EFFICACY AND SAFETY OF UP-SCALED DOSAGE OF 60 MG/KG PRAZIQUANTEL IN CONTROL OF *SCHISTOSOMA MANSONI* IN SCHOOL GOING CHILDREN IN KIRINYAGA COUNTY, KENYA

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Efficacy and Safety of Up-Scaled Dosage of 60 Mg/Kg Praziquantel in Control of *Schistosoma Mansoni* in School Going Children in Kirinyaga County, Kenya

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DECLARATION

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

Signature……..……..Date……..

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This thesis has been submitted for examination with our approval as university supervisors.

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DEDICATION

I dedicate this thesis to my grand parents Mr and Mrs Kirubi Njuguna, and to my aunt Jane Kirubi.

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LIST OF ABBREVIATIONS AND ACRONYMS

AE	Adverse events
DNA	Deoxyribonucleic Acid
Epg	Eggs per gram of faeces
Hb	Haemoglobin
HCG	Human chorionic gonadotropin
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KEMRI	Kenya Medical Research Institute
MDA	Mass Drug Administration
NTD	Neglected tropical diseases
PZQ	Praziquantel
SAC	School-aged children
SERU	Scientific Review Unit
TCBZ	The benzimidazole triclabendazole
WBCs	White blood cells
WHO	World Health Organization

ABSTRACT

Chemotherapy with praziquantel has been the core treatment strategy for schistosomiasis. There has been concerns on tolerance and resistance to praziquantel. Socio-economic activities in Kirinyaga County exposes the community to fresh water snails that harbor the schistosome parasite. Various studies have reported increased prevalence and high infection intensity in the area despite ongoing mass drug administration (MDA) interventions. The study area was in Kirinyaga County, in central Kenya where the prevalence of schistosomiasis in school going children was first determined. A sample size of 190 subjects aged between 4-17 year infected with *S. mansoni* were randomly allocated in Group A (40 mg/kg) and Group B (60 mg/kg). Stool samples were collected for baseline prevalence, cure rate and egg reduction rate post-treatment. Frequency and severity of adverse events (AE) at 4 and 24 hrs post-treatment were also determined. Peripheral blood was collected for haemoglobin, liver function test and eosinophil levels assays. Prevalence of *S. mansoni* infection was 52.8%. Cure rates at 21 days post-treatment were 92% for 40 mg/kg while that of 60 mg/kg was at 94% which did not represent any significant difference between the two treatment groups ($p>0.05$). Mean hemoglobin levels at 21 days post-treatment were 10.96 g/dl and 11.19 g/dl respectively representing a non-significant difference ($p>0.05$). Eosinophil levels implied a significant decrease after treatment in both groups although no significant difference was observed between the treatment groups ($p>0.05$). Adverse events recorded in the 60 mg/kg and 40 mg/kg groups showed that mild abdominal pain was the most frequent AE for the 2 dosages while anoxia was the least occurring at 4 hrs and 24 hrs post-treatment. Both 40 mg/kg and up-scaled 60 mg/kg treatment dosages were proven to offer substantial cure to *S. mansoni* but the difference in cure rates was found to be insignificant.

CHAPTER ONE

INTRODUCTION

1.1 Background of information

Schistosomiasis is a water-borne disease that affects more than 250 million people 94% of which are on the African continent (WHO, 2014; Savioli et al., 1997; Steinmann et al., 2006). Schistosomiasis has an estimated global disease burden of 24-56 million disability-adjusted life-years lost (Fenwick, 2012; King, 2010). Human schistosomiasis is transmitted by a parasitic flatworm of the genus *Schistosoma*. The two major schistosome species that transmit human Schistosomiasis in Kenya are *S. haematobium,* and *S. mansoni* and are responsible for disease burden in Sub-Saharan Africa (Utzinger et al., 2009).

Contamination of open water with human excreta containing the parasite's eggs initiate human to-snail transmission when miracidia released from hatching eggs penetrate into the appropriate fresh water snails species, which serve as intermediate hosts. Schistosome infections are linked to permanent organ damage of spleen and liver, increased morbidity, poor childhood development, and reduction in adult productivity. Severe form of the infection has also resulted in death (Conteh et al., 2010).

Schistosomiasis control has traditionally relied on chemotherapy, intermediate vector elimination, improved sanitation and public health education (WHO, 2014; WHO, 2010). Chemotherapy is the gold standard in the global strategy for schistosomiasis elimination (Doenhoff *et al.,* 2008). No vaccine is available in the market hence the disease is controlled with anti-schistosomal drugs which include praziquantel (PZQ), oxamniquine, hycanthone and niridazole. However, PZQ has been found to be the most effective anti-schistosomal drug, the least expensive and most readily available of all (Hagan *et al.,* 2004). It is also extremely effective against all schistosome species that infect humans, is

well-tolerated thereby suitable for mass drug administration (MDA) campaigns in school going children deworming programme that are a high-risk group for infection in the population (Cioli *et al.,* 1995; Sudtida *et al.,* 2006).

In 2014, WHO announced a 'roadmap' for the eradication of schistosomiasis as a public health concern in multiple African countries by the year 2020 and globally by the year 2025 (WHO, 2014; Lancet, 2014). This inspired a global alliance of 22 partners including the World Bank, WHO, The Bill and Melinda Gates Foundation and major pharmaceutical companies to announce a sustainable program to control schistosomiasis by the year 2020 (http://unitingtocombatntds.org). Praziquantel use has increased over the years, not only in intensity but also in frequency. The acceptable dose of PZQ for treatment of *S. mansoni* ranges between 30 to 60 mg/Kg and the optimum dose ranges between 40 to 60 mg/kg. The dosage of below 30mg/Kg is termed as sub-curative while that of above 60 mg/kg is overdose (Zwang and Olliaro, 2014; Montresor *et al.,* 2001). The WHO recommended treatment dose of PZQ is 40 mg/kg. Even though the efficacy of the drug at this dose is high, reported cure rates commonly range from 70 to 95%. A high proportion of infected population occurs in endemic areas like Kirinyaga County, Kenya where the major economic activity is irrigation farming. This exposes the community to fresh water snails that harbor the infectious schistosome parasite. It is estimated that approximately six million people are infected with schistosomiasis in Kenya (Chitsulo, 2000). School-aged children are mostly devastated with the morbidity and mortality associated with schistosomiais (Rollinson *et al.,* 2013). This has led to current treatment and control measures of schistosomiasis in Africa especially in Kenya that target only school children aiming at curing or reducing the morbidity of Schistosomiasis. School-age children with schistosomiasis are mostly physically and intellectually compromised, most of them suffering from concurrent anaemia, fatigue, weakness, learning disabilities, attention deficits, impairment of memory and cognitive reasoning, school absenteeism and higher dropout rates (Guyatt *et al.,* 2001; WHO, 2002; WHO, 2013). This has resulted in poor academic performance, thereby resulting to limited potential of infected children and

socio-economic burden of the society (Houweling *et al.,* 2016; Gray *et al.,* 2011; Conteh *et al.,* 2010). Continous annual mass drug administration (MDA) of PZQ has been taking place in Kirinyaga County. Despite these interventions, the prevalence of Schistosomiasis has increased over the years from 47% in 2011 (Kihara *et al.,* 2011) to 53% in 2015 (Masaku *et al.,* 2015). In addition, concern arises that continued PZQ use will likely lead in drug resistance or reduced susceptibility due to drug pressure (Gray *et al.,* 2011; Ismail *et al.,* 1996). Studies to establish the efficacy and safety of up-scaled dosage to 60 mg/kg PZQ in Kirinyaga County are therefore justified.

1.2 Problem Statement

Praziquantel (PZQ) is currently the WHO recommended anti-Schistosomal drug which is effective against all schistosome species. It is readily available and well-tolerated making it suitable for mass treatment campaigns. World Health Organization (WHO) widely advocates treatment dose of PZQ is 40 mg/kg. Even though the efficacy of the drug at this dose is high, reported cure rates ranges from 70 to 95%. The use of PZQ has increased over the years, not only in intensity but also in frequency.There is alarm that continued PZQ use will likely result in drug resistance or reduced susceptibility due to drug pressure. This is evident in Kirinyaga County where the prevalence of Schistosomiasis has increased over the years from 47% in 2011 (Kihara *et al.,* 2011) to 53% in 2015 (Masaku *et al.,* 2015), despite the annual interventions through mass drug administration (MDA) programs. Alarmingly, low cure rates in response to the standard dose of 40 mg/kg began to appear 10-15 years after its MDA in Egypt (Ismail *et al.,* 1996) and evidence of emerging resistance has been documented (Bennett *et al.,* 2017). The study proposes the investigation of the efficacy and safety for increased dose of 60 mg/kg PZQ in Schistosomiasis treatment in Kirinyaga County.

1.3 Justification

A high proportion of infected population occur in endemic areas like Kirinyaga County where the major economic activity is irrigation farming exposing the community to fresh water snails that harbor the infectious schistosome parasite. Children are the most vulnerable to Schistosomiasis, and infected school-age children are often physically and intellectually compromised (Guyatt *et al.*, 2001; WHO, 2002). Praziquantel is known to be efficacious at acceptable dose range of between 30 to 60 mg/Kg (Zwang and Olliaro, 2014; Montresor *et al.*, 2001) with a standard single oral dose of 40 mg/kg for uniformity in treatment of both urinary and intestinal Schistosomiasis (WHO, 2014). Due to increased drug pressure over the years under the MDA program, the prevalence of schistosomiasis in Kirinyaga County has astoundingly risen from 47% to 53% in a span of 5 years. There is an urgent need therefore to establish whether there is a significant difference in efficacy by up-scaling the treatment dosage to 60 mg/kg. Present study will determine the difference in the efficacy and safety profiles of 40 mg/kg and 60 mg/kg dosages in management of schistosomiasis.

1.4 Research questions

1. Does an up-scaled dosage of 60 mg/kg PZQ have improved efficacy compared to the standard 40 mg/kg?
2. Is there a difference in morbidity of increased dosage of PZQ 60 mg/kg in comparison with a standard dosage of 40 mg/kg?
3. Is there a difference in the safety and adverse events of PZQ 60 mg/kg when compared to 40 mg/kg?

1.5 Null Hypothesis

There is no significant difference in efficacy and safety of 60 mg/kg dosage of PZQ as compared to the 40 mg/kg dosage of PZQ in treatment of *S. mansoni* in school children in Kirinyaga County.

1.6 Study Objectives

1.6.1 General objective

To determine the efficacy and safety of 60 mg/kg dose up-scaled from 40 mg/kg in school going children in Kirinyaga County, as a strategy for improved management of *S. mansoni*.

1.6.2 Specific Objectives

1. To determine efficacy of 60 mg/kg increased dosage of PZQ in comparison with a standard dosage of 40 mg/kg of PZQ in the treatment of *S. mansoni* in school going children in Kirinyaga County.
2. To determine morbidity in increased dosage of PZQ 60 mg/kg in comparison with a standard dosage of 40 mg/kg of PZQ in the treatment of *S. mansoni* in school going children in Kirinyaga County.
3. To establish safety and adverse events of PZQ 60 mg/kg in comparison with a standard dosage of 40 mg/kg of PZQ in the treatment of *S. mansoni* in school going children in Kirinyaga County.

CHAPTER TWO

LITERATURE REVIEW

2.1 Epidemiology and transmission of schistosomiasis

Schistosomiasis is the second most significant tropical disease in terms of incidence of morbidity and mortality second to malaria (WHO, 2012). It was first discovered in 1890 by Theodor Bilharz in Egypt (Nelson, 1989). Schistosomiasis is of great public health and socio-economic concern in developing countries. It is estimated that more than 250 million people are infected with schistosomiasis with majority of them residing in rural communities (Table 1). 700 million people in the world are at risk of infection with 91.4% requiring treatment for schistosomiasis reside in Africa (Brunn *et al.*, 2008; WHO, 2014).

Schistosomiasis infection is contributed by human contact with water contaminated with parasite in daily water activities. These activities include farming, bathing and fetching water for domestic use. Other activities of school-age children include swimming and fishing in parasite infested water. These activities are mainly linked to poverty, ignorance, poor hygiene practices. These factors make them vulnerable to infection (Mostafa *et al.*, 1999; WHO 2014).

Schistosomiasis is snail transmitted and prevalent in tropical and sub-tropical areas, especially in communities with limited access to safe drinking water and ample sanitation. Its infection spreads rapidly due to social dislocation or massive migrations caused by famine, drought, war, labour migration and other man-made ecological changes that include creation of dams and the cultivation of rice in paddy fields. These produce expanses of water which are suitable breeding grounds for vector snails (Morgan *et al.*, 2005; Malek, 1981).

Transmission of infection requires contaminated water by excreta from infected definitive hosts to get incontact with specific fresh water snails that are intermediate hosts for

transmitting the infective stage (cercariae). The cycle of transmission is completed by human contact with contaminated water through activities such as farming, fishing, and domestic chores (WHO, 2013).

Distribution of schistosomiasis is mainly determined by the geographical distribution of snail intermediate hosts which differ in their habitat preferences. Availability of suitable snail host, the potential of infected humans to contaminate the local water and human activities like flood irrigation of crops in the contaminated water determines the endemicity of the particular species of *Schistosoma*. Schistosomiasis has a worldwide distribution as shown in Table 1. The widely distributed species include *S. haematobium* (identified in 1852), *S. japonicum* (1904), *S. mansoni* (1907), *S. intercalatum* (1934), and *S. mekongi* (1978).

Table 2.1: Parasite species and geographical distribution of schistosomiasis

Form of schistosomiasis (CDC, 2013; WHO, 2013)	***Schistosoma* species**	**Species Geographical distribution**
Intestinal schistosomiasis	*Schistosoma mansoni* (identified in 1907)	Africa, Brazil the Middle East, Venezuela,Caribbean
	Schistosoma japonicum (identified in 1904)	China, Philippines, Indonesia
	Schistosoma mekongi (identified in 1978)	Cambodia and the Lao People's Democratic Republic
	Schistosoma guineensis and related *S. Intercalatum* (identified in 1934)	Central Africa regions
Urinary schistosomiasis	*Schistosoma haematobium* (identified in 1852)	Africa, the Middle East

2.2 The Human Schistosomes

The human schistosome, a parasitic flatworm of the genus *Schistosoma*, causes Schistosomiasis which is a neglected tropical disease known to affect a global estimate of 700 million people (WHO, 2014; King, 2010; Colley *et al.*, 2014). Schistosome infections may lead to permanent organ damage, increased morbidity, tragic effects on childhood development and reducted adult productivity. Severe infection has also resulted in death (Fenwick *et al.*, 2009; King, 2010; Hotez and Fenwick 2009).

Two major schistosome species responsible for causing human schistosomiasis in Kenya include *S. haematobium*, and *S. mansoni*. *Schistosoma mansoni* is mostly endemic in East Africa including Kenya. It is also found in Malasyia, Arabia, Egypt, South America and the Carribean Islands (Colley *et al.*, 2014; CDC, 2013). In Kenya, major factors that contribute to distribution of *S. mansoni* include scattered distribution of intermediate hosts

especially in Mwea irrigation scheme, Coastal region and Kisumu region along the lake basin (Mwangi *et al.,* 2014; Masake *et al.,* 2015). Snail development is favoured by tropical climate and availability of slow-moving or still water (WHO, 2002).

Schistosoma mansoni infection is transmitted by fresh water pulmonate snails of the genus *Biomphalaria* (Rozendaal, 1997) which serve as the intermediate hosts in disease transmission. In Africa, 12 Biomphalaria species exist and majority are known to be susceptible to the natural infection with *Schistosoma mansoni* (Rozendaal, 1997). There are three Biomphalaria species namely; *Biomphalaria pteifferi*, *Biomphalaria sudanica* and *Biomphalaria choamphala* which occur in areas endemic with *S. mansoni* (Rozendaal, 1997; CDC, 2013). *Biomphalaria pteifferi* is the most wide-spread in Africa especially in south of Sahara and the Lake Victoria region.

Parasites are transmitted in the hosts by motile aquatic stages of miracidia and cercaria which dynamically seek intermediate and definitive hosts respectively. The miracidia penetrates the soft tissues of the snail, develop forming sporocysts which bursts releasing fork-tailed cercariae. The cercariae periodically swim to the surface of the water and then sink to the bottom for up to three days and when they come into contact with the prospective definitive host, they attach and penetrate the skin within minutes, shedding their tails in the process (CDC, 2013).

Optimum transmission is subject to factors that affect any or all stages of transmission including egg hatching, miracidial survival and penetration, host seeking, sporocyst development, cercariae shedding and penetration into the definitive host. Physical, chemical and biological characteristics of water such as temperature, ion balance, dissolved gases and food availability affect suitability of the water for snail development (Morgan *et al.,* 2005; Colley *et al.,* 2014).

2.3 Life cycle, biology and infection of *Schistosoma mansoni*

Schistosomes are unisexual trematodes with indirect digenetic life-cycle which involve sexual reproduction in vertebrate definitive host and asexual reproduction in snail intermediate vector hosts. Adult *Schistosoma mansoni* reside in the mesenteric veins draining sigmoido-rectal region. In human and other susceptible vertebrate hosts, the male and female schistosomes pair up and the female is held in the gynaecophoric canal of the male from extending its anterior end far into the smallest venules where it deposits the eggs one at a time. Female schistosomes produce numerous eggs between 200-3000 per day (CDC, 2013). Eggs then move through the vessels and the mucosa of the large intestines and enter the lumen of the intestines. The embryonated eggs are then released with feaces into fresh water. Under suitable conditions the eggs hatch into free swimming miracidia which are viable for several hours while actively seeking suitable intermediate hosts using chemotaxis and phototaxis. When the miracidia encounter the snail host, they penetrate into the soft tissue, making their way into the snail's liver. In the snail host, the miracidia losses cilia and develops into a first stage sporocyst. They then develop into second stage sporocyst with free swimming fork- tailed cercariae. The cercariae are then released into the water 4 weeks after the infection (CDC, 2013). The complete life cycle of schistosomiasis is highlighted in Figure 1.

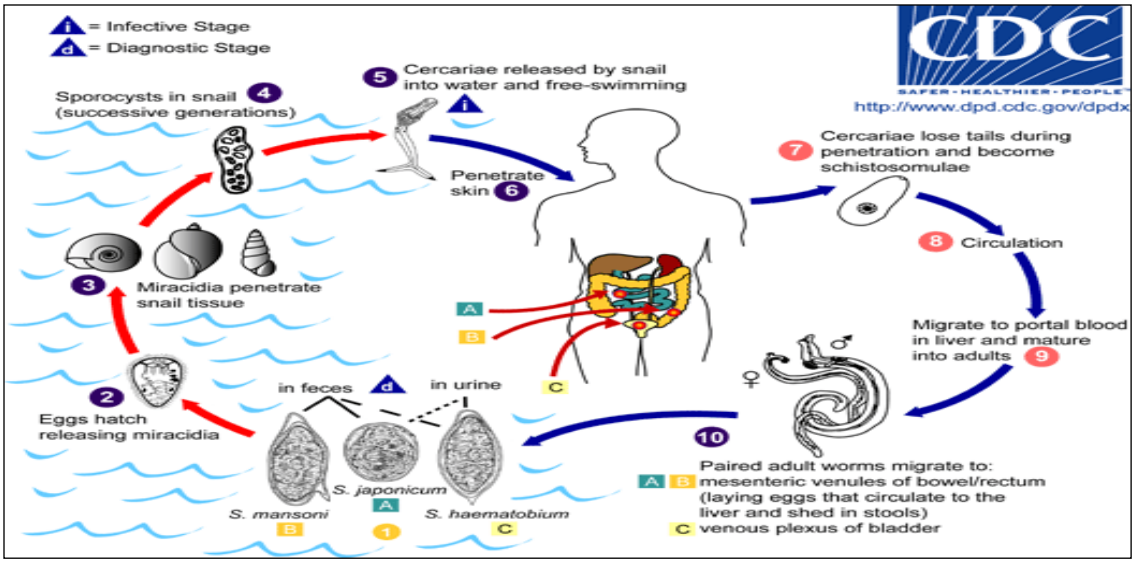

Figure 2.1: The schistosome life cycle, (1) Egg (2) larval stage-miracidia (3) Intermediate host -snail (5) Larval stage-cercaria (6) definitive host-human (10) Adult stage-worms (http://www.dpd.cdc.gov/dpdx)

2.4 Diagnosis of schistosomiasis by microscopy and Kato Kartz technique

Schistosomiasis infections are diagnosed by the detection of schistosome eggs in fecal samples by microscopic examination as the gold standard of *S. mansoni* produced by the adult worms (Gray, 2011). Since the shedding of eggs vary, for effective diagnosis, atleast three specimens of the first stool of the day, for three consecutive days are required in some patients (Arora and Arora, 2002). The diagnosis process involves a simple stool smear and staining technique using Lugol's iodine where the characteristic eggs are identified and counted under light microscopy. Direct smears are done to identify the positive samples. *Schistosoma mansoni* eggs are large (114-180µ long by 40-70µ wide), brown or yellow in colour with a thin smooth shell, with rounded posterior and the anterior ends pointed and curved (Figure 2.2). It also has a characteristic prominent lateral spine at the posterior end (Arora *et al.*, 2002).

Figure 2.2: *Schistosoma mansoni* egg that show rounded posterior end and the anterior end with characteristic prominent lateral spine at the posterior end (Source: CDC, 2013).

Kato Kartz technique is a rapid and inexpensive method recommended by WHO for diagnosis of *S. mansoni* particularly in high intensity of infection. It requires 40-50 mg of stool sample (Kartz *et al.,* 1972). It has a high specificity but the sensivity varies with prevalence and intensity of infection (WHO, 2014; Gryseels *et al.*, 2006). *Schistosoma mansoni* intensity of infection is categorized as light, moderate or heavy based on the number of eggs per gram (EPG) of the stool sample (WHO, 2002). From population studies, the mean egg burdens is linked to the severity of the disease (Gryseels *et al.*, 2006). In light infections, false negative results are common thereby leading to underestimation of the prevalence and intensity of infection (De-Vlas *et al.,* 1992).

2.5 Control and treatment of schistosomiasis

Control of schistosomiasis is normally designed at reducing infections and morbidity by interrupting the parasite life-cycle. This can be achieved through different methods directed on the hosts, parasites and the environment (CDC, 2013). Schistosomiasis control has been attempted in several ways: Snail intermediate hosts control, public health

education and improved sanitation, vaccination and chemotherapy (WHO, 2010, Daumerie and Savioli, 2010).

2.5.1 Snail intermediate hosts control

The intermediate hosts control is significant in reducing reinfection. Intermediate host control is mainly done using molluscicides to control schistosomiasis though a perfect molluscicide does not exist (Katsivo, 1993). Molluscicides are however toxic to humans and fish. Their application also depend on peak seasons of snail populations for maximum kill of the snails. Major desirable characteristics for molluscicides includes; selective toxicity to snails in low concentrations, the lack of toxicity to mammals, absence of adverse effects upon entering the food chain and their storage stability of at least 18 months (WHO, 2002; Souza, 1995). A molluscicide known as niclosamide is safe to handle and use, and is effective against snails and their eggs. Niclosamide has been widely effective in Egypt, China and Morocco but its usage is limited due to its high cost (Yang *et al.,* 2010). The berries of endod (*Phytolacca dodecandra*) have been determined to posses natural molluscicide activity as observed in Ethiopia contributing to significant reduction of snail population (Sharma *et al.,* 2009; Adema *at al.,* 2006).

Other forms of intermediate hosts control include biological control of snails by means of predators and competitor snails. Most competitor snails used are *Marisa comuarietis, Melanoides tuberculata* and *Thiara granifera* which compete for food with the intermediate hosts and prey on snail eggs. These snails have been effective in Brazil as a control means (Giovanelli *et al.,* 2002). Cray fish *Procambarus clakii* is cultured snail eating fish introduced in infected water has contributed to significant success (Mkoji *et al.*, 1999; Zhou *et al.*, 2010).

Other environmental management practices such as changing the rate of water flow through clearing vegetation in the irrigation canals, seepage control have been

successfully applied making the snail habitat unsuitable because snails prefer stagnant water (CDC, 2013).

2.5.2 Public health education

According to WHO, public health education has to a greater extent been effective in controlling schiostosomiasis. This include proper disposal of human waste, advocating for dangers of swimming, bathing and household chores (washing clothes or fetching water) in canals and slow moving streams and wearing of shoes and protective gears in the fields (WHO, 2014; Lancet, 2014). Countries with high prevalence of schistosomiasis such as Kenya, Tanzania, Egypt and Brazil have reduced infections in human hosts by significantly reducing infected water contact activities and deterrence of contamination of the environment with infected person's human waste. Nevertheless, this has been held back by high illiteracy levels, lack of social amenities such as toilets, and treated water for domestic use (CDC, 2013; WHO, 2014). Community based behavioural interventions should primarily target school going children, parents and population to embody ample behavior change by sensitizing the importance of periodic deworming to reduce worm burden (Utzinger *et al.,* 2009; Musuva *et al.,* 2014).

2.5.3 Use of vaccination

Currently, vaccines for schistosomiasis are not available; however, research is currently underway to develop a vaccine for schistosomiasis (WHO, 2014). Vaccine may reduce worm virulence thereby preventing infection and re-infection in humans and other reservoir (McManus and Loukas, 2008). In the past three decades, various vaccine candidates have been developed that are based on recombinant-derived schistosome proteins (Loukas *et al.,* 2007). Other candidate vacines are based on radiation-attenuated schistosome larval stages and DNA- derived proteins (Lin *et al.,* 2011; McManus *et al.,* 2008; Bickle, 2009). A number of the vaccines candidates have attained significant protection against various schistosome species of host reservoirs, only few such as

recombinant rSh28GST antigen and Sm 14/FABP antigen have been utilised in clinical trials (Webster *et al.,* 2010; Tendler and Simpson, 2008).

2.5.4 Chemotherapy

Chemotherapy has been the vital means in the global control approach against Schistosomiasis (Doenhoff *et al.,* 2008; Bartley, 2017). This is done through administration of PZQ and oxamniquine to eliminate the parasite from the humans who are definitive hosts (Cioli *et al.,* 2014). PZQ is an isoquinoline-pyrazine derivative with a broad anti-parasitic effect which was discovered in 1972 by E. Merck and Bayer AG, Germany (Andrews *et al,* 1983; Bartley, 2017).

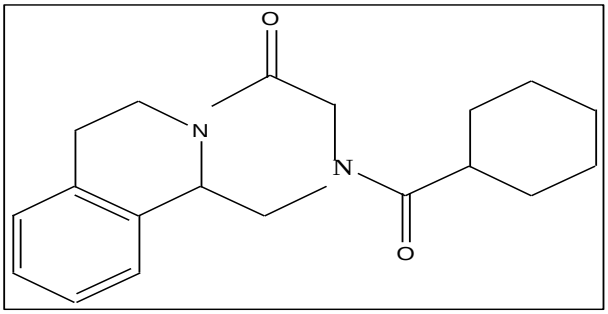

Figure 2.3: The structure of PZQ.

Clinical studies in man have shown that PZQ is effective against all human schistosome infections. Drugs used in treatment of schistosomiasis include artemisinins derivatives, oxamniquine and hycanthone which have been shown to have anti-helminthic activity (Keiser and Utzinger, 2012). These anti-schistosomal drugs, however, are not available in most parts of the world. Treatment and control of schistosomiasis thereby relies almost exclusively on PZQ. Use of PZQ has been relied in the past because of its effectiveness against all human schistosome species, while oxamniquine and hycanthone are not (Cioli *et al.,* 2014; Cioli *et al.,* 1995).

Orally administered PZQ is rapidly absorbed (80%) from the gastro-intestinal tract reaching maximum plasma concentration within 1-2 hours after administration (Chai, 2013). It is metabolized by the liver by the cytochrome P450 system and excreted through the urine and feces (Bylund *et al.*, 1977). Praziquantel mechanism of action involves initiating severe spasms and paralysis of the schistosome muscles thereby exposing the worm antigens and subsequently leading to an attack by the body immune system. The drug achieves 60-90% cure rates with egg reduction of 90-95% in those not cured (WHO, 2012). However, the drug is not effective against eggs and juvenile schistosomes. PZQ cannot be used for chemo-prophylaxis because it has a short half-life (1-1.5 hours) and cannot kill schistosomula that are between 3-21 days old (CDC, 2013).

Regular treatment especially MDA of high risk groups in endemic areas, particularly school going children with PZQ is highly recommended for reducing morbidity and mortality rates in endemic schistosomiasis infested regions (WHO, 2002). Repeated annual treatment with continuous evaluation to assess the extent of the control interventions in high transmission areas has significantly reduced the infection and re-infection rates (WHO, 2014). People of all ages can get re-infected after treatment, although older people re-acquire the infections at slower rates than in children (Kabatereine *et al.*, 2004).

2.6 Praziquantel Recommended Dosage

The acceptable dose of PZQ for treatment of *S. mansoni* ranges between 30 to 60 mg/kg with optimum dose ranging between 40 to 60 mg/kg (Zwang and Olliaro, 2014). Dosage of below 30mg/kg is termed as sub-curative while that of above 60 mg/kg is overdose as described in table 2. However, WHO advocates PZQ to be used as a standard single oral dose of 40 mg/kg for treatment of urinary and intestinal schistosomiasis (WHO, 2002).

Table 2.2: Acceptable and optimal dosages of PZQ as defined appropriate (Zwang and Olliaro, 2014; Doenhoff, 1998; WHO, 2002)

Treatment	Dosage
Acceptable dose	30-60 mg/kg
Excessive/overdose	>60 mg/kg
Sub curative/insufficient dose	<30mg/kg
Standard dose	40 mg/kg

2.7 Schistosomes Response to Praziquantel

Praziquantel use has increased over the years, not only in intensity but also in frequency. In 2006, approximately 12 million people had been treated with PZQ. By 2012, the number of treated individuals had reached up to 42 million (WHO, 2014). There is concern that with increase in PZQ use, drug resistance is likely to emerge with reduced susceptibility resulting in low cure rates (Cioli *et al.,* 2014; Botros *et al.,* 2005). PZQ is highly effective against sexually mature forms of *S. mansoni* but is not effective against the juvenile schistosomes of less than 3 weeks old (Aragon *et al.,* 2009; Pica-Mattoccia and Cioli, 2004). Administration of PZQ is mostly through MDA to children after months or years between treatments. This result to a significant reservoir of schistosomes in the untreated community at risk thereby making re-infections a certainty (Danso-Appiah *et al.,* 2002; Sudtida *et al.,* 2006). After continuing exposure to the parasite, PZQ can often provide short-term relief from infection. Despite this shortcoming, PZQ is the only readily existing treatment for Schistosomiasis.

Alarming low cure rates in response to the standard dose of 40 mg/kg of PZQ in the field began to appear 10-15 years after the commencement of its use in MDA in Egypt (Ismail *et al.,* 1999). Some isolates from uncured patients when tested in mice were harder to kill than worms from laboratory strains known to be susceptible to the drug. These findings suggest that some cases of PZQ failure could be linked to the developing drug resistance

in the schistosome worms with heavy worm burdens, host factors and pre-patent infections being potential contributing factors (Ismail *et al.,* 1999; Sudtida *et al.,* 2006). Reports of low cure rates with PZQ were also found in new highly endemic areas for *S. mansoni* in northern Senegal (Stelma *et al.,* 1997; Gryseels *et al.,* 2006), where high intensity of transmission among a non-immune population played a role. Schistosome Control Initiative which supported treatment programs in Uganda, Burkina Faso, Niger, Tanzania and Zambia has indicated that the number of persons treated with PZQ in these countries increased from ~500,000 in 2003 to >10 million in 2008 (Fenwick *et al.*,2009).

Additional evidence for isolates presenting resistance to PZQ has been established in Kenya. Miracidia hatched from eggs excreted by individuals with *S. mansoni* infections in Lake Victoria were assay in an *in vtro* study showed decreased susceptibility to PZQ (Mwangi *et al.,* 2014). Miracidia hatched from patients eggs had variable PZQ sensitivity with lowered sensitivity to the drug in patients who had previously been treated with PZQ. Adult worms derived from these patients' eggs were less sensitive to PZQ (Melman *et al.,* 2009; Mwangi *et al.,* 2014). There has been reported incidents of failure of PZQ to cure *S. haematobium* infections (Herwaldt *et al.,* 1995), however, there is currently no evidence for heritable resistance (Alonso *et al.* 2006; Boray *et al.,* 1983). Evidence of PZQ drug failure has been documented in other trematodes that include liver fluke *Fasciola hepatica* (Boray *et al.,* 1983).

2.8 Schistosomiasis Enhanced Control Strategies

Since 2003, several MDA campaigns have been effected in sub-Saharan Africa resulting in millions of school-aged children (SAC) treated for Schistosomiasis with PZQ. These interventions primarily focus in provision of free treatment with PZQ to SAC that range between 5–16 years of age (WHO, 2014; WHO, 2006; Fenwick *et al.,* 2009). These strategies have resulted in huge impact in reduction of morbidity suffered by millions of SAC.

The WHO has initiated a plan for eradication of 17 neglected tropical diseases (NTD) including schistosomiasis (WHO, 2012). This plan was to eliminate schistosomiasis as a public health concern in African countries by the year 2020 and globally by the year 2025. This inspired an alliance of 22 global partners comprising of the World Bank, WHO, The Bill and Melinda Gates Foundation and pharmaceutical companies to initiate a sustained program for elimination of schistosomiasis by the year 2020 (http://unitingtocombatntds.org). these patners have achieved to provide approximately 42 million PZQ tablets which were dispensed in 2012 (WHO, 2014). The number of PZQ tablets is likely to increase prominently to meet the immediate aim of disease eradication. Pharmaceutical companies have greatly assisted in the fight for eradication especially Merck KGaA will freely produce 250 million PZQ tablets per year. Other manufacturers expected to contribute tablets to increase the supply.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study design

This study was a portion of a larger longitudinal on-going study, following the treatment-reinfection study design commonly applied in human helminth field studies. The study involved a cross-sectional study at baseline and a follow-up after 21 days. Recruited participants were screened at baseline for *S. mansoni* infections and morbidity. The experimental set up was to determine if higher doses of PZQ 60 mg/kg would improve treatment efficacy of *S. mansoni* with an adequate safety profile, in comparison with the standard dosage of PZQ 40 mg/kg in school going children.

3.2 Study Site

The study was primarily conducted in Mianya Primary school (0°38'08.9"S 37°19'25.1"E) in Kirinyaga County in the Central region of Kenya where *S. mansoni*, the causative agent of Schistosomiasis is endemic. Inhabitants of the study area are mainly subsistence farmers and fishermen. These communities depend on irrigation scheme water for domestic and social economic needs. The study area was selected based on preliminary evidence of water contact activities such as rice farming, water collection for domestic use, bathing and swimming. The population in Mwea is estimated at 176,261 (Kenya Population Census, 2009) and *S. mansoni* prevalence is at approximately 53% (Masaku *et al.*, 2015).

3.3 Study Population

The study was conducted on school going children from Mianya Primary school in Kirinyaga County, Kenya - within the Mwea rice irrigation scheme - aged between 4 and 17 years. This group was chosen because school age children are heavily susceptible to

infection by *S. mansoni* because of the school locality and water contact activities. They would therefore be the most appropriate to receive an increased dosage of PZQ and consequently determine the safety profile of the up-scaled dosage in this age group.

3.4 Sample Size Determination

The sample size calculation was determined according to Sakpal, (2010).

$$n = \frac{(Z\alpha/2 + Z\beta)2 \times \{(P1\,(1-P1) + (P2\,(1-P2))\}}{(P1 - P2)2}$$

Where;

n = sample size,

P_2 = proportion of subject cured by concentration B (60 mg/kg) = 0.8,

P_1-P_2 = clinically significant difference = 0.2

$Z_{\alpha/2}$: This depends on level of significance, for 5% this is 1.96

Z_{β}: This depends on power, for 80% this is 0.84.

The sample size required was calculated to approximately 80 patients per treatment group supposing the cure rate of the standard dose (40 mg/kg) is 60% and cure rates of PZQ at 60 mg/kg is 80% at 95% confidence, totalling to 160 study subjects. However, considering a drop out of about 20% due to loss to follow-up or medical reasons, this resulted in 192 study participants.

3.5 Inclusion criteria

The following individuals were selected for the study:-

- *Schistosoma mansoni* infected individuals of either sex
- Aged between 4 -17 years who provided stool samples
- Filled the written informed consent
- Able and willing to be examined on follow-up visits

3.6 Exclusion criteria

The following individuals were not selected for the study:-

- Children with previous history of adverse reaction to PZQ, Children Children on any other medication that may affect the results of the trial (e.g. antibiotics) within the past week,
- Children with history of treatment in the past 30 days with PZQ.
- Children under 4 years of age. PZQ is safe for children from four years of age (Parker *et al.,* 2008, WHO, 2002).

3.7 Baseline Laboratory and Clinical Procedures

3.7.1 Stool

At baseline, each participant recruited in the study provided a stool sample where duplicate Kato Katz slides (2 slides) were prepared in the field (using the 41.7 mg faecal template) from each stool sample (Katz *et al.,* 1972). The prepared Kato slides in the field were subjected to quantitative microscopic examination for *S. mansoni* ova identification.

3.7.2 Blood collection

The left hand of each participant was carefully tied at the fore with a tourniquet and cleaned thoroughly with methylated spirit. Venous blood was gently aspirated by 5 ml syringe and needle. 5 ml of blood was drawn from the vein of each participant and 2 ml was put in a special Ethylene Diamine Tetra-acetic Acid (EDTA) vial and rotated to mix.

The EDTA blood was put in 8 ^{0}C cooler box for transportation and analysis in the laboratory. The remaining 3 ml of blood was put in a plain bottle and allowed to clot for 1 hour. Serum was collected from the clot using a Pasteur pipette into a smaller vial. The sera were then stored at -80^{0}C for later biochemical analysis.

3.7.3 Weight

Weight was measured at baseline using a digital electronic balance.

3.8 Treatment with PZQ and Sample Collection

Two random groups were recruited and categorized into current treatment group (Group A) and up-scaled treatment group (Group B). Each individual of either group was diagnosed for *S. mansoni* by Kato-Katz technique at baseline. Those who fulfilled the inclusion criteria for enrolment in the trial were randomly allocated to groups to receive treatment with PZQ. The first group were treated with 40 mg/kg PZQ while second group received 60 mg/kg PZQ as shown on figure 4. Appendix I, table 9 contains the dose chart of 40 versus 60 mg/kg. Efficacy of the 40 versus 60 mg/kg PZQ was determined during follow up. The intensity of infection at Day 0 and Day 21 of patients given PZQ 60 or 40 mg/kg was also compared. The intensity of infection for positive individuals was categorized as light, moderate and heavy infection according to WHO (2002a) whereby; light (1–100 eggs per gram of faeces); moderate (101–400 epg) and heavy (>400 epg). Participants with positive *Schistosoma spp.* diagnosis who do not fulfill the inclusion and exclusion criteria were treated with the standard treatment of a single dose of PZQ 40 mg/kg. Patients diagnosed with other soil transmitted helminthes were treated with albendazole after 21 days post-treatment.

Individual stool samples were obtained and prepared for Kato Katz quantitative microscopic examination for *S. mansoni* ova. Blood samples were obtained by

venipuncture in heparinized syringes after 21 days post-treatment for blood hemoglobin, eosinophil counts and biochemical analysis.

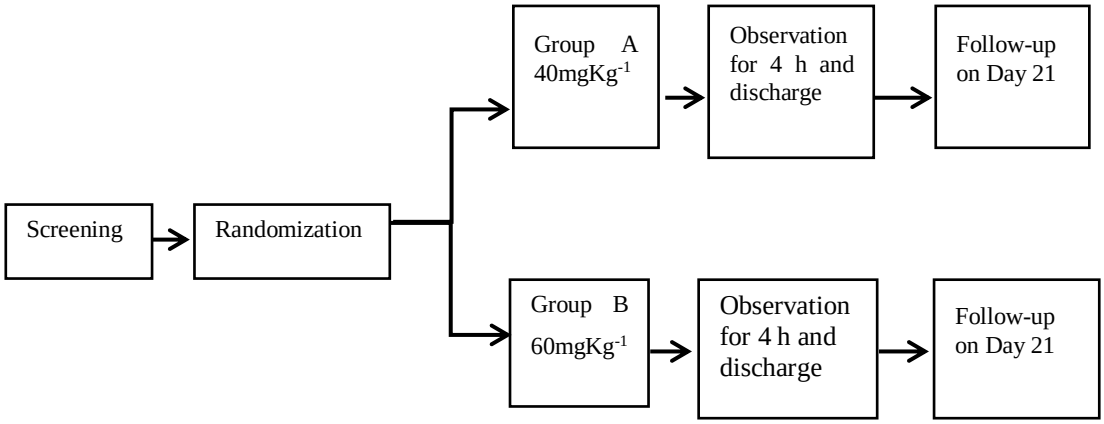

Figure 3.1: Flow chart illustrating the study design. It involves screening of the participants, randomization, grouping and follow up 21 days post-treatment. Treatment was on day 1 with a follow-up for adverse events after 4 and 24 hours. There was a follow-up for efficacy and morbidity determination on Day 21.

3.9 Determination of Efficacy of 60 from 40 mg/kg PZQ

Two stool samples collected from each participant and thick smear was prepared by using 42-mg plastic templates. The prepared Kato slides in the field were subjected to quantitative microscopic examination for *S. mansoni* ova identification. Cure rate at 21 days after treatment of the participants with 40 and 60 mg/kg PZQ respectively was estimated as the proportion of subjects with negative result at 21 days after treatment. Egg reduction rate at 21 days after treatment for the 40 and 60 mg/kg groups was estimated through the following formula:

$$[1 - (epg2 / epg1) \times 100]$$

Where: epg1 and epg2 are the geometric mean of log10 transformed (x+1) numbers of epg of faeces at the baseline and the Day 21 post-treatment respectively.

3.10 Determination of Morbidity

3.10.1 Anaemia Determination

Venous blood of about 10 µl was placed in using a portable haemoglobinometer for haemoglobin (Hb) concentration estimation in g/dl according to manufacturer's instructions (HemoCue Hb 301, HemoCue®, Sweden). A new standard cuvette supplied together with the haemoglobinometer was used for quality control. Participants with Hb of less than 11 g/dL were considered anaemic (WHO, 2014).

3.10.2 Blood cell count

A thin smear blood film was prepared from the venous blood sample from each participant and stained with Giemsa as described by Cheesbrough (2011). Differential cell count was done on 100 white blood cells (WBCs) in each film and the numbers of each type of white blood cells expressed as a percentage of the total 100 white blood cells. Eosinophilia was defined as eosinophils above 7% of WBCs on the blood film (Njaanake *et al.,* 2015).

3.10.3 Determination of serum liver function tests

Serum liver function tests (LFTs) were done by using Cobas Integra 400 plus biochemistry analyser machine (Roche, Berlin, Germany). The upper limit normal values of the machine was 37 U/L for aspartate aminotransferase 42 U/L for alanine aminotransferase and 17.0 µmol/L for bilirubin.

3.11 Determination of Safety (Adverse Events)

Occurrence and severity of adverse events (AEs) at 4 hrs and Day 1 after treatment was collected. An AE was defined as any unfavourable and unintended sign, symptom, or disease temporally associated with the use of a medicinal or investigational product (WHO, 2002). Treatment with PZQ was done in the morning of Day 0 and observations

were done until 18.00 hr of the same day. Participants administered with PZQ were observed for at least 4 hrs before leaving. All participants enrolled in the study were assessed after treatment for the following signs and symptoms: abdominal pain, headache, vomiting, nausea, fever, diarrhoea, anorexia, dizziness and allergic reaction. Each symptom was graded as described in appendix II. Each participant's data of adverse events included date and time of onset, duration and severity to PZQ treatment. This was repeated on the following day (Day 1), that is, 24 hrs post-treatment to assess to see if they experience adverse events.

3.12 Ethical Concerns and Biosafety Issues

The study was approved by the KEMRI Scientific Review Unit (SERU) approval number KEMRI/SER/CBRD/0162/3398 (Appendix XII). The study was conducted according to applicable regulatory requirements as per the Helsinki declaration and the KEMRI Scientific and Ethics Review Unit (SERU) guidelines. In addition to this, the study was carried out in compliance with the protocol, and Good Clinical Practice. At the beginning of the study, meetings were organized with parents, teachers and earmarked communities whereby detailed information was provided by the research team about the aims, procedures, benefits and potential risks of the study. The study was explained to participants before they were randomly selected and invited to participate. They received an information sheet and a consent form, which they were asked to return the following day with a signature of their parent/guardian and individual assent if less than 17 years of age (Appendix IV and V). Informed consent agreement was also sought from the head teacher (Appendix VI). Participation in the study was on voluntary terms and that an individual was free to withdraw from the study at any time. The selected children were registered and their age, sex and participation at the last MDA recorded. The informed consent document outlining all the benefits and risks associated with the study are attached in appendix III and the translated informed consent local language attached in appendices VIII, IX, X and XI.

3.13 Statistical Analysis

Baseline characteristics were compared by Student's t-test and Chi-square. In the efficacy evaluation, cure rates of the participants were compared using Chi-square test. The egg reduction rate was assessed by determining geometric mean egg counts (GMECs) at baseline and day 21. Analysis of variance (ANOVA) was utilized to determine difference in the mean log egg counts between the two groups at baseline and day 21 post-treatment. For safety assessment, Chi-square test was used to determine if the prevalence of adverse events was significantly different between the treatment groups.

CHAPTER FOUR

RESULTS

4.1 Prevalence and intensity of infection of *S. mansoni*

The study had a total of 192 pre-primary and primary school going children of ages 4 -17 years. Of the infected population, 48.95% were boys while 51.05% were girls. The mean age of participants with *S. mansoni* was 10.5±3.1 years. The point prevalence of schistosomiasis of the school children after screening was 52.8% and the mean egg per gram (epg) was 270.65 and 233.56 for 40 mg/kg and 60 mg/kg respectively (Table 4.1). The overall infection intensity was light infection (40%); moderate infection (41.58%) and heavy infection (18.42%). The prevalence of infection after treatment was negative infection at 94% and light infection at 6% (figure 4.1).

Table 4.1: Baseline assessment of sex distribution and arithmetic and geometric means of 40 and 60 mg/kg PZQ groups.

Parameters		**40 mg/kg**	**60 mg/kg**	**P value***
Sex	Male	48	45	0.16
	Female	47	50	
EPG mean±SEM		270.65±62.58	233.56±34.89	0.33
Geometric EPG (CI 95%)		2.01 (1.08 – 3.71)	1.98 (1.08 – 3.23)	0.33

n: number of children; EPG: egg per gram of stool; SEM: standard error of the mean; CI: confidence interval; *Level of significance was set at 0.05.

Table 3.2: Cure rate (CR), Egg Reduction rate (ERR), arithmetic and geometric means of *S. mansoni* at 21 days post-treatment

Parameters	40 mg/kg	60 mg/kg	P value*
EPG mean±SEM	1.89±0.81	1.58±0.78	0.7
Cure rate	92%	94%	
Egg reduction rate	96%	96%	
Geometric EPG (CI 95%)	0.08 (0.00 – 1.78)	0.08 (0.00 – 1.78)	0.7

n: number of children; EPG: egg per gram of stool; SEM: standard error of the mean; CI: confidence interval; *Level of significance was set at 0.05.

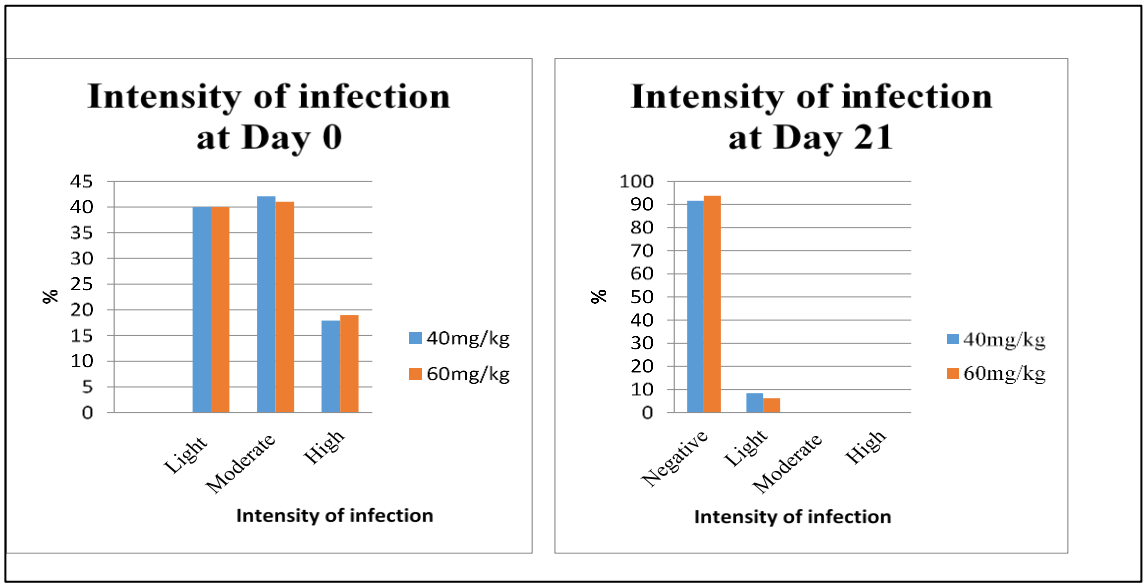

Figure 4.1: Intensity of infection at baseline and 21 days post treatment. Light infection (1-100 epg); moderate infection (101-400 epg) and heavy infection (>400 epg).

4.2 Efficacy of 40 mg/kg and 60 mg/kg PZQ

There was a significant reduction of *S. mansoni* infections in both treatment groups. Parasitological cure rates at Day 21 post-treatment were 92% for 40 mg/kg and 94% for 60 mg/kg as shown in Table 4.2 and Figure 4.1. There was no significant difference in cure rates after treatment in sex and age group in both treatment groups ($p>0.05$) (Table 4.2). The cure rate of PZQ in relation to infection intensity differed significantly (Figure 4.1). A higher cure rate in light and moderate infected participants was observed when compared to heavy infected participants. There was no significant difference in egg reduction rate at day 21 post-treatment for both 40 mg/kg and 60 mg/kg groups (Figure 4.2). The percentage reduction rate was 96% for both 40 mg/kg and 60 mg/kg.

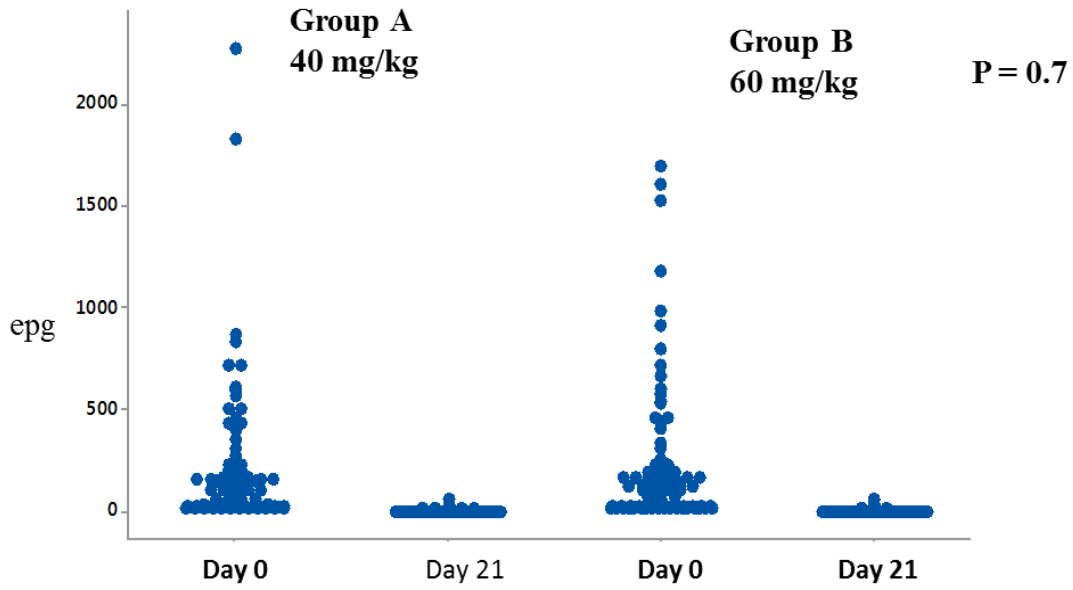

Figure 4.2: Individual Value Plot of egg per gram (epg) of *S. mansoni* eggs in Group A (40 mg/kg) and Group B (60 mg/kg) at baseline and post-treatment of PZQ. Mean epg of group A was 270.65±62.58 and group B was 233.56±34.89 which reduced to 1.89±0.81 and 1.58±0.78, respectively.

4.3 Morbidity of 40 mg/kg and 60 mg/kg PZQ

4.3.1 Anaemia

The baseline mean Hb levels were 10.96±0.15and 11.19±0.10 g/dl respectively. Hemoglobin levels at 21 days post-treatment were 11.26 and 11.34 g/dl respectively but the difference was not significant. Prevalence of anaemia among the two dose groups showed no significant difference at baseline and after treatment (Table 4.3).

4.3.2 Blood cell count

Eosinophil levels showed a significant decrease after 21 days post-treatment in both treatment groups although no difference was observed between the 40 and 60 mg/kg

treatment groups. Eosinophilia (eosinophils ≥7%) was still high at 88% after treatment in both treatment groups as shown in Table 4.3.

4.3.3 Serum liver function tests

After 21 days post treatment, the serum enzyme assays showed that a higher percentage of the participants had mild increased levels of AST whereas ALT and bilirubin levels were within the normal ranges (Table 4.3).

Table 4.3: Baseline and post-treatment assessment of morbidity parameters of 40 and 60 mg/kg PZQ groups

Parameters	40 mg/kg	60 mg/kg	P value*
Baseline			
Hemoglobin mean±SEM	10.96±0.15	11.19±0.10	0.052
Anaemia	44%	42%	
Eosinophil count	12.67±0.45	13.11±0.36	0.39
Eosinophilia (<7%)	96%	100%	
Elevated AST (> 37 units/L)	70.2%	73.4%	
Elevated ALT (> 42 units/L)	2.4%	2.8%	
Elevated bilirubin (> 17 µmol/L)	2.0%	2.5%	
21 Days post-treatment			
Hemoglobin mean±SEM	11.26±0.13	11.34±0.09	0.062
Anaemia	37.9%	37.9%	
Eosinophil count mean±SEM	12.67±0.45	10.05±0.27	0.25
Eosinophilia (<7%)	88%	88%	
Elevated AST (> 37 units/L)	68.5%	72.5%	
Elevated ALT (> 42 units/L)	1.8%	2.1%	
Elevated bilirubin (> 17 µmol/L)	1.4%	1.4%	

n: number of children; EPG: egg per gram of stool; SEM: standard error of the mean; CI: confidence interval; *Level of significance was set at 0.05.

4.4 Safety of 40 mg/kg and 60 mg/kg PZQ

Most frequent adverse events observed in the treatment groups were abdominal pain, headache and nausea whereas the least occurring were anorexia, fever and allergic reaction (figure 4.3 and 4.4). No significant difference in the adverse events was observed between the treatment groups, however the cumulative adverse events (participants with at least one adverse event) was higher in 60 mg/kg than 40 mg/kg (Table 4.4).

Table 4.4: Adverse Events of 40 and 60 mg/Kg post-treatment with PZQ

Parameter	**Severity of Adverse Events**			**P value***
	All subjects (190 subjects)	**40 mg/Kg (95 subjects)**	**60 mg/Kg (95 subjects)**	
Nausea	28.25%	25.5%	31.0%	
Fever	1.5%	1.0%	2.0%	
Headache	31.5%	28.0%	34.5%	
Allergic reaction	1.91%	1.94%	1.88%	
Abdominal Pain	35.5%	35.0%	36.0%	
Vomiting	7.8%	5.4%	10.2%	
Diarrhoea	18.1%	18.8%	17.4%	
Anorexia	0.36%	0.34%	0.38%	
Dizziness	8.0%	8.4%	7.6%	
Cumulative adverse events		53%	59%	0.17

* Level of significance was set at 0.05.

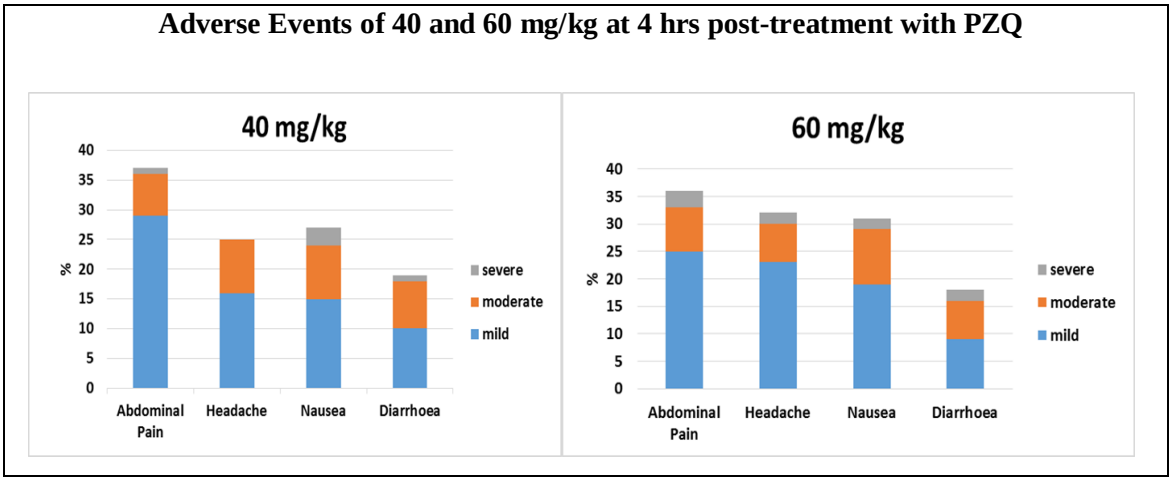

Figure 4.3: Severity of Adverse Events of 40 mg/kg and 60 mg/kg at 4 hrs post-treatment with PZQ. The most frequent AEs were abdominal pain (37%), headache (25%) and nausea (27%) for 40 mg/kg and abdominal pain (36%), headache (32%) and nausea (31%) for 60 mg/kg.

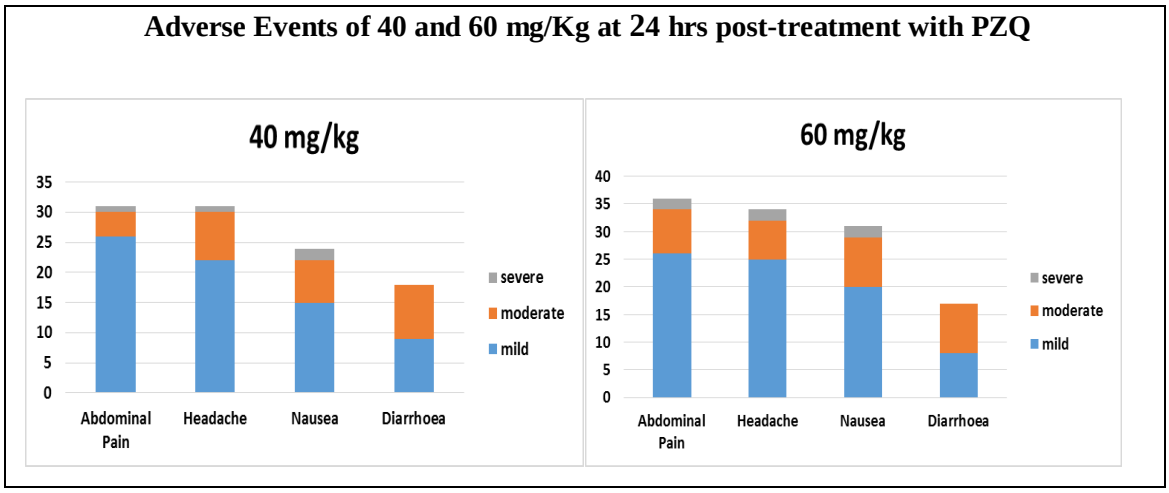

Figure 4.4: Severity of Adverse Events of 40 mg/Kg and 60 mg/Kg at 24 hrs post-treatment with PZQ. The most frequent AEs were abdominal pain (31%), headache (31%) and nausea (24%) for 40 mg/kg and abdominal pain (36%), headache (34%) and nausea (31%) for 60 mg/kg.

CHAPTER FIVE

DISCUSSION

5.1 Prevalence and Infection intensity of *S. mansoni* in Mwea irrigation scheme

The prevalence of schistosomiasis in Kenya especially in endemic region of Mwea irrigation scheme has resulted to recurrent infections. In the present study, the established prevalence of 53.2% of *S. mansoni* infection in Kirinyaga County is considered high according to WHO classification (WHO, 2014). Trends in the 1970s and 1980s recorded in the Mwea region showed that the prevalence of *S. mansoni* was 60% and 80% respectively (Oomen *et al.*, 1979; Waiyaki, 1987). In recent studies in Kirinyaga County, high prevalence of up to 75% was recorded (Masaku *et al.,* 2015; Lelo *et al.,* 2014; Kihara *et al.,* 2012). It has been observed that despite the ongoing mass drug administration (MDA) interventions in the region for several years, there has been no significant reduction of prevalence. This can also be attributed to the decline in susceptibility of the parasite to the drug, exclusion of adult population treatment in ongoing MDA which may result to high re-infection rate. In addition, the frequent use of PZQ in MDA programs may result in drug pressure thereby leading to resistance (Mwangi *et al.,* 2014; Bennett *et al.,* 2017). Kirinyaga County in the Mwea irrigation scheme is characterized by low socio-economic status and poor hygiene conditions that include lack of clean water supply, latrines and sewage disposal facilities. The target group was school going children of Mianya Primary School who come from families with river water contact activities such as rice farming, river water collection for domestic use, bathing and swimming. The majority of the participants in the study (80 %) had light to moderate intensity of infection. This can be attributed to the ongoing annual MDA exercises in the region (Masaku *et al.,* 2015). In spite of the national deworming programme in Kenya, the prevalence of *S. mansoni* in Kirinyaga County are of public health concern.

Children between 4-17 years of age are important for comparative analysis of infection prevalence and are in the age for formal schooling in Kenya and many developing countries where schistosomiasis is endemic. Praziquantel is safe for children from four years of age (WHO, 2012). In the study there was no difference in prevalence between age groups and sex of the study participants even though studies in the region have shown varying water contact activities. Common water contact activities including swimming, playing, fishing and bathing in cercariae infested water bodies are male dominated, whereas, females are also are exposed as they are predominantly responsible for household chores like fetching water and washing clothes and utensils at these infested water sources (Masaku *et al.,* 2015; Lai *et al.,* 2015). A study carried out by Anto *et al.,* (2013) also determined that there is a direct link between water contact activities and transmission of schistosomiasis resulting to higher prevalence.

5.2 Efficacy of increased dosage of PZQ 60 mg/kg compared to 40 mg/kg of PZQ

The study showed that there was no significant difference in cure and egg reduction rates between the two treatment groups 21 days post treatment. These findings in Kirinyaga County in Kenya are comparable to similar findings from a study based on multicentre randomized controlled trials in the Philippines, Mauritania, Tanzania and Brazil which also concluded that there was no significant difference between 60 and 40 mg/kg PZQ (Olliaro *et al.,* 2011). The study present is also in agreement with a similar study in Mauritania (Ouldabdallahi *et al.,* 2013) and a community-wide study in Brazil (Galvão *et al.,* 2010) which found no difference between the two treatment groups. Although WHO expert committee (WHO, 1993) had predicted that an up-scaled dosage of 60 mg/kg would provide a higher cure rate than the standard 40mg/kg, this study concludes that this is not the case.

The cure rate of PZQ at 40 mg/kg (92%) in this study was comparable with studies in Philippines (92%), Mauritania (91%) and Ethiopia (93%) (Olliaro *et al.*, 2011; Bajiro *et al.*, 2016). It was however higher when compared to Tanzania (87%), Ethiopia (89%) and

Egypt (63%) (Dejenie *et al.,* 2009; Botros *et al.,* 2005; Olliaro *et al.,* 2011). Other studies in Brazil have shown a higher cure rate of 100% and 98% (Olliaro *et al.,* 2011; Galvão *et al.,* 2010). The differences in efficacy may be attributed to infection intensity, and the presence of immature parasite (Bajiro *et al.,* 2016).

5.3 Morbidity of increased dosage of 60 mg/kg compared to 40 mg/kg of PZQ

Low Hb levels were recorded at baseline and were relative to the intensity of infection. The baseline Hb levels for both 40 and 60 mg/kg treatment groups of 11.66 and 11.19 g/dl respectively and post-treatment levels of 11.26 and 11.34 g/dl respectively showed there was no observed difference. There was a reduction in the number of participants with anaemia in both treatment groups. *Schistosoma mansoni* infection has been linked to significant loss of blood and iron leading to anaemia (Guyatt *et al.,* 2001; WHO, 2013). Treatment with both dosages of PZQ which led to a significant reduction in infection may be attributed to the reduced anaemic status. The high eosinophil levels observed may be due to immunological cell response to *S. mansoni* infection where they play important role in host defence against helminth infections. The particular roles of eosinophils in schistosomiasis vis-à-vis immunopathology remains unclear but it is believed that eosinophils participate in antibody-dependent protective immune, and individuals with acute schistosomiasis show increased levels of circulating eosinophils (Silveira-Lemos *et al.,* 2008).

Bilirubin is an endogenous anion derived from hemoglobin degradation from the red blood cells. In underlying liver diseases such as schistosomiasis, and acute viral hepatitis, elevated levels of bilirubin can be as a result of hepatic failure and jaundice which is highly associated with increased mortality (Bendezu *et al.,* 2013; Agrawal *et al.,* 2016). The aminotransferases are the most commonly utilized and specific indicators of hepato-cellular necrosis. These enzymes include aspartate aminotransferase (AST) and alanine amino transferase (ALT); they are involved in the catalysis of the transfer of the α-amino acids of aspartate and alanine respectively to the α-keto group of ketoglutaric acid. Alanine

amino transferase is predominantly localized in the liver but the AST is present in extensive variety of tissues such as the liver, heart, kidney, skeletal muscles and brain (Bendezu *et al.,* 2013; Agrawal *et al.,* 2016). Normal human bilirubin levels range between 0 - 20 U/L while that of ALT and AST are 5.3 – 47 U/L and 10.7 – 43 U/L respectively (Rai, 2016). The elevation of AST was observed in both 40 mg/kg and 60 mg/kg groups with no significant difference between the groups. The levels of ALT and bilirubin were within the normal ranges. This may be attributed to the fact that the majority of the infected participants had light to moderate infections which may have not resulted in severe liver damage. Infection of schistosomiasis is associated with damage to the hepatic cells leading to a significant increase in serum levels of ALT, AST and bilirubin which upon PZQ treatment has been shown to normalize these levels and consequently improving liver function (Lee *et al.,* 2012; Luo *et al.,* 2017). The elevation of AST may be linked to the extent of hepatocellular necrosis that occurs as a result of *S. mansoni* infections (Lee *et al.,* 2012). Other studies have determined that levels of ALT highly elevated as compared to AST in chronic liver diseases (Woreta and Alqahtani, 2014; Katkov *et al.,* 1993).

5.4 Safety and adverse events of 60 mg/kg compared to 40 mg/kg of PZQ

The occurrence of adverse events (AEs) in the participants may be explained by drug effects in the body, however, it can also be attributed to parasitic infections (Li *et al.,* 2002). Most of the participants (40%) in both treatment groups experienced lower abdominal pain as the major adverse event. This is due to the granulomatous reaction from the trapped eggs interacting with host immunity and associated toxicity from dying schistosome worms (Shuja *et al.,* 2018). Other events were transient and mild but none reported severe reactions that required special treatment. Most AEs cleared after 24 hrs after drug administration. This is in agreement with other studies conducted among school children in other endemic regions in Kenya which concluded that abdominal pain to be the most frequently reported AEs after treatment with PZQ (Jaoko *et al.,* 1996, Njomo *et al.,* 2010). Other frequently observed AEs include headache and nausea which have

previously been reported by Li *et al.*, (2002). In comparing the cumulative adverse events between the groups, a higher proportion (74%) of participants in the 60 mg/kg group experienced at least one or more adverse events compared to the standard dose (68%). Administration of higher doses of PZQ has been attributed to higher reports of adverse events (WHO, 2014).

5.6 Conclusion

Based on the findings of this study, it can be concluded that the up-scaled dosage of 60 mg/kg praziquantel (PZQ) in mass chemotherapy campaigns of *S. mansoni* treatment offers no significant advantage over the World Health Organization (WHO) recommended 40 mg/kg. In addition, no significant difference in cure rates and egg reduction rates between the two treatment groups was observed. The 60 mg/kg dosage was associated with higher cumulative adverse events than 40 mg/kg dosage but the difference was not significant. Morbidity markers in both dosages showed a significant reduction in eosinophil counts but eosinophilia was still high post-treatment. No significant difference was observed in haemoglobin levels post-treatment in both dosages.

5.7 Recommendation

Based on the conclusions that have been made in this study, it is recommended that the use of 40 mg/kg dosage should continue given the treatment with 60 mg/kg did not translate into a significant advantage in efficacy in this study. Kirinyaga County has been under Kenya National School-Based Deworming Programme since 2009; however, the prevalence of schistosomiasis has not reduced. There is therefore need for drastic public health and enviromental intervention measures such as vector/snail control, basic sanitation, clean water supply and health education. Given that the MDA programmes only target school going children which leaves most of the adult population untreated, this may result to high reinfection rates, and developing PZQ resistance due to drug pressure

It is therefore recommended that entire community should undergo MDA treatment with PZQ.

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APPENDICES

Appendix I: Number of PZQ tablet to be given to subjectsunder the 40 mg/kg and 60 mg/kg regimens

40 mg/kg regimen		60 mg/kg regimen	
(kg)	**Number of tablets**	**(kg)**	**Number of tablets**
13-15	1	10	1
16-18	1 1/4	10-12.5	1 1/4
19-22	1 1/2	12.6-15	1 1/4
23-25	1 3/4	15.1-17.5	1 1/2
26-29	2	17.6-20	1 3/4
30-33	2 1/4	20.1-22.5	2
34-37	2 1/2	22.6-25	2 1/4
38-40	2 3/4	25.1-27.5	2 3/4
41-44	3	27.6-30	3
45-48	3 1/4	30.1-32.5	3 1/4
49-52	3 1/2	32.6-35	3 1/2
53-55	3 3/4	35.1-37.5	3 3/4
56-59	4	37.6-40	4
60-63	4 1/4	40.1-42.5	4 1/4
64-66	4 1/2	42.6-45	4 1/2
67-70	4 3/4	45.6-47.5	4 3/4
71-75	5	47.6-50	5
		50.1-52.5	5 1/4
		52.6-55	5 1/2
		55.1-57.5	5 3/4
		57.6-60	6

In this table, 13-15 means 13.1 kg to 15.9 kg. The same applies to other weight ranges

Appendix II: Grading scale for determination of the severity of adverse effects

Clinical sign/symptom	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**
Nausea (1)	Mild discomfort; maintains reasonable intake	Moderate discomfort; intake decreased significantly; same activity limited	Severe discomfort; no significant intake; activities limited	Minimal fluid intake
Fever (1)	37.7-38.5 °C	38.6-39.5 °C	39.6-40.5 °C	>40.5 °C
Headache (1)	Mild, no therapy required	Transient. Moderate; therapy required	Severe; responds to initial narcotic therapy	Intractable; required repeated narcotic therapy
Allergic reaction (1)	Pruritus without rash	Localized urticarial	Generalized urticarial; angioedema	Anaphyliaxis
Abdominal Pain (2)	Mild	Moderate- no treatment needed	Moderate-treatment needed	Severe-hospitalization for treatment
Vomiting (2)	1 episode/day	2-3 episodes/day	4-6 episodes/day	>6 episodes /day or intractable vomiting
Diarrhoea (2)	Slight change in consistency and/or	Liquid stools	Liquid stools >4X the amount or number normal	Liquid stools greater than 8X the amount of the number

	frequency of stool			normal for this child
Anoxia (2)	----------	Decreased appetite	Appetite very decrease, no solid food taken	No solid or liquid taken
Dizziness (3)	Not interfering with function	Interfering with function, but not interfering with activities of daily living	Interfering with activities of daily living	Bedridden of disabling

Adopted from (1) WHO Toxicity Grading Scale, (2) Division of Microbiology and Infectious Disease (DMID/NIH) Toxicity Tables, and (3) National Cancer Institute (NCI) Toxicity Grading Scale (WHO, 2002; Olliaro *et al.*, 2011).

Severity grading for other Adverse Events.

Grade 1	**Mild.** Transient or mild discomfort (<48hs); no medical intervention/therapy required
Grade 2	**Moderate.** Mild to moderate limitation in activity – some assistance may be needed; no or mini al medication intervention/therapy required
Grade 3	**Severe.** Marked limitation in activity, some assistance usually required; medical intervention/therapy required, hospitalization possible
Grade 4	**Life threatening.** Extreme limitation I activity, significant assistance required; significant medical intervention/therapy required, hospitalization or hospice care probable

(WHO, 2002; Olliaro *et al.*, 2011)

Appendix III: Informed Consent Explanation

PROJECT TITLE: **Efficacy and Safety of Up-scaled Dosage of 60 mg/kg Praziquantel in Control of *Schistosoma mansoni* in School Going Children in Kirinyaga County**

PRINCIPAL INVESTIGATOR: ***Elvis Kirubi Muthoni****, Centre for Biotechnology Research and Development*

INTRODUCTION: The study being carried out is on bilharzia, a disease caused by worms that live in human beings, and which are transmitted through freshwater snails. The study aims to understand better how infected people respond to praziquantel, the medication used to treat people infected with the bilharzia worms. A study like this will help us find out if adding the dose for praziquantel will be beneficial for treating the bilharzia disease. Bilharzia causes sickness to millions of people all over the world including Kenya. Bilharzia parasites will be collected from from bilharzia infected persons from Kirinyaga County, Central Kenya. In order to get the parasite samples we require for the study, we will need to identify people who have bilharzia who can provide stool samples from which we will obtain the bilharzia parasite eggs from, for our research. The study investigators are requesting you to take part in this study by giving us stool samples from which the bilharzia worms, eggs will be obtained. Participating in this study is voluntary. If you accept to take part in the study, then you or your parent/guardian will have to give permission (that is, consent) to take part in this study. Even when you or your parent/guardian has given permission to participate in this study, it is still possible for you to leave the study, if you decide to do so, at any time in the future, without suffering any penalty or losing the benefits which you have been promised through participating in this study. Please take time to read this information sheet about the study, and when you have read, feel free to ask questions or to seek clarification on any issues related to this study or your participation in it, both, now or anytime, later. This study has already been approved by the KEMRIs' Scientific and Ethics Unit (SERU)

PURPOSE OF THE STUDY: The purpose of this study is to see how bilharzia parasites (which cause disease in people) respond when treated with increased dosage of praziquantel, the bilharzia medication. The aim is to have a better understanding of how the medicine works in killing the parasite, and how sometimes it fails. When it is clearly understood how the medication works, then it may be possible to increase the dosage of the drug to prevent the spread of, and control the bilharzia disease. In this study, you will be asked to give a small stool samples to see if you are infected with the bilharzia worms or other parasites, and incase you are infected with the bilharzia worms, you will be treated with the praziquantel and you will be asked to give more samples at a later date to confirm the success or failure of treatment.

PROCEDURES TO BE USED: you may be included in this study only if you or your parent/guardian signs a consent form, giving permission for you to take part in the study. Any person under 18 years old may also be included in the study only if they accept to take part, and if written consent has been provided by a parent/ guardian.

BENEFITS: If you are found to have bilharzia, a qualified doctor will give you praziquantel, the medication for bilharzia, free of charge. If you have other intestinal parasites, you will also receive medication for these infections as well, regardless of your bilharzia infection status, also, free of charge. If the doctor discovers that you have other medical conditions, he/she will refer you to a health clinic or hospital for further medical attention. However, you or your parent/guardian will be responsible for buying any other medications the doctor may prescribe for you for the other ailments.

RISKS, HAZARDS AND DISCOMFORTS ASSOCIATED WITH THE PROCEDURES: Giving stool samples will not cause any harm in you. The medications you will be given for treatment of bilharzia or other parasitic infections found in the stool you give, are known to be safe. However, in some people, they may cause some side effects which may include dizziness, headaches, stomach pain, but these are mild and last only for a brief period.

CONFIDENTIALITY: Your identity and test results will remain confidential. As a study participant, you will be assigned a number, and yourself and the test results that will be carried out on samples obtained from you, will remain confidential. All information and medical records will remain confidential, and will be kept in a lockable cabinet and will only be accessible to the people carrying out this study.

CONTACT OF SITE PRINCIPAL INVESTIGATOR: If you need more information about the study, please call: Elvis Kirubi Muthoni -Cell Phone: 0729 253208.

CONTACT OF THE KEMRI'S SCIENTIFIC AND ETHICS REVIEW UNIT (SERU): If you have questions about your rights as a research participant, please contact: The Secretary, KEMRI Ethics Review Committee, PO Box 54840-00200, Nairobi; Phone: 020-2722541, 0722-205901, 0733-400003; e-mail: erc@kemri.org

Appendix IV: Informed Consent Agreement for Parents/Guardians

I, Mr./Mrs/Miss _____, being an adult aged 18 years and over, and being the parent/guardian of: Master/Miss (Child's Name) _____Aged _____, who attends _____School, do hereby give permission to **Mr. Elvis K. Muthoni** for my child to take part in the new study entitled **"Efficacy and Safety of Up-scaled Dosage of 60 mg/kg Praziquantel in Control of *Schistosoma mansoni* in School Going Children in Kirinyaga County"** which has been explained clearly to you. I have been explained to about the tests to be done on my child, the benefits for taking part in the study, and the medications my child will receive if found to be sick with bilharzia or other intestinal illnesses caused by parasites, and the side effects the child could suffer, which I understand are mild and temporary. I have been given opportunity to ask questions and to seek a clarification of the issues I had not understood clearly, and I am satisfied with the answers and the explanations I was given. I have also, been informed that if I have additional questions or concerns about the study later, I can contact the researcher in charge of the study, or the Scientific and Ethics Review Unit (SERU) at KEMRI.

I accept that my child can take part in this study, and agree that he/she can provide stool samples for the tests needed in this study. I have been informed that my child can leave the study any time he/she decides to do so, and I have been assured that he/she will not suffer any penalty or loss of benefits that he/she should get in this study.

_____ _____

Signature (or Thumb Print) of Participant/Guardian Date

Witnessed by

_____ _____

Name of PI or study coordinator Date

Appendix V: Assent for Children (13-17 Year Olds)

You are being asked to take part in a study entitled **"Efficacy and Safety of Up-scaled Dosage of 60 mg/kg Praziquantel in Control of *Schistosoma mansoni* inSchool Going Children in Kirinyaga County"** The study aims at finding out how people infected with bilharzias disease are affected by increased dosage of the medication (praziquantel) used to treat the bilharzia disease. If we can get to know more about the effects of this medication on the bilharzias, then, researchers will be able to discover new ways of treating or controlling the disease. For this study to be carried out, you will be asked to provide stool samples for checking if you have the eggs of bilharzia worms in your body. If the eggs of these worms are found in your stool sample, you will be asked to provide another stool sample so that we can isolate the eggs for doing diagnosis and analysis. If we find eggs of bilharzia worms or any other parasites in you, you will be given medication by the doctor

You do not have to provide a samples for this study, if you don't want to, but there will be no harm if you do. Do you agree to take part in this study and give stool samples?

If you **agree to take part in this study and give stool samples, please** put a tick (√) next to the answer **"YES"**, in the space given below:

YES_____I agree to take part in this study and provide stool samples.

_____ _____ _____

Name of the Child Signature or Thumb Print Date

Witnessed by

Name of PI or study coordinator	Date

Appendix VI: Informed Consent Agreement for Head Teacher

I, Mr./Mrs/Miss _____, being an adult aged 18 years and over, and being the head teacher of: Master/Miss (Child's Name) _____Aged__, who attends _____School, do hereby give permission to **Mr. Elvis K. Muthoni** for my pupil to take part in the new study entitled **"Efficacy and Safety of Up-scaled Dosage of 60 mg/kg Praziquantel in Control of *Schistosoma mansoni* in School Going Children in Kirinyaga County"** that has been explained clearly to me. I have been explained to about the tests to be done on the children in this school, the benefits for taking part in the study, the medications the children will receive if found to be sick with bilharzia or other intestinal illnesses caused by parasites, and the side effects the children may suffer from, which I understand are mild and temporary. I have been given opportunity to ask questions and to seek a clarification of the issues I had not understood clearly, and I am satisfied with the answers and the explanations. I have also, been informed that if I have additional questions or concerns about the study later, I can contact the researcher in charge of the study, or the Scientific and Ethics Review Unit (SERU) at KEMRI

I accept that the children in my school can take part in this study, and agree that they can provide stool samples for the tests needed in this study. I have been informed that the children can leave the study any time they decide to do so, and I have been assured that they will not suffer any penalty or loss of benefits that they should get in this study.

_____ _____

Signature (or Thumb Print) of Participant/Guardian Date

Witnessed by

_____ _____

Name of PI or study coordinator Date

Appendix VII: Certificate of Translation

Protocol Title: "Efficacy and Safety of Up-scaled Dosage of 60 mg/kg Praziquantel in Control of *Schistosoma mansoni* in School Going Children in Kirinyaga County"

Principal Investigator: Elvis K. Muthoni

To Whom It May Concern

I _____do hereby testify that I translated the **English version of the Assent form, Version 1.2 dated 06 Jan 2017** into **Kikuyu** for the above named study. I certify that this is an accurate and true translation to the best of my ability.

SIGNED:_____ DATE: _____

ADDRESS:_____TEL NO:_____

EMAIL ADDRESS:_____

I_____do hereby testify that I have reviewed the **Kikuyu** translation **of the Assent form, Version 1.2 dated 6 Jan 2017** for the above named study. I have compared it with the **English** version of the same document, and found that the translation indicates that the assent form for the above named study has been properly translated into Kikuyu

SIGNED:_____ DATE: _____

ADDRESS:_____ TEL NO:_____

EMAIL ADDRESS:_____

Appendix VIII: utariria niguo ugie na umenyo ukiheana rutha kiongo kia utuiria: kurigiriria murimu wa mbilhacia thiinie wa county ya kirinyaga gutumira dawa na uthuthuria wa githimo kia iguru kia dawa icio

mUTHUTHURIA:Elvis Kirubi Muthoni wa ruhonge rwa uthuthuria wa kiriu wa muturire na uthii wanambere, ruhonge rwa uthuthuria wa mirimu rwa Kenya (KEMRI)

KIAMBIRIRIA: Ruhonge rwa uthuthuria wa mirimu rwa Kenya (KEMRI) ni mareka uthuthuria iguru wa murimu wa bilharzia uria urehagwo ni minyoo iria iikaraga thiini wa andu. Minyoo ino igwatanagio kugerera ndinoho iria iikaraga ma-ini matheru. Uthuthuria uyu worotithiirio gutaukirwo makiria uria minyoo ino iikaraga thutha wa urigiti na dawa ya Praziquantel iria ihuthikaga kurigita andu aria mena minyoo ya mbirihacia. Uthuthuria ta uyu ni ugututeithiriria kugia na njira cia kwagiria urigitani wa murimu wa mbirihacia. Minyoo ya bilharzias ni ikonganio kuma a kuri andu aria mena murimu wa bilharzia akuma Kirinyaga County theini wa Kenya. Nigetha tuike utuiria uyu ni tukubatara andu mena murimu wa mbirihacia nigetha matuhe kioro nigetha turore matumbe ma minyoo ya mbirihacia kuma ho. Ithui, andu aria mareka uthuthuria uyu ni tugukuria unyitanire naithui thiini wa utuiria uyu na gutuhe kioro kiria tukuhuthira kuthima murimu wa mbirihacia. Kunyita itemi thiini wa uthuthuria uyu ni wa kwiyendera na gutiri mundu ugugutindikiriria. Ona kuhana uguo, wetikira kunyita itemi, we kana muciari/mumenyereri waku ni ekuhoyana rutha nigetha, wee unyite itemi utuiria-ini uyu. Ona kungituika ati we, muciari kana mukumenyereri ni aheyanite rutha, no kuhoteke ume uthuthuria-ini uyu, ungiciria gwika uguo mahinda omothe thuthaini utekuhurwo baini oyothe orona kana gute uteithio uria wiriirwo ni gukorwo no unyitite itemi uthuthuria-ini uyu. Niukurio ugie na ihinda ria guthoma iratathi riri rigie uthuthuria uyu na thuthaini no urie ciuria kana uhoro makiria undu-ini wigie uthuthuria uyu. Kana unyiti waku wa itemi kwa ihinda riri ona kana thutha-ini. Niukwendwo umenye ati uthuthuria uyu niuhitukitio ni KEMRI

GITUMI KIA UTHUTHURIA: Gitumi kia uthuthuria uyu ni kuona uria minyoo ya mbirihacia (iria irehaga mirimu kuri andu). Iikaraga thutha wa urigiti na dawa ya Praziquantel iria iingataga murimu wa mbirihacia. Mworoto ni kugia na umenyo muiganu uria dawa ino irutaga wira ikiuraga minyoo na uria rimwe na rimwe iremagwo ni kuruta wira. Twarikia kugia na umenyo wa uria urigitani uyu urutaga wira wa kuraga minyoo, oro na kana gitumi kia kuremwo kuraga, ni tukuhota kugudura njira ingi njega cia kurigiriria kana kugitira murimu wa mbirihacia. Uthuthuriaini uyu, ni tugukuria wambe utuhe kioro gitari kiingi nigetha tumenye kana wina minyoo ya mbirihacia.

MUTARATARA URIA UKUHUTHIRWO: Tugugwitikiria uthuthuria-ini uyu angikorwo we kana muciari/mumenyereri ni egwikira kirore kana thairi iratathi-ini ria gwitikira ati ni aheana rutha rwaku ati unyite itemi uthuthuria-ini uyu. Mundu owothe wa thi ya ukuru wa miaka 18 onake no etikirio uthuthuria-ini uyu angitikira kunyita itemi, na iratathi ria gwitikira ni iheyanitwo ni muciari/mumenyereri. Ungiitikira kunyita itemi uthuthuria-ini uyu, dagitari ni egukurora na thutha ucio orio uheane kioro gitari kiingi gia guceka kana wina matumbe ma minyoo ya mbirihacia kana minyoo ingi o yothe. Ungikorwo wina minyoo ya mbirihacia no urio uheyane kioro ringi na ringi nigetha turute matumbi ma minyoo ya mbirihacia. Na, niukuheo dawa ya mbirihacia.

UGUNI: Ungioneka na mbirihacia kana minyoo oingi yothe dagitari mukinyaniru niegukuhe dawa ya Praziquantel tuhu. Ungikorwo na minyoo ingi o yothe ya nda ni ukurigitwo kumana na minyoo ino oronayo hatari kurora kana wina minyoo ya bilharzia kana nduri, hatari marihi. Dagitari angiona wina mathina mangi ma mwiri, ni egugutuma thibitari uthii ukarorwo makiria no orona kuhana ouguo, we muciari/mumenyereri niwe ukurugamirira urigiti o wothe dagitari agiona ni ukwagiriire kumana na mirimu iyo ingi.

MOGWATI, MITINO, NAKWAGA KUIGANIRA KURIA KUNGIUMANA NA MUTARATARA UYU: Kuheana kioro gutingiguthukira na njira o yothe. Urigiti uria ukuheyo ni undu wa murimu wa mbirihacia oro kana minyoo o yothe ingioneka kioroini kiria ukuheana ni yuikaine ati nduri ugwati ungirehe. Ona kuhana o oguo andu-ini amwe

dawa ici no irehe kwaga kuiganira thiini wa mwiri ta guthiururuka, kurio ni mutwe, kurio ni nda, na maundu maya ni mahuthu na nima kahinda kanini.

UGITIRI WA UHORO WAKU: Uhoro wa we wi uu na moimirira na uthuthuria wa kioro giaku uguikara wi mugitire. Wita munyiti iteme uthuthuria-ini uyu ni ukuheyo namba na we kana moimirira ma uthuthuria wa kioro uheanite iritanagio na namba ino thiini wa macokio kana gucabithio kwa moimirira na uthuthuria uyu. Uhoro wothe na rekodi cia urigitani cigutura ciri ngitire na cigwo thiini wa ndiroo hinge thiini wa wabici ya muthuthuria Ibrahim Ndungu iri i KEMRI na cingionekana no andu aria mareka uthuthuria uyu.

NAMBA CIA MUNENE WA UTHUTHURIA: Ungienda uhoro makiria ukonie uthuthuria uyu no uhurire Ibrahim Ndungu kuhitukiria namba ya wabici, 020-2727231 EXT 2243 kana thimu ya guoko 0720 400713.

NAMBA YA KAMITI YA KEMRI IGIE MIRUTIRE MIEGA YA WIRA: Ungikorwo na kiuria kiigie kihoto giaku wita munyiti itemi wa uthuthuria uyu, nyitanira na mwandiki was Kamiti ya KEMRI igie mirutire miega ya wira kuhitukira njira ici: Ithanduku ria marua 54840-00200, Nairobi, Namba ya thimu 020-2722541, 0722-205901, 0733-400003, e-mail erc@kemri.org

Appendix IX: Rutha Ruiguanire Rwa Muciari/Mumenyereri

Nii, Mr./Mrs./Miss.:_____, Ndi mundu mugima wa miaka ikumi na inana guthii na iguru,na ndi muciari/mumenyereri wa:

Master/Miss (Ritwa ria mwana)_____Wa ukuru wa _____ Uria uthomagira cukuru wa_____ nindaheana rutha kuri Prof/Dr./Mr./Mrs./Miss. _____kuri mwana wakwa akorwo ari umwe wa uthomi mweru witwagwo "**Kurigiririamurimu wa mbilhacia Thiinie wa County ya Kirinyaga gutumira dawa na uthuthuria wa githimo kia iguru kia dawa icio"** ta uria menyithitio wega, na ruthiomi rwa gikuyu ruria njaragia na ngaigua wega, na riu ni menyete gitumi gia githomo giki.Nimandariirie uthomi uyu, utuiria uria magwika na mwana wakwa, na uguni hari mwana wakwa ekwo uthomi uyu,urigiti uria angiheo onekana ena mbirihacia kana mirimu ingi ya nda iria ingirehwo ni minyoo,kana undu angiona thutha wa kuheo ndawa na ninjiritwo itiri na uuru na itingirehe ugwati hari mwana wakwa. Nindaheirwo kamweke ga kuria ciuria na kweretherwo wega maundu maria itanyitite wega makonie githomo giki na ninjiganirite na uria ndatariirio.Ninjiritwo ingikorwo na ciuria ingi ikonii githomo giki thuthaini no twaranirie na mutuiria murugamiriri wa githomo giki, kana ingikorwo na ciuria ikonii haki cia mwana wakwa githomoini giki,no njararanirie na; Mwandiki wa kamiti ya urori wa utuiria ya KEMRI, Kenya Medical Research Institute,P.o Box 54840- 00200 Nairobi, Thimu 020 2722541,0722 205901, 0733 400003, e- mail erc@kemri.org .

Nindetikiria mwana wakwa akorwo ari umwe githomoini giki, na nindetikira no aheane kioro kiria gikwendeka utuiriaini wa githomo giki. Ninjiritwo ati mwana wakwa no oime githomoini giki ihinda o riothe angienda na ngaiguithio ndari kindu angitio kana kindu angitee kiria angionire hari githomo giki. Maundu maya mothe nitutariirio na mwana wakwa na ruthiomi ruria twararagia na tukaigua wega.

_____

Thairi (kana Kirore) ya muciari/mumenyereri.

_____

Mweri

_____

Ritwa ria muhoi wa rutha na thairi

_____

Ritwa na thairi ya muira

Appendix X: Rutha Ruiguanire Rwa Mwarimu

Nii, Mr./Mrs./Miss.:_____, Ndi mundu mugima wa miaka ikumi na inana guthii na iguru,na ndi mwarimu wa:

Master/Miss (Ritwa ria mwana)_____Wa ukuru wa _____ Uria uthomagira cukuru wa_____ nindaheana rutha kuri Prof/Dr./Mr./Mrs./Miss. _____kuri mwana wakwa akorwo ari umwe wa uthomi mweru witwagwo "**Kurigiririamurimu wa mbilhacia Thiinie wa County ya Kirinyaga gutumira dawa na uthuthuria wa githimo kia iguru kia dawa icio"** ta uria menyithitio wega, na ruthiomi rwa gikuyu ruria njaragia na ngaigua wega, na riu ni menyete gitumi gia githomo giki.Nimandariirie uthomi uyu, utuiria uria magwika na mwana wakwa, na uguni hari mwana wakwa ekwo uthomi uyu,urigiti uria angiheo onekana ena mbirihacia kana mirimu ingi ya nda iria ingirehwo ni minyoo,kana undu angiona thutha wa kuheo ndawa na ninjiritwo itiri na uuru na itingirehe ugwati hari mwana wakwa. Nindaheirwo

kamweke ga kuria ciuria na kweretherwo wega maundu maria itanyitite wega makonie githomo giki na ninjiganirite na uria ndatariirio.Ninjiritwo ingikorwo na ciuria ingi ikonii githomo giki thuthaini no twaranirie na mutuiria murugamiriri wa githomo giki, kana ingikorwo na ciuria ikonii haki cia mwana wakwa githomoini giki,no njararanirie na; Mwandiki wa kamiti ya urori wa utuiria ya KEMRI, Kenya Medical Research Institute,P.o Box 54840- 00200 Nairobi, Thimu 020 2722541,0722 205901, 0733 400003, e-mail erc@kemri.org.

Nindetikiria mwana wakwa akorwo ari umwe githomoini giki, na nindetikira no aheane kioro kiria gikwendeka utuiriaini wa githomo giki. Ninjiritwo ati mwana wakwa no oime githomoini giki ihinda o riothe angienda na ngaiguithio ndari kindu angitio kana kindu angitee kiria angionire hari githomo giki. Maundu maya mothe nitutariirio na mwana wakwa na ruthiomi ruria twararagia na tukaigua wega.

_____

Thairi (kana Kirore) ya muciari/mumenyereri.

_____

Mweri

_____

Ritwa ria muhoi wa rutha na thairi

_____

Ritwa na thairi ya muira

Appendix XI: Guitikira Gwa Ciana Cia (Ukuru Wa Miaka 13-17)

Niuritio ukorwo uri umwe githomo-ini gia **"Kurigiririamurimu wa mbilhacia Thiinie wa County ya Kirinyaga gutumira dawa na uthuthuria wa githimo kia iguru kia dawa icio"**kiria kirekwo ni atuiria a kuma ruhonge rwa utuiria wa mirimu rwa Kenya Medical Research Institute (KEMRI). Muoroto wa githomo giki ni kumenya uria minyoo ino (iria irehaga murimu kuri andu, makiria ciana) ikagwo thutha wa urigitani na dawa ya Praziquantel iria ihuthiragwo guthondeka murimu wa mbirihacia. Tungimenya makiria ugaruruku uria wonekaga thutha wa urigitani kuri minyoo ya mbirihacia, muthenya umwe, athuthuria nimakahota kumenya njira njeru cia kurigita kana kugiririria murimu uyu. Nigetha tuhote utuiria uyu nitukumuria muheane kioro nigetha turore kana mwina matumbi ma minyoo ya mbirihacia thiini wa miiri yanyu.. Tungiona matumbi ma minyoo ya mbirihacia kana minyoo ingi o yothe thiini waku , niukurigitwo ni ndagitari nigetha minyoo ithire nawe uigue wega. To nginya uheane kioro kiingi utuiria-ini uyu, angikorwo ndukwenda kuhehana kioro utuiriaini uyu, no hatiri na thina na thina ungiheana. Kwoguo ugiheana nitukumenya kana wina minyoo iria itumaga andu marware. Makiria, waheana kioro nitukuruta matumbi ma mbirihacia maria tubatairio nimo utuiria-ini uyu. kwaria uhoro wama, kuheana kioro gutingiguthukia na njiira o yothe.

Niwetikira gukorwo uri umwe utuiria-ini uyu na kuheana kioro?

Akorwo **niwetikiragukorwo uri umwe utuiria-ini uyu na kuheana kioro ikira** ruri rwa gwitikira (√) mbere ya anja ya **ïi"** iyo iheanitwo hau kianda.

Ii____ nindetikira gutuika umwe utuiria-ini na kuheana kioro niundu wa utuiria.

_____

Ritwa ria mwana Thaini kana kirore

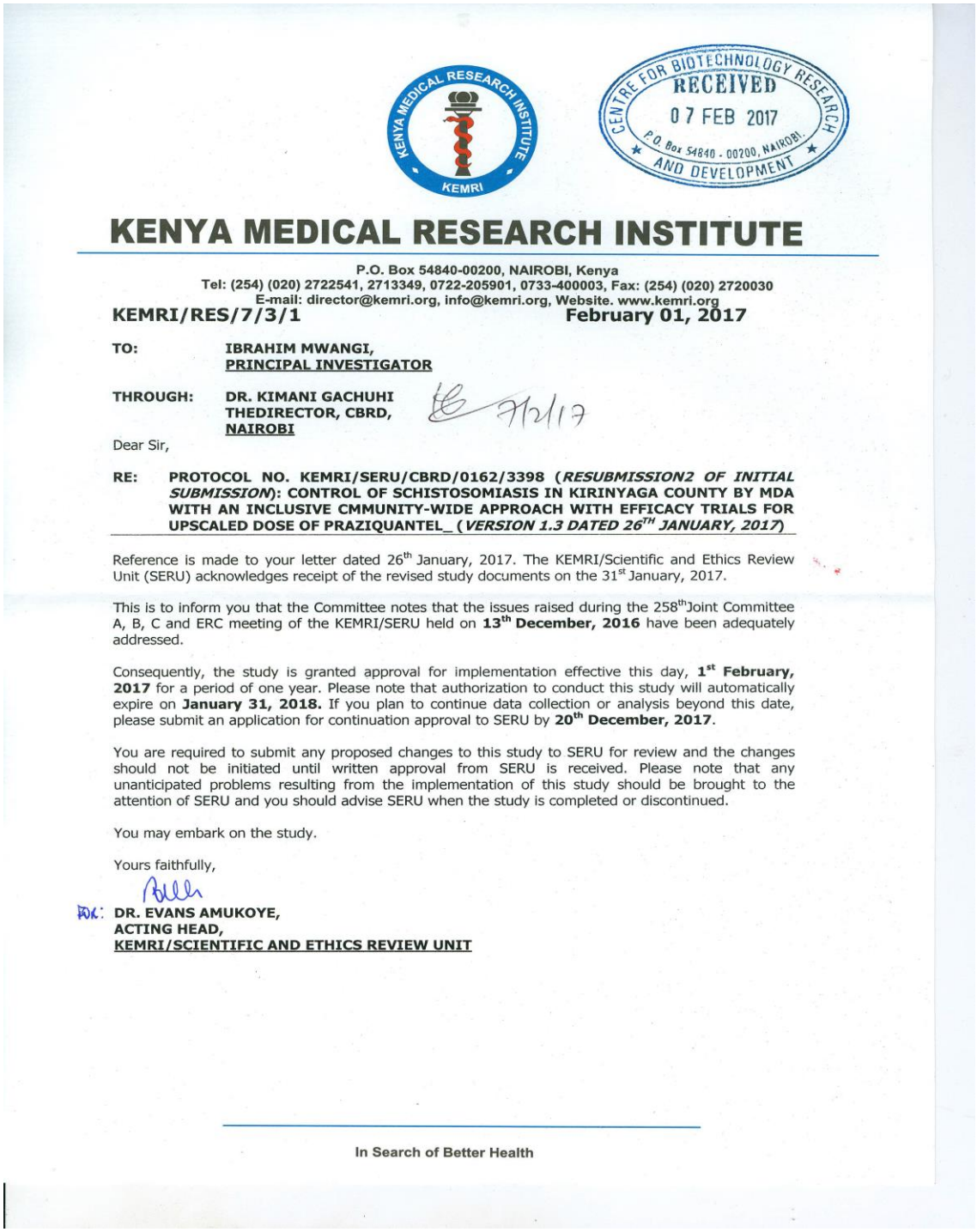
_____

Ritwa ria mundu uria uraheo rutha Thaini kana kirore

Ritwa na thaini (kana kirore) ya muira

MUHURI

Appendix XII: Seru Approval Letter



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KEMRI/RES/7/3/1 **February 01, 2017**

TO: **IBRAHIM MWANGI,**
PRINCIPAL INVESTIGATOR

THROUGH: **DR. KIMANI GACHUHI**
THEDIRECTOR, CBRD,
NAIROBI

Dear Sir,

RE: **PROTOCOL NO. KEMRI/SERU/CBRD/0162/3398 (*RESUBMISSION2 OF INITIAL SUBMISSION*): CONTROL OF SCHISTOSOMIASIS IN KIRINYAGA COUNTY BY MDA WITH AN INCLUSIVE CMMUNITY-WIDE APPROACH WITH EFFICACY TRIALS FOR UPSCALED DOSE OF PRAZIQUANTEL_ (*VERSION 1.3 DATED 26TH JANUARY, 2017*)**

Reference is made to your letter dated 26th January, 2017. The KEMRI/Scientific and Ethics Review Unit (SERU) acknowledges receipt of the revised study documents on the 31st January, 2017.

This is to inform you that the Committee notes that the issues raised during the 258thJoint Committee A, B, C and ERC meeting of the KEMRI/SERU held on **13th December, 2016** have been adequately addressed.

Consequently, the study is granted approval for implementation effective this day, **1st February, 2017** for a period of one year. Please note that authorization to conduct this study will automatically expire on **January 31, 2018.** If you plan to continue data collection or analysis beyond this date, please submit an application for continuation approval to SERU by **20th December, 2017**.

You are required to submit any proposed changes to this study to SERU for review and the changes should not be initiated until written approval from SERU is received. Please note that any unanticipated problems resulting from the implementation of this study should be brought to the attention of SERU and you should advise SERU when the study is completed or discontinued.

You may embark on the study.

Yours faithfully,

DR. EVANS AMUKOYE,
ACTING HEAD,
KEMRI/SCIENTIFIC AND ETHICS REVIEW UNIT

In Search of Better Health