtained from individual adult participants while parents or guardians consented on behalf of children. A sterile and less- paining sharp lancet was used to prick the participants. All information obtained from

participants was kept confidential. Participants suffering any minor ailment like clinical malaria, anaemia, diarrhoea and others were treated according to the national guidelines.

4.3 Results

4.3.1 Demographic characteristics of study participants

Although the calculated sample size was 552, some people were absent, thus the results presented here are from 446 people who were recruited at baseline. None of these participants reported to have ever taken praziquantel. There were 229 males, 217 females with a mean age of 26 years (SD ± 16, range 7-76 years). There were 161 (36.1%) farmers, 70 (15.7%) fishermen, 177 (39.7%) students, while 38 (8.5%) were of other occupations. With regard to tribe, 140 (31.4%) were Basoga, 130 (29.1%) were Japadhola, 59 (13.2%) were Ateso, 35 (7.8%) were Samia, 27 (6.1%) were Bagwere and 55 (12.3%) were of other tribes.

4.3.2 Levels of *S. mansoni* infection

Table 4.1 shows *S. mansoni* infection prevalence in relation to sex, age and occupation. The overall prevalence of *S. mansoni* was 88.6% (95% CI: 85.6 – 91.5). Figure 4.1 shows the distribution of prevalence of infection according to sex for different age groups. The overall GMI was moderate (100-399 epg), with males exhibiting a significantly higher GMI than females ($t = ^{-}6.03$, $P < 0.001$), (table 4.2). Generally, the GMI increased with age up to 19 years, thereafter it started to decline. However, there was an increase within the male age group of 25 – 29 years. For females, the GMI of those aged less than 10 years or more than 29 years were significantly different from that of 10 – 29 years of age ($F = 4.36$, $P = 0.038$ & $F = 57.08$, $P < 0.001$ respectively). For males only those above 40 years of age had a significantly lower GMI than the other age groups ($F = 43.3$, $P < 0.001$) (figure 4.2). Occupation of fishing exhibited significantly higher GMI ($F = 26.8$, $P < 0.001$) than the rest. Generally, the proportions of heavily, moderately and lightly infected persons were 39%, 22.2% and 27.4% respectively. Among females light infections accounted for 35%.

Table 4. 1: Prevalence of *S. mansoni* infection by sex, age and occupation

Characteristic	No. exam'd	No. infected	Prevalence (%)	95% CI	OR
Overall	446	395	88.6	85.6 – 91.5	
Sex					
Female	217	173	79.9	74.3 – 85.1	0.82
Male	229	222	96.9	94.7 – 99.2	1
Age category					
Children	153	148	96.7	93.9 – 99.6	1.15
Adults	293	247	84.3	80.1 – 88.5	1
Age group by sex					
Female					
7 - 9 years	26	23	88.5	75.3 – 101.6	1.47
10 - 14	41	40	97.6	92.6 – 102.5	1.63
15 – 19	25	23	92.0	80.6 – 103.4	1.53
20 – 24	25	17	68.0	48.3 – 87.7	1.13
25 – 29	27	21	77.8	61.0 – 94.5	1.30
30 – 39	38	28	73.7	59.0 – 88.4	1.23
40+	35	21	60.0	42.9 – 77.1	1
Male					
7 - 9 years	39	39	100.0	100.0 – 100.0	1.06
10 - 14	47	46	97.9	93.6 – 102.2	1.03
15 – 19	21	21	100.0	100.0 – 100.0	1.08
20 – 24	16	16	100.0	100.0 – 100.0	1.06
25 – 29	29	29	100.0	100.0 – 100.0	1.06
30 – 39	39	35	89.7	79.8 – 99.7	0.95
40+	38	36	94.7	87.3 – 102.2	1
Occupation					
Farmers	161	126	78.3	71.8 – 84.7	0.98
Fishing	70	66	94.3	88.7 – 99.9	1.19
Students	176	172	97.7	95.5 – 100.0	1.23
Other	39	31	79.5	66.2 – 92.7	1

OR = Odds ratio. 95% CI = Confidence interval for prevalence.

Table 4. 2: *S. mansoni* infection intensity by sex, age and occupation

Characteristic	No. +ve	*GMI	95% CI
Overall	395	236.2	198.5 – 460.9
Sex			
Female	173	132.6	102.5 – 171.5
Male	222	370.2	297.5 – 460.9
Age category			
Children	148	276.3	214.1 – 356.7
Adults	247	215.0	170.4 – 271.1
Age group by sex			
Female			
7 - 9 years	23	76.5	40.5 – 144.6
10 - 14	40	275.3	169.9 – 446.2
15 – 19	23	327.9	187.2 – 144.6
20 – 24	17	170.2	72.8 – 398.0
25 – 29	21	223.5	120.4 – 414.8
30 – 39	28	54.9	27.3 – 110.4
40+	21	35.1	17.1 – 72.3
Male			
7 - 9 years	39	284.6	178.0 – 455.0
10 - 14	46	513.7	336.6 – 783.8
15 – 19	21	700.7	338.7 – 1449.6
20 – 24	16	671.9	254.4 – 1774.3
25 – 29	29	793.1	523.0 – 1202.5
30 – 39	35	421.3	234.0 – 758.6
40+	36	81.9	49.2 – 136.3
Occupation			
Farmers	126	98.8	72.3 – 135.1
Fishing	66	806.4	581.7 – 1117.8
Students	172	295.1	233.8 – 372.5
Other	31	173.1	92.0 – 325.7

*GMI was calculated from positive cases only. 95% CI = Confidence interval for GMI.

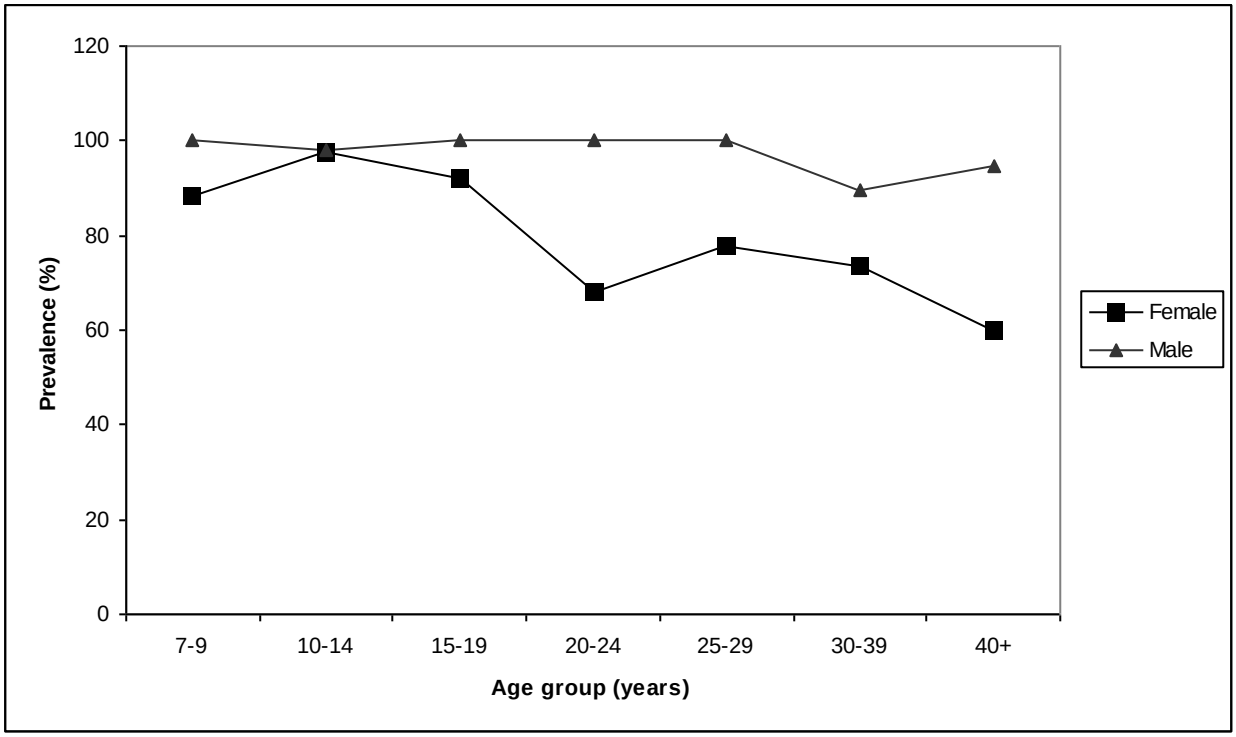

Figure 4. 1: Age distribution pattern of *S. mansoni* infection prevalence by sex

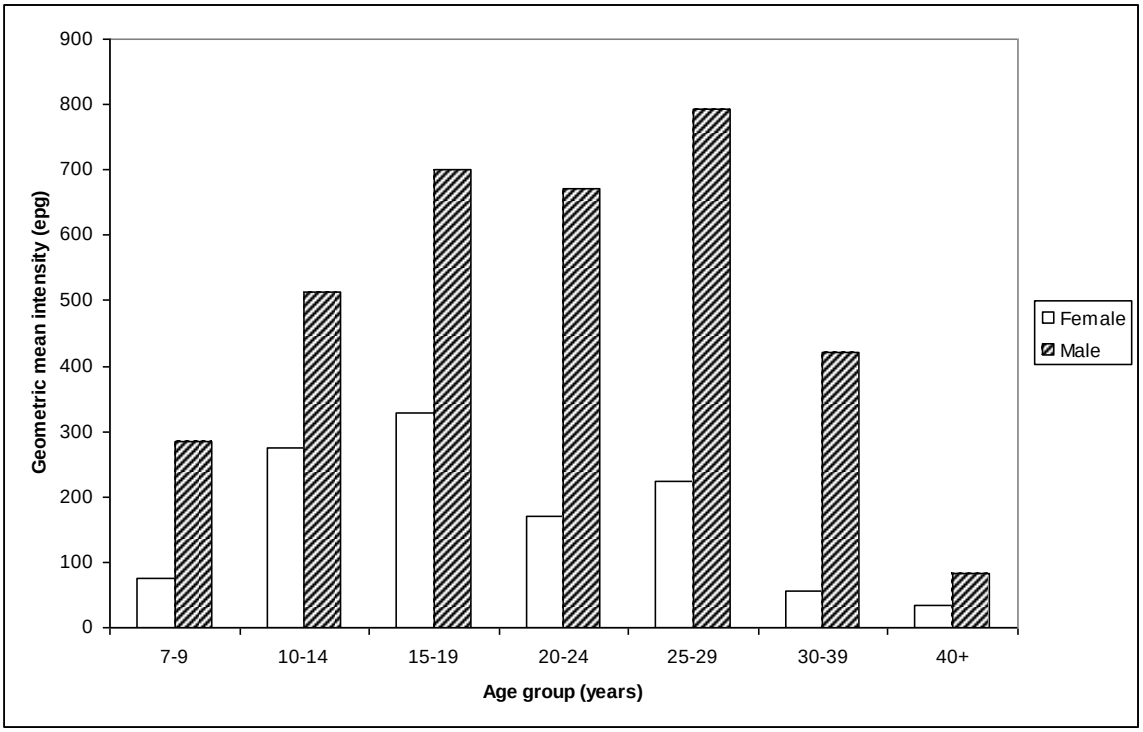

Figure 4. 2: *S. mansoni* infection intensity by sex and age group

4.3.3 Water contact

The water contact activities reported in this study were washing clothes or utensils, fetching water, swimming, bathing and fishing. Two hundred (52.1%) females and 184 (47.9%) males reported that they go to the lake. There were no significant differences in prevalence (table 4.3) and intensity of infection (table 4.4) between those who reported to go and those who reported not to go to the lake.

Considering the frequency of exposure, 73.4 % of the people reported to go to the lake more than 5 days in a week while 13.6% and 13.0% went there 1-2 days and 3-4 days in a week, respectively. Generally, *S. mansoni* infection intensity increased with number of days of going to the lake (table 4.4). A larger proportion of people (53.3%) went to swim, wash clothes and fetch water, while 26.2% went to fetch water only and 4.2% went to fish only. Of those who went to swim, wash and fetch water, 65.1% were children and 47.5% were adults. Comparing water contact activities with *S. mansoni* prevalence of infection, there was no significant effect of the type of activity on prevalence (table 4.3). However, the GMI of *S. mansoni* for those who reported to do all the mentioned activities in the lake was high.

Table 4. 3: *S. mansoni* infection prevalence in relation to water contact

Characteristic	No. examined	No. infected (%)	95% CI	OR
Go to the lake				
Yes	384	340 (88.5)	85.3 – 91.7	1.00
No	61	54 (88.5)	80.3 – 96.3	1
Number of days per week in contact with water				
1 – 2	44	35 (79.5)	67.1 – 92.0	0.89
3 – 4	42	37 (88.1)	77.9 – 98.3	0.98
5 – 7	237	213 (89.9)	86.0 – 93.7	1
Activity in the water				
Fishing	16	16 (100)	100.0 – 100.0	1.05
Fetch water	100	80 (80)	72.0 – 88.0	0.84
Swim/wash/fetch	203	182 (89.7)	85.4 – 93.9	0.94
All activities	62	59 (95.2)	89.7 – 100.7	1

95% CI = Confidence interval for prevalence.

Table 4. 4: *S. mansoni* infection intensity in relation to water contact

Characteristic	GMI – epg (N)	95% CI
Go to the lake		
Yes	229.4 (34)	189.8 – 227.4
No	292.2 (54)	188.9 – 452.1
Number of days per week in contact with water		
Females		
1 – 2	73.6 (14)	27.7 – 195.2
3 – 4	97.2 (14)	36.1 – 261.6
5 – 7	169.2 (106)	121.0 – 236.5
Males		
1 – 2	144.9 (21)	59.2 – 354.9
3 – 4	443.4 (23)	230.1 – 854.6
5 – 7	450.2 (107)	333.4 – 607.8
Activity in the water		
Fishing	590.7 (16)	226.7 – 1538.8
Fetch water	108.2 (80)	74.5 – 157.0
Swim/wash/fetch	195.6 (182)	151.1 – 253.2
All activities	792.4 (59)	581.0 – 1080.8

*GMI was calculated from positive cases only. 95% CI = Confidence interval for GMI.

4.3.4 Other infections

The overall prevalence of hookworm infection was 43.3% (95% CI: 38.7 – 47.9). The number of people infected with hookworms was 102 (47%) females and 91 (39.7%) males, not statistically significant. Although farmers had higher prevalence of hookworm infection (61.5%) there was no significant difference in prevalence among the different occupation categories. Prevalence of hookworm was significantly higher in adults than in children for both males (OR = 0.44, 95% CI: 0.24 - 0.80, P = 0.006) and females (OR = 0.42, 95% CI: 0.22 - 0.79, P = 0.006). Out of 446 people, 166 (37.2%) had *S. mansoni* and hookworm co-infection.

Malaria affected 291 (65.2%) of the participants with an overall arithmetic mean parasite density of 571.4 (95% CI: 430.8 – 712.1) parasites/μL of blood. Children had significantly higher prevalence of malaria (87.6%) than adults (53.6%) (OR = 1.63, 95% CI: 1.19 – 2.24, P = 0.002). Malaria density of infection was also significantly different between children (884.8, 95% CI: 600.3 – 1169.3 parasites/ μL of blood) and adults (303.9, 95% CI: 226.4 –

381.5 parasites/ μL of blood) (t = 4.16, df = 289, $P < 0.001$). Neither the prevalence nor the parasite density of malaria was significantly different between males and females (data not shown). Of those infected with *S. mansoni*, 265 (91.1%) people were co-infected with malaria. A logistic regression model of *S. mansoni* against malaria and hookworm infection revealed no association.

4.3.5 Organomegaly

Table 4.5 shows the distribution of splenomegaly, hepatomegaly and hepatosplenomegaly detected by clinical examination. Hepatosplenomegaly was the most common presentation and it was significantly more common in children than in adults (OR = 1.73, 95% CI: 1.15 – 2.60, $P = 0.008$). Among those with enlarged organs, the majority had firm organs (Table 4.6). More adults had firm livers than children (OR = 1.65, 95% CI: 1.18 – 2.31, $P = 0.003$). The proportion of those with firm spleens was higher among adults (58.7%) than among children (41.3%) (OR = 1.63, 95% CI: 1.15 – 2.31, $P = 0.005$). There was no significant difference in occurrence of organomegaly between *S. mansoni* infected and non-infected people. Controlling for malaria, *S. mansoni* intensity was not correlated to liver or spleen size. However, when malaria and *S. mansoni* co-infections were compared with the liver size, malaria had a weak correlation with the liver size measured through mid-clavicular line (MCL) (coefficient = 0.14, $P = 0.004$) and through the mid-sternal line (MSL) (coefficient = 0.12, $P = 0.014$). Implying that malaria infection accounted for 14% and 12% of the liver enlargement measured through the MCL and MSL respectively.

Table 4. 5: Liver and spleen size measured by clinical palpation

	Number with organomegaly* (%)				
	Score 0	**Score 1**	**Score 2**	**Score 3**	**Score 4**
Overall	167 (37.4)	14 (3.1)	22 (4.9)	108 (24.2)	135 (30.3)
Sex					
Female	79 (36.4)	10 (4.6)	13 (6.0)	51 (23.5)	64 (29.5)
Male	88 (38.4)	4 (1.7)	9 (3.9)	57 (24.9)	71 (31.0)
Age category					
Child	27 (17.6)	9 (5.9)	7 (4.6)	46 (30.1)	64 (41.8)
Adult	140 (47.8)	5 (1.7)	15 (5.1)	62 (21.2)	71 (24.2)
***S. mansoni* prevalence of infection**					
Positive	144 (36.5)	14 (3.5)	20 (5.1)	96 (24.3)	121 (30.6)
Negative	23 (45.1)	0	2 (3.9)	12 (23.5)	14 (27.5)
***S. mansoni* infection intensity group**					
1- 99 epg	43 (35.2)	3 (2.5)	9 (7.4)	32 (26.2)	35 (28.7)
100- 399 epg	40 (40.4)	1 (1.0)	4 (4.0)	30 (30.3)	24 (24.2)
≥400 epg	61 (35.1)	10 (5.7)	7 (4.0)	34 (19.5)	62 (35. 6)

* Organomegaly is based on scores as described in the clinical form (appendix II)

Score 0 = No organomegaly. Score 1 = Splenomegaly or hepatomegaly or both but with soft consistency (mainly related to malaria infection). Score 2 = Splenomegaly. Score 3 = Hepatomegaly. Score 4 = Hepatosplenomegaly.

Table 4. 6: Consistency of enlarged livers and enlarged spleens

Characteristic	**Measure**	**Number of enlarged livers (%)**		**Number of enlarged spleens (%)**	
		***S. mansoni* positive**	***S. mansoni* negative**	***S. mansoni* positive**	***S. mansoni* negative**
Consistency	Soft	17 (7.5)	2 (7.7)	9 (4.4)	3 (12.0)
	Firm	205 (89.9)	24 (92.3)	189 (92.7)	22 (88.0)
	Hard	6 (2.6)	0 (0)	6 (2.9)	0 (0)

4.3.6 Ultra sound examinations

Periportal fibrosis (liver texture patterns C-F, see appendix III) was rare. A total of 423 (94.8%) and 3 (0.7%) had patterns A and B respectively, while ten (2.2%) and one (0.2 %) people had liver image patterns C and D respectively. Eight persons (1.8%) had liver image pattern Z and one person (0.2%) had Y liver image pattern. Patterns Y and Z are not related

to schistosomiasis induced pathology. Forty six people (10.3%) had moderately dilated portal vein diameters while 2(0.5%) had marked dilated portal vein diameters. When controlling for malaria, *S. mansoni* infection intensity was associated with portal vein diameter (coefficient = 0.14, *P* = 0.006) and splenic length (coefficient = 0.159, *P* = 0.003). Other than 7 (13.7%) non-infected people with moderately dilated portal vein diameter, all people whose portal vein diameter was dilated were infected with *S. mansoni*. Table 4.7 shows the *S. mansoni* infection intensity by portal vein diameter dilatation. The two people who had markedly dilated portal vein diameter were males. One of them was a fisherman aged 23 years and had liver image pattern C while the other one had liver image pattern Z, which is not related to schistosomiasis infection. Two hundred nine people (47%) had moderately enlarged spleens while 97 (21.8%) had severely enlarged spleens. Among the 209 with moderately abnormal splenic length, 186 (89%) were infected with *S. mansoni* while 89 (91.8%) out of 97 with severely abnormal splenic length were positive for *S. mansoni* (table 4.8).

Table 4. 7: *S. mansoni* infection intensity in relation to portal vein diameter measured by ultrasound

Portal vein diameter	N	GMI	95% CI
Normal	354	220.4	183.9 – 264.3
Moderately dilated	39	401.9	220.8 – 731.4
Markedly dilated	2	1469.2	4.1 – 532,239.4

*GMI was calculated from positive cases only. 95% CI = Confidence interval for GMI.

Table 4. 8: *S. mansoni* infection intensity in relation to spleen length measured by ultrasound

Spleen length	N	GMI	95% CI
Normal	119	168.2	121.7 – 232.4
Moderately abnormal	186	234.8	184.2 – 299.2
Severely abnormal	89	371.8	254.7 – 542.8

*GMI was calculated from positive cases only. 95% CI = Confidence interval for GMI.

4.3.7 Haemoglobin levels and anaemia

The mean haemoglobin level was 12.6 g/dL (95% CI: 12.4 – 12.8). There was no significant difference in haemoglobin levels between *S. mansoni* infected and non-infected persons (t = ¯0.19, *P* = 0.851). There was a highly significant difference in haemoglobin levels with regard to sex (t = ¯5.59, *P* < 0.001) and age (t = ¯5.45, *P* < 0.001). There was no significant difference in haemoglobin levels between those infected and non-infected with hookworms (t = ¯1.17, *P* = 0.244). From pair wise correlations analysis of haemoglobin, malaria, *S. mansoni* and hookworms; occurrence of malaria had a significant association with haemoglobin concentrations (coefficient = ¯0.16, *P* = 0.001). The overall proportion of anaemic people was 36.4% (95% CI: 31.7 – 41.1). Generally there was no association of anaemia with sex, age and *S. mansoni* prevalence and intensity of infection. Individual malaria and hookworm infections did not correlate with anaemia and a logistic regression model of anaemia against *S. mansoni*, malaria and hookworm infection revealed no association with any of the predictors.

4.3.8 *S. mansoni* infection and growth indicators for children below 15 years of age

The mean height was 133.6 cm (95% CI: 131.4 – 135.8) and the mean weight was 30.2 Kg (95% CI: 28.8 – 31.5). Comparing *S. mansoni* infected and un-infected, there was no significant difference in height (t = ¯1.76, *P*=0.081) and weight (t = 1.40, *P* = 0.164). There was no significant difference in prevalence of *S. mansoni* and any of the growth indicators (table 4.9). Intensity of *S. mansoni* significantly affected the level of stunting (t = ¯2.86, *P* = 0.005) but there was no evidence of the effect of intensity on wasting and underweight (table 4.10).

Table 4. 9: Prevalence of *S. mansoni* infection by growth indicators

Indicator	Number examined	Number infected (%)	95% CI	OR
HAZ				
Normal	123	120 (97.6)	94.8 – 100.3	1.01
Stunted	30	29 (96.7)	89.8 – 103.5	1
WAZ				
Normal	143	139 (97.2)	94.5 – 99.9	0.97
Wasted	12	12 (100)	100.0 – 100.0	1
BMI				
Normal	132	129 (97.7)	95.2 – 100.3	1.02
Underweight	23	22 (95.7)	86.6 – 104.7	1

95% CI = Confidence interval for prevalence.

Table 4. 10: *S. mansoni* infection intensity by growth indicators

Indicator	GMI (N)	95% CI
HAZ		
Normal	227.4 (120)	170.8 – 302.8
Stunted	571.3 (29)	330.3 – 988.2
WAZ		
Normal	262.3 (139)	201.3 – 341.8
Wasted	463.1 (12)	160.5 – 1336.8
BMI		
Normal	274.3 (129)	209.0 – 359.9
Underweight	275.2 (22)	124.6 – 608.0

*GMI was calculated from positive cases only. 95% CI = Confidence interval for GMI.

4.4 Discussion and conclusion

The high *S. mansoni* prevalence and infection intensity found in this study are typical for an endemic area like Lake Victoria (Kardorff *et al*., 1997; Karanja *et al*., 2002; Malenganisho, 2005; Odogwu *et al*., 2006). In agreement with findings elsewhere, the intensity of infection was higher in males than females (Ongom & Bradley, 1972; Bundy, 1988; Kabatereine *et al*., 1996b; 2004a, 2004b; Barakat *et al*., 2000; Kabatereine, 2000; Naus *et al*., 2003). This could be due to occupation-related factors (Gryseels & Nkulikyinka, 1988; Jordan & Webbe, 1993) such as duration of exposure to cercariae-infested water (Kabatereine *et al*., 2003; Scott *et al*., 2003; Booth *et al*., 2004a). In our study, most of the highly infected males were fishermen who usually spend long hours in contaminated water, hence getting more infected than females.

The peak intensity occurring in the 15-19 years age group is similar to observations elsewhere (Ongom & Bradley, 1972; Butterworth *et al*., 1988 & 1991; Stelma *et al*., 1993; Boisier *et al*., 1995; Kabatereine *et al*., 1996b, 2004a, 2004b; Fulford *et al*., 1998; Naus *et al*., 1999; Barakat *et al*., 2000; Kabatereine, 2000; Scott *et al*., 2003; Conlon, 2005), where the highest infection levels were observed in an age range of 10-19 years. Several explanations have been suggested for this trend, among which is water contact. However, water contact does not necessarily imply that one is exposed to schistosome infection. Various issues such as the time of day when one gets into contact with cercariae-infested water, duration of exposure and size of the body surface that gets into contact with water may influence the levels of cercarial penetration (Butterworth *et al*., 1988; Chandiwana & Woolhouse, 1991; Fulford *et al*., 1996; Scott *et al*., 2003; Conlon, 2005). Unlike previous water contact studies that have reported observed individual water contact activities at infective sites (Chandiwana & Woolhouse, 1991; Fulford *et al*., 1996), we based water contact study on individual reports. This could have affected the accuracy of our findings as noted elsewhere (Fulford *et al*., 1996; Friedman *et al*., 2001) in that participants may not have recalled or properly estimated the time periods they were exposed to water. We found out that more children than adults were engaged in swimming, washing and fetching water than adults. Swimming exposes larger parts of their bodies to contaminated water and provides a wider area for cercarial penetration (Butterworth *et al*., 1988). The age group of

25-29 years who showed very high intensity of infection were fishermen whose occupation exposes them to contaminated water for long periods.

On the other hand, exposure alone may not explain this age difference in infection as depicted in studies of a fishing community along Lake Albert where the same age-related schistosomiasis distribution pattern was realised and yet adults were more exposed to infested water than children (Kabatereine *et al*., 2004b). This could be due to lack of acquired immunity to schistosomiasis infection, which usually develops after approximately 10 – 15 years of infection (Gryseels, 1994; Dunne *et al*., 1992; Corrêa-Oliveira *et al*., 2000). It is documented that in an endemic area, people acquire immunity in response to parasite antigens and this immunity is influenced by age (Rihet *et al*., 1991; Corrêa-Oliveira *et al*., 2000; Fitzsimmons *et al*., 2004) or exposure levels such that prolonged exposure to infection increases parasite antigens in the body of the host, which in turn trigger the release of antibodies, like IgE (Rihet *et al*., 1991; Dunne *et al*., 1992; Webster *et al*., 1998; Naus *et al*., 1999 & 2003). IgE is associated with resistance to infection and is usually higher in adults than in children (Naus *et al*., 1999; Vereecken *et al*., 2007). Another explanation for infection peaking in the second decade of life could be due to physiological changes at puberty (Butterworth *et al*., 1988; Fulford *et al*., 1998). It has been reported that hormonal changes during puberty such as increase in skin thickness or deposition of fat increase resistance to *S. mansoni* infection by reducing cercarial penetration (Gryseels, 1994; Dunne & Mountford, 2001).

In agreement with results found in a study among Kenyan school children (Vennervald *et al*., 2004), we observed hepatosplenomegaly without fibrosis. More children had hepatosplenomegaly than adults, which is comparable to other studies (Gryseels & Polderman, 1987; Gryseels, 1988; Yazdanpanah *et al*., 1997). Organomegaly may be caused by *S. mansoni* eggs that get trapped in the presinusoidal periportal spaces. The eggs contain a developing miracidium, which releases proteolytic enzymes that give rise to typical eosinophilic inflammatory and granulomatous reactions (Mitchell, 1990; Gryseels *et al*., 2006). The granuloma may shrink when the miracidium in the egg dies and the affected area starts healing. The healing process may lead to formation of fibrotic lesions around the

portal venules, which together with the granulomatous reactions may result in hepatosplenic schistosomiasis. Thus, the age difference in prevalence of hepatosplenomegaly could be due to regulation of inflammatory immune responses that increase with age.

Contrary to other studies where hepatosplenomegaly was associated with prevalence of *S. mansoni* (Homeida, 1988; Fulford *et al*., 1991) and intensity of infection (Ongom & Bradley, 1972; Friis & Byskov, 1987; Gryseels & Polderman, 1987; Gryseels & Nkulikyinka 1990; Corbett *et al*., 1992; Boisier *et al*., 1995; Kardorff *et al*., 1997; Vennervald *et al*., 2004), clinically detected enlarged spleens and livers in our study were not associated with prevalence of *S. mansoni*. Our findings are comparable to the findings by Wilson *et al*., (2007) among Kenyan school children where no significant difference in hepatosplenomegaly between *S. mansoni* infected and un-infected children was found. Contrary to a study in Kenya (Fulford et al., 1991) where organomegaly was not associated with malaria infection, organomegaly in our study could have been affected by malaria since we observed a weak correlation between malaria infection and liver size.

There were very few persons with liver image patterns C and D which are typical for fibrosis. Periportal fibrosis (PPF) was also very limited. This is contrary to findings in two communities along Lake Victoria in Tanzania (Malenganisho *et al*., 2008) and in a fishing community along Lake Albert (Kabatereine *et al*., 2000) where PPF was observed in a relatively high proportion of the individuals examined. In the study from Tanzania, the prevalence of PPF varied between two villages and was related to infection intensity (Malenganisho *et al*., 2008). The intensity levels of infection in these villages were much lower than what we observed in our study and yet we realised minimal fibrosis. Thus intensity of infection may not explain the minimal PPF in our study.

In another study, variation in the prevalence of PPF was attributed to difference in duration of exposure to infection (Booth *et al*., 2004a). Adults who had resided in the village for more than 15 years had increased risk of fibrosis than those who had lived there less than 15 years. Duration of exposure is not likely to have affected morbidity in our study since

most people were born in the village and almost everyone gets exposed to contaminated water, which is the only source of water for all purposes. Probably factors like parasite genetic differences (Yazdanpanah *et al*., 1997; Dunne & Pearce, 1999) could have influenced the levels of PPF in our study. Human genetic factors may influence the immune response that mediates the granulomatous reactions and the fibrosis that develops around the deposited eggs in the tissue. Alcohol consumption may moderate schistosomiasis related liver pathology as was found in a study by Kabatereine (2000). Kabatereine *et al*., (2000) reported a significant difference in liver enlargement between *S. mansoni* infected and un-infected persons among non-alcohol consumers whereas the difference was not significant in alcoholics. Though not purposively measured, from observations, the level of alcohol consumption in our study population was very low. Portal vein dilatation and abnormal spleen length measured by ultrasound were associated with *S. mansoni* intensity, whereas the spleen size measured by clinical examination was not associated with intensity. This may indicate that ultrasound examination yields better organ measurements than clinical examination. Whereas ultrasonography should be the gold standard for evaluation of hepatosplenic schistosomiasis (Marinho *et al*., 2006), no purposeful comparison of organ sizes measured by clinical palpations or ultraultrasonographically was performed. This is likely not to have affected our results since ultrasonography is known to be good for detection of peri-portal fibrosis (Yazdanpanah *et al*., 1997; Ritchter *et al*., 2003) while clinical examination is good for determination of the degree of organ enlargement and consistency (Vennervald *et al*., 2004).

It is documented that intestinal schistosomiasis and other parasitic infections such as malaria and hookworms may cause anaemia (Gryseels & Polderman, 1987; Stephenson, 1993; Sturrock *et al*., 1996; Friedman *et al*., 2005; Koukounari *et al*., 2008). However, other factors such as poor nutritional diet or inadequate dietary in-take without iron supplements, poverty and haemoglobinopathies may have an impact on haemoglobin levels and lead to anaemia (Stephenson, 1993; Crawley, 2004; Koukounari *et al*., 2008). Mild anaemia was highly prevalent in Musoli village. Comparable to studies elsewhere (Gryseels & Polderman, 1987; Sturrock *et al*., 1996; Olsen *et al*., 1998), we observed no relationship between anaemia and *S. mansoni* infection. In the Kenyan study by Olsen *et al*., (1998) the

lack of relationship between haemoglobin and intensity of *S. mansoni* was probably due to the low intensity of *S. mansoni* infection. However, the intensity of *S. mansoni* in our study was high as compared to that by Olsen *et al*., (1998), thus intensity of infection may not fully explain the lack of a relationship between *S. mansoni* and anaemia in our study. Anaemia also had no relationship with neither malaria nor hookworm prevalence. This is contrary to previous studies which report an association between malaria and anaemia (Olsen *et al*., 1998; Koukounari *et al*., 2008) yet malaria parasitaemia was as prevalent in our study as it was in these previous studies. Our results suggest that anaemia was probably the result of poor nutritional status or haemoglobinopathies (Stephen, 1993). On the other hand, anaemia in our study could have been due to splenomegaly that might have increased haemolysis in the spleen (Friis *et al*., 2003).

Growth of children was determined using nutritional status parameters of stunting, wasting and underweight as have been used in previous studies (Parraga *et al*., 1996; Stoltzfus *et al*., 1997; Assis *et al*., 2004; Zhou *et al*., 2005). Those heavily infected with *S. mansoni* were more stunted than those with moderate and light infections. This is in agreement with a study of Brazilian children, where stunting in the heavily infected ones was more than twice as much as that of the uninfected ones (Assis *et al*., 2004). It is reported elsewhere that many other factors like lack of adequate food, poverty, poor sanitation, parasitic infections, individual and environmental conditions as well as minimum health care can all aggravate malnutrition, mainly of school-age children who are in a period of physical and intellectual growth, in the developing world (Rigaud *et al*., 1994; Assis *et al*., 1998; WHO, 2006). It is possible that stunting was influenced by poverty and inadequate food intake.

Immunological factors triggered by parasitic infections can also explain why the heavily *S. mansoni* infected ones were more stunted than those with less infection levels. McDermott *et al*., (2006) in their study of mice infected with *Trichinella spiralis* reported significant reduction of food intake at the peak of parasite induced intestinal inflammation. Schistosomiasis causes inflammation of the mucosa, hyperplasia of cells and gut ulceration (Friis *et al*., 1996), which in turn decreases food intake and nutrient utilisation within the

host body (Stephenson & Holland, 1987; Stephenson, 1993; King *et al*., 2005), all of which contribute to childhood stunting (Campbell *et al*., 2003).

In conclusion, *S. mansoni* infection is prevalent in Musoli village. Whereas there is evidence from this study that a person's age, sex and occupation determine the level of *S. mansoni* intensity of infection, there was none to show that intensity has an influence on morbidity. Likewise, *S. mansoni* infection seemed not to impact on haemoglobin levels, instead other factors such as nutritional status may have slowly affected the levels of haemoglobin. It is documented that the current strategy to control schistosomiasis is morbidity control by mass chemotherapy using a single annual dose of praziquantel, targeted to mainly school age children (WHO, 2002). Our findings of higher intensity and morbidity exhibited by children than adults add more support to the existing strategy. With the high prevalence and intensity of schistosomiasis infection and its related morbidity observed in this study, more information about the effect of different doses of praziquantel on schistosomiasis is required.

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Chapter 5: Comparative efficacy of one and two doses of praziquantel on *Schistosoma mansoni* infection in Musoli village along Lake Victoria in Uganda

Summary

To investigate the effect of one versus two doses of praziquantel on cure rate and intensity of infection, 395 schistosomiasis infected people were treated with a standard dose of praziquantel (Distocide® 600 mg, Shin Poong Pharmaceuticals, Seoul Republic of Korea, 40mg/kg body weight). After this treatment, the participants were randomly divided into two groups. One of the groups received a second standard dose of praziquantel two weeks after the first treatment while the second group was not treated again. The cure rate and intensity of *S. mansoni* infections were assessed nine weeks after the first treatment using standard parasitological procedures. Those who received two doses were more likely to be cured (69.7%) compared to those who received a single dose (47.9%) (Relative Risk [RR] 1.7, 95% Confidence interval [95% CI]: 1.3 - 2.2). Among those not cured, the geometric mean intensity (GMI) of infection measured as eggs per gram of faeces (epg) was lower among those who received two doses (12.0 epg, 95% CI: 8.9 – 16.1) compared to those who received a single dose (22.1 epg, 95% CI: 16.9 – 28.8). Our results suggest that a second dose of praziquantel given two weeks after the first dose improves cure rate and reduces *S. mansoni* infection intensity.

5.1 Introduction

The major causative organism of schistosomiasis in Uganda is *S. mansoni*. The disease which was reported to be more endemic in north western Uganda (Nelson, 1958) and around the greater lakes (Prentice, 1972; Doumenge *et al*., 1987) is now widespread in more than 38 districts (Kabatereine *et al*., 2006). Approximately 16.7 million people are at risk of being infected with schistosomiasis and 4 million estimated to be infected in Uganda (Kabatereine *et al*., 2006). The major target in controlling schistosomiasis is to reduce morbidity to levels of no public health importance. Mass drug administration with praziquantel as a single annual standard dose (40 mg/kg body weight) is currently the treatment strategy recommended to control schistosomiasis related morbidity in highly endemic areas (WHO, 2002). However, several studies have reported low cure rates ranging between 18 – 58% using a standard single dose of praziquantel (Stelma *et al*., 1995; Gryseels *et al*., 2001; Tchuem Tchuente *et al*., 2001). The low cure rates may be attributable to high intensity of infection, high level of transmission and low sensitivity to praziquantel especially for *S. mansoni* (Danso-Appiah & De Vlas, 2002). Increasing the cure rates and reducing the intensity of infection by therapeutic evaluation of different praziquantel dose regimens is a research priority especially in schistosomiasis high transmission areas (Butterworth *et al*., 1991; Guise *et al*., 1997; Cioli 1998; Engels *et al*., 2002; Ferrari *et al*., 2003; N'Goran *et al*., 2003; Utzinger *et al*., 2003). Since *S. mansoni* related morbidity is mainly caused by inflammatory and immunological reactions induced by eggs lodged in the host's tissue (Vennervald & Dunne, 2004), increased cure rate and reduction in intensity of infection is thought to lead to more morbidity regression (Frenzel *et al*., 1999).

The hypothesis of this study was that the efficacy of one dose of praziquantel is not different from that of two doses given two weeks apart. We compared cure rates of a single versus double standard dose (given two weeks apart) of praziquantel on individuals infected with *S. mansoni* in a community living in a high transmission area along Lake Victoria in Uganda. The hypothesis of giving the second dose two weeks later is based on the time (4-6 weeks) the infective *S. mansoni* parasite takes to mature into adult worms, a stage that is susceptible to praziquantel. It was assumed that the first dose would clear only

the mature adult schistosomes, while the juvenile worms mature within two weeks and become susceptible to the second dose of praziquantel.

5.2 Material and methods

5.2.1 Study area and population

The study was conducted in Musoli village along Lake Victoria, in Mayuge district of South East Uganda where transmission of schistosomiasis is stable and high throughout the year (Kabatereine *et al*., 2003). The main economic activities in Musoli village are fishing and subsitence farming. Lake Victoria is the only source of water in Musoli and this exposes the people to schistosomiasis infection.

5.2.2 Study design

The study consisted of a cross sectional baseline survey followed by a randomised intervention follow up survey. The cross sectional study was conducted to collect pre-treatment levels of *S. mansoni* infection after which all participants were treated. The study was single-blinded in that the participants knew how many doses of praziquantel they received while all the examiners were blinded to which treatment group the participants belonged. It was hypothesised that two doses of praziquantel given two weeks apart would yield the same cure rate as a single dose.

5.2.3 Sample size determination and sampling procedure

The aim of the whole study was to compare the effect of one versus two doses of praziquantel on cure rate, re-infection and morbidity regression of *S. mansoni*. The sample size calculation of the cohort was based on expected difference in cure rate between the two study groups as well as the expected loss to follow-up. Sample size was calculated using Kirkwood's 1988 modified formula for comparing two rates (Hardon *et al*., 1994). Basing the calculations on cure rates obtained from a study of communities living along the shores of Lake Albert (Kabatereine *et al*., 2003) of 41.9% and 69.1% in the first treatment and second treatment respectively, the sample size at 95% significance level and power of 90% was calculated as follows:

$$n = \frac{(u + v)^2 (r_1 + r_2)}{(r_1 - r_2)^2},$$

Where r = cure rate; u = two-sided percentage point of the normal distribution corresponding to the power of 90%; v = percentage point of the normal distribution corresponding to the significance of 95%.

The sample size per group: $\frac{(1.64 + 1.96)^2 (0.42 + 0.69)}{(0.42 - 0.69)^2} = 197$

In order to allow for a 20% annual loss to follow-up (total loss 40% in two years) 79 people were added and each group had 276 participants, giving a total sample size of 552 people to be recruited in the study. It should be noted that this sample size was for the entire intervention study up two years of follow up. However, the follow up period for this part of the study was only nine weeks after treatment, hence the sample size calculation did not require an allowance for an annual loss to follow up. Thus to detect a true difference in cure rates between the two treatment groups, the sample size was calculated using Statcalc calculator of Epi Info, version 6.04 (Centers for Disease Control and Prevention, USA). Using the above stated cure rate of 41.9% for a single dose, power of 80% and a probability of 95%, the sample size obtained for each dose group was 161 people. The sample used for the entire study was therefore big enough to significantly detect any efficacy difference between the two doses.

The sampling frame comprised of all the inhabitants in Musoli village who were registered within their homes. Each household had a unique number and every member of a household carried the household number followed by an individual number. For instance, a seventh member from household numbered 95, had an identification number of 95-07. Data were entered in the computer and computer generated random numbers were used to select a representative cohort stratified for age and sex. Sample selection was performed upon the individuals in the whole sampling frame. After the baseline data collection, a list of all the examined participants was drawn. Using computer generated random numbers, stratified randomisation was used to allocate participants to treatment regimens. Odd numbers in each stratum were located to single treatment group while even numbers were located to

double treatment group. Randomisation was performed by a Scientist, who was independent of all the examinations.

5.2.4 Data collection methods and procedures

5.2.4.1 Stool examination

For improvement of the diagnostic sensitivity, each participant produced an early morning stool specimen on three consecutive days (Cheever *et al*., 1994; Booth *et al*., 2003). From each specimen; two slides were prepared using the modified Kato Katz thick smear technique (Katz *et al*., 1972) with a 50 mg template, giving rise to six slides per participant. Two experienced technicians, all blinded to the participants' treatment group, examined the slides under the microscope (10x). In order to minimise intra-observer variation, both technicians read slides prepared from the same sample in such a way that each technician read only one of the duplicate slides. The number of *S. mansoni* eggs observed per slide were recorded. Stool examination was repeated nine weeks after the first treatment to assess levels of *S. mansoni* infection and cure rates.

5.2.4.2 Treatment

After all stool specimens were collected, each participant was treated with a single standard dose of praziquantel (40 mg/kg body weight) and one tablet of albendazole 400 mg. The brands of albendazole and praziquantel used were Alzental® 400mg and Distocide® 600 mg respectively all manufactured by Shin Poong Pharmaceuticals, Seoul Republic of Korea. Two weeks later, one of the groups received another standard dose of praziquantel while the other did not receive any treatment. Treatment was performed by and under direct observation of an experienced nurse. Participants were kept at the field research station for two hours after treatment to observe and manage any possible adverse events.

5.2.5 Data management and analysis

5.2.5.1 Quality control

Results from different observers were not compared, instead stool quality control was performed by an independent experienced microscopist reading a random 10% of the

slides, after which the results were compared and where there was controversy, all the six slides of the same individual were read by two different experienced technicians and the counts were harmonised. Data were double-entered by two experienced data entry clerks using excel computer programme and the two files were cleaned, compared and a master file compiled, which was used for all analyses.

5.2.5.2 Data management

Data were imported from Excel computer programme to Stata for windows (Intercooled Stata 10.1, Stata Corporation, USA) for analysis. Analysis was performed for only those who were positive before treatment and were present at nine weeks after treatment. The cure rate was calculated as the proportion of treated persons who were egg-positive at baseline but became negative nine weeks after treatment. The arithmetic mean of *S. mansoni* egg counts for each participant was calculated from the counts all the six slide and multiplied by 20 to obtain individual eggs per gram (epg) of faeces i.e. intensity of *S. mansoni* infection. Histograms, with normal curves, of *S. mansoni* intensity according to various predictors of infection were generated before analysis to check whether intensity was normally distributed. Intensity was found to be skewed, thus the data was normalised using base 10 logarithm transformation. The geometric mean intensity (GMI) of *S. mansoni* infection was obtained as the antilog of the mean of the transformed egg counts and is reported as eggs per gram (epg) of faeces. The egg reduction rate (ERR) for those who remained positive was calculated as: [1 – (GMI after treatment/GMI before treatment)] x 100. Intensity of infection was categorised low for GMI of 1-99 epg, moderate for GMI of 100-399 epg and heavy for GMI ≥400 epg (WHO, 2002).

5.2.5.3 Data analysis

Analysis was performed for only those who were positive at baseline. Student`s t test and ANOVA were applied to compare the effect of the two treatment regimens on mean intensity of infection. Chi square was used to compare baseline characteristics between the two treatment groups. Cure rates of the two treatment groups were compared using risk ratios (RR) with their corresponding 95% confidence interval (95% CI). The efficacy of the two treatment regimens on GMI were also compared across various strata of sex, age,

occupation and baseline intensity of infection. A *P* value <0.05 was used to determine statistical significance in all analyses.

5.2.6 Ethical considerations

Ethical approval was obtained from the Higher Degrees Research and Ethics Committee of the School of Public Health, Makerere University. Ethical clearance was obtained from the Uganda National Council of Science and Technology. This study was supported by DANIDA, thus clearance was also sought from the Danish National Committee on Biomedical Research Ethics in Denmark. Informed consent was obtained from individual adult participants while parents or guardians consented on behalf of children. All information obtained from participants was kept confidential.

To minimise occurrence of possible side effects of praziquantel when taken on an empty stomach, it is advisable to take the tablets after at least a light meal. Thus, a snack and a soft drink were given before treatment. A trained nurse was available during treatment to attend to any adverse event. Other minor ailments like laboratory diagnosed malaria, anaemia, diarrhoea and others were treated according to the national guidelines. Adhering to the National Schistosomiasis and Worm Control Programme strategy of mass treatment in endemic areas, the rest of the community was treated after the second treatment of the cohort.

5.3 Results

5.3.1 Characteristics of study participants

This chapter was mainly for comparison of the efficacy of one and two doses of praziquantel on *S. mansoni* infection. Three hundred ninety five people infected with *S. mansoni* at baseline were treated with praziquantel. At nine weeks of follow-up 376 (95.2%) individuals positive at baseline were present for re-examination (figure 5.1). Comparing baseline characteristics of those who were positive before treatment and were present at nine weeks, there were no significant differences between the two treatment groups in terms of sex, age, occupation and *S. mansoni* infection intensity (table 5.1). The loss to follow-up was similar in the two treatment groups.

Table 5. 1: A comparison of two treatment groups at nine weeks of follow-up

	Number, single dose	**Number, double dose**	**Test statistic**	***P***
Lost to follow up (%)	9 (57.1)	6 (42.9)	Fisher's exact,	0.787
*Sex (male) (%)	102 (48.3)	109 (51.7)	$\chi^2 = 0.53$	0.467
*Occupation (fishing) (%)	30 (45.5)	36 (54.5)	$\chi^2 = 0.66$	0.416
*Baseline GMI ± SD (epg)	196 (287.9 ± 5.6)	194 (249.2 ± 5.9)	t = ¯0.66	0.511
*Mean age ± SD (years)	196 (20.3 ± 1.9)	194 (19.6 ± 1.9)	t = 0.57	0.571

All numbers include those who were *S. mansoni* positive at baseline. * includes those who were present at nine weeks after treatment.

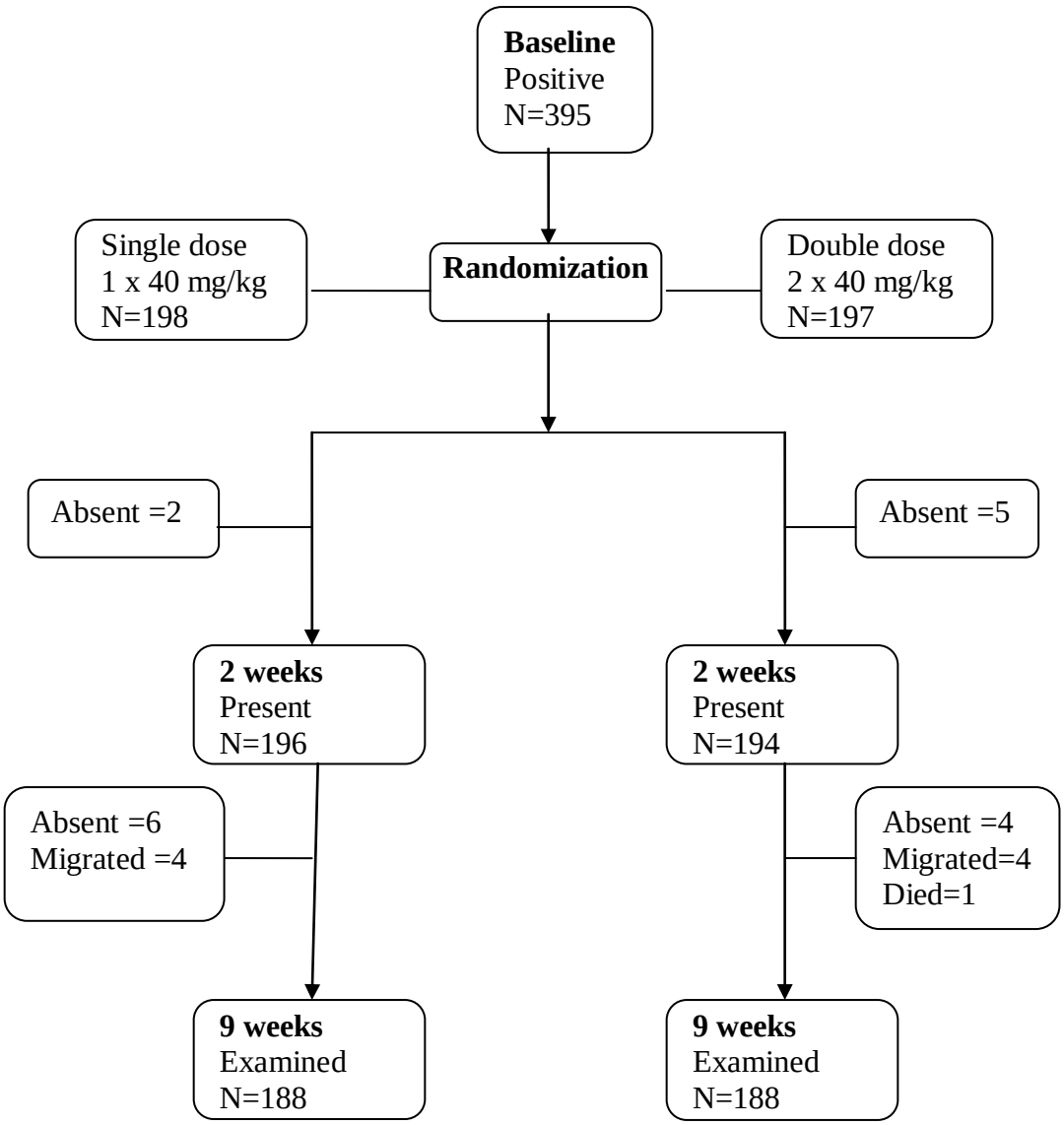

Figure 5. 1: Trial profile

5.3.2 Cure rates

Of the 376 who were infected at baseline and were followed up at nine weeks, 221 (58.8%) became egg negative. In the single dose group, 90 (47.9%) people were cured compared to 131 (69.7%) for the two dose group. Thus double dose was more likely to lead to higher cure rate than single dose (Risk ratio [RR]=1.7, 95% CI :1.3 – 2.2, $P < 0.001$). The superiority of double dose compared to single dose in terms of cure rate remained after stratification regarding sex, age, occupation and *S. mansoni* infection intensity at baseline (table 5.2). Regarding pre-treatment intensity double dose appears not to be particularly efficacious for those who had light infection.

Table 5. 2: Comparison of cure rate of two treatment regimens of praziquantel with regard to sex, age, occupation and baseline *S. mansoni* infection intensity

	N	Number cured (%)		RR	95% CI
		Single dose	Double dose		
Overall	376	90 (47.9)	131 (69.7)	1.7	1.3 – 2.2
Sex					
Males	211	48 (47.1)	80 (73.4)	2.0	1.4 – 2.9
Females	165	42 (48.8)	51 (64.6)	1.4	1.0 – 2.1
Age (years)					
7 – 14	141	38 (55.1)	52 (72.2)	1.6	1.0 – 2.6
≥15	235	52 (43.7)	79 (68.1)	1.8	1.3 – 2.2
Occupation					
Farmer	117	30 (44.8)	31 (62.0)	1.4	1.0 – 2.2
Fishing	66	11 (36.7)	29 (80.6)	3.2	1.6 – 6.7
Student	165	41 (52.6)	61 (70.1)	1.6	1.1 – 2.4
Other	28	8 (61.5)	10 (66.7)	1.1	0.4 – 3.1
Baseline intensity (epg)					
Low (1-99)	114	36 (61.0)	33 (60.0)	1.0	0.6 – 1.5
Medium (100-399)	93	17 (35.4)	34 (75.6)	2.6	1.5 – 4.5
Heavy (≥400)	169	37 (45.7)	64 (72.7)	2.0	1.3 – 2.9

RR = Risk ratios, given as the recipricols of the calculated RR. 95% CI = Confidence interval for risk ratios

5.3.3 Measuring the impact of the treatment

Table 5.3: Efficacy of praziquantel using two regimens

Regimen	Cured	Not cured	Total
Two doses (exp)	57	131	188
Single dose (cont)	98	90	188
Total	155	221	376

Relative risk (RR) = $\frac{\text{Incidence}_{exp}}{\text{Incidence}_{cont}} = \frac{57}{188} \times \frac{188}{98} = 0.58$

Relative risk reduction (efficacy) = $\frac{\text{Incidence}_{exp} - \text{Incidence}_{exp}}{\text{Incidence}_{cont}}$

Efficacy = $\frac{\text{Incidence}_{cont}}{\text{Incidence}_{cont}} - \frac{\text{Incidence}_{exp}}{\text{Incidence}_{cont}} = 1\text{-RR}$

Efficacy: 1 - 0.58 = 0.42

95% CI_{RR} = RR/exp[1.96 x s.e.(lnRR)] to RR x exp[1.96 x s.e.(lnRR)]

But exp[1.96 x s.e.(lnRR) = error factor (EF), thus 95% CI_{RR} = RR/EF to RR x EF

s.e.(lnRR) = $\sqrt{[1/d_1 - 1/n_1 + 1/d_0 - 1/n_0]}$

s.e(lnRR) = $\sqrt{[1/57 - 1/188 + 1/98 - 1/188]} = 1.14$

EF = exp(1.96 x 1.14) = 9.341

95% CI_{RR} = 0.58/9.341 to 0.58 x 9.341 = 0.06 to 5.42

Since efficacy = 1 – RR, its 95% CI is obtained by subtracting RR confidence limits from one.

95% $CI_{efficacy}$ = 1 – RR x EF to 1 – RR/EF = 1 – 5.42 to 1 – 0.06
= -4.42 to 0.94

Thus the efficacy of two doses of praziquantel = 0.42 (95% CI: -4.42 – 0.94).

Absolute risk reduction (ARR) = $I_{cont} - I_{exp}$

ARR: 0.52 – 0.30 = 0.22

Number needed to treat (NNT) = 1/ARR; NNT: 1/0.22 = 5

5.3.4 Effect of the two treatment regimens on *S. mansoni* infection intensity

The results reported here are for those who were positive at baseline and remained infected with *S. mansoni* nine weeks after treatment. At baseline, the GMI for single dose was 227.5 epg (95% CI: 176.9 – 292.7) and it reduced to 22.1 epg (95% CI: 16.9 – 28.8) after treatment. Double dose reduced the GMI from 256.7 epg (95% CI: 198.6 – 331.7) before treatment to 12.0 epg (95% CI: 8.9 – 16.1) (table 5.4).

Table 5. 4: *S. mansoni* geometric mean intensity at nine weeks after treatment with regard to sex, age, occupation and baseline *S. mansoni* infection intensity across the two treatment groups

Characteristic	Praziquantel dose	Baseline		9 weeks after treatment	
		GMI (N)	95% CI	GMI (N)	95% CI
Overall	Single	227.5 (188)	176.9 – 292.7	22.1 (98)	16.9 – 28.8
	Double	256.7 (188)	198.6 – 331.7	12.0 (57)	8.9 – 16.1
Sex					
Female	Single	144.5 (86)	101.2 – 206.4	23.0 (44)	15.2 – 34.7
	Double	126.1 (79)	83.7 – 189.9	12.7 (28)	7.9 – 20.1
Male	Single	333.6 (102)	237.1 – 469.5	21.3 (54)	14.9 – 30.6
	Double	429.7 (109)	319.3 – 578.3	11.3 (29)	7.6 – 16.8
Age (years)					
7 – 14	Single	257.0 (69)	179.8 – 367.4	20.8 (31)	12.4 – 35.1
	Double	308.2 (72)	208.2 – 456.0	12.8 (20)	7.7 – 21.3
≥ 15	Single	212.0 (119)	150.5 – 298.8	22.6 (67)	7.9 – 16.8
	Double	229.1 (116)	163.1 – 321.8	11.5 (37)	7.9 – 16.8
Occupation					
Farmer	Single	103.8 (67)	66.5 – 162.0	20.8 (37)	13.8 – 31.4
	Double	92.5 (50)	56.3 – 152.0	12.6 (19)	6.9 – 22.8
Fishing	Single	752.3 (30)	444.4 – 1273.7	13.9 (19)	8.8 – 21.9
	Double	854.3 (36)	555.3 – 1314.6	7.0 (7)	3.5 – 14.0
Student	Single	273.1 (78)	194.5 – 383.5	27.2 (37)	16.3 – 45.4
	Double	328.5 (87)	233.7 – 461.6	15.1 (26)	9.6 – 23.8
Other	Single	275.8 (13)	100.6 – 756.3	42.4 (5)	7.9 – 226.3
	Double	102.9 (15)	37.6 – 281.3	6.2 (5)	3.8 – 10.3
Baseline *S. mansoni* intensity (epg)					
Low (1-99)	Single	27.3 (59)	21.2 – 35.0	16.6 (23)	10.4 – 26.7
	Double	26.7 (55)	20.7 – 34.5	12.4 (22)	7.3 – 20.9
Medium (100-399)	Single	198.8 (48)	177.7 – 222.3	23.4 (31)	15.3 – 35.5
	Double	200.4 (45)	175.3 – 229.2	14.8 (11)	7.2 – 30.6
Heavy (≥400)	Single	1155.8 (81)	1004.7 – 1329.6	24.6 (44)	15.3 – 39.4
	Double	1198.5 (88)	1032.2 – 1391.5	10.5 (24)	6.6 – 16.6

The overall egg reduction rate was 92.9%. The egg reduction rate of the single dose (92.3%) was not significantly different from that of double dose group (93.9%) ($P = 0.6$). Even when the two treatment groups were stratified by age, sex and occupation, the difference in egg reduction rate was not significant (results not shown).

5.4 Discussion and conclusion

The major findings of this study are that two doses of praziquantel given two weeks apart increases the cure rate and leads to a greater reduction in intensity of *S. mansoni* infection compared to single dose. This is supported by the obtained efficacy which implies that the risk of not curing with two doses is 42% of that of the single dose; and the number needed to treat of 5, in that by treating with two doses, one adverse invent would be prevented for every 5 people treated. The improved cure and reduction in intensity of infection is particularly marked among those with heavy infection. Although these results are similar to what has been observed elsewhere (Picquet *et al*., 1998; Kabatereine *et al*., 2003; N'Goran *et al*., 2003), most of these other studies assessed different time intervals between the two doses. The time interval of two weeks used in this study and the higher cure rate with double dose than single dose could be explained based on the life cycle of *S. mansoni* in relation to praziquantel action on mature schistosome parasites. *S. mansoni* parasites take 4-6 weeks from infective stage to adult mature worms that can lay eggs (Jordan & Webbe, 1993; Sturrock, 2001). In a high transmission area, people are found to be infected with all the stages of schistosomes. It is likely that at the time of first treatment, people in our study were harbouring significant numbers of juvenile worms from pre-patent infections. Noting that juvenile schistosomes are insensitive to praziquantel (Sabah *et al*., 1986; Renganathan & Cioli, 1998), the first treatment could have killed only the mature worms. Within two weeks, the worms that were immature at first treatment matured and became susceptible to praziquantel, and were killed by the second dose. Based on the same principle and similar to findings elsewhere (Kabatereine *et al*., 2003), the cure rate of double dose group was significantly higher than that of single dose after stratification by sex, age, occupation and pre-treatment intensity of infection. Among the uncured, and in consistency with other studies (Tchuem Tchuente *et al*., 2001; Kabatereine *et al.*, 2003; N'Goran *et al*., 2003) both treatment regimens greatly reduced the mean intensity of *S. mansoni* infection.

Considering the standard range of schistosomiasis cure rate of 60 – 90% for a single dose (Cioli *et al*., 1995; WHO, 2002; Raso *et al*., 2004), the cure rates obtained, even for double dose in the present study are below the expected range. However, our cure rates are superior to those reported in Senegal (Stelma *et al*., 1995; Van Lieshout *et al*., 1999; Stelma

et al., 1995; Gryseels *et al*., 2001; Danso-Appiah & De Vlas, 2002). In a study in Senegal, Guisse *et al*., (1997) treated one group of people with 40 mg/Kg body weight and six weeks later only 36% were cured. Another group was treated with two doses of 30 mg/Kg at an interval of 6 hours and six weeks later the cure rate was 49%. Similar findings were also reported in Côte d'Ivoire (Utzinger *et al*., 2000). They attributed the low cure rates to high pre-treatment intensity of infection. It is likely that the rather low cure rates in our study were due to high intensity of *S. mansoni* infection. It should also be noted that we did not carry out viability studies, and may be some of the detected eggs after treatment were dead, hence low cure rates. On the other hand, the highly sensitive method used in our study of taking three stool samples could have yielded low cure rates as compared to other studies that realised similar results but used a single stool sample whereby the light infections after treatment could have been missed. For instance, a study carried out in Senegalese children, used a single stool sample and reported a GMI of 478 epg (Picquet *et al*., 1998). The children were treated twice with standard doses given 40 days apart. After the first dose, the cure rate was 42.5% and the egg reduction rate was 70.7% while the cure rate and egg reduction rate for the second dose were 76.1% and 88.1% respectively.

In conclusion, double dose has advantages over single dose e.g. increased cure rate and higher reduction in intensity of *S. mansoni* infection. However, it is likely to have implementation disadvantages in a control programme such as high operational cost, reduced compliance and logistical problems. We cannot recommend use of two doses in the control of schistosomiasis based on only cure rates. There is need to balance the merits and de-merits based on further research to compare the effectiveness of two doses on morbidity regression, growth among children and incidence of re-infection. We followed up this cohort to compare the effect of double versus single dose treatment on these parameters.

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Chapter 6: A comparison of the effect of treatment with one or two doses of praziquantel on re-infection with S. *mansoni* along Lake Victoria in Uganda

Summary

To compare the effect of treatment with one and two doses on re-infection, 395 people infected with *S. mansoni* were treated with a standard dose of praziquantel (Distocide® 600 mg, Shin Poong Pharmaceuticals, Seoul Republic of Korea, 40mg/kg body weight). Two weeks after the initial treatment, one half of the study participants received another dose of praziquantel. Re-infection levels were assessed 8 months and 24 months after treatment.

At eight months follow up, the incidence of re-infection for those who were given two doses (61.6%, 95% confidence interval [95% CI]: 50.2 – 73.1) was not significantly different from that of the ones that received a single dose (68.3%, 95% CI: 59.9 – 76.8). The incidence of re-infection 24 months after treatment for those given a single dose (73.2%, 95% CI: 62.7 – 83.8) was also not significantly different from that of the double dose group (70.8%, 95% CI: 62.3 – 79.3). There was no significant difference in GMI of re-infection for single (33.8 epg, 95% CI: 23.2 – 49.3) and double (34.5 epg, 95% CI: 24.7 – 48.1) dose groups 8 months after treatment. Twenty four months after treatment, the GMI of re-infection for single dose group (57.5 epg, 95% CI: 33.9 – 97.5) and double dose group (42.2 epg, 95% CI: 29.9 – 59.6) were not significantly different. Our results showed that the effect of two doses of praziquantel on re-infection is not significantly different from that of a single dose.

6.1 Introduction

The major public health importance of schistosomiasis infection is the morbidity that the disease inflicts onto the affected community. Control of schistosomiasis by chemotherapy aims at reducing morbidity, which is mainly caused by schistosome eggs deposited in the host's tissue (WHO, 2002). Treatment of schistosomiasis using praziquantel targets to reduce or temporarily eliminate schistosome parasites from infected populations but people living in highly endemic areas frequently get re-infected within a year after treatment.

It has been documented that the immunological responses to schistosome antigens that enhance resistance to re-infection can be boosted after treatment with praziquantel. Treatment increases the amount of IL-4 and IL-5 Th2 cytokines released in response to worm antigen which in turn trigger release of IgE antibodies. IgE antibodies are known to be associated with resistance to schistosomiasis (re)infection (Rihet *et al*., 1991; Farghaly, 1993; Mutapi, 2001; Secor, 2005). It has also been reported that praziquantel temporarily immunises against schistosomiasis (re)infection (Fallon *et al*., 1992; Karanja *et al*., 2002; Colley & Secor, 2004). Thus repeated chemotherapy is expected to enhance "passive vaccination" effect and increase the levels of schistosome worm antigens released in the blood circulation of the host, thereby increase resistance to re-infection. This may in turn have an impact on intensities of re-infection (Richter, 2003).

Although mass treatment of schistosomiasis using praziquantel has proved to be effective in controlling schistosomiasis related morbidity, evidence has shown that in high transmission areas, re-infection with schistosomes after chemotherapy is high especially in children and young adolescents (Boisier *et al*., 1995; Fulford *et al*., 1998; Corrêa-Oliveira *et al*., 2000; Barakat *et al*., 2000; Kabatereine, 2000; Kabatereine *et al*., 2004; Conlon, 2005; Vereecken *et al*., 2007). If treatment can enhance resistance to re-infection, two doses would be expected to increase the level of resistance more than a single dose. However, information comparing the effect of two doses of praziquantel to re-infection is limited. In this study, we compared the effect of one and two doses of praziquantel given two weeks apart on *S. mansoni* re-infection.

6.2 Material and methods

6.2.1 Study area and population

The study was carried out along Lake Victoria in Musoli village community, Mayuge district. As reported along Lake Albert in Uganda (Kabatereine *et al*., 2003), transmission of schistosomiasis in this area is stable and high throughout the year. Besides fishing being the major economic activity in this village that exposes people to infection, there is no any source of safe water other than Lake Victoria, which also exposes the community to schistosomiasis infection.

6.2.2 Study design

The study was a cross sectional baseline survey followed by longitudinal randomised intervention follow up surveys. The cross sectional study was conducted to collect pre-treatment levels of *S. mansoni* infection after which all participants were treated.

6.2.3 Sample size determination and sampling procedure

To detect a true difference of the effect of one dose and two doses on re-infection levels with *S. mansoni*, Statcalc calculator of Epi Info, version 6.04 (Centers for Disease Control and Prevention, USA) was used to calculate the sample size. Using re-infection levels of 51.3% (Butterworth *et al*., 1985), power of 90% and 95% probability, the obtained sample size was 79 people per treatment group. Allowing for an annual loss to follow up of 20%, at two years follow up, the loss would be 40% of the calculated sample size. Thus 32 more people were required per group to cater for the loss, resulting into 111 people per dose group. However, this report is part of a study that assessed the effect of two doses of praziquantel on cure rate, re-infection and morbidity regression, thus the sample size calculation for the whole study was based on the expected difference in cure rate between the two study groups as well as the expected loss to follow-up. Sample size was calculated using Kirkwood's 1988 modified formula for comparing two rates (Hardon *et al*., 1994). Basing the calculations on cure rates obtained from a study of communities living along the shores of Lake Albert (Kabatereine *et al*., 2003) of 41.9% and 69.1% for a single dose and two doses of praziquantel respectively, the sample size at 95% significance level and power of 90% was calculated as follows:

$$n = \frac{(u + v)^2 (r_1 + r_2)}{(r_1 - r_2)^2},$$

Where r = cure rate; u = two-sided percentage point of the normal distribution corresponding to the power of 90%; v = percentage point of the normal distribution corresponding to the significance of 95%.

The sample size per group: $\frac{(1.64 + 1.96)^2 (0.42 + 0.69)}{(0.42 - 0.69)^2} = 197$

In order to allow for a 20% annual loss to follow-up (total loss 40% in two years) 79 people were added and each group had 276 participants, giving a total sample size of 552 people to be recruited in the study. Thus, the used sample size for the whole study was big enough to detect a true difference in the effect of the two treatment doses on *S. mansoni* re-infection.

All the inhabitants in Musoli village were registered and this made a sampling frame. A unique number was assigned to each household and every member of the household carried this number followed by his/her individual number. Data were entered in the computer and computer generated random numbers were used to select a representative cohort stratified for age and sex. Sample selection was performed upon the individuals in the whole sampling frame. After the baseline data collection, a list of all the examined participants was drawn. Again using computer generated random numbers, odd numbers in each stratum were located to single treatment group while even numbers were located to double treatment group. Randomisation was performed by a Scientist, who was independent of all the examinations.

6.2.4 Data collection methods and procedures

6.2.4.1 Stool examination

Stool was collected using the modified Kato Katz thick smear technique (Katz *et al*., 1972) with a 50 mg template. In order to improve diagnostic sensitivity, three early morning stool specimens were taken from each participant on three consecutive days (Cheever *et al*., 1994; Utzinger *et al*., 2000; Booth *et al*., 2003). From each specimen; two slides were prepared, giving rise to six slides per participant. Two experienced technicians examined the slides under the microscope (10x). In order to minimise intra-observer variation, both

technicians read slides from the same sample in such a way that each technician read one of the duplicate slides. The numbers of *S. mansoni* eggs observed per slide were recorded. The technicians were blinded to the participant's treatment group. Stool examination was repeated nine weeks after the first treatment to determine those who were stool egg negative and again at eight and twenty four months to determine the re-infection levels.

6.2.4.2 Treatment

After all stool specimens were collected, each participant was treated with a single dose of praziquantel (40 mg/kg body weight) and one tablet of albendazole 400 mg to clear other intestinal helminths. The brands of albendazole and praziquantel used were Alzental® 400mg and Distocide® 600 mg respectively all manufactured by Shin Poong Pharmaceuticals, Seoul Republic of Korea. Two weeks later, one of the groups received another dose of praziquantel while the other did not receive any treatment. Treatment was performed by and under direct observation of an experienced nurse. Participants were kept at the field research station for two hours after treatment to observe and manage any possible adverse events.

6.2.5 Data management and analysis

6.2.5.1 Quality control

Results from different observers were not compared, instead stool quality control was done by an independent experienced microscopist reading a random 10% of the slides, after which the results were compared and where there was controversy, all the six slides of the same individual were read by two different experienced technicians and the counts were harmonised. Data were double-entered by two experienced data entry clerks using excel computer programme and the two files were cleaned, compared and a master file compiled, which was used for all analyses.

6.2.5.2 Data management

Data were imported from Excel computer programme to Stata for windows (Intercooled Stata 10.1, Stata Corporation, USA) for analysis. Two age categories, for children (<15 years) and adults (15 years and above) were created and these were used in analysis.

Analysis for re-infection was performed for only those who were positive before treatment and were stool egg negative nine weeks after treatment but later became positive again. The arithmetic mean of the individual *S. mansoni* egg counts was calculated from all the six slide results and multiplied by 20 to obtain individual eggs per gram (epg) faeces i.e. *S. mansoni* infection intensity. Histograms, with normal curves, of *S. mansoni* intensity according to various predictors of infection were generated before analysis to check whether intensity was normally distributed. Intensity was found to be skewed, thus the data was normalised using base 10 logarithm transformations. The geometric mean intensity (GMI) of *S. mansoni* infection of only the positive ones was obtained as the antilog of the mean of the transformed egg counts and is reported as eggs per gram (epg) of faeces.

Intensity of infection was categorised based on World Health Organisation criteria as: 1-99, 100-399 and ≥400, defined as low, moderate and heavy intensities of infection respectively (WHO, 2002). Re-infection was defined as persons who were positive before treatment that became egg negative nine weeks later but were detected egg positive eight or 24 months after treatment.

6.2.5.3 Data analysis

Student's t test was applied and 95% confidence interval (CI) of GMI used to compare the effect of the two treatment regimens on mean intensity of re-infection. Chi square test was performed to compare proportions of various parameters and of those lost to follow up among the two treatment groups. Proportions of incidence of re-infection and their 95% CI were used to compare re-infection between the two treatment groups. A *P* value <0.05 was used to determine statistical significance in all analyses. Confounding was not controlled for during analysis because the confounders were randomised between the two treatment groups.

6.2.6 Ethical considerations

Ethical approval was obtained from the Higher Degrees Research and Ethics Committee of the School of Public Health, Makerere University. Ethical clearance was obtained from the Uganda National Council of Science and Technology. This study was supported by

DANIDA, thus clearance was also sought from the Danish National Committee on Biomedical Research Ethics in Denmark. Informed consent was obtained from individual adult participants while parents or guardians consented on behalf of children. All information obtained from participants was kept confidential.

To minimise occurrence of possible side effects of praziquantel when taken on an empty stomach, a snack and a soft drink were given before treatment. A trained nurse was available during treatment to attend to any adverse event. Other minor ailments like laboratory diagnosed malaria, anaemia, diarrhoea and others were treated according to the national guidelines. Adhering to the National Schistosomiasis and Worm Control Programme strategy of mass treatment in endemic areas, the rest of the community was treated after the second treatment of the cohort.

6.3 Results

6.3.1 Characteristics of the study population

Re-infection being defined as people who were *S. mansoni* positive before treatment and became egg-negative after treatment but later got infected again; the baseline characteristics of those who became re-infected with *S. mansoni* were compared between the two treatment groups as shown in table 6.1. The mean age of children below 15 years of age was 10.0 years (SD ± 2.3, range 7 – 14 years) while that of adults was 32.5 years (SD ± 14.4, range 15 – 76 years). However, at follow up points, some people were absent, figure 6.1. For the ones lost to follow up at 8 months after treatment, there was no significant difference in: pre-treatment prevalence of *S. mansoni* infection (χ^2 = 1.59, *P* = 0.207), pre-treatment infection intensity (t = -1.11, df = 43, *P* = 0.272), sex distribution (χ^2 = 0.09, *P* = 0.754) and age distribution (χ^2 = 0.19, *P* = 0.656) among the two treatment groups. Likewise, at 24 months after treatment, the ones lost to follow up were not significantly different with regard to: pre-treatment prevalence of *S. mansoni* infection (χ^2 = 0.25, *P* = 0.615), pre-treatment infection intensity (t = 0.53, df = 83, *P* = 0.59), sex distribution (χ^2 = 0.02, *P* = 0.875) and age distribution (χ^2 = 0.50, *P* = 0.479) among the two treatment groups.

Table 6. 1: Comparison of baseline characteristics between the two treatment groups for people who were infected with *S. mansoni* before treatment but became negative nine weeks after treatment.

(a) At eight months

Characteristic	**Number, single dose**	**Number, double dose**	**Test statistic**	***P***
Sex (Male) (%)	38 (52.1)	71 (59.2)	$\chi^2 = 0.93$	0.334
Occupation (Fishing) (%)	10 (13.7)	24 (20.0)	$\chi^2 = 1.24$	0.265
Baseline GMI ± SD (epg)	73 (178.2 ± 6.9)	120 (276.4 ± 5.5)	t = ¯1.65	0.100
Mean age ± SD (years)	73 (24.1 ± 16.7)	120 (23.3 ± 16.1)	t = 0.33	0.745

(b) At twenty four months

Characteristic	**Number, single dose**	**Number, double dose**	**Test statistic**	***P***
Sex (Male) (%)	39 (54.9)	72 (63.7)	$\chi^2 = 1.41$	0.236
Occupation (Fishing) (%)	10 (14.1)	26 (23.0)	$\chi^2 = 2.21$	0.137
Baseline GMI ± SD (epg)	71 (184.1 ± 6.5)	113 (301.0 ± 5.1)	t = ¯1.88	0.062
Mean age ± SD (years)	71 (24.1 ± 16.4)	113 (23.9 ± 16.3)	t = ¯0.06	0.956

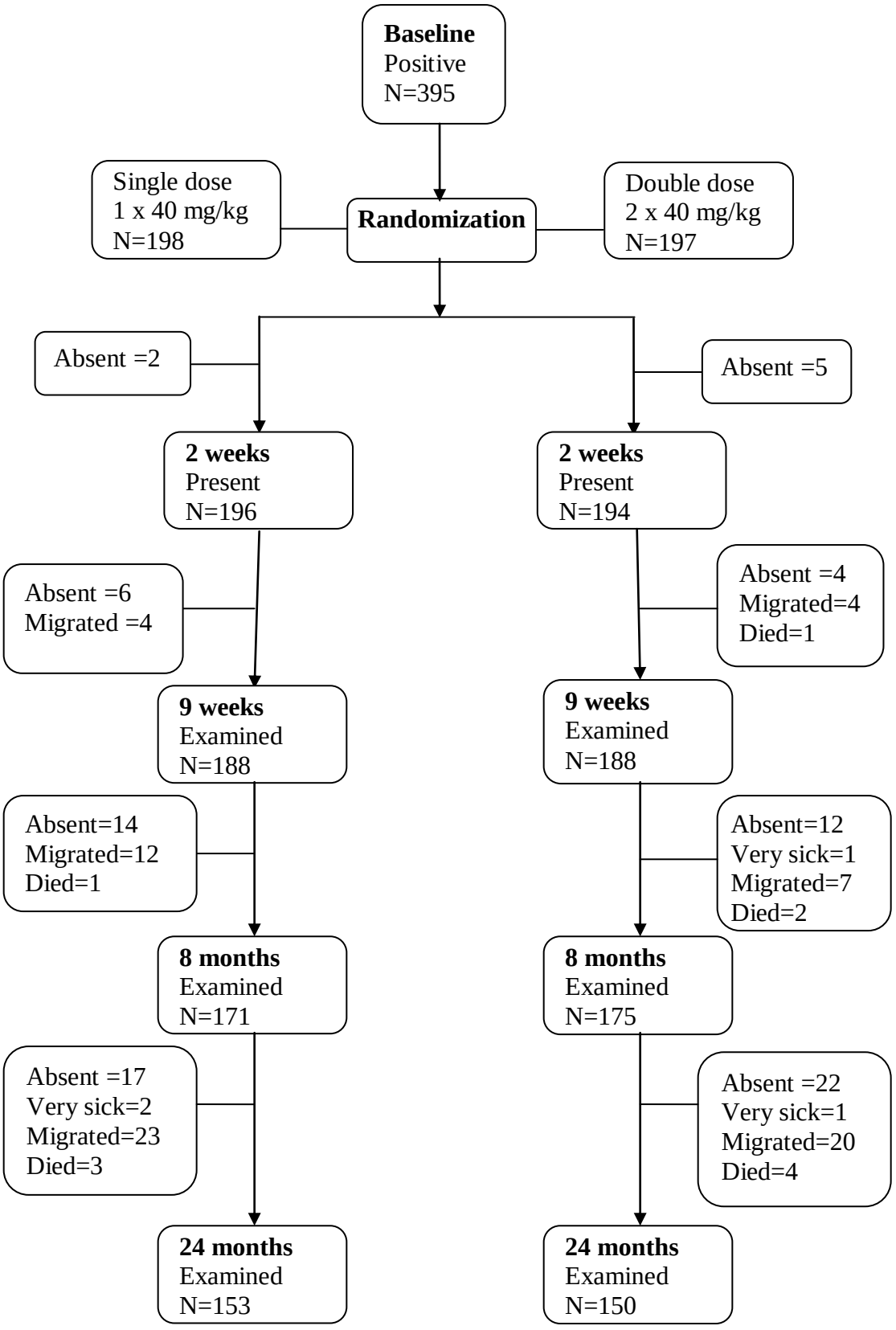

Figure 6. 1: Profile showing *S. mansoni* infection levels at different time points

6.3.2 Incidence of re-infection with *S. mansoni* at eight and twenty four months after treatment

Overall, 221(58.8%) people were cured at nine weeks. Of those cured at nine weeks, 127 (65.8%) and 132 (71.1%) became re-infected at eight and 24 months after treatment respectively. Table 6.2 compares *S. mansoni* re-infection levels among the two treatment groups at eight and 24 months with regard to sex, age, occupation and pre-treatment *S. mansoni* infection intensity. No significant difference in incidence of *S. mansoni* re-infection was observed when the two treatment groups were compared at the two follow up points. Even after stratification with regard to sex the difference was not significant. When incidence of re-infection was stratified by age and occupation, there was a significant difference between the two treatment groups for children ($\chi^2 = 4.5$, P = 0.031) and students ($\chi^2 = 4.6$, P = 0.032) at eight months (table 6.2a).

Table 6. 2: Comparison of incidence of re-infection between the two treatment groups with regard to sex, age, occupation and pre-treatment *S. mansoni* infection intensity

(a) At eight months

Characteristic	**Single dose**		**Double dose**	
	No.*re-infected (%)	**95% CI**	**No. *re-infected (%)**	**95% CI**
Overall	45 (61.6)	50.2 – 73.1	82 (68.3)	59.9 – 76.8
Sex				
Female	17 (48.6)	31.2 – 66.0	30 (61.2)	47.1 – 75.4
Male	28 (73.7)	59.0 – 88.4	52 (73.2)	62.7 – 83.8
Age				
< 15 years	20 (64.5)	46.7 – 82.3	41 (85.4)	75.1 – 95.8
≥ 15 years	25 (59.5)	44.0 – 75.0	41 (56.9)	45.2 – 68.7
Occupation				
Farmer	13 (54.2)	32.7 – 75.7	18 (58.1)	40.0 – 76.5
Fisherman	8 (80.0)	49.8 – 100	14 (58.3)	37.1 – 79.6
Student	21 (61.8)	44.6 – 79.0	46 (82.1)	71.8 – 92.5
Other	3 (60.0)	8.0 – 100	4 (44.4)	3.9 – 85.0
Baseline intensity of *S. mansoni* (epg)				
Low (1-99)	13 (41.9)	23.5 – 60.3	18 (56.3)	38.1 – 74.4
Moderate (100-399)	10 (100)	100 – 100	22 (73.3)	56.5 – 90.1
Heavy (≥400)	22 (68.8)	51.8 – 85.7	42 (72.4)	60.6 – 84.3

*Re-infection is defined as those people who were positive for *S. mansoni* before treatment and became egg negative at nine weeks after treatment but later became infected again.

(b) At twenty four months

Characteristic	Single dose		Double dose	
	No. *re-infected (%)	95% CI	No. *re-infected (%)	95% CI
Overall	52 (73.2)	62.7 – 83.8	80 (70.8)	62.3 – 79.3
Sex				
Female	21 (65.6)	48.2 – 83.0	26 (63.4)	48.0 – 78.8
Male	31 (79.5)	66.2 – 92.7	54 (75.0)	64.8 – 85.2
Age				
< 15 years	28 (90.3)	79.3 – 100	42 (93.3)	85.8 – 100
≥ 15 years	24 (60.0)	44.1 – 75.9	38 (55.9)	43.8 – 68.0
Occupation				
Farmer	12 (52.2)	30.1 – 74.3	14 (51.9)	31.7 – 72.0
Fisherman	8 (80.0)	49.8 – 100	17 (65.4)	45.8 – 85.0
Student	29 (87.9)	76.1 – 99.6	46 (88.5)	79.5 – 97.4
Other	3 (60.0)	8.0 – 100	3 (37.5)	5.8 – 80.8
Baseline intensity of *S. mansoni* (epg)				
Low (1-99)	15 (51.7)	32.4 – 71.1	13 (46.4)	26.7 – 66.1
Moderate (100-399)	9 (81.8)	54.6 – 100	25 (83.3)	69.2 – 97.5
Heavy (≥400)	28 (90.3)	79.3 – 100	42 (76.4)	64.8 – 88.0

*Re-infection is defined as those people who were positive for *S. mansoni* before treatment and became egg negative at nine weeks after treatment but later became infected again.

6.3.3 *S. mansoni* re-infection intensity at eight and twenty four months after treatment

The *S. mansoni* re-infection intensity was not significantly different among the two dose groups throughout the study. Even after stratification by sex, age, occupation and pre-treatment intensity of infection, the difference was not significant (table 6.3). Comparing GMI of re-infection 24 months after treatment with regard to pre-treatment intensity levels, there was no significant difference between the pre-treatment heavily infected ones (68.3

epg) and the moderately infected ones (40.5epg) (t = ¯1.47, *P* = 0.146). Those who had light intensity of infection before treatment had significantly lower GMI of re-infection (23.7 epg) than the ones that were heavily infected (t = ¯2.89, *P* = 0.005).

Table 6. 3: Comparison of *S. mansoni* re-infection intensity between the two treatment groups at eight months according to sex, age, occupation and pre-treatment intensity of infection

(a) At eight months

Characteristic	**GMI (epg), single dose**		**GMI (epg), double dose**	
	GMI (N)	**95% CI**	**GMI (N)**	**95% CI**
Overall	33.8 (45)	23.2 – 49.3	34.5 (82)	24.7 – 48.1
Sex				
Female	31.1 (17)	15.8 – 61.0	23.8 (30)	15.0 – 37.8
Male	35.6 (28)	22.0 – 57.6	42.7 (52)	27.2 – 67.2
Age				
< 15 years	44.7 (20)	25.4 – 78.9	36.0 (41)	23.3 – 55.4
≥ 15 years	27.1 (25)	16.0 – 45.6	33.1 (41)	19.6 – 55.9
Occupation				
Farmer	24.1 (13)	10.2 – 57.0	19.3 (18)	10.3 – 36.1
Fisherman	34.7 (8)	14.0 – 86.1	51.0 (4)	6.0 – 162.3
Student	42.8 (21)	24.8 – 73.9	35.0 (46)	23.0 – 53.2
Other	26.2 (3)	2.2 – 1383.2	20.2 (4)	17.1 – 608.5
Baseline *S. mansoni* infection intensity (epg)				
Low (1-99)	22.7 (13)	9.5 – 54.1	19.5 (18)	9.3 – 41.0
Moderate (100-399)	23.3 (10)	10.7 – 50.0	36.3 (22)	19.2 – 68.5
Heavy (≥400)	50.8 (22)	30.8 – 84.0	42.9 (42)	26.6 – 69.2

(b) At twenty four months

Characteristic	GMI (epg), single dose		GMI (epg), double dose	
	GMI (N)	**95% CI**	**GMI (N)**	**95% CI**
Overall	57.5 (52)	33.9 – 97.5	42.2 (80)	29.9 – 59.6
Sex				
Female	30.5 (21)	14.0 – 66.5	28.3 (26)	16.0 – 49.9
Male	88.3 (31)	43.6 – 178.8	51.2 (54)	33.2 – 79.1
Age				
< 15 years	81.2 (28)	39.3 – 167.5	63.0 (42)	41.1 – 96.8
≥ 15 years	38.4 (24)	17.3 – 85.4	27.1 (38)	15.9 – 46.3
Occupation				
Farmer	30.2 (12)	9.3 – 98.4	16.8 (14)	7.7 – 36.4
Fisherman	68.9 (8)	11.5 – 414.0	41.5 (17)	18.3 – 94.2
Student	77.1 (29)	38.0 – 156.1	55.0 (46)	35.7 – 84.7
Other	61.0 (30)	5.0 – 3697.5	27.1 (3)	14.3 – 5323.5
Baseline *S. mansoni* infection intensity (epg)				
Low (1-99)	24.6 (15)	10.0 – 55.3	22.7 (13)	9.5 – 55.3
Moderate (100-399)	76.4 (9)	18.3 – 319.2	32.2 (25)	16.4 – 63.1
Heavy (≥400)	82.6 (28)	37.6 – 181.4	60.2 (42)	38.0 – 95.2

6.4 Discussion and conclusion

In this part of the study we assessed infection levels after treatment and compared the effect of a single dose versus two doses of praziquantel treatment on re-infection of *S. mansoni*. There was no significant difference in incidence and intensity of re-infection between the two treatment groups at eight and 24 months after treatment, which conforms to findings by Sacko, (2006). This could be due to high transmission of schistosomiasis in our study area coupled with the temporary action (about two months) of praziquantel as a 'passive vaccine' towards *S. mansoni* infection (Rihet *et al*., 1991; Dunne *et al*., 1992; Fallon *et al*., 1992; Farghaly, 1993; Mutapi, 2001; Karanja *et al*., 2002; Colley & Secor, 2004; Fitzsimmons *et al*., 2004; Secor, 2005). This implies that probably 2 – 3 months after treatment, people from both treatment groups were equally exposed to *S. mansoni* re-infection. With high transmission of schistosomiasis and high water contact (chapter 4) in this area, both groups were likely to be re-infected to similar levels. More evidence to the continued transmission is shown by higher incidence and intensity of re-infection 24 months after treatment than eight months after treatment.

Other than comparing the effect of the two treatment doses on re-infection, a number of factors could have influenced levels of re-infection in our study. These are mainly sex, age and pre-treatment *S. mansoni* infection intensity. Similar to observations elsewhere (Ongom & Bradley, 1972; Jordan & Webbe, 1993; Kabatereine *et al*., 1999; Kabatereine, 2000) males had higher re-infection levels than females throughout the study. Children also had higher intensity of re-infection than adults throughout the study period (Ongom & Bradley, 1972; Bensted-Smith *et al*., 1987; Butterworth *et al*., 1988 & 1991; Boisier *et al*., 1995; Kabatereine *et al*., 1996, 1999; Fulford *et al*., 1998; Barakat *et al*., 2000; Corrêa-Oliveira *et al*., 2000; Kabatereine, 2000; Scott *et al*., 2003; Conlon 2005; Vereecken *et al*., 2007). This could be due to the duration and type of water contact activities that expose males and children to schistosomiasis infested water. In support to this, Scott *et al*., (2003) in their study in Senegal noted that even though females were more frequently in contact with water than males, males spent longer time in water exposing larger parts of their bodies than females. Likewise in our study, as reported in chapter four, females went to the lake more often than males but females did not show an association between GMI of *S.*

mansoni and the frequency of going to the lake while males exhibited a significant difference in GMI with regard to the number of days they went to the lake.

The association of re-infection and exposure to contaminated water has been further explained by the extent and nature of exposure (Butterworth *et al*., 1988) in that children expose larger parts of their bodies and increase the area for cercarial penetration than adults. In agreement to this, we observed that more children than adults went to the lake to swim, wash and fetch water (chapter 4). However, some studies elsewhere reported similar age patterns with high levels of re-infection in children despite the fact that adults were even more exposed to contaminated water than children (Butterworth *et al*., 1988; Kabatereine *et al*., 1999). This implies that children's high re-infection levels go beyond the magnitude of exposure to contaminated water and could probably be due to immunological and physiological factors.

Children may get more re-infected than adults due to lack of acquired immunity to schistosomiasis infection (Gryseels, 1994; Dunne *et al*., 1992; Naus *et al*., 1999; Fitzsimmons *et al*., 2004; Joseph *et al*., 2004; Dunne *et al*., 2006). Reports show that acquired immunity to schistosome re-infection increases with age (Rihet *et al*., 1991; Dunne *et al*., 1992; Corrêa-Oliveira, 2000; Fitzsimmons *et al*., 2004). The principal here is that schistosome antigens trigger the release of IL-4 and IL-5 Th2 cytokines and these cytokines stimulate the release of IgE antibodies, the antibodies that are associated with resistance to (re)infection (Rihet *et al*., 1991; Dunne *et al*., 1992; Webster *et al*., 1998; Naus *et al*., 1999). However, children below 15 years of age are reported not to exhibit an increase in IL-4 and IL-5 release (Dunne *et al*., 1992; Corrêa-Oliveira, 2000; Fitzsimmons *et al*., 2004; Secor, 2005) and some studies have shown that IgE levels increase with age (Naus *et al*., 1999; Vereecken *et al*., 2007). With low levels of cytokine release by children and its effect on the amount of IgE antibody release in the host body, it is possible that children lacked adequate immunity to schistosomiasis re-infection.

Another explanation to the age re-infection variation may be pubertal physiological changes such as increased skin thickness or fat deposition, all of which increase resistance

to re-infection by reducing cercarial penetration (Butterworth *et al*., 1988; Fulford *et al*., 1998; Dunne & Mountford, 2001). Since we observed that adults in Musoli frequently get in contact with the lake water and yet their re-infection levels are much lower than children's levels it is possible that the rate of cercarial penetration in adults is generally less than that in children.

High levels of re-infection in males and children could also be attributed to their high pre-treatment *S. mansoni* infection intensity (Bensted-Smith *et al*., 1987; Butterworth *et al*., 1988; Tingley *et al*., 1988; Webster *et al*., 1998; Karanja *et al*., 2002). These studies noted that the pre-treatment heavily infected people were more heavily re-infected than those who had light infections before treatment. Similarly, in our study the individuals who were heavily infected at baseline had the highest GMI of re-infection. It should be also noted that males and children in our study had higher pre-treatment intensity of infection than females and adults respectively.

Whereas it may be hypothesised that three or more doses of praziquantel per year may reduce the risk of re-infection, this may not be necessary since the major goal of schistosomiasis control is to reduce morbidity which is mainly associated with intensity of infection. Since in our study we realised no significant difference in intensity of re-infection between a single and double dose, implying that a single dose may be sufficient to keep morbidity levels low. Besides, three doses may have operational constraints in mass treatment control programme that relies on external funding with regard to implementation costs. The programme also depends on voluntary medicine distributors who usually complain about their time put into the single annual treatment programme and increasing the frequency of implementation may cause more fatigue.

In conclusion, incidence and GMI of re-infection was not significantly different between those who received one and two doses of praziquantel. Thus, two doses cannot be recommended for control of schistosomiasis based on ability to prevent re-infection. Nonetheless, having realised higher re-infection levels in children than in adults, our findings add more evidence to the strategy for control of schistosomiasis of mass treatment

targeting school-age children using a single annual dose of praziquantel. This is not only beneficial in terms of reducing intensity of infection, hence risk of morbidity development, but also reducing schistosomiasis transmission to the community since children contribute significantly in the spread of the disease by indiscriminately defaecating in the environment. Nonetheless, the loss to follow up was high and the number of re-infected people was low in that the sample sizes compared were small. This could have affected the detection of a difference in incidence of re-infection in our study.

Although there was no significant difference in the re-infection levels between the two treatment groups, there was a general reduction in intensity of *S. mansoni* eight and twenty four months after treatment. From our findings, it is evident that *S. mansoni* re-infection intensity remains low within two years after treatment. This finding could be applied in the control of schistosomiasis to massively treat some endemic communities every other year instead of annual treatments. On the other hand, reduction in intensity of infection is an indirect indicator of reduced worm load, which would result in fewer eggs being deposited in the host tissue. Since schistosomiasis-related morbidity is caused by egg granulomatous and inflammatory reactions, treatment with praziquantel is expected to reduce morbidity related to schistosomiasis including children's growth. As part of an assessment of the effect of treatment on schistosomiasis-related morbidity, we went ahead and compared the effect of one and two doses of praziquantel on children's growth.

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Chapter 7: Comparison of the effect of one and two doses of praziquantel on anthropometric indices among children living along Lake Victoria, Uganda

Summary

To compare the effect of one and two doses of praziquantel on children's growth, a longitudinal randomized intervention study was carried out in Musoli village along Lake Victoria in Uganda. Growth parameters of 146 *S. mansoni* infected children, mean age 10.2 (SD ± 2.3, range 7 – 14) years were compared between the two treatment regimens.

Anthropometric measurements for determination of growth and stool examinations for assessment of *Schistosoma mansoni* infection were performed prior to treatment. Body Mass Index (BMI), z-scores of Height for Age (HAZ) and Weight for Age (WAZ) were calculated and used to determine growth patterns described as underweight, stunting and wasting respectively. On completion of all examinations, all participants were treated with a standard dose of praziquantel (40mg/kg body weight). Two weeks later, one half of the children were given a second standard dose of praziquantel while the others were not given another dose. The effect of treatment on anthropometric measurements was assessed eight months and 24 months after the first treatment. Stool examinations were also repeated during the two follow up points.

No significant differences in height and weight measurements were found between the two treatment groups throughout the study. There was no significant difference in growth patterns between the two dose groups.

7.1 Introduction

Schistosomiasis is an important and highly prevalent poverty-related health problem in many parts of sub-Saharan Africa. Helminth infections, including schistosomiasis, coupled with malnutrition are public health problems in poor developing countries (Latham *et al*., 1983; Stephenson & Holland, 1987; Olsen, 1999; WHO, 2001). Although schistosomiasis can infect a whole population in an endemic area, school-age children are more vulnerable and at a higher risk of infection than adults (Jordan & Webbe, 1993; Chitsulo *et al*., 2000; Gryseels *et al*., 2006) and they harbour more intense infections than adults (Verle *et al*., 1994; Fulford *et al*., 1998; Talat *et al*., 1999). In high transmission areas, children also exhibit morbidity and retarded growth due to schistosomiasis (Corbett *et al*., 1992; Bosompem *et al*., 2004; Odogwu, *et al*., 2006). It has been reported that schistosomiasis may cause malnutrition (Stephenson & Holland, 1987; Friis *et al*., 2003; King *et al*., 2005; Zhou *et al*., 2005) but the cause effect relationship is not very clear since malnutrition also increases susceptibility to infections (Stephenson, 1993). The common forms of malnutrition caused by schistosomiasis and other helminth infections like hookworms in the developing world are low protein-energy and blood iron-deficiency, which are the major causes of stunting (Stephenson, 1993; Assis *et al*., 2004).

Impaired growth in children, as one of the schistosomiasis related morbidity symptom, has been reported in many studies (Doehring-Schwerdtfeger, 1990; Corbett *et al*., 1992; Parraga *et al*., 1996; Friis *et al*. 2003; Koukounari *et al*., 2006; Leenstra *et al*., 2006, Odogwu *et al*., 2006). Information from these studies contributed to the basis upon which the World Health Organisation developed a strategy for morbidity control of schistosomiasis of periodically treating endemic populations targeting mainly school-age children using praziquantel (WHO, 2002, 2006).

Trapped eggs in intestinal walls mediate an immune reaction that leads to inflammation and formation of scars around the trapped eggs. This triggers release of cytokines, which in turn stimulate protein metabolism and result into weight loss, as observed by Campbell *et al*., (2003), where immunological inflammation was associated with childhood stunting. Praziquantel, the current drug of choice for the control of schistosomiasis, mainly attacks

the adult worms. If treatment with praziquantel kills adult worms, then there would be fewer eggs deposited in the human tissue and this would result in reduced protein metabolism. It is therefore expected that a double dose of praziquantel administered two weeks apart would clear more worms than a single dose from the host body, implying that fewer eggs are deposited in the tissues hence reduced level of protein metabolism. To assess this effect, we compared the effects of a single dose (40 mg/kg body weight) versus two doses (2 x 40 mg/kg body weight) of praziquantel on growth parameters of children at eight and 24 months after first treatment.

7.2 Material and methods

7.2.1 Study area and population

This study was carried out along Lake Victoria in Musoli village, Mayuge district South East Uganda. Schistosomiasis is highly prevalent in this area. Lake Victoria is the only source of water here and this exposes the people to continuous schistosomiasis infection. Most of the children in our study go to school but they still spend a lot of time in the lake swimming, playing and hooking fish.

7.2.2 Study design

The study consisted of a cross sectional baseline survey followed by longitudinal randomised intervention follow-up surveys. The cross sectional study was conducted to collect pre-treatment levels of *S. mansoni* infection and anthropometric measurements after which all participants were treated. The longitudinal study assessed the effect of praziquantel treatment on children's growth.

7.2.3 Sample size determination and sampling procedure

To detect a true difference of the effect of one dose and two doses on growth parameters, the function of sample size and power determination for two sample comparison of means of Stata, (Intercooled Stata 10.1, Stata Corporation, USA) was used to calculate the sample size. Using an increase in mean weight of 3.7 kg for treated children and 3.0 kg for the control (Assis *et al*., 1998), power of 80% and 90% probability, the obtained sample size was 74 people per treatment group. Allowing for an annual loss to follow up of 20%, i.e.

40% loss within two years, 30 more people were required per group, resulting into 104 people per dose group.

However, this report is part of a wider study that compared among others the effect of one and two doses of praziquantel on cure rate and morbidity regression of *S. mansoni*. The sample size of the whole study was calculated based on the expected difference in cure rate between the two study groups as well as the expected loss to follow-up. Kirkwood's 1988 modified formula for comparing two rates (Hardon *et al*., 1994) was used. Using cure rates obtained from a study of communities living along the shores of Lake Albert (Kabatereine *et al*., 2003) of 41.9% and 69.1% in the first treatment and second treatment respectively, the sample size at 95% significance level and power of 90% was calculated as follows:

$$n = \frac{(u + v)^2 (r_1 + r_2)}{(r_1 - r_2)^2},$$

Where r = cure rate; u = two-sided percentage point of the normal distribution corresponding to the power of 90%; v = percentage point of the normal distribution corresponding to the significance of 95%.

The sample size per group: $\frac{(1.64 + 1.96)^2 (0.42 + 0.69)}{(0.42 - 0.69)^2} = 197$

In order to allow for a 20% annual loss to follow-up (total loss 40% in two years) 79 people were added and each group had 276 participants, giving a total sample size of 552 people to be recruited in the study. Nonetheless, this part of the study covered only schistosomiasis infected children below 15 years of age selected from the wider cohort. This is because schistosomiasis infection affects growth of younger children more than older people. This implies that the actual sample size used was too small to detect a difference in growth after treatment.

All the inhabitants in Musoli village were registered and data were entered in the computer. Computer generated random numbers were used to select a representative cohort stratified for age and sex. After the baseline data collection, a list of all the examined participants was drawn and the cohort stratified according to sex. Again using computer generated

random numbers, odd numbers in each stratum were located to single treatment group while even numbers were located to double treatment group.

7.2.4 Data collection methods and procedures

7.2.4.1 Stool examination

Stool was collected using the modified Kato Katz thick smear technique (Katz *et al*., 1972) with a 50 mg template. In order to improve diagnostic sensitivity, three early morning stool specimens were taken from each participant on three consecutive days (Cheever *et al*., 1994; Utzinger *et al*., 2001; Booth *et al*., 2003). From each specimen; two slides were prepared, giving rise to six slides per participant. Two experienced technicians examined the slides under the microscope magnification 10x using natural light. In order to minimise intra-observer variation, both technicians read slides from the same sample in such a way that each technician read one of the duplicate slides. The technicians were blinded to the participant's treatment group. Stool examination was repeated eight and twenty four months after treatment to determine post-treatment infection levels.

7.2.4.2 Anthropometric measurements

Before treatment, standing in an upright position and without shoes, each individual's height and weight measurements were taken. The height was measured to the nearest 0.1 centimetres using a portable stadiometer and weight measured to the nearest 0.1 kg using a Seca portable digital scale. A waxed paper insertion tape and a calliper were used to measure mid upper arm circumference (nearest to 0.1 cm) and triceps skinfold thickness (nearest to 0.1mm) respectively. These were taken between the bony points of the shoulder and elbow of the left arm hanging relaxed. Anthropometry assessment was repeated eight and 24 months after treatment. A single set of anthropometric measurements was taken at all time points. The person taking the measurements was blinded to the participant's treatment group allocation.

7.2.4.3 Treatment

After all stool specimens were collected and anthropometric measurements taken, each participant was treated with a single dose of praziquantel (40 mg/kg body weight). The

brand of praziquantel used was Distocide® 600 mg manufactured by Shin Poong Pharmaceuticals, Seoul Republic of Korea. Two weeks later, one half of the children received another dose of praziquantel while the other did not receive any treatment. Treatment was performed by and under direct observation of an experienced nurse. Participants were kept at the field research station for two hours after treatment to observe and manage any possible adverse events.

7.2.5 Data management and analysis

7.2.5.1 Quality control

Results from different observers were not compared, instead an independent experienced microscopist read a random 10% of the stool slides, after which the results were compared and where there was controversy, all the six slides of the same individual were read by two different experienced technicians and the counts were harmonised. Data were double-entered by two experienced data entry clerks using excel computer programme and the two files were cleaned, compared and a master file compiled, which was used for all analyses.

7.2.5.2 Data management

Data were imported from Excel computer programme to Stata for windows (Intercooled Stata 10.1, Stata Corporation, USA) for analysis. *S. mansoni* eggs were quantified and intensity of infection was obtained as the geometric mean of the total egg counts, per gram of faeces (epg). Four categories of *S. mansoni* intensity groups were developed as: (i) negative for those who were not infected (ii) light for those with 1-99 epg (iii) moderate for those who had 100 – 399 epg (iv) heavy for those who had ≥ 400 egp (WHO, 2002).

Growth was analysed for only children below 15 years of age because schistosomiasis mainly impairs growth of children (Corbett *et al*., 1992; Stephenson, 1993; Parraga *et al*., 1996; Assis *et al*., 1998; King *et al*., 2005; Zhou *et al*., 2005; Leenstra *et al*., 2006). Growth reference curve from American children collected by the National Centre for Health Statistics was used to derive standard deviation scores (z-scores). Measurements of height and weight were related to references as z-scores of height-for-age (HAZ); weight-for-age (WAZ), obtained as the difference between the value for an individual and median value of

the reference for the same age and sex, divided by the standard deviation of the reference population (Kirkwood & Sterne, 2003). Z-scores were calculated using Nutritional Index Calculator, Epi Info, Version 6.04 (Centers for Disease Control and Prevention, USA). The nutritional index calculator flags any implausible z-scores and WHO recommends such scores to be treated as missing values. We used ¯2 standard deviations (SD) from the reference mean because 95% of the measured variable lies within 2 SD of a standard normal distribution curve, and since African children are expected to be less developed than American ones; using 2 SD would cover most of the African children. Thus, HAZ and WAZ values less than ¯2 SD were considered as stunting and wasting respectively as described by WHO (1995). Body Mass Index (BMI) of each child was calculated as weight in kilograms divided by the square of height in meters (kg/m^2). In our study, like Sacko (2006), BMI <15 kg/m^2 was considered as underweight, otherwise BMI beyond 15 kg/m^2 would have rendered all the children in our study to be underweight.

7.2.5.3 Data analysis

The analysis was performed for only those who were positive before treatment. The mean values of height and weight were compared among the two treatment groups using Student's t test and ANOVA. The mean differences of height and weight between baseline and eight months, baseline and 24 months and between eight months and 24 months were compared using paired t tests. Chi-square tests were used to compare proportions of stunting, wasting and underweight among the two treatment groups. *P* value <0.05 was used to determine statistical significance.

7.2.6 Ethical considerations

Ethical approval was obtained from the Higher Degrees Research and Ethics Committee of the School of Public Health, Makerere University. Ethical clearance was obtained from the Uganda National Council of Science and Technology. This study was supported by DANIDA, thus clearance was also sought from the Danish National Committee on Biomedical Research Ethics in Denmark. Informed consent was obtained from individual adult participants while parents or guardians consented on behalf of children. All information obtained from participants was kept confidential.

To minimise occurrence of known side effects of praziquantel when taken on an empty stomach, a snack and a soft drink were given before treatment. A trained nurse was available during treatment to attend to any adverse event. Other minor ailments like laboratory diagnosed malaria, anaemia, diarrhoea and others were treated according to the national guidelines. Adhering to the National Schistosomiasis and Worm Control Programme strategy of mass treatment in endemic areas, the rest of the community was treated after the second treatment of the cohort.

7.3 Results

7.3.1 Characteristics of the study population

The study cohort comprised of 146 children, mean age 10.2 (SD ± 2.3, range 7 – 14) years who were recruited at baseline and were all treated. Some children were lost to follow up mainly because they joined boarding schools, migrated with their parents or were just absent at the time of follow up. The study profile of the pre-treatment *S. mansoni* infected children is shown in figure 7.1. Before treatment, the cohort was balanced between the two treatment groups with regard to sex, age and baseline intensity of *S. mansoni* infection. The ones lost to follow up at eight and 24 months after treatment were not different among the two treatment groups with regard to sex, age and *S. mansoni* intensity of infection. For the ones present at eight and 24 months after treatment; age, sex, *S. mansoni* prevalence and intensity of infections at baseline were not significantly different between the two treatment groups (table 7.1).

At baseline, mean HAZ for single dose was ¯1.07 (95% confidence interval [95% CI]: ¯1.35 - ¯0.79) while the double dose group had mean HAZ of ¯0.91 (95% CI: ¯1.2 - ¯0.6), no significant difference. The mean WAZ for single dose was ¯0.83 (95% CI: ¯1.04 - ¯0.61) while the double dose group had mean WAZ of ¯0.73 (95% CI: ¯0.94 - ¯0.52).

Table 7. 1: Post treatment comparison of baseline characteristics between the two treatment regimens

Characteristics	Single dose	Double dose	Test statistic	*P* value
Eight months				
Number of males (%)	28 (43.8)	39 (59.1)	$\chi^2 = 3.06$	0.080
GMI ± SD (epg)	284.3 ± 5.0	269.9 ± 4.9	t = 0.186	0.852
	N=64	N=66		
Mean height ± SD (cm)	133.1 ± 11.4	134.4 ± 15.5	t = -0.55	0.583
Mean weight ± SD (kg)	29.0 ± 6.8	31.2 ± 10.1	t = -1.48	0.142
Mean age ± SD (years)	10.6 ± 2.3	10.2 ± 2.2	t = 0.81	0.420
Twenty four months				
Number of males (%)	29 (52.7)	32 (61.5)	$\chi^2 = 0.85$	0.357
GMI ± SD (epg)	282.1 ± 5.3	281.0 ± 5.0	t = -0.01	0.990
	N= 55	N=52		
Mean height ± SD (cm)	133.7 ± 11.1	133.0 ± 14.7	t = 0.27	0.788
Mean weight ± SD (kg)	29.2 ± 6.7	30.1 ± 8.8	t = -0.64	0.521
Mean age ± SD (years)	10.2 ± 2.4	10.2 ± 2.2	t = 0.07	0.947

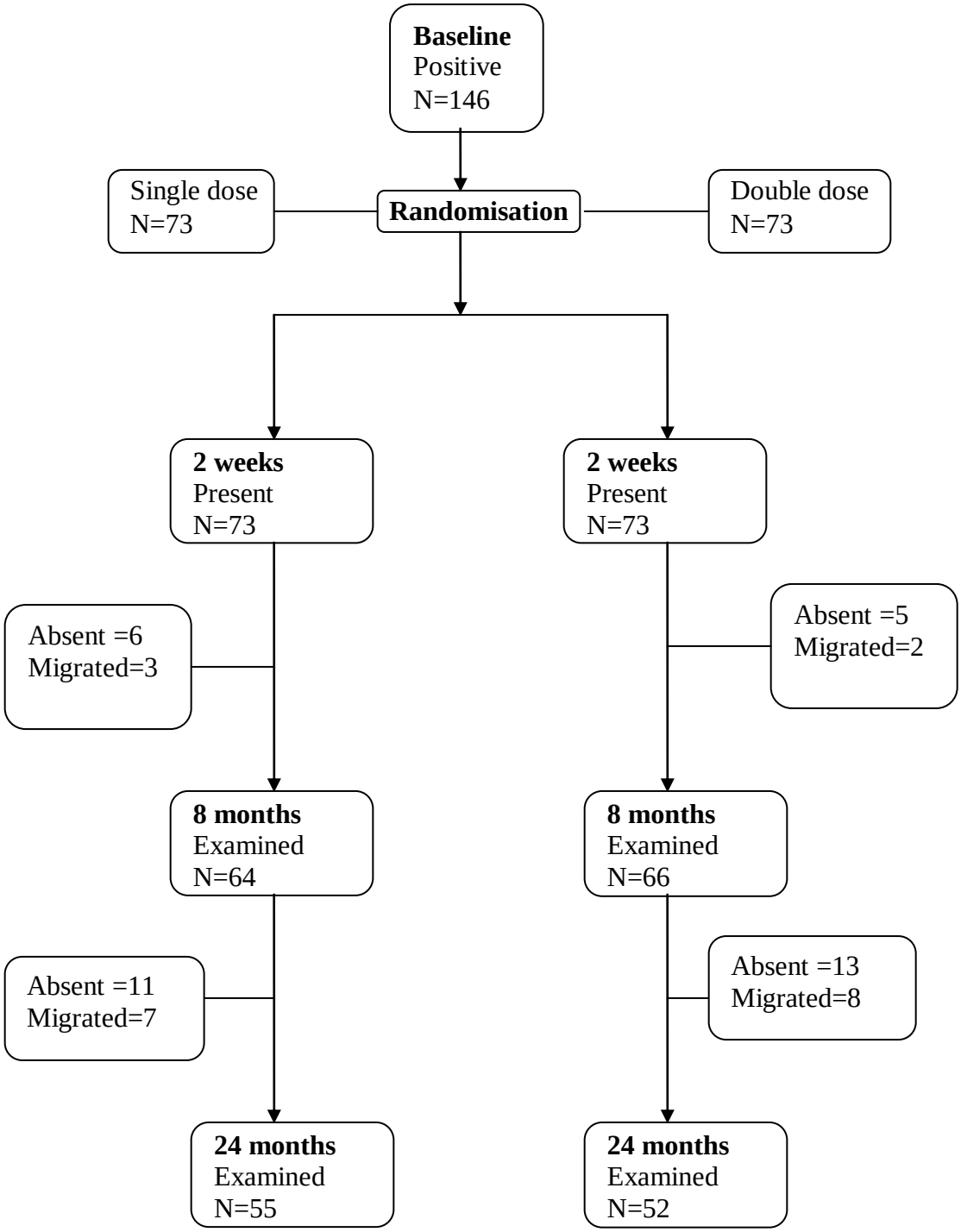

Figure 7. 1: Trial profile for children examined at different time points

7.3.2 Mean weight and mean height

The mean height (table 7.2) and mean weight (table 7.3) of the single dose group were not significantly different from those who received a double dose throughout the study period. Comparing mean height and mean weight between baseline and eight or 24 months after treatment, within each dose group, there was no significant difference. Both height and weight increased over time (figures 7.2 & 7.3).

Table 7. 2: Comparison of mean height of children according to treatment regimen at baseline, eight and twenty four months after treatment

Time point	Single dose (N)		Double dose (N)	
	Mean height in cm	95% CI	Mean height in cm	95% CI
Baseline	133.3 (73)	130.5 – 136.1	134.6 (73)	131.0 – 138.3
8 months	137.1 (64)	134.2 – 140.1	139.5 (66)	135.7 – 143.4
24 months	144.1 (55)	141.2 – 143.3	143.3 (52)	139.5 – 147.2

95% CI = 95 % confidence interval of the mean height.

Table 7. 3: Comparison of mean weight of children according to treatment regimen at baseline, eight and twenty four months after treatment

Time point	Single dose (N)		Double dose (N)	
	Mean weight in kg	95% CI	Mean weight in kg	95% CI
Baseline	29.2 (73)	27.5 – 30.9	31.5 (73)	29.2 – 33.9
8 months	30.6 (64)	28.6 – 32.6	33.7 (66)	30.9 – 36.4
24 months	36.2 (55)	33.8 – 38.5	37.0 (52)	34.0 – 40.9

95% CI = 95 % confidence interval of the mean weight.

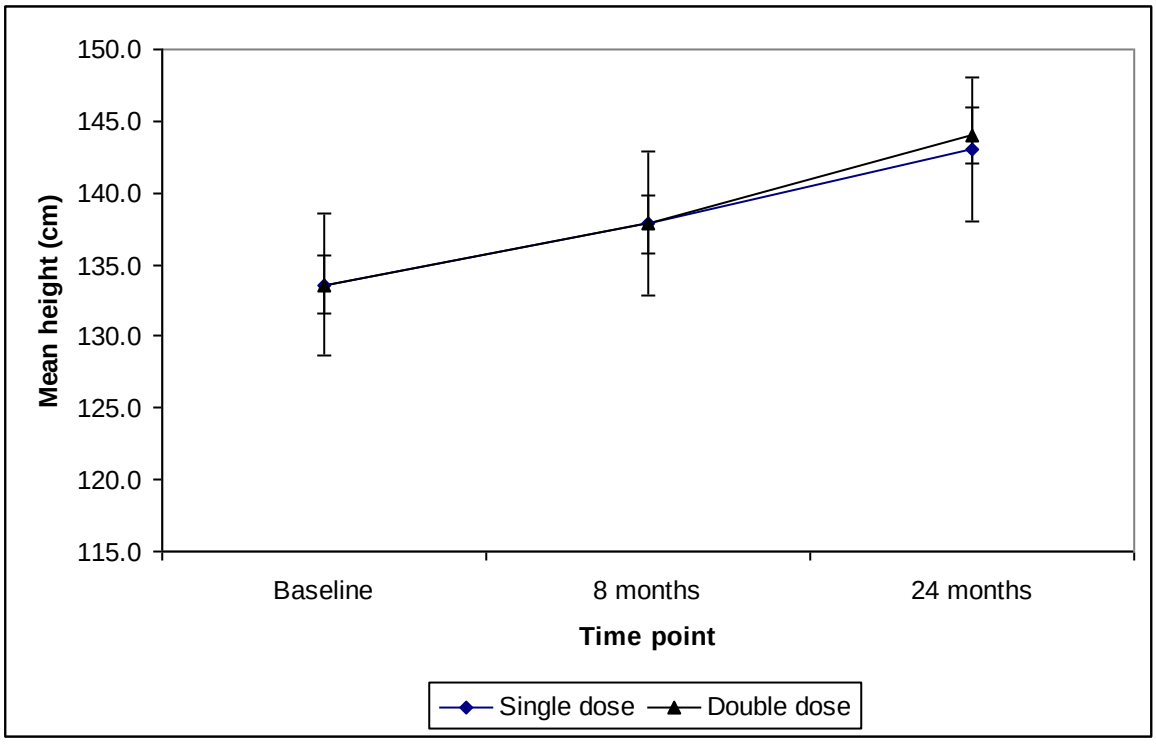

Figure 7. 2: Height (mean ± SD) compared by treatment regimen at baseline, eight and twenty four months after treatment, bars represent standard deviation

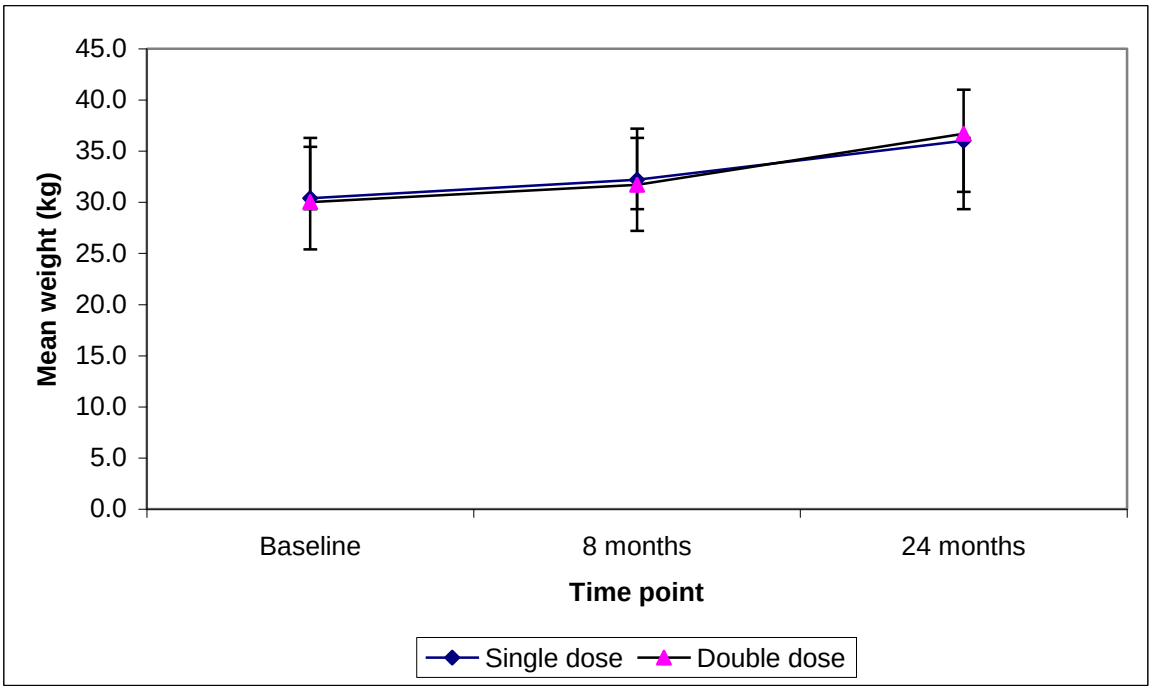

Figure 7. 3: Weight (mean ± SD) compared by treatment regimen at baseline, eight and twenty four months after treatment, bars represent standard deviation

7.3.3 Comparison of the effect of one and two doses of praziquantel on anthropometric indices

Anthropometric indices were used to express growth patterns of stunting, wasting and underweight. Prior treatment, more of the heavily infected ones (30.3%) were stunted than the moderately (15.0%) and lightly (7.9%) infected ones ($\chi^2_{trend} = 8.9$, $P = 0.006$). Wasting and underweight did not show any association with intensity of infection. The effect of the two treatment regimens on growth indices are shown in table 7.4. Generally, there was no significant difference in the growth patterns between the two treatment groups. Even when children were stratified by age groups 7-11 years and 12-14 years separate for males and females, the difference between the two dose groups was not significant.

Table 7. 4: Comparison of growth patterns, depicted as stunting (HAZ<⁻2SD), wasting (WAZ <⁻2SD) and underweight (BMI<15), between one and two doses of praziquantel at baseline, eight and twenty four months after treatment

Characteristic	Single dose		Double dose	
	Number (%)	**95% CI**	**Number (%)**	**95% CI**
Stunted				
Baseline	13 (18.3)	9.1 – 27.5	16 (21.9)	12.2 – 31.6
8 months	11 (20.0)	9.1 – 30.9	10 (17.9)	7.5 – 28.2
24 months	7 (17.5)	5.2 – 29.8	7 (18.0)	5.9 – 33.0
Wasted				
Baseline	7 (9.6)	2.7 – 16.5	5 (6.9)	0.9 – 12.8
8 months	10 (18.2)	7.7 – 28.7	8 (13.6)	4.6 – 22.6
24 months	5 (11.1)	1.6 – 21.6	4 (9.8)	⁻1.1 – 17.3
Underweight				
Baseline	14 (19.2)	9.9 – 28.4	8 (11.0)	3.6 – 18.3
8 months	18 (28.1)	16.8 – 39.4	10 (15.2)	6.3 – 24.0
24 months	2 (3.6)	⁻1.5 – 8.7	2 (3.8)	⁻1.6 – 9.3

Number = children who were positive before treatment. 95% CI = 95% confidence interval of proportions.

7.4 Discussion and conclusion

Schistosomiasis is among the parasitic diseases that have a negative impact on children's growth and nutritional status in developing countries (Corbett *et al*., 1992; Stephenson, 1993; Parraga *et al*., 1996; Assis *et al*., 1998; King *et al*., 2005; Zhou *et al*., 2005; Leenstra *et al*., 2006). Like studies carried out elsewhere (Parraga *et al*., 1996; Stoltzfus *et al*., 1997; Assis *et al*., 2004; Zhou *et al*., 2005), nutritional indices of stunting, wasting and underweight were used to determine growth status of children. However, there is limited information on comparison of different treatment regimens on children's growth. The study reported here was carried out to compare the effect of one and two doses of praziquantel on nutritional status of children over a period of two years.

Determining the effect of treatment on growth needs an un-treated control group, which due to ethical reasons, we did not have. Similar to Sacko (2006) findings in Mali, we found no significant difference in growth parameters between the two treatment groups. Explaining the lack of a difference in the two treatment groups is rather difficult because there was no control group. Since it is the trapped eggs that stimulate protein metabolism and lead to weight loss (Campbell *et al*., 2003) and as observed in our study (chapter 4) where intensity of *S. mansoni* was associated with only stunting, lack of a difference in growth indices between the two groups could be attributed to both treatment regimens realizing marked *S. mansoni* egg reduction (chapter 5). In addition to this, intensity of re-infection was not different among the two dose groups, and it is likely that the trapped eggs in both dose groups had similar effects on children's growth indices.

Also to be noted is that our sample size in each treatment group was much less than the calculated one which was expected to detect a significant difference and yet growth changes after an intervention are usually fairly small, which requires adequate sample size and a long period of follow up to detect small differences (McGarvey *et al*., 1996). Thus small sample size in our study could have contributed to lack of a difference in growth parameters between the two treatment regimens. Whereas inadequate food, socio-economic status and malaria infection can influence growth, these factors were balanced between the two groups by the randomisation.

In conclusion, a single dose of praziquantel did not exhibit a significantly different effect on children's growth as compared to two doses. Thus we cannot recommend use of two doses of praziquantel for children on the basis of growth changes. Nonetheless, praziquantel might have a significant effect on other morbidity indicators such as haemoglobin levels, liver and/or spleen measurements. To assess this, we compared the effect of one and two doses of praziquantel on morbidity indicators.

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Chapter 8: Comparison of the effect of treatment with one and two doses of praziquantel on S. *mansoni* associated morbidity along Lake Victoria in Uganda

Summary

To compare the effect of treatment with one and two doses of praziquantel on schistosomiasis related morbidity, a longitudinal randomised intervention study was carried out. Three hundred ninety five people infected with *S. mansoni* were treated with a standard dose of praziquantel (Distocide® 600 mg, Shin Poong Pharmaceuticals, Seoul Republic of Korea, 40mg/kg body weight). A half of the study participants received another standard dose of praziquantel two weeks later. Morbidity indicators measured were spleen size and its consistency; liver size, its consistency and its texture pattern; portal vein diameter and haemoglobin levels. Morbidity was assessed before treatment, eight months and 24 months after treatment.

From clinical palpation, the liver and spleen consistencies of those given a single dose were not significantly different from those of the ones that received two doses throughout the study. Proportions of those with splenomegaly and hepatomegaly were not significantly different between the two dose groups at eight and 24 months after treatment. Proportions of those with hepatosplenomegaly for the double dose group (7.2%, 95% CI: 3.5 – 10.9) at 24 months after treatment were significantly less than those of the single dose group (13.8%, 95% CI: 8.9 – 18.6).

Proportions with abnormal spleen length and portal vein diameter measured by ultrasound were not significantly different between the two dose groups throughout the study. Haemoglobin levels and anaemia proportions were not significantly different between the two dose groups throughout the study. Our results showed that one dose and two doses of praziquantel all reduce schistosomiasis-related morbidity, with no significant difference in their effect on morbidity. Two doses given two weeks apart seem not to add any benefit to morbidity regression. Thus a single annual dose is good enough to control schistosomiasis *mansoni*.

8.1 Introduction

Schistosomiasis is one of the parasitic infections that causes morbidity and mortality in the developing world (van der Werf *et al*., 2003; King *et al*., 2005). Van der Werf *et al*., (2003) reported that *Schistosoma mansoni* causes hepatomegaly and splenomegaly in 8.5 and 6.3 million people respectively in sub-Saharan Africa. Mortality rate due to schistosomiasis has been estimated to about 200,000 deaths per year worldwide (Conlon, 2005) but predictions based on standardised data from sub-Saharan Africa show that mortality rates due to urinary and intestinal schistosomiasis are 150,000 and 130,000 persons per year respectively (van der Werf *et al*., 2003). Studies have shown that *S. mansoni* is the major cause of schistosomiasis related morbidity in Uganda (Ongom & Bradley, 1972; Ongom *et al*., 1972; Frenzel *et al*., 1999; Kabatereine, 2000; Kabatereine *et al*., 2003) including anaemia (Sturrock *et al*., 1996; Friedman *et al*., 2005; Koukounari *et al*., 2006).

With schistosomiasis *mansoni*, approximately 50% of the laid eggs are excreted in faeces while the rest are trapped and retained in the host tissue (Gryseels & Nkulikyinka, 1988). Eggs that are trapped in the micro-vascular host's tissue induce a granulomatous inflammatory response around them which results in schistosomiasis related pathology and morbidity such as hepatomegaly, splenomegaly, hepatosplenomegaly, portal hypertension and liver fibrosis. Organomegaly related to *S. mansoni* infection can be detected by clinical examination (Vennervald *et al*., 2004) and ultrasound examination (Homeida *et al*., 1988; Doehring-Schwerdtfeger *et al*., 1990, 1992; Hatz *et al*., 1992). Classical manifestations of hepatosplenic schistosomiasis arise from periportal thickening of the liver, ultrasonographically seen as echogenic portal vein wall thickening. However, *S. mansoni* related morbidity is not manifested by every infected individual (Savioli *et al*., 1997; Utzinger *et al*., 2001; van der Werf *et al*., 2003). The degree of morbidity can be influenced by intensity of infection, immunological factors, age and gender (Doehring-Schwerdtfeger, 1992; Dunne & Pearce, 1999; Booth *et al*., 2004a).

Treatment with a standard dose of praziquantel (40 mg/kg body weight) reduces schistosomiasis prevalence and intensity of infection (WHO, 2002; Utzinger *et al*., 2003). This affects the number of eggs deposited in the host tissue, and reduces the inflammatory

responses induced by the schistosome eggs and may translate into reduced morbidity. If treatment with praziquantel can interrupt morbidity progression and/or induce its regression, two doses are likely to increase morbidity regression more than a single dose. Generally, there is insufficient information about the impact of treatment on schistosomiasis related morbidity and in particular comparing the effect of one and two doses of praziquantel. In this study, we compared the effect of one and two doses of praziquantel given two weeks apart on *S. mansoni* related morbidity.

8.2 Material and methods

8.2.1 Study area and population

This study was conducted in a community living along Lake Victoria, Mayuge district. This area is highly endemic for schistosomiasis as reported elsewhere in Uganda (Kabatereine *et al*., 2003; Booth *et al*., 2004a; Fitzsimmons *et al*., 2004). Fishing and peasant farming are the major economic activities in this village. Lake Victoria is the only source of water for domestic use and this exposes the people to schistosomiasis infection.

8.2.2 Study design

This study consisted of a cross sectional baseline survey followed by longitudinal randomised intervention follow-up surveys. The cross sectional study was conducted to collect pre-treatment levels of *S. mansoni* infection and its related morbidity. After initial treatment, participants were randomised to two groups in that two weeks after first dose, one group received a second dose of praziquantel (40 mg/kg body weight) while the other was not given any more treatment. Follow up examinations were performed eight and 24 months after treatment.

8.2.3 Sample size determination and sampling procedure

To detect a true difference of the effect of one dose and two doses of praziquantel on schistosomiasis related morbidity, Statcalc calculator of Epi Info, version 6.04 (Centers for Disease Control and Prevention, USA) was used to calculate the sample size. Using frequency of hepatosplenomegaly after treatment of 35.6% (Kabatereine, 2000), power of 80% and 95% probability, the obtained sample size was 132 people per treatment group.

Allowing for a 20% loss to follow up per year, i.e. 40% loss after two years, 26 more people were required per group to cater for the loss. Thus the sample size for each dose group was 158. However, this report is part of a study that assessed the effect of two doses of praziquantel on cure rate, re-infection and morbidity regression, thus the overall sample size calculation was based on the expected difference in cure rate between the two study groups as well as the expected loss to follow-up. Sample size was calculated using Kirkwood's, 1988 modified formula for comparing two rates (Hardon *et al*., 1994). Basing the calculations on cure rates obtained from a study of communities living along the shores of Lake Albert (Kabatereine *et al*., 2003) of 41.9% and 69.1% for a single and two doses of praziquantel respectively, the sample size at 95% significance level and power of 90% was calculated as follows:

$$n = \frac{(u + v)^2 (r_1 + r_2)}{(r_1 - r_2)^2},$$

Where r = cure rate; u = two-sided percentage point of the normal distribution corresponding to the power of 90%; v = percentage point of the normal distribution corresponding to the significance of 95%.

The sample size per group: $\frac{(1.64 + 1.96)^2 (0.42 + 0.69)}{(0.42 - 0.69)^2} = 197$

In order to allow for a 20% annual loss to follow-up (total loss 40% in two years) 79 people were added and each group had 276 participants, giving a total sample size of 552 people. Thus, the used sample size for the whole study was big enough to detect a true difference in the effect of the two treatment doses on *S. mansoni* morbidity.

All the inhabitants in Musoli village were registered and data was entered in the computer. Computer generated random numbers were used to select a representative cohort stratified for sex. After the baseline data collection, a list of all the examined participants was drawn and the cohort stratified according to sex. Again using computer generated random numbers, odd numbers in each stratum were located to single dose group while even numbers were located to double dose group.

8.2.4 Data collection methods and procedures

8.2.4.1 Stool examination

Stool was collected using the modified Kato Katz thick smear technique (Katz *et al*., 1972) with a 50 mg template. Three early morning stool specimens were taken from each participant on three consecutive days so as to improve diagnostic sensitivity (Cheever *et al*., 1994; Utzinger *et al*., 2001; Booth *et al*., 2003). Two slides were prepared from each specimen, giving rise to six slides per participant. Two experienced technicians examined the slides under the microscope (10x). In order to minimise intra-observer variation, both technicians read slides from the same sample in such a way that each technician read one of the duplicate slides. The technicians were blinded to the participant's treatment group.

8.2.4.2 Blood sampling for haemoglobin levels

A finger prick blood sample was taken from each subject. A drop of blood was absorbed into a micro-cuvette, inserted into a portable photometer (HemoCue Hb 201^{+} Analyser manufactured by Quest Diagnostics Company, Norrköping - Sweden) and haemoglobin readings taken directly from the machine. Blood sampling was repeated eight and 24 months after the second treatment to assess haemoglobin change.

8.2.4.3 Clinical examination

For consistency, each participant was clinically palpated by three experienced examiners, i.e. one physician and two nurses. The examiners were all blinded to which treatment group the subjects belonged. The examination was performed by having the subject lie on an examination table, with knees bent so as to relax the abdominal muscles. Then the abdomen was palpated as described by Vennervald *et al*., (2004). Using a tape measure, the following measurements were taken: the extension of the left liver lobe beneath the sternum was measured in the mid sternal line (MSL); the extension of the right liver lobe beneath the rib cage was measured in the right mid clavicular line (MCL); the extension of the spleen below the rib cage was measured both in the left MCL and left mid axillary line (MAL). The liver and spleen data were graded as described by Vennervald *et al*., (2004) and shown in the clinical form (Appendix II). The findings were translated into a clinical score reflecting the degree of organomegaly, as shown in chapter 4 and appendix II. Each

subject was palpated by all the examiners independently and the order of examination was randomised such that each examiner had a chance of being the first. When all the three finished the examination, the obtained measurements were discussed and a final measurement agreed upon and recorded in the clinical form. If the measurements of the three examiners greatly varied, all the examiners repeated the examination and reached a consensus about the final measurements. Using the same protocol, all participants were clinically examined again eight and 24 months after treatment to assess the effect of the two doses on morbidity.

8.2.4.4 Ultrasound examination

Ultrasonography examination was performed on each study participant using a portable ultrasound machine (SSD 500 Aloka with 3.5 MHz curvlinear - 60% probe). The examination was conducted by two experienced ultrasonographers working alternatively and they were both blinded to the subject's treatment group. Each study subject was examined by one ultrasonographer, who would consult the other ultrasonographer in case of unclear liver texture pattern.. The subjects were examined in a supine position lying with their legs stretched on an examination table. The liver size (measured in the parasternal and mid-clavicular longitudinal lines), the spleen length, the portal vein diameter measured at the point of entrance into porta hepatis at the ventral lower end of the caudate lobe as described by Wahab & Esmat (1992) and the liver image patterns were examined. The measurements were recorded in an ultrasound form (appendix III). Liver texture patterns were graded as described in chapter 4 and according to WHO guidelines (Richter *et al*., 2000). Using the same protocol, ultrasonography was repeated eight and 24 months after treatment to assess morbidity changes.

8.2.4.5 Treatment

After all baseline examinations were completed, all study participants were treated with a single dose of praziquantel (40 mg/kg body weight) and one tablet of albendazole 400 mg. The brands of albendazole and praziquantel used were Alzental® 400mg and Distocide® 600 mg respectively all manufactured by Shin Poong Pharmaceuticals, Seoul Republic of Korea. Two weeks later, one of the groups received another standard dose of praziquantel

while the other group did not receive any treatment. Treatment was performed by and under direct observation of an experienced nurse. Participants were kept at the field research station for two hours after treatment to observe and manage any possible adverse events.

8.2.5 Data management and analysis

8.2.5.1 Quality control

Results from different observers were not compared, instead stool quality control was performed by an independent experienced microscopist reading a random 10% of the stool slides, after which the results were compared and where there was controversy, all slides of the same individual were read by different experienced technicians. Clinical and ultrasound examinations were performed by more than one person and results compared. Data were double-entered by two experienced data entry clerks using excel computer programme and the two files were cleaned, compared and a master file compiled, which was used for all analyses.

8.2.5.2 Data management

Data were imported from Excel computer programme to Stata for windows (Intercooled Stata 10.1, Stata Corporation, USA) for analysis. Two age categories, for children (<15 years) and adults (15 years and above) were created and these were used in analysis.

The normal range of organ dimensions is closely related to age and body length. Thus, height categories, matched to those of the Senegalese non-infected population (Yazdanpanah *et al*., 1997), were created as follows: 110-129.9, 130-149.9, and 150-169.9, ≥170 cm. Individual spleen length and portal vein diameter values were expressed as standard deviations (SD) away from the mean of the height-category. Portal vein diameter and spleen length values below or equal to the mean + 2 SD were classified as normal; if they were > mean + 2 SD but ≤ mean + 4 SD they were classified as moderately abnormal and they were considered severely abnormal if they were > mean + 4 SD of the values for the corresponding height groups from a Senegalese non-infected population (Yazdanpanah *et al*., 1997) as suggested by Richter *et al*., (2000). The individual spleen length and portal vein diameter were standardised upon these height categories and classified as normal,

moderately abnormal and markedly abnormal using normograms of the Senegalese population (Richter *et al.*, 2000). Anaemia, based on haemoglobin levels, was calculated according to age and sex and was defined as: Hb < 115 g/L for children 5 – 11 years; Hb <120 g/L for children 12 – 14 years; Hb <120 g/L for non-pregnant women ≥ 15 years; Hb <110 g/L for pregnant women; Hb <130 g/L for men ≥ 15 years, (WHO, 2001).

8.2.5.3 Data analysis

Analysis was performed for only participants who were positive before treatment. Student`s t test was applied to compare baseline mean age and intensity of *S. mansoni* infection between the two dose groups and the effect of the two treatment regimens on organ sizes and haemoglobin levels. Chi square tests and confidence intervals were used to compare proportions of organ consistency, organomegaly and anaemia between the two dose groups. A *P* value <0.05 was used to determine statistical significance in all analyses.

8.2.6 Ethical considerations

Ethical approval was obtained from the Higher Degrees Research and Ethics Committee of the School of Public Health, Makerere University. Ethical clearance was obtained from the Uganda National Council of Science and Technology. This study was supported by DANIDA, thus clearance was also sought from the Danish National Committee on Biomedical Research Ethics in Denmark. Informed consent was obtained from individual adult participants while parents or guardians consented on behalf of children. All information obtained from participants was kept confidential.

To minimise occurrence of known side effects of praziquantel when taken on an empty stomach, a snack and a soft drink were given before treatment. A trained nurse was available during treatment to attend to any adverse event. Other minor ailments like laboratory diagnosed malaria, anaemia, diarrhoea and others were treated according to the national guidelines. Adhering to the National Schistosomiasis and Worm Control Programme strategy of mass treatment in endemic areas, the rest of the community was treated after the second treatment of the cohort.

8.3 Results

8.3.1 Characteristics of the study population

The major comparisons in this chapter were changes of morbidity indicators among the two treatment groups. Baseline characteristics of those who were positive before treatment and were present at eight and 24 months after treatment were compared between the two dose groups and there was no significant difference in terms of sex, age and intensity of *S. mansoni* infection (table 8.1). At eight and 24 months follow-up, 346 (87.6%) and 303 (76.7%) individuals respectively were re-examined (figure 8.1). The mean age of children was 10.2 years (SD ± 2.2, range 7 – 14 years) while that of adults was 33.0 years (SD ± 14.5, range 15 – 76 years).

However, 49 and 92 people were lost to follow up at eight months and 24 months after treatment respectively (figure 8.1). The loss to follow-up was similar in the two treatment groups throughout the study. For the ones lost to follow up at eight months after treatment, there was no significant difference in: pre-treatment *S. mansoni* infection intensity (t = -0.96, $P = 0.341$), sex distribution ($\chi^2 = 0.65$, $P = 0.419$) and age distribution (t = 0.04, $P = 0.972$) among the two treatment groups. Those lost at 24 months after treatment, the two dose groups were not significantly different in terms of pre-treatment *S. mansoni* infection intensity (t = 0.58, $P = 0.567$), sex distribution ($\chi^2 = 0.07$, $P = 0.786$) and age distribution (t = 1.08, $P = 0.283$).

Table 8. 1: A comparison of two treatment groups at follow-up points

	Number, single dose	**Number, double dose**	**Test statistic**	***P***
8 months				
Sex (male) (%)	88 (51.5)	101 (58.1)	χ^2 = 1.51	0.219
Baseline GMI ± SD (epg)	171 (230.4 ± 5.8)	174 (239.9 ± 6.2)	t = -0.21	0.835
Mean age ± SD (years)	171 (24.7 ± 15.4)	174 (24.4 ± 16.9)	t = 0.17	0.864
24 months				
Sex (male) (%)	83 (55.0)	94 (62.7)	χ^2 = 1.84	0.175
Baseline GMI ± SD (epg)	151 (230.3 ± 5.7)	150 (280.5 ± 5.7)	t = ¯0.98	0.326
Mean age ± SD (years)	151 (24.8 ± 15.7)	150 (25.1 ± 16.9)	t = ¯0.17	0.866

GMI = geometric mean intensity. Epg = eggs per gram of faeces.

Numbers include those who were *S. mansoni* positive at baseline.

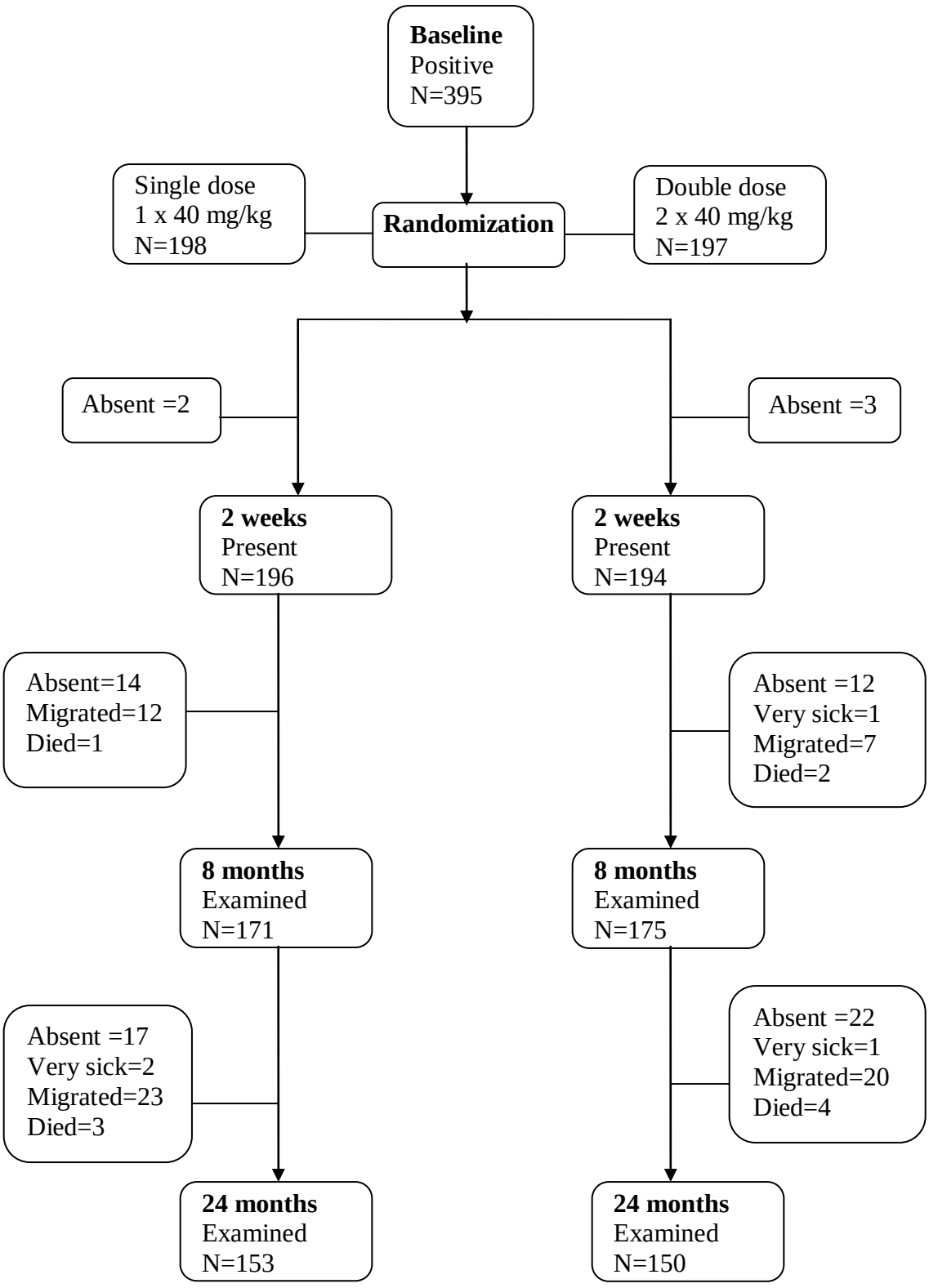

Figure 8. 1: Trial profile

8.3.2 Clinical observations

8.3.2.1 Liver assessment

Liver size measurements compared between infected and un-infected participants were not significantly different, chapter 4. The right and left liver lobe measurements showed no significant difference between the two treatment groups throughout the study. Comparison of liver consistency between single and two doses is shown in table 8.2 and there was no significant difference between the two dose groups at all time points. Normal liver consistency significantly increased over time within each dose group and there was a significant decrease in proportions of firm and hard livers in both dose groups throughout the study. However, proportions of firm livers slightly increased again 24 months after treatment in both groups but not up to pre-treatment levels.

8.3.2.2 Spleen assessment

The spleen size measured through the mid-clavicular line (MCL) and the mid-axillary line (MAL) was not associated with *S. mansoni* infection status neither in children nor in adults, chapter 4. Proportions of spleen consistency by dose group are compared in table 8.3. Before treatment, the spleen consistency was similar in the two dose groups. Eight and 24 months later, the proportions of those with normal spleens in the double dose group were significantly higher than those in the single dose group. Nonetheless, there was a markedly significant increase in normal spleens over time within each dose group. Proportions of hard spleens significantly decreased over time while firm spleens decreased at eight months and increased again 24 months after treatment but below the pre-treatment levels (table 8.3).

Table 8. 2: Comparison of liver consistency among treatment groups at baseline, eight and twenty four months after treatment

	Single dose		Double dose	
	Number (%)	**95% CI**	**Number (%)**	**95% CI**
Baseline				
Normal	78 (39.8)	32.9 – 46.7	87 (44.9)	37.8 – 51.9
Soft	7 (3.6)	1.0 – 6.2	10 (5.2)	2.0 – 8.3
Firm	106 (54.1)	47.0 – 61.1	96 (49.5)	42.4 – 56.6
Hard	5 (2.6)	0.3 – 4.8	1 (0.5)	-0.5 -1.5
8 months				
Normal	131 (76.6)	70.2 – 83.0	124 (70.9)	64.1 – 77.7
Soft	21 (10.7)	6.3 – 15.1	39 (20.1)	14.4 – 25.8
Firm	19 (11.1)	6.4 – 15.9	12 (6.9)	3.1 – 10.6
Hard	0	NA	0	NA
24 months				
Normal	109 (71.2)	64.0 – 78.5	116 (77.3)	70.6 – 84.1
Soft	3 (1.5)	0.2 – 3.3	0	NA
Firm	41 (26.8)	19.7 – 33.9	34 (22.7)	15.9 – 29.4
Hard	0	NA	0	NA

NA = Not applicable. Number = those infected with *S. mansoni* before treatment, including those that became egg negative nine weeks after treatment.

Table 8. 3: Comparison of spleen consistency between treatment groups at baseline, eight and twenty four months after treatment

	Single dose		Double dose	
	Number (%)	**95% CI**	**Number (%)**	**95% CI**
Baseline				
Normal	87 (44.4)	37.4 – 51.4	102 (52.6)	45.5 – 59.7
Soft	4 (2.0)	0 – 4.0	5 (2.6)	0.3 – 4.8
Firm	100 (51.0)	44.0 – 58.1	86 (44.3)	37.3 – 51.4
Hard	5 (2.6)	0.3 – 4.8	1 (0.5)	-0.5 – 1.5
8 months				
Normal	113 (66.9)	59.7 – 74.0	141 (80.6)	74.7 – 86.5
Soft	33 (16.8)	11.6 – 22.1	24 (12.4)	7.7 – 17.0
Firm	23 (11.7)	7.2 – 16.3	9 (4.6)	1.7 – 7.0
Hard	0	NA	1 (0.5)	-0.5 – 1.5
24 months				
Normal	103 (70.5)	65.1 – 76.0	122 (81.9)	76.6 – 88.1
Soft	2 (1.0)	0.4 – 2.4	0	NA
Firm	41 (20.9)	15.2 – 26.7	26 (13.4)	8.6 – 18.2
Hard	0	NA	1 (0.5)	-0.5 – 1.5

NA = Not applicable. Number = all those that were infected with *S. mansoni* before treatment, including those that became egg negative nine weeks after treatment.

8.3.2.3 Organomegaly

Table 8.4 shows proportions of splenomegaly, hepatomegaly or hepatosplenomegaly by treatment groups. Generally, organomegaly reduced after treatment with either single or double dose. However, the proportion of only hepatosplenomegaly was significantly lower for those that received two doses than those who got a single dose at 24 months after treatment, whereas hepatomegaly and splenomegaly were not significantly different in the two treatment groups throughout the study.

Table 8. 4: Comparison of clinically measured organomegaly among treatment groups at baseline, eight and twenty four months after treatment

	Single dose		Double dose	
	Number (%)	**95% CI**	**Number (%)**	**95% CI**
Baseline				
Normal	71 (36.2)	29.4 – 43.0	86 (44.3)	37.3 – 51.4
Splenomegaly	9 (4.6)	1.6 – 7.5	10 (5.2)	2.0 – 8.3
Hepatomegaly	49 (25.0)	18.9 – 31.1	46 (23.7)	17.7 – 29.7
Hepatosplenomegaly	67 (34.2)	27.5 – 40.9	52 (26.8)	20.5 – 33.1
8 months				
Normal	134 (78.4)	72.1 – 84.6	145 (82.9)	77.2 – 88.5
Splenomegaly	11 (5.6)	2.4 – 8.9	6 (3.1)	0.6 – 5.6
Hepatomegaly	13 (6.6)	3.1 – 10.1	17 (8.8)	4.7 – 12.8
Hepatosplenomegaly	13 (6.6)	3.1 – 10.1	7 (3.6)	1.0 – 6.3
24 months				
Normal	95 (62.1)	54.3 – 69.9	107 (72.3)	65.0 – 79.6
Splenomegaly	14 (7.1)	3.5 – 10.8	7 (3.6)	1.0 – 6.3
Hepatomegaly	16 (8.2)	4.3 – 12.0	20 (10.3)	6.0 – 14.6
Hepatosplenomegaly	28 (18.3)	14.1 – 24.5	14 (9.3)	4.6 – 13.0

Number = those that were infected with *S. mansoni* before treatment, including those that became egg negative nine weeks after treatment.

8.3.3 Ultrasound observations

8.3.3.1 Spleen measurement

Sonographically measured and height-standardised spleen length was categorized as normal, moderately enlarged and markedly enlarged. There was no significant difference in spleen enlargement between the two treatment groups at baseline and eight months after treatment (table 8.5). However, the normal and moderately enlarged spleens at 24 months after treatment were significantly different between the two dose groups. Within each dose

group, the normal spleens increased over time while the markedly enlarged spleens decreased.

Table 8. 5: Comparison of proportions of sonographically measured spleen length between treatment groups at baseline, eight and twenty four months after treatment

Splenic length	Single dose		Double dose	
	Number (%)	95% CI	Number (%)	95% CI
Baseline				
Normal	61 (31.3)	24.7 – 37.8	56 (28.9)	22.4 – 35.3
Moderately enlarged	86 (44.1)	37.1 – 51.1	99 (51.0)	43.9 – 58.1
Markedly enlarged	48 (24.6)	18.5 – 30.7	39 (20.1)	14.4 – 25.8
8 months				
Normal	59 (34.7)	27.5 – 41.9	66 (37.7)	30.5 – 45.0
Moderately enlarged	83 (48.8)	41.2 – 56.4	81 (46.3)	38.8 – 53.7
Markedly enlarged	28 (16.5)	10.8 – 22.1	28 (16.0)	10.5 – 21.5
24 months				
Normal	66 (44.0)	36.0 – 52.0	101 (69.7)	62.1 – 77.2
Moderately enlarged	67 (44.7)	36.6 – 52.7	35 (24.1)	17.1 – 31.2
Markedly enlarged	17 (11.3)	0.62 – 16.5	9 (6.21)	2.2 – 10.2

8.3.3.2 Liver image patterns

Periportal fibrosis, based on the liver image pattern types B-F as described in ultrasound form (appendix III) was low throughout the study. At baseline, of the positive ones that received one dose, 2(0.9%) had pattern B, 2(0.9%) had pattern C and 1(0.5%) had pattern D. For the two dose group, 1 (0.5%) had pattern B and 8(3.7%) had pattern C. At eight months, of those who got one dose, 1(0.5%) had pattern B and 8(3.7%) had pattern C, while in the two dose group, 1(0.5%) had pattern B, 5(2.6%) had pattern C and 1(0.5%) had pattern D. At 24 months, the proportions for the single dose group were 9(5.3%), 6(3.5%), 2(1.2%) and 1(0.6%) with patterns B, C, D and E respectively. For those who got two

doses, 10(5.9%), 10(5.9) and 3(1.8%) had patterns B, C and D respectively. The numbers were too small to carry out a statistical comparison between treatment groups.

8.3.3.3 Portal vein diameter

At baseline, while controlling for malaria, *S. mansoni* intensity of infection was associated with the dilated portal vein diameter ($\chi^2_{\text{trend}} = 9.57$, $P = 0.029$). Only two people had markedly dilated portal vein diameter before treatment and both of them were heavily infected, chapter 4. The difference in dilated portal vein diameters between the two dose groups was not significant throughout the study (table 8.6). Within each treatment group, proportions of normal portal vein diameter increased eight months but decreased 24 months after treatment. Those with moderately dilated portal vein diameters decreased at eight months but increased again 24 months after treatment.

Table 8. 6: Comparison of portal vein diameter between treatment groups at baseline, eight and twenty four months after treatment

Portal vein diameter	Single dose		Double dose	
	Number (%)	95% CI	Number (%)	95% CI
Baseline				
Normal	172 (87.8)	83.1 – 92.4	177 (91.2)	87.2 – 95.3
Moderately dilated	23 (11.7)	7.2 – 16.3	16 (8.3)	4.3 – 12.2
Markedly dilated	1 (0.5)	-0.5 – 1.5	1 (0.5)	-0.5 – 1.5
8 months				
Normal	157 (92.4)	88.3 – 96.4	161 (92.5)	88.6 – 96.5
Moderately dilated	11 (6.5)	2.7 – 10.2	13 (7.5)	3.5 – 11.4
Markedly dilated	2 (1.8)	-0.5 – 2.8	0	NA
24 months				
Normal	122 (80.8)	74.4 – 87.1	116 (80.0)	73.4 – 86.6
Moderately dilated	27 (17.9)	11.7 – 24.1	29 (20.0)	13.4 – 26.6
Markedly dilated	2 (1.3)	-0.5 – 3.2	0	NA

8.3.4 Haemoglobin levels and anaemia

At baseline, the single and double dose groups had haemoglobin levels of 125.4 g/dL and 122.7 g/dL respectively, the difference was not significant (*P*=0.360). haemoglobin levels at eight months increased to 129.7 g/dL for single dose group and to 131.1 g/dL for double dose group and the difference was still not significant (*P*=0.422). Twenty four months after treatment, the haemoglobin levels of the single dose group (137.2 g/dL) was not significantly different from that of the double dose group (136.9 g/dL) (*P*=0.919). Likewise, there was no significant difference in prevalence of anaemia among the two dose groups throughout the study, table 8.7. However, the prevalence of anaemia decreased throughout the study within each treatment group.

Table 8. 7: Comparison of proportions of anaemia between treatment groups at baseline, eight and twenty four months after treatment

	Single dose		**Double dose**	
	Number (%)	**95% CI**	**Number (%)**	**95% CI**
Baseline				
Not anaemic	114 (63.3)	56.2 – 70.4	110 (63.2)	56.0 – 70.5
Anaemic	66 (36.7)	29.6 – 43.8	64 (36.8)	29.5 – 44.1
8 months				
Not anaemic	121 (71.2)	64.3 – 78.1	130 (74.7)	68.2 – 81.2
Anaemic	49 (28.8)	21.9 – 35.7	44 (25.3)	18.8 – 31.8
24 months				
Not anaemic	128 (83.7)	77.7 – 89.6	122 (81.3)	75.0 – 87.6)
Anaemic	25 (16.3)	10.4 – 22.3	28 (18.7)	12.4 – 25.0

8.4 Discussion and conclusion

Schistosomiasis related morbidity is mainly caused by retained eggs in the host's liver periportal spaces. The developing miracidium in the eggs releases proteolytic enzymes, which give rise to inflammatory and granulomatous reactions that result in granuloma formation (Gryseels *et al*., 2006). When the miracidium in the egg dies, enzyme release decreases and the granuloma may shrink, leading to formation of fibrotic lesions around the portal venules. It is the granulomatous reactions and the fibrotic lesions that may result in hepatosplenic schistosomiasis morbidity, sometimes coupled with increased portal pressure (Vennervald *et al*., 2004; Conlon, 2005; Gryseels *et al*., 2006). However, treatment with praziquantel reduces the worm load in individuals infected with schistosomiasis (WHO, 2002; Fenwick *et al*., 2003; Kabatereine *et al*., 2003), thereby reducing the amount of eggs deposited in the host tissue. This may result in reduction of egg-mediated inflammatory reactions that cause schistosomiasis-related morbidity. On the other hand, in high transmission areas, re-infection occurs and this may result in re-occurrence of morbidity.

We assessed schistosomiasis related morbidity pre- and post-treatment and compared the effect of a single dose versus two doses of praziquantel on schistosomiasis related morbidity using both clinical palpations and ultrasound examinations. Although clinical palpation is known to have limited accuracy as compared to ultrasound examinations, it is documented that hepatosplenic schistosomiasis in field-based studies can be identified by abdominal palpations (Gerspacher-Lara *et al*., 1998). It has also been noted that detection of schistosomiasis morbidity using ultrasonography needs to be combined with clinical palpations and epidemiological data (Martins *et al*., 1998). Besides, we correlated the spleen size taken in the MAL and MCL by clinical palpation with the spleen length measured by ultrasound and there were strong associations throughout the study. However, correlation of MCL liver size measurements examined by clinical palpation with those measured by ultrasound were not associated throughout the study.

In our study, we observed no difference in occurrence of organomegaly between single and two doses. This finding is difficult to explain but since morbidity is mediated by retained schistosome eggs in the host tissue, this could be due to lack of a difference in intensity of

re-infection between the two treatment regimens that we observed in chapter 6. Whereas no difference in occurrence of organomegaly in the two dose groups would probably be attributed to lack of association between organ sizes and *S. mansoni* prevalence of infection in our study (chapter 4), proportions of organomegaly reduced after treatment within each dose group. This indicates that praziquantel had an effect on organomegaly, which indirectly implies that *S. mansoni* influenced levels of organomegaly. This is supported by findings from randomized placebo-controlled trials where splenomegaly and hepatomegaly regressed in only the treated ones but increased in the placebo group (Stephenson *et al*., 1985; Olds *et al*., 1999). Nonetheless, we realised a slight increase in proportions of organomegaly from eight to 24 months after treatment, implying there was rebound morbidity. This is comparable to findings elsewhere (Doehring-Schwerdtfeger *et al*., 1992), where hepatomegaly reduced at eight months but increased again at 24 months after treatment. Rebound morbidity is typical for high transmission areas as reported by Olds *et al*., (1996) in a study of *S. japonicum* in Philippines. In their study, liver enlargement reversed two years post-treatment with praziquantel, after which liver enlargement re-occurred and they attributed it to high schistosomiasis transmission and delayed repeat treatment. Hotez *et al*., (2008) also reported that if repeat treatment is interrupted, schistosomiasis re-infection may occur within 12-24 months after treatment resulting into severe rebound morbidity of hepatosplenic disease. This rebound morbidity, mainly caused by re-occurrence of schistosome egg–induced inflammation in the host tissue, was likely due to re-infection observed in our study (chapter 6).

On the other hand, whereas prevalence of splenomegaly reduced at eight months and increased again at 24 months after treatment for those that received two doses, it increased throughout for those who were treated with a single dose. Similar findings were reported from Sudan where splenomegaly remained unchanged or increased seven months (Doehring-Schwerdtfeger *et al*., 1992) and 23 months after treatment (Mohamed-Ali *et al*., 1991). The authors suggested that the persistent splenomegaly could have been due to malaria infection. Persistent splenomegaly was also reported in Madagascar (Boisier *et al*., 1998), Burundi (Gryseels, 1988) and Tanzania (Malenganisho, 2005) and in all these studies un-regressed splenomegaly was attributed to confounding effect of malaria

infection. Similarly, malaria could have contributed to the progression of splenomegaly in our study since we detected a high prevalence of malaria parasitemia at all time points. This is also supported by studies in Kenya (Booth *et al*., 2004b) where the prevalence of hard enlarged spleens was highest at all time points in those with high levels of malaria antibodies.

Despite the high levels of organomegaly realized in our study, it should be noted that hepatosplenic schistosomiasis may occur with or without periportal fibrosis (Vennervald *et al*., 2004). Where periportal fibrosis occurs, it is expected to increase with intensity of infection (Gryseels, 1992). Unlike observations elsewhere in high transmission areas, with more less similar intensity of infection like our study, (Kabatereine, 2000; Malenganisho, 2005) liver image patterns C-E related to periportal fibrosis was so minimal in our study that no statistical comparison could be made between the two treatment groups. Probably, this limited occurrence of periportal fibrosis could be due to genetic factors of the community (Dunne and Pearce, 1999) or a different focal parasite strain and may be, toxins (Yazdanpanah *et al*., 1997) or limited alcohol consumption in our study, unlike what is reported in other studies (Kabatereine, 2000; Malenganisho, 2005).

Other than organ morbidity indicators, we realized no difference in proportions of anaemic people among the two treatment groups throughout the study. It is documented that anaemia can be influenced by other parasitic infections such as hookworms (Sturrock *et al*., 1996; Friedman *et al*., 2005). However, we could not attribute lack of a difference in occurrence of anaemia to hookworm infection because this was balanced in the two groups by treating all the study subjects with albendazole. Besides, when anaemia was regressed against *S. mansoni,* malaria and hookworm infections, there was no significant association with any of the predictors (chapter 4). Nonetheless, anaemia systematically reduced throughout the study in both dose groups, indicating that *S. mansoni* is probably partially responsible for anaemia and treatment enhanced haemoglobin levels. This is in agreement to Olds *et al*., 1999) placebo-controlled study, where haemoglobin levels significantly increased in only the treated children.

Considering that the major strategy for schistosomiasis control is morbidity control, it is vital to obtain more knowledge about the effect of praziquantel treatment on schistosomiasis-related morbidity. Our findings indicate that one dose and two doses of praziquantel all reduce morbidity and they exhibit no significant difference in morbidity indicators after treatment. Two doses given two weeks apart seem not to add any benefit to morbidity regression. It is also evident from our study that morbidity re-appears between eight and 24 months after treatment. Thus, a single annual dose of praziquantel appears to be sufficient in the control of schistosomiasis-related morbidity and this supports the current WHO recommendation of a single annual dose for the control of schistosomiasis. However, since malaria is known to influence morbidity related to schistosomiasis and malaria is prevalent in most schistosomiasis endemic areas of Uganda, research needs to be carried out to determine the effect of schistosomiasis and malaria co-infection on schistosomiasis related morbidity.

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Chapter 9: General discussion

9.1 Introduction

The current study was carried out mainly to enhance our knowledge about the use of different praziquantel treatment regimens in the control of schistosomiasis *mansoni*. The effect of one dose (40 mg/kg body weight) and two doses (2 x 40 mg/kg body weight given two weeks apart) of praziquantel on schistosomiasis cure rate, re-infection, children's growth and schistosomiasis related morbidity was compared in a community living along Lake Victoria, Uganda.

9.2 Discussion

The high prevalence and GMI of *S. mansoni* infection obtained in this study are typical for an endemic area as reported in other studies along Lake Victoria (Kardorff *et al*., 1997; Karanja *et al*., 2002; Malenganisho, 2005; Odogwu *et al*., 2006) and the Albertine region (Odongo-Aginya *et al*., 1994; Kabatereine *et al*., 1996; Frenzel *et al*., 1999; Kabatereine, 2000; Naus *et al*., 2003; Kabatereine *et al*., 2004b). Similar to what other studies have observed (Ongom & Bradley, 1972; Kabatereine *et al*., 1996; 2004a, 2004b; Barakat *et al*., 2000; Kabatereine, 2000; Naus *et al*., 2003), males had higher prevalence and intensity of infection than females. This has been attributed to various factors such as occupation whereby males are more involved in water-related activities like fishing than females (Gryseels & Nkulikyinka, 1988; Jordan & Webbe, 1993). It should be noted that Lake Victoria is the only source of water in our study area and women also frequently go there to fetch water as was observed in our water contact study where there were more females than males reported to go to the lake, chapter 4. However, the duration and nature of exposure to contaminated water varies with the activity. Thus another factor that could explain the gender difference is the duration of exposure to contaminated water (Kabatereine *et al*., 2003; Scott *et al*., 2003; Booth *et al*., 2004a). Most of the highly infected males were fishermen, an activity that exposes them to contaminated water for long periods, hence getting more infected than females.

We observed a common age-dependent trend of peaking in the age group of 10-14 years (Ongom & Bradley, 1972; Butterworth *et al*., 1988 & 1991; Stelma *et al*., 1993; Boisier *et*

al., 1995; Kabatereine *et al*., 1996, 2004a, 2004b; Fulford *et al*., 1998; Naus *et al*., 1999; Barakat *et al*., 2000; Kabatereine, 2000; Scott *et al*., 2003; Conlon, 2005). The age range of 10-19 years in our study had the highest infection levels. We suggested that this pattern of infection could probably have been influenced by the time of day when children get into contact with cercariae-infested water, duration of exposure and size of the body that gets into contact with water (Butterworth *et al*., 1988; Chandiwana & Woolhouse, 1991; Fulford *et al*., 1996; Scott *et al*., 2003; Conlon, 2005).

Nonetheless, the age-dependant infection patterns could have been due to undeveloped naturally acquired immunity to schistosomiasis infection that develops within a period of 10 – 15 years after infection (Rihet *et al*., 1991; Dunne *et al*., 1992; Gryseels, 1994; Corrêa-Oliveira *et al*., 2000; Fitzsimmons *et al*., 2004). This implies that the age group of 10 – 19 years might not have been infected long enough to acquire natural immunity to schistosomiasis infection. Besides immunity, physiological changes at puberty such as increase in skin thickness or increased fat deposition, which all increase resistance to *S. mansoni* cercarial penetration, could also have contributed to infection peaking in the second decade of life (Butterworth *et al*., 1988; Gryseels, 1994; Fulford *et al*., 1998; Dunne & Mountford, 2001).

High *S. mansoni* infections may or may not cause morbidity. Schistosomiasis related organomegaly in our study was not as prevalent as what was reported in other endemic communities in Uganda (Frenzel *et al*., 1999; Kabatereine *et al*., 2004b). We observed hepatomegaly and hepatosplenomegaly but with limited periportal fibrosis, similar to observations in Kenyan school children (Vennervald *et al*., 2004). Contrary to findings elsewhere (Ongom & Bradley, 1972; Friis & Byskov, 1987; Gryseels & Polderman, 1987; Gryseels & Nkulikyinka 1990; Fulford *et al*., 1991; Corbett *et al*., 1992; Boisier *et al*., 1995; Kardorff *et al*., 1997; Vennervald *et al*., 2004), splenomegaly and hepatomegaly were not associated with prevalence of *S. mansoni*. This suggests the possibility of the observed organomegaly not being due to schistosomiasis alone but probably influenced by other factors such as co-infection of schistosomiasis with malaria.

Comparable to other studies (Gryseels & Polderman, 1987; Gryseels, 1988; Yazdanpanah *et al*., 1997), hepatomegaly and hepatosplenomegaly were more prevalent in children than adults. It is documented that hepatosplenomic schistosomiasis may be a result of inflammatory and granulomatous reactions caused by schistosome eggs trapped in the host tissue (Mitchell, 1990; Vennervald & Dunne, 2004; Gryseels *et al*., 2006). Corrêa-Oliveira *et al*., (2000) and Fitzsimmons *et al*., (2004) noted that regulation of inflammatory immune responses increase with age. Considering malaria as a possible factor influencing organomegaly and our study area being endemic for malaria, which was more prevalent in children than in adults, could explain why organomegaly was more prevalent in children than in adults.

Portal hypertension indicators were minimal with less than 3% people presenting with liver image patterns typical for fibrosis and yet periportal fibrosis is expected to increase with intensity of infection (Gryseels, 1992). This is contrary to findings of Kabatereine *et al*., (2004b) in a fishing community along Lake Albert where the prevalence of periportal fibrosis was high and yet the prevalence and intensity of *S. mansoni* were almost similar to those of our study. In another study along Lake Victoria in Tanzania where the GMI was much less than what we obtained, periportal fibrosis was prevalent (Malenganisho *et al*., 2008). This implies that intensity of infection may not explain lack of periportal fibrosis in our study. It is not clear as to why periportal fibrosis was observed in communities along Lake Victoria in Tanzania (Malenganisho, 2005) but not in the Ugandan community.

There are other factors that could affect levels of periportal fibrosis. Booth *et al*., (2004a) noted that duration of infection influenced the prevalence of fibrosis in two adjacent villages along Lake Albert. Adults who had resided in the village for more than 15 years had increased risk of fibrosis than those who had lived there less than 15 years. Duration of exposure is not likely to have affected periportal fibrosis in our study since most people were born in the village and they all use lake water for all purposes. Probably fibrosis in our study was influenced by factors like parasite strain differences and concomitant infections or exposure to toxins (Yazdanpanah *et al*., 1997; Dunne & Pearce, 1999), which were not in our scope and we did not examine them.

Anaemia was one of the morbidity parameters that we assessed in our study and it was found to be mild but highly prevalent. Contrary to studies elsewhere (Gryseels & Polderman, 1987; Sturrock *et al*., 1996; Olsen *et al*., 1998), anaemia had no relationship with *S. mansoni* infection. This observation was not different from that reported in Kenya where there was no relationship between haemoglobin and intensity of *S. mansoni* infection (Olsen *et al*., 1998). Whereas it is documented that schistosomiasis *mansoni* and other parasitic infections such as malaria and hookworms may cause anaemia (Gryseels & Polderman, 1987; Stephenson, 1993; Sturrock *et al*., 1996; Ezeamama *et al*., 2005; Friedman *et al*., 2005; King *et al*., 2005; Brito *et al*., 2006; Koukounari *et al*., 2006), in our study anaemia had no relationship with malaria prevalence. Studies have shown that other factors such as poor nutritional diet or inadequate dietary in-take without iron supplements, poverty, haemoglobinopathies may have an impact on haemoglobin levels and lead to anaemia (Stephenson, 1993; Crawley, 2004; Koukounari *et al*., 2006). Poor nutritional status, hookworm intensity of infection and haemoglobinopathies are likely to have influenced anaemia levels in our study.

Schistosomiasis has been reported to impact negatively on children's growth (Assis *et al*., 2004). In our study, the heavily infected ones were more stunted than those with moderate and light infections. We did not detect any association between growth indicators and *S. mansoni* prevalence. It was predicted that growth could have been affected by other factors such as inadequate food, poverty, poor sanitation, other parasitic infections, individual and environmental conditions.

The main focus of this study was to compare the effect of one and two doses of praziquantel on various schistosomiasis indicators. Nine weeks after treatment, the prevalence and intensity of *S. mansoni* infection for the two treatment groups remarkably reduced. Double dose given two weeks apart realised a significantly higher cure rate than single dose, which is comparable to other studies in high transmission areas (Kabatereine *et al*., 2003; N'Goran *et al*., 2003). Nonetheless, the cure rate for single dose was lower than the expected 60-90% rate with a single dose (Cioli *et al*., 1995; WHO, 2002), while that of double dose was just slightly above the lower limit of the expected rate. Similar low

cure rates were reported elsewhere (Polderman *et al*., 1984; Guisse *et al*., 1997; Kabatereine *et al*., 2003). Based on the fact that praziquantel kills only the mature worms, it is suggested that the double dose treatment exhibited significantly higher cure rate than single dose probably because the second dose killed more worms that were not yet mature at first treatment. Those who remained infected with schistosomiasis after the treatment had their GMI of infection greatly reduced in accordance to the recommended rates (WHO, 2002) in both treatment groups, which is also in agreement with other studies (Kabatereine *et al*., 2003; N'Goran *et al*., 2003). Although there was no significant difference in egg reduction rate between the two treatment groups, two doses lead to slightly greater reduction in *S. mansoni* infection intensity. This is more less similar to findings elsewhere (Tchuem Tchuente *et al*., 2001; Kabatereine *et al*., 2003; N'Goran *et al*., 2003).

Comparing the effect of the two doses on incidence and intensity of *S. mansoni* re-infection, no significant difference was realised. Similar results were reported in Mali (Sacko, 2006). The two doses exhibited no difference probably because of high schistosomiasis transmission in our study area coupled with the temporary action (about two months) of praziquantel as a 'vaccine' towards *S. mansoni* re-infection (Rihet *et al*., 1991; Dunne *et al*., 1992; Fallon *et al*., 1992; Farghaly *et al*., 1993; Mutapi, 2001; Karanja *et al*., 2002; Colley & Secor, 2004; Fitzsimmons *et al*., 2004; Secor, 2005). Perhaps the short-lived "vaccination" effect of praziquantel suppressed the difference in infection levels between the two doses in that by the time of assessment the vaccination effect of the two treatment doses was counterbalanced and with high transmission in the area accompanied by high water contact, both groups got re-infected almost to similar levels.

Re-infection levels were also assessed among other parameters. Comparable to observations elsewhere (Ongom & Bradley, 1972; Jordan & Webbe, 1993; Kabatereine *et al*., 1999; Kabatereine, 2000), males were more re-infected than females at all follow up points. Considering age, children were more re-infected than adults (Ongom & Bradley, 1972; Bensted-Smith *et al*., 1987; Butterworth *et al*., 1988 & 1991; Boisier *et al*., 1995; Kabatereine *et al*., 1996, 1999; Fulford *et al*., 1998; Barakat *et al*., 2000; Corrêa-Oliveira *et al*., 2000; Kabatereine, 2000; Scott *et al*., 2003; Conlon 2005; Vereecken *et al*., 2007). Re-

infection following the same pattern as observed before treatment, suggests that re-infection is influenced by the same factors as those before treatment with regard to age and gender. These include nature and duration of exposure, immunity and physiological changes as earlier mentioned. The re-infection difference based on age and gender could have also been influenced by pre-treatment intensity of infection as noted in earlier studies (Bensted-Smith *et al*., 1987; Butterworth *et al*., 1988; Webster *et al*., 1998; Karanja *et al*., 2002) where there was a positive association between pre-treatment intensity of infection and level of re-infection. This is supported by the fact that males and children had higher pre-treatment intensity of infection than females and adults respectively (Chapter 4).

Schistosomiasis being one of the parasitic diseases that may retard children's growth in developing countries (Corbett *et al*., 1992; Stephenson, 1993; Parraga *et al*., 1996; Assis *et al*., 1998; King *et al*., 2005; Zhou *et al*., 2005; Leenstra *et al*., 2006), we compared the effect of one versus two doses of praziquantel on children's growth indicators based on height and weight. There was no significant difference in z-scores of height for age and weight for age as well as in body mass index between the two treatment groups, which is in agreement with Sacko (2006) findings in their study of children in Mali. It should be noted that growth changes after any intervention are usually small and in order to detect any difference, it requires a control group and a large sample. Our study had limitations in that we had a small sample of children and due to ethical reasons, we had no control group. Small numbers in our study could have suppressed a detection of any smallest difference in growth indicators between the two treatment groups. In conformity with reports in other studies (Corbett *et al*., 1992; Assis *et a*l., 2004), there was no significant correlation between *S. mansoni* infection and the growth indices. The trapped eggs in host tissue stimulate protein metabolism which in turn leads to weight loss (Campbell *et al*., 2003). This could explain the lack of a difference in growth between the two dose groups because both treatment regimens exhibited marked egg reduction.

On comparing the effect of a single dose and two doses of praziquantel on schistosomiasis related morbidity, some parameters were significantly different while others were not. Prevalence of splenomegaly and hepatomegaly did not significantly differ between the

treatment groups whereas hepasplenomegaly was different 24 months after treatment. These add more support to the suggestion that the observed splenomegaly and hepatomegaly were probably not related to schistosomiasis but rather to other parasitic infections like malaria as reported elsewhere (Gryseels, 1988; Boisier *et al*., 1998; Mwatha *et al*., 2003; Booth *et al*., 2004b; Malenganisho, 2005; Wilson *et al*., 2007). On the other hand, within each treatment group, proportions of organomegaly reduced after treatment, indicating that praziquantel had an effect on organomegaly. This is supported by findings from randomized placebo-controlled trials where splenomegaly and hepatomegaly regressed in only the treated ones but increased in the placebo group (Stephenson *et al*., 1985; Olds *et al*., 1999). However, since schistosomiasis morbidity is mediated by eggs in the host tissue, the similarity in intensity of re-infection in the two groups (chapter 6) could have contributed to lack of a difference in some organomegaly parameters. Those given two doses had a significantly higher proportion of soft livers, and fewer firm livers than those treated once. The prevalence of firm livers in both treatment groups significantly decreased at eight months and increased again 24 months after treatment. This is an indication of rebound morbidity that was probably aggravated by re-infection (Olds *et al*., 1996; Hotez *et al*., 2008).

Considered among schistosomiasis related morbidity indicators, haemoglobin concentrations and occurrence of anaemia were not significantly different between the two treatment groups throughout the study. This could be explained by haemoglobin levels not being associated with *S. mansoni* infection and further supports our prediction of haemoglobin being influenced by other factors such as malnutrition and haemoglobinopathies. Whereas malaria and hookworm infections influence haemoglobin levels, lack of a difference in proportions of anaemia between the treatment groups could not be explained by these infections because their effects were balanced in the two treatment groups by treating all of them for hookworm with albendazole while malaria had no intervention in both groups. Our findings could be supported by Sturrock *et al*., (1996) observations in Kenyan children where anaemia was not significantly related to *S. mansoni* and it was attributed to other parasitic infections like malaria or haemoglobinopathies and poor nutrition with low iron intake.

From our study, it is likely that schistosomiasis related morbidity was also influenced by other parasitic and socio-economic factors. This leaves an information gap about the effect of these confounding factors on schistosomiasis morbidity, a factor that needs to be studied.

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Chapter 10: Conclusion

This study describes the epidemiology and morbidity related to schistosomiasis in a community of Musoli village. It also elucidates the impact of two doses of praziquantel on schistosomiasis infection and morbidity. However, our study was affected by a number of limitations. A single set of anthropometric measurements were taken and clinical examination measurements were based on the examiners' consensus agreement for the final value. This is likely to have created a measurement bias. Other confounding factors such as malaria, nutritional status and socio-economic factors could have affected the associations between *S. mansoni* infection and its related morbidity. There was great loss to follow up, which resulted into small sample sizes and this could have contributed to the lack significant differences in the measured parameters between the two treatment regimens. Nonetheless, our parasitological results show that Musoli village is highly endemic for schistosomiasis, with intensity of infection and morbidity being more prevalent in children than adults. Even though *S. mansoni* infection intensity was high, its effect on schistosomiasis related morbidity was not evident. Hepatomegaly and splenomegaly showed no association with schistosomiasis. Clinically detected hepatosplenic schistosomiasis but with minimal periportal fibrosis was prevalent. Growth retardation measured by growth indicators of stunting, wasting and underweight, was common in children but no association was detected between growth parameters and *S. mansoni* infection. No association was detected between anaemia and *S. mansoni*, indicating that anaemia is influenced by some factors other than schistosomiasis.

Comparing the effect of two doses versus one dose of praziquantel on schistosomiasis infection, two doses yielded higher cure rate than single dose. However, it is rather difficult to recommend usage of two doses in a control programme as it may have high operational costs and logistical implications. Re-infection with *S. mansoni* in Musoli community is high, with children being more re-infected than adults. This indicates that transmission in this focus is intense and everyone exposed to the lake water is susceptible to infection. Having observed high (re)infection levels in children in our study, it adds more support to the control of schistosomiasis targeting school-age children as an appropriate and beneficial

strategy not only in terms of reducing intensity of infection but also reducing transmission in an area since children contribute significantly in the spread of schistosomiasis by indiscriminately defaecating in the environment. However, two doses have no advantage over a single dose with regard to prevalence and intensity of re-infection. It is also evident that *S. mansoni* infection intensity remains low even two years after treatment with either one or two doses. This may have a policy implication where mass treatment can be carried out in alternate years.

Our findings have indicated that there is no benefit in using two doses of praziquantel to reduce morbidity, since treatment with either one or two doses reduced levels of organomegaly without any significant difference. This implies that the current single annual dose of praziquantel is effective in combating schistosomiasis related morbidity, even in highly endemic areas. Nonetheless praziquantel being the only drug that is massively used to control schistosomiasis, parasite resistance is likely to build up. Therefore there should be mechanisms to monitor parasite tolerance to praziquantel.

From this study, the following are recommended:

- Malaria seems to have influenced most of the schistosomiasis related morbidity in our study and yet it is prevalent in most schistosomiasis endemic areas of Uganda. Thus research needs to be carried out to determine the effect of schistosomiasis and malaria co-infection on schistosomiasis related morbidity.
- Having realised low cure rates in our study and also in other studies elsewhere in endemic areas, further studies should be conducted to assess if there is a resistant strain of schistosomes that require a different drug or to set standard cure rates in relation to the target population and schistosomiasis endemicity. The level of morbidity along Lake Albert and along Lake Victoria in Tanzania seems to be higher than what we observed. Thus a study of parasite strains would also answer why there is this difference.
- Two doses of praziquantel were not superior to one dose in reducing morbidity or re-infection levels. The national mass treatment of endemic communities has not stopped transmission either. Thus there is need to tackle schistosomiasis control in

an integrated manner. Chemotherapy should be supplemented preventive measures such as improved access to safe water, enhanced excreta disposal, health education and snail control where feasible.

- To assess the relationship between *S. mansoni* and children's growth and the impact of treatment on growth, a study with a relatively large sample needs to be carried out over a long period of time.
- The height standardisation of morbidity indicators based on the Senegalese population may not be adequate for all endemic communities. Different ethnic groups may have varying reference values due to different genetic backgrounds, nutritional and other environmental factors. Thus there is need to define standard heights of a local schistosomiasis-free population that would compare with the local infected population better than that of the Senegalese population.
- Clinical palpation is good for determining the degree of liver or spleen enlargement and their consistency, while ultrasound examination is good for detection of peri-portal fibrosis and any sign of portal hypertension. Thus, clinical examinations should always supplement ultrasound examinations in monitoring and evaluating the health impact of schistosomiasis control programmes.

Appendices

Appendix I: Questionnaire

We would like to ask you some simple questions about your health and your activities in the lake. The answers to these questions will help in our study of bilharzia.

Do you agree to answer these questions (Y/N)?

Name…….. HH/ID…..…… Sex ……

Tribe …… Age…….

District …… Sub county ……...Village……

1. Were you born in this village (Y/N/DK)? ……..
2. How long have you been living in the village? ……..
3. Please tell us where else you have lived (>6 months), and for how long you were there.

Place	How long (years)	Doing what?

4. What is your occupation? …….
5. Have you ever been treated for worms? (Y/N/DK) ….
   If "yes" when? ……
6. Have you ever heard of bilharzia? (Y/N/DK) ……
   If "yes", how do you think one can get it? ……

7. Have you ever been treated for bilharzia? (Y/N/DK) ……. If "yes" when? ……
8. Have you ever vomited blood (not coughed)? (Y/N/DK) ……. If "yes" when? …….
9. Have you had the following in the last one month?
   (i) Diarrhoea only (Y/N/DK) ……
   (ii) Diarrhoea with blood (Y/N/DK) ……
   (iii) Persistent stomach-ache (Y/N/DK) ……
10. Which diseases have you suffered from in the last one month?
    (i) …… (ii) ……. (iii) ……..
11. Do you have a pit latrine at home? (Y/N/DK) …..
12. When at home where do you ease yourself? ……..……..
13. When away from home, where do you ease yourself? …….

Activities in the lake

Activity	(Yes/ no)	How often (days per week)	Usual time of day (Morning, afternoon, evening, night)	From which part of the lake.
Fishing near the shore				
Fishing far away from the shore				
Swimming/bathing				
Washing clothes or utensils				
Fetching water				
Buying fish				
Transport				
Other:				

Thank you.

Appendix II: Clinical form.

Date:___ / ___ / ___ Area:_____

Study number (HH, id): _____ ____ Name:_____

Sex *(1=male, 0=female)*: ____ Year of birth: _____

Anthropometric parameters

Height (cm): _____ Weight (kg): _____

MUAC (cm): _____ Triceps skinfold (mm): _____ _____

Clinical examination

Performed by (initials): _____ _____ _____ _____

Time of examination: _____ Time since last full meal (hours): _____

Abdomen palpable *(0=no 1=yes, 2=with difficulty)*: _____

Liver

MSL (cm): _____ MCL (cm): _____

Tender? *(0=no, 1=yes)*: _____

Consistency *(0=not palpable, 1=soft, 2=firm, 3=hard):* _____

Irregular? *(0=no, 1=yes):* _____

Spleen

MCL (cm): _____ MAL (cm): _____ tipped? *(0=no, 1=yes)*: _____

Consistency *(0=not palpable, 1=soft, 2=firm, 3=hard)*: _____

Other _____ _____ _____ _____

(*One or more scores possible: 0=nothing, 1=ascites, 2=other abdominal swelling, 3=umbilical collaterals, 4=febrile, 5=scars, 6=pregnant*)

Score: Preferred: _____ Alternative: _____

0 = no organomegaly

1 = hepatomegaly, splenomegaly or both but with *soft* consistency. Liver may be tender.

2= spleen enlarged with firm or hard consistency. No hepatomegaly.

3 = liver enlarged, especially along the MSL. May be tender, firm or hard. No splenomegaly.

4 = firm or hard splenomegaly plus firm or hard hepatomegaly, may be irregular.

5 = massive hepatosplenomegaly, with or without ascites and collaterals.

Appendix III: Ultrasound form

Performed by (initials): _____

Spleen

Spleen length (cm): _____

Splenic vein diameter (mm): _____

Echogenicity *(0=normal, 1=hypoechoic, 2=hyper echoic)*: _____

Texture (*0=homogenous, 1=coarse, 2=hyper echoic foci)* : _____

Liver

PSL (cm): _____ MCL (cm): _____

Shape *(0=convex-concave, 1=bi-convex)*: _____

Surface *(0=normal, 1=irregular)* _____

Echogenicity *(0=normal, 1=hypoechoic, 2=hyperechoic)* _____

Gall Bladder

Wall thickness (*0= not measurable*) mm: _____

Shape of wall *(0=normal, 1=irregular)*: _____

Liver texture pattern (tick one box):

□ A= normal

□ B = feather streaks □ B1 = flying saucers □ B2 = spider thickening

□ C1= more prominent peripheral rings □ C2 = more prominent pipe stems

□ D = ruff □ Dc

□ E = patches □ Ec

□ F = birds claw □ Fc

Not classifiable: □ X = cirrhosis like □ Y = fatty liver like □ Z = other

Segmental portal branch walls (left portal branch)

External (mm): _____ _____ Mean: _____

Internal (mm): _____ _____ Mean: _____

Portal vein diameter, deep inspiration (mm): _____

Porto-systemic collaterals *(0=not detected, 1=detected)*: _____ If present, specify type: _____

Ascites *(0=absent, 1=present):* _____

Picture taken *(0=no, 1=yes):* _____