INTESTINAL SCHISTOSOMIASIS AND THE ASSOCIATED TRANSMISSION FACTORS IN PRE-SCHOOL AGED CHILDREN IN VILLAGES SURROUNDING LAKE RWERU IN BUGESERA DISTRICT, RWANDA

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Intestinal Schistosomiasis and the Associated Transmission Factors in Pre-School Aged Children In Villages Surrounding Lake Rweru in Bugesera District, Rwanda

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DECLARATION

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

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DEDICATION

I dedicate this dissertation to my parents, brothers and sisters for their love, care, encouragements empowered me to succeed in my studies.

I also dedicate it to my beloved fiancée Françoise Uwase.

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ABBREVIATION AND ACRONYMS

CCA: Circulating Cathodic Antigen

DALYs: Disability-adjusted life years

ELISA: enzyme linked immunosorbent assay

EPG: Egg Per Gram

Ig: Immunoglobulin

KK: Kato-Katz

NTD: Neglected Tropical Diseases

PCR: Polymerase reaction chain

POC-CCA: Point Of Care- Circulating Cathodic Antigen

PSAC: Pre-school aged children

RBC: Rwanda Biomedical Center

SAC: school aged children

STH: Soil transmitted helminthes

WHO: World Health Organization

ABSTRACT

Schistosomiasis is a waterborne snail-transmitted parasitic disease and is a major public health problem in sub-Saharan Africa, affecting populations living where water supply and sanitation are poor and inadequate. Pre-school aged children (PSAC) have often been forgotten in disease control and prevention programs, as result very little information is available on the prevalence or intensity of schistosomiasis in this age group. This study was conducted to determine the prevalence and intensity of intestinal schistosomiasis among PSAC, and determine factors associated with infection transmission in PSAC in villages surrounding Lake Rweru in Bugesera District, Rwanda. This was a cross-sectional study involving 256 PSAC aged 12-59 months. Double Kato-Katz (KK) stool smears and the point of care circulating cathodic antigen (CCA) test were used to diagnose *Schistosome mansoni* in stool and urine samples, respectively. A questionnaire in the local language (Kinyarwanda) was administered to parents/guardians of the PSAC to determine factors associated with schistosomiasis transmission in the area. Based on the KK stool smears, no *Schistosome mansoni* infection in any of the PSAC tested. However, using the point of care CCA test, 16.9% of the PSAC tested positive, when trace was considered as positive. The information collected on questionnaire showed a significant association between infection with *Schistosome mansoni* (based on the CCA test) and visits to the lake especially, when the children accompanied their parents/guardians, or older children ($P<0.000$). The prevalence of *Schistosome mansoni* in the study population was light on CCA test and the infection was associated with going to the lake by the children when they accompanied their parents/guardians or older siblings. These results call for the need to consider including a point of care screening tool for *Schistosome mansoni* infections among preschool children as well as a prevention program targeting this age group.

CHAPTER ONE

INTRODUCTION

1.1 Background

Schistosomiasis is a waterborne snail-transmitted parasitic diseases and a major public health problem in sub-Saharan Africa, mostly, affecting populations living in low-resource countries where, water supply and sanitation are poor and inadequate (Chitsulo *et al*., 2000). It is considered among the neglected tropical diseases (NTDs), and is one of the diseases targeted for elimination through global efforts, which involve preventive chemotherapy (Liese *et al*., 2010).

It is estimated that more than 200 million people worldwide, mostly in tropical and subtropical countries are infected (Gryseels *et al*., 2006; King *et al*., 2005; Sady *et al*., 2013). It is difficult to accurately estimate the disease burden due to schistosomiasis, but recent work suggests a loss of 3-70 million disability-adjusted life years (DALYs) is attributed to the disease (King *et al*., 2005). Significant reduction of infection and morbidity has been achieved in some countries of Africa through global efforts to eliminate schistosomiasis and other NTDs by deworming(Kabatereine *et al*., 2007). However, because of the development of irrigation schemes and other water resources development projects that increase the habitats for snail intermediate hosts, the disease distribution and transmission continue to increase in the endemic areas.

In Rwanda several studies have been done on intestinal schistosomiasis especially in school children where the prevalence in endemic areas ranges from 10 to 69.5% and

no case of urinary schistosomiasis was found (Rwanda Ministry of Health, 2008; Mupfasoni *et al*., 2009; Ruberanziza *et al*., 2015).

For a long time, it was generally believed, that schistosomiasis prevalence and intensity curves show peaks in school aged children (SAC) aged 6-15 years, and it has been resourceful in providing an index for assessing community prevalence. Accordingly, epidemiological studies and control interventions on schistosomiasis are frequently directed towards school-aged children by regular administration of Praziquantel(Kabatereine *et al*., 2007).

The lower frequency of active water contact of pre-school-aged children (PSAC) [<6 years]compared to SAC and the development of an acquired protective immunity against schistosomiasis in adolescents and adults (≥16 years), PSAC have often been ignored in disease control and prevention programs (Verani *et al*., 2011). As a result very little information is available on the prevalence or intensity of schistosomiasis in infants and PSAC in much of the endemic areas of the world including Rwanda. There is emerging evidence that, in moderate to high schistosomiasis transmission areas, infants and PSAC are also infected (Odogwu *et al*., 2006).

The detection of *Schistosome mansoni* eggs in stool using Kato-Katz (KK) method is the most commonly used method, and its benefits include high specificity, low cost, and relatively simple technological requirements. However, the sensitivity of this method is low particularly in areas of low endemicity, and low-infection intensities (e.g. in young children) (Knopp *et al.*, 2008), and may be affected by day to day variability in the rate of egg excretion(Barreto *et al*. 1978; Teesdale *et al*., 1985).

Immunodiagnosis, on the other hand, is generally more sensitive than examination of stool samples, particularly in low transmission areas where infection intensities are light *(*Shane *et al*., 2011). Studies done in Uganda and Kenya to assess a Circulating Cathodic Antigen (CCA) urine dipstick and a Point Of Care-Circulating Cathodic Antigen (POC-CCA) cassettetest in PSAC, recommended these rapid tests as a useful technique for the detection of *Schistosome mansoni* in that age group (Verani *et al*., 2011; Sousa-Figueiredo *et al*., 2013).In one study conducted in Ivory Coast on *Schistosome mansoni* in SAC, a single POC-CCA cassette test was shown to have similar sensitivity as triplicate KK thick smears *(*Coulibaly *et al.*, 2011).

However, many studies have shown day-to-day fluctuation in *Schistosome mansoni* CCA test scores and egg counts by KK, and they have recommended a collection of several samples from individuals on consecutive days to increase the sensitivity of these methods (Coulibaly *et al*., 2013; Sousa-Figueiredo *et al*., 2013; Degarege *et al*., 2014). But this may have some limitations like cost and getting stool specimens from individuals on different days which would be challenging (Knopp *et al*., 2008), so it is easier to use double Kato- Katz smears for single stool sample and even a single urine-CCA cassette test would be enough for mapping and screening of *Schistosome mansoni* infection at a reasonable cost (Degarege *et al*.. 2014).As the degree of exposure and intensity of infection increases, it is necessary to document the prevalence and intensity of infection in PSAC in schistosomiasis endemic areas so that they can benefit from large scale disease control programs.

The aim of this study was to determine the prevalence, intensity of *S. mansoni* infection and the factors associated with intestinal schistosomiasis transmission in

pre-school-aged children in areas surrounding Lake Rweru in Bugesera district using both KK as specific test and POC-CCA cassette test as sensitive test.

1.2 Problem Statement

In schistosomiasis endemic areas like villages surrounding Lake Rweru in Bugesera district, Rwanda, PSAC are among those at risk of exposure to *Schistosome* infection, because of the passive and/or active contact with contaminated water bodies as they are bathed by their mothers or when they play in the water. In most schistosomiasis control efforts, the PSAC are never considered, and no studies have previously been done in the villages surrounding Lake Rweru to determine the prevalence and intensity of intestinal schistosomiasis infection in this age group.

1.3 Justification

Although intestinal schistosomiasis is prevalent in most of sub-Sahara African countries including Rwanda, its prevalence in pre-school aged children remains relatively unknown among this age group, which is often overlooked and ignored by disease control and prevention programs (Verani *et al*., 2011), probably, partly because it is commonly assumed that water contact levels in this age group is insufficient to be a risk factor for infection.

However, emerging evidence suggests that, in moderate to high schistosomiasis transmission areas, pre-school aged children are also infected (Odogwu *et al*., 2006; Opara *et al*., 2007), providing sufficient justification for further investigations into the extent of the problem in other endemic areas including Rwanda.

1.4 Research Questions

i. What is the prevalence of intestinal schistosomiasis in pre-school aged children in villages surrounding Lake Rweru in Bugesera district?

ii. What is the intensity of intestinal schistosomiasis in pre-school aged children in villages surrounding Lake Rweru in Bugesera district?

iii. What are the factors associated with intestinal schistosomiasis transmission in pre-school aged children in villages surrounding Lake Rweru in Bugesera district?

1.5 Objectives

1.5.1 General objective

To determine the prevalence, the intensity of infection and factors associated with intestinal schistosomiasis transmission in pre-school aged children in villages surrounding Lake Rweru in Bugesera district.

1.5.2 Specific objectives

i. To determine the prevalence of intestinal schistosomiasis among pre-school aged children in villages surrounding Lake Rweru in Bugesera district.

ii. To determine the intensity of intestinal schistosomiasis among pre-school aged children in villages surrounding Lake Rweru in Bugesera district.

iii. To determine factors associated with intestinal schistosomiasis transmission in pre-school aged children in villages surrounding Lake Rweru in Bugesera district.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview

Schistosomiasis (also known as bilharziasis) is an infection caused by blood flukes of the genus *Schistosome*. This parasitic infection affects either the gastro-intestinal or urinary tracts depending on the causative agent (Gray *et al*., 2011).According to WHO, schistosomiasis is the third after malaria and soil transmitted helminthiasis (STH), most devastating tropical disease worldwide and is a main cause of morbidity and mortality for developing countries mostly in Africa (WHO, 2002).

Five species infect humans, namely: *Schistosome haematobium, Schistosome mansoni, Schistosome japonicum, Schistosome mekongi,* and *Schistosome intercalatum* (Olveda *et al*., 2013). The first three species account for the majority of human disease and the burden of disease attributable to them is estimated to be between 24-29 million disability adjusted life years (King *et al*., 2005).

2.2 Transmission and Biology of Schistosomes

Figure 2.1 shows the life cycle of human *Schistosome* species. The life cycle is complex because of many different morphology stages in definitive host and the involvement of a snail as intermediate host. Definitive hosts get infected when they come in contact with fresh water contaminated by cercariae (infective stage) which penetrate the host's skin and become schistosomula, by blood circulation schistosomula migrate to the lungs and travelling in the hepatic portal system where the two sexes pair and mature to adult worms (Gryseels*et al*., 2006; Olveda *et al.* 2013). For *Schistosome haematobium*, the worms finally pass to the vesical venules

around the bladder whereas *Schistosome mansoni, Schistosome japonicum, Schistosome intercalatum,* and *Schistosome mekongi* reside in the mesenteric venules(Walker, 2011; Olveda *et al*., 2013).

After copulation the female begins to lay eggs within the mesenteric or pelvic vessels, a proportion of which escape from the host via the gut (e.g. *Schistosome mansoni, Schistosome japonicum*) or bladder wall (*Schistosome haematobium*) to enter the excreta. Eggs not voided become trapped in organs (e.g. liver) causing immune reactions that result in human schistosomiasis(Gryseels*et al*., 2006; Olveda *et al.*, 2013).

When excreted eggs contact water, they hatch into ciliated larval forms called miracidia that can penetrate the intermediate hosts (snails). Inside the snail host, miracidia undergo asexual development and transform into cercariae which emerge from the snails and seek out the definitive host (Gryseels *et al*., 2006; Olveda *et al*., 2013). The *Schistosome* do not multiply in the human host and the intensity of infection in humans is basically determined by the level at which new worms are acquired through contact with cercariae-infected water.

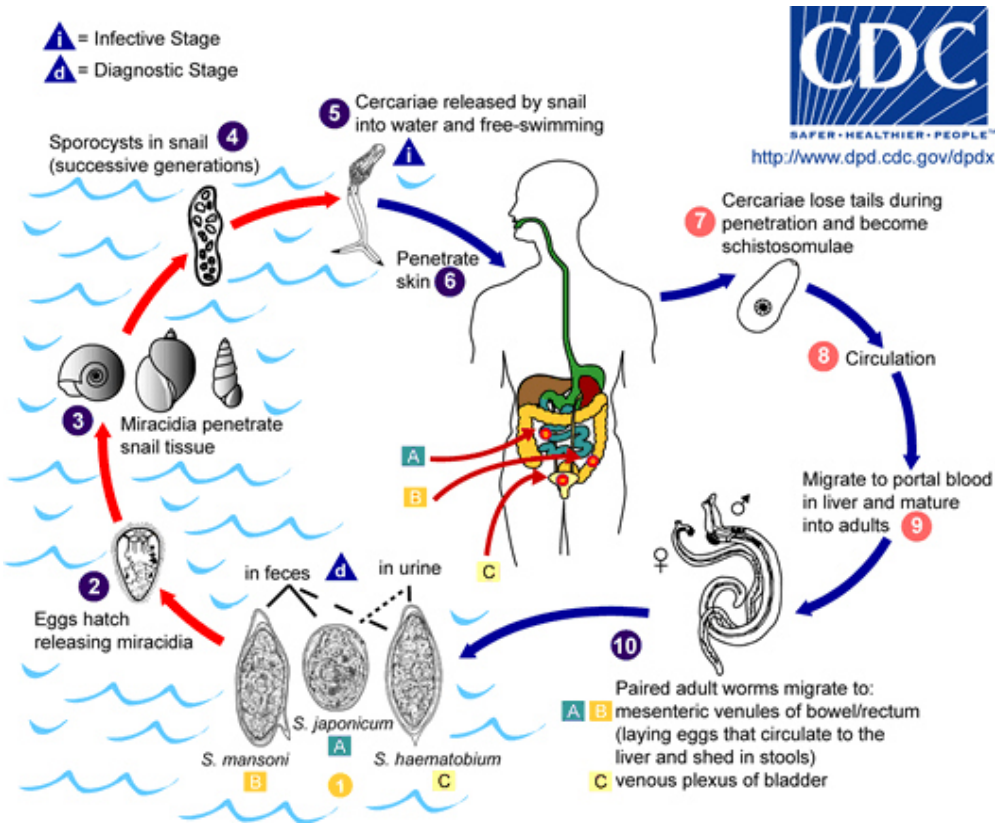

Figure 2.1: Life cycle of *Schistosome* species
Source: http://www.scienceinschool.org

2.3 Prevalence and Intensity of Intestinal Schistosomiasis PSAC

Intestinal schistosomiasis has been studied highly in SAC, and numerous of these researches have reported both prevalence and intensity of infection peak between 10 and 15 years of age. For example in study done in Kenya prevalence increased from 31.4% among children aged 5–7 years to peak at 61.8% among children aged 11–16 years before diminishing in the 17–19 year old group(Odiere *et al.*, 2012). In a national prevalence survey on intestinal parasites conducted in Rwanda, it has been shown that the prevalence of intestinal schistosomiasis in school-aged children in endemic areas was ranged between 10%- 69.5% and no case of urinary schistosomiasis was found (Rwanda Ministry of Health, 2008).

The intensity of infection is classified as light, moderate or heavy infection according to the number of eggs per gram of stool, it means 1-99 eggs/gram, 100-399 eggs/gram, and ≥400 eggs/gram for light, moderate and heavy infection respectively. Most people experience light infection and few people have heavy infection (WHO, 2001).

The pattern of age, infection rates and intensity may be attributed to the levels of exposure to the cercariae-contaminated water among the members of community, and the development of an acquired protective immunity against infection in older adolescents and adults *(*Roberts *et al*., 1993).Intestinal schistosomiasis infection with has been recognized to cause anemia, growth retardation, impaired cognitive development, portal hypertension and liver failure in children(Jukes *et al*., 2002). Based on the pattern of age, infection rates and intensity, school-aged children have been at the focus of schistosomiasis infection control by periodically administration of Praziquental and it has shown a considerable achievement (Kabatereine *et al*., 2007; WHO, 2001).

Based on the assumptions that water contact levels of pre-school aged children were not enough for a considerable risk of infection, pre-school aged children have often been ignored in disease control and prevention programs (Verani *et al*., 2011). But some studies done in schistosomiasis endemic areas have shown that the pre-school aged children are also infected, for instance in a study done in 2 localities in Ivory Coast, the prevalence of schistosomiasis in PSAC was 23.1% using quadruplicate Kato-Katz and 34.3% using CCA in which trace results considered as negative (Coulibaly *et al*., 2013). In a study done in Uganda along the shoreline of Lake Victoria in infants aged under 3 years using KK and CCA has shown that the

intestinal schistosomiasis prevalence is 7% on KK and 40% using CCA (Odogwu *et al*., 2006).

Many other studies have been carried out in PSAC to study the urinary and/or intestinal schistosomiasis infection in Kenya, Ethiopia, and Sudan and have shown that the prevalence ranged between 8.8%-58.1% (Shane *et al*., 2011; Ghiwot *et al*., 2014; Ekpo *et al*., 2010).

2.4 Diagnosis of Intestinal Schistosomiasis

Appropriate diagnosis of *Schistosome* infection permits accurate prevalence estimation for deciding about the disease control and prevention measures to communities at risk, by control programs (Sturrock, 2001). There are many methods that can be used in diagnosis of intestinal schistosomiasis.

2.4.1 Kato-Katz technique

WHO recommends to most control programs and epidemiologic studies on *Schistosome mansoni* infection the Kato-Katz parasitological technique as the method of choice, which depends on detection of parasite eggs in the human feces(WHO, 2002). This technique is simple and cheap but requires a minimal of laboratory equipment and well-trained laboratory technicians (Speich *et al*., 2010).

In an early stage of a control program, when its objective is to control morbidity, infection prevalence and intensity are usually high, and hereafter Kato-Katz technique show reasonable accuracy (Coulibaly *et al*., 2011). However, there are several limitations of this method. There is an intra-specimen and day-to-day variation on faecal egg output, and by consequence a single Kato–Katz thick smear

examination underestimate the true prevalence of *Schistosome mansoni*(Utzinger *et al*., 2001; Lin *et al*. 2008). Even though its sensitivity can be improved by increasing the number of stool samples with at least duplicate Kato-Katz smears per sample, getting stool specimens from individuals on different days would be challenging *(*Knopp *et al*., 2008).

2.4.2 Immunodiagnosis

In order to overcome some limitations of parasitological techniques, immunological methods have been developed which are based on antibodies or antigens detection in blood or urine specimens (van Lieshout *et al*., 2000; Doenhoff *et al*., 2004). These methods have higher sensitivity than Kato-Katz technique for example in 1998 during an epidemiological study of *Schistosome mansoni* in low endemic area in Brazil, according to Kato-Katz the prevalence was 1.6% whereas according to IgG-Indirect immunofluorescence test the prevalence was 33.2% (Burlandy*et al*., 2003).

The need for an expensive and complex instruments, qualified staffs and delicate reagents limits the use of these techniques in endemic areas. The main limitations with antibodies detection methods, is that are not quantitative, so it is challenging to differentiate between light and heavy infections. Furthermore, antibody levels remain high for long time after treatment, which means that it is not possible to differentiate active from cured infections. Lastly, in areas with co-endemicity of *Schistosome* and other trematode infections there might be a high degree of cross-reactivity (Bergquist *et al*., 2009; Johansen *et al*., 2010).

Detection of *Schistosome* antigens, such as Circulating Cathodic Antigen (CCA) in blood or urine, using enzyme-linked immunosorbent assays (ELISA) or dipstick

platforms (Van Dam *et al*., 2004; van Lieshout *et al*., 2000) have been the most widely studied methods and hold numerous advantages over antibody detection. Most notably, they are of high specificity, positive correlation with worm burden, the possibility for estimation of infection intensity, and disappear rapidly after treatment, therefore be used for assessment of cure (Shane *et al*., 2011).

Circulating Cathodic Antigen cassette test detects a circulating *Schistosome mansoni* antigen which is the glycoprotein regurgitated in bloodstream by viable adult worms during feeding and continuously clearance in the host's kidneys and pass in urine(Ashton *et al*., 2011). The principle of the test is based on a lateral-flow assay using a nitrocellulose strip of the sample with a colloidal carbon conjugate of anti-CCA monoclonal antibodies (Van Dam *et al*., 2004).

2.4.3 Molecular Diagnosis

During the last decade, polymerase chain reaction (PCR)-based assays have become more available and have been used in the diagnosis of infectious and parasitic diseases. In the specific case of human schistosomiasis, the first application of PCR as a diagnostic tool in an endemic area was a PCR assay for *Schistosome mansoni* DNA detection in stool samples of Brazilian endemic area inhabitants, the assay showed high sensitivity and absence of cross-reaction (Pontes *et al*., 2002; Pontes *et al*., 2003). The method is based on the amplification of a highly repeated DNA sequence, first extract the DNA, and then denaturation and finally amplification (Lodh *et al*., 2013).

The role of PCR-based assays in schistosomiasis diagnosis apart from research situations remains to be well-defined, despite their significant potential in low

transmission locations and in situations where high sensitivity and specificity are required. PCR assays are expected to become more affordable and may constitute a new available tool for Schistosomiasis diagnosis.

2.5 Control Strategies of Schistosomiasis

The current strategy recommended by World Health Organization (WHO) of controlling schistosomiasis is regularly administration of a single oral dose of 40mg/kg (body weight) of Praziquental to the group at risk of infection in endemic areas (WHO, 2001). This has objective to reverse the morbidity that may result from schistosomiasis like periportalfibrosis, hepatomegaly and splenomegaly (Bruno *et al*., 2006). The sooner treatment is given the higher are the chances of reversing the organ damage.

The frequency of chemotherapy is directed by prevalence of schistosomiasis in school-aged children. If the prevalence of *Schistosome* infection in school-aged children is 50% or higher, entire communities should receive treatment once every year; if the prevalence is between 10% and 50%, only school-aged children should receive treatment once every two years; if the prevalence is below 10%, school-aged children should be treated twice, at school entry and again before they finish schooling (WHO, 2012).

Table 2.1: Recommended treatment strategies by WHO for *Schistosome* infection

Community category	Prevalence in school survey	Intervention in school
High prevalence	≥30% visible haematuria (*S. h.* by questionnaire) Or ≥50% infected (S. m. And S.h by parasitological methods)	Targeted treatment of school- aged children once year
Moderate prevalence	<30% visible haematuria (S. h. by questionnaire) or ≥10% but <50% infected (S. m. and S. h. by parasit-ological methods)	Targeted treatment of school-aged children once every two years.
Low prevalence	<10% infected (S. m. and S. h. by parasitological methods)	Targeted treatment of school-aged children twice during primary schooling (once on entry, again on leaving).

S. m.*: Schistosome mansoni;* S. h*: Schistosome haematobium*

In most endemic areas, yet persons other than school aged children are infected and are overlooked by control programs including pre-school aged children (Hodges *et al*., 2012; Stothard *et al*., 2011, 2013), there is evidence that these PSAC in some areas are also infected with *Schistosome*. Even though the information on prevalence and intensity of schistosomiasis in pre-school aged children is scant, there is also a Praziquental treatment gap because of the absence of a suitable Praziquental pediatric formulation (Stothard *et al*., 2011; Ekpo *et al*., 2012). Currently WHO recommends the use of crushed or broken Praziquental tablet to treat infected pre-school aged children where a need is shown (Coulibaly *et al*., 2012; WHO 2010; Navaratnam *et al*., 2012).

In Rwanda the NTD control program has great achievement in controlling schistosomiasis and other intestinal parasites in school aged children, as the nationwide MDA coverage is above 90%, however still have challenges, WHO estimates that in 2012, only 16% of all people at risk of schistosomiasis infection in Rwanda were reported to have received treatment (WHO, 2014).

Other components of schistosomiasis control include access to safe water, adequate sanitation, health education and snail control (WHO, 2014). However, water, sanitation, and hygiene are inadequate in large parts of low- and middle-income countries, where schistosomiasis is endemic (Bruno *et al*., 2006; Colley *et al*., 2014; Grimes *et al*. 2015). Continuously using of Praziquantel in preventive chemotherapy with rapid re-infection after treatment, this may lead to the possibility of emerging of resistance to Praziquental; so there is no doubt that an effective vaccine against human schistosomiasiswould be a very effective control tool (Utzinger *et al*., 2009).

2.6 Factor Associated With Exposure to Schistosomiasis in PSAC

Intestinal schistosomiasis infection in adults is mainly connected with occupational activities that make people in contact with contaminated water like agriculture practices in irrigation schemes, fishing, washing clothes and utensils, washing cars and motorcycles and watering animals (Sady *et al*., 2013). Infection in school-aged children is associated with recreational events such as swimming, playing, and their participation in domestic tasks like fetching of water from unsafe sources (Rudge *et al*., 2008; Stothard and Gabrielli 2007).

A limited number of studies have assessed factors associated with intestinal schistosomiasis in pre-school aged children, and those that have been done depended

on administering questionnaire to the parents/guardians of children (Bosompem *et al.* 2014; Mafiana *et al.*, 2003). In these studies, it has been shown that the parents/guardians used water from contaminated stream, river, or pond for bathing their children and older pre-school children went themselves to water bodies for swimming, bathing and fetching the water (Ekpo *et al.*, 2010). By consequence the preschoolers with an age above 24 months are more infected than preschoolers under 24 months of age as it has been shown in south Côte d'Ivoire (Coulibaly *et al.*, 2013).

CHAPTER THREE

MATERIALS AND METHOD

3.1. Study Area

The Lake Rweru is located in Bugesera district in Eastern province of Rwanda as it is indicated in figure 3.1, at an altitude of 1350 m. The total surface area of Lake Rweru covers some 100 km^2, of which 20% is in Rwanda and the remaining lie in Burundi as it is shown in figure 3.2. The mean depth is around 2.1 m with a maximum of 3.9 m (Ruberanziza *et al*., 2010). Compared to other regions of the country, Bugesera's climate is dry with a temperature varying between 20°C and 30°C with an average ranging between 26 and 29°C. The district has two dry periods and two rainy periods; a short dry season (January to mid-March), long rainy season (mid- March to June), long dry season (mid-June to September), a short rainy season (mid-October to December). This climate influences agriculture in Bugesera. About water and sanitation in this district, a big proportion still use water from rivers and lakes (27%) and 7.9% households don't have toilet facilities (Republic of Rwanda, 2012).

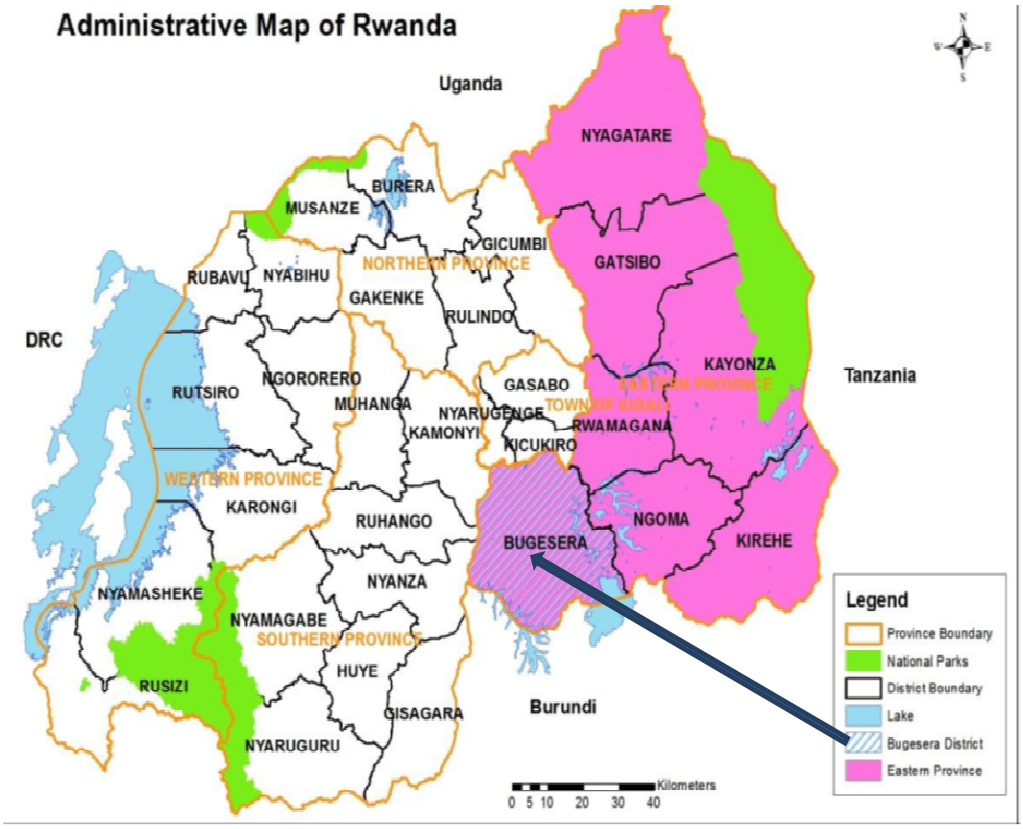

Figure 3.1: Administrative map of Rwanda (Source: Bugesera district development plan 2013-2018 document)

Figure 3.2: Administrative map of Bugesera district(Source: Bugesera district development plan 2013-2018 document)

3.2. Study Design

A cross-sectional descriptive study was undertaken from November to December 2015 to determine the prevalence, intensity and factors associated with intestinal schistosomiasis transmission in pre-school-aged children in villages surrounding Lake Rweru in Bugesera district.

3.3. Study Population

The study population was the pre-school aged children in villages surrounding Lake Rweru in Bugesera district who were present during the study period. The primary sources for obtaining the children's age were their birth certificate or the child's

vaccination card, the age of children without any of these two official documents, were declared by the parents/guardians.

3.3.1. Inclusion criteria

Pre-school children aged of 12 to 59 months old who have been living in the study area for at least three months whose parents or guardians had signed a written consent form and willing to respond to the questionnaire were included in the study.

3.3.2. Exclusion criteria

Pre-school children less than 12 months and above 59 months old, children who didn't live in the study area for at least three months, and those whose parents and guardians refused to sign a written consent form were not included in the study.

3.4. Sample Size Determination

In this study, the sampling units were pre-school aged children in different villages surrounding Lake Rweru. In determination of the sample size, a 21.1% prevalence of *Schistosome mansoni* infection, obtained in lake's Rweru shore inhabitants, including children and adults (Ruberanziza *et al*., 2010) was used to calculate the sample size. Sample size determination for study estimating population prevalence was calculated by using the following formula (Naing *et al*., 2006).

$$N = \frac{Z^2 P(1-P)}{D^2}$$

Where; N= the estimated sample size, Z= 1.96=the confidence interval (95%), D= 0.05 = is the margin of error, P= estimated proportion of lake's Rweru shore

inhabitants with *Schistosome mansoni* infection from previous study (Ruberanziza *et al*., 2010).

$$N = \frac{1.96^2 * 0.211\ (1-0.211)}{0.05^2} = 255.8 \approx 256$$

Therefore, the minimum number of pre-school aged children in villages surrounding Lake Rweru in Bugesera district included in this study was 256 pre-school children.

3.5. Sampling Procedure

A sampling frame of all pre-school aged children for every village surrounding Lake Rweru was prepared with the help of community health workers and then the proportional simple random sampling was used to select the participants of this study in each village, it means that the village with high number of target population had a high number of study participants. After making the sampling frame in every village, the proportion (fraction) of every village in total target population was determined. Unique number was assigned toevery member in each village; a blind draw method (lottery method) was used to select the study participants.

3.6. Data Collection Procedure

3.6.1. Specimen collection

The day before sample collection, two empty containers were given to children's parents/guardians, one for stool and the other for urine collection, labeled with unique IDs for each child and the code number of the container and the name of the parent or guardian who took that particular container was recorded in a note book to avoid the accidental exchange of specimen. Because of the difficulty to collect

biological samples in this age group, parents/guardians were allowed to obtain samples from their young children at any time of the day (usually in the early morning hours).

3.6.2. Laboratory procedures

Stool and urine samples were transported to a laboratory in the Nzangwa health center. Double Kato-Katz thick smears,and CCA cassette test were performed respectively.

3.6.2.1 Kato-Katz technique

Hydrophilic cellophane strips measuring 25 x 35mm in size were soaked in 50% glycerol-malachite green solution for about 24 hours before use. For each collected specimen, a gram of stool was transferred onto a paper using an applicator stick. A nylon mesh was then pressed on top of the sample and a flat-sided wooden applicator stick used to scrape across the upper surface of the mesh to sieve the stool sample. The sieved sample was used to fill the hole of 41.7 mg template placed on a clean labeled microscope slide. This was then levelled with the applicator stick and the template removed carefully to leave a cylindrical sieved stool sample on the slide. The sample on the slide was covered with the treated cellophane strip, inverted, and the sample firmly pressed against the cellophane strip on a smooth surface to evenly spread out. The prepared slide was then placed in a slide holder for about 30minutes (mins) to clear, after which it was examined by experienced laboratory technician for eggs using the x10 objectives of the Olympus light microscope. Observed eggs were identified, counted and recorded. The mean of eggs counted in 2 Kato-Katz smears from one stool was calculated and then multiplied with 24 to obtain the number of

eggs per gram of stool(Katz *et al*., 1972).To ensure quality control, 10% of all examined slides were selected randomly and reexamination by more experienced laboratory technician in blindness manner. In the case of intestinal schistosomiasis, intensity was measured as total egg count in one gram of stool and classified as either light (1–99epg), moderate (100–399epg) or heavy (>400epg) infections (WHO, 2001).Any child who was determined to be *Schistosome* or STH positive (by stool) was treated with the appropriate dose of praziquantel or albendazole, respectively.

3.6.2.2 Circulating Cathodic Antigen cassette test

CCA urine cassette assays were obtained from Rapid Medical Diagnostics (Pretoria, South Africa) and performed at ambient temperature to detect the *Schistosome mansoni* circulating cathodic antigen in urine, following the manufacturer's instructions. Briefly, one drop of urine was added to the well of the testing cassette and allowed to absorb. Once fully absorbed, one drop of buffer (provided with the CCA test kits) was added. The test results were read 20 mins after adding the buffer. In case the control bands did not develop, the test was considered as invalid. Valid tests were scored as either negative or positive, the latter further stratified into trace, 1+, 2+, or 3+ according to the visibility of the color reaction. All tests were read independently by two blinded laboratory technicians and in case of discordant results discussed with the PI until agreement was reached.

3.6.3. Questionnaire survey

A questionnaire in the local language (Kinyarwanda) was administered to parents/guardians of pre-school–aged children to determine factors associated with schistosomiasis. These factors were socio-demographic data of the parents/guardians

of study participant, hygiene, sanitation, and lake's water contact of study participant.

3.7. Data Management

Data collected were entered and stored in data collection form and Excel sheet based databases; data was cleaned for errors due to inconsistent entry. Data collected in hard copies were kept in lockable cabinets where only the researcher had access to maintain confidentially. Information stored in Microsoft excel based databases was protected from unauthorized individual with the use of a password. All records were identified by study ID numbers to maintain confidentiality. Names and other personal identifiers werenot disclosed.

3.8. Statistical Analysis

Double data entry were performed in Microsoft Excel 2010 spreadsheet, and checked for entry errors. The STATA version 11 (Stata Corp.; College Station, Texas) was used in analysis. The mean and standard deviation of age variable were described and categorized in age groups: 12-24, 25-36, 37-48, and 49-59 months. Binary variables were compared using the Chi- square test ($\chi 2$) or the Fisher exact test, where appropriate. Socio-demographic, hygiene, sanitation, and lake's water contact of study participant to the Schistosomiasis were assessed using logistic regression.Non-overlapping 95% confidence intervals (CI) or p-values ≤ 0.05 was considered as statistical significance.

3.9. Ethical Consideration

The study was approved by Rwanda National Ethic Committee (RNEC) No.331/RNEC/2015 and Malaria & OPDD-RBC. Before starting the study, meetings

were held at village's level with parents whom have pre-school aged children to describe the purpose of the study, the procedures to be followed, and the risks and benefits to the participation. Written informed consent was obtained from the parents/guardians of children before their enrolment into the study. The study had no risks to the participants, because the samples (stool and urine) that were used are obtained in normal life processes. Praziquantel and albendazole at the recommended doses were administered to the infected children under the supervision of a qualified clinician. Information obtained on the questionnaire was treated in confidential way and the records were stored in a locked cabinet and data were entered in a password protected file in a computer.Detailed report regarding the outcome results of the study was shared to the Neglected Tropical Diseases (NTD) unit-Malaria and other parasitic diseases division/Rwanda Biomedical Center and Ministry of Health, and the findings has been published in peer review journal.

CHAPTER FOUR

RESULTS

Introduction

This chapter presents the findings of the study. The findings are presented and interpreted based on the objectives of the study. A total of 251 pre-school aged children participated in the study in villages surrounding Lake Rweru, Bugesera district, Rwanda. The respondents were drawn from 10 villages. The results are presented in sections that cover: socio-demographic characteristics of participants, prevalence of *Schistosoma mansoni* and STH, intensity of *Schistosoma mansoni* and STH, and factors associated with transmission of *Schistosoma mansoni*. The results are presented in tables and graphs form.

4.1 Socio-Demographic Characteristics of Participants

4.1.1 Socio-demographic characteristics of PSAC

A total of 251 pre-school aged children were included in the study (the level of participation in this study was 98.5%). As indicated in table 4.1, the mean age of participant was 35.3 months (range: 12 to 59 months) with standard deviation of 13, and among these, 130 (51.8%) were males and 121 (48.2%) were females. The PSAC surveyed, were placed in four groups according to age with the youngest being in the range of 12-24 months (25.4%) and the oldest 49-59 months (20.4%). The PSAC that participated in this study were sampled from 10 villages located around Lake Rweru, with the lowest percentage of 3.6% for PSAC recorded in Rukira village, and the highest at 19.2%) in Ruzo village (Table 4.2).

Table 4.1: The Pre-school aged children participant by sex and age group

	Children Gender					
	Female		Male			
Age groups (months)	Number	Percentage	Number	Percentage	Mean	S.D
12-24	32	12.7%	32	12.7%		
25-36	34	13.5%	33	13.1%		
37-48	30	12.0%	39	15.5%		
49-59	25	10.0%	26	10.4%		
Total	**121**	**48.2%**	**130**	**51.8%**	**35.37**	**13**

Table 4.2: The pre-school aged children participants organized by villages

Villages	Sex			
	Female	Male	Total	
	No	No	No	%
Kigina	24	19	43	**17.1**
Mugina	17	15	32	**12.7**
Mujwiri	11	5	16	**6.4**
Nyiragiseke	6	13	19	**7.6**
Ruzo	19	30	49	**19.5**
Sharita	14	14	28	**11.2**
Karizinge	7	12	19	**7.6**
Rukira	3	6	9	**3.6**
Gasasa	8	5	13	**5.2**
Rusenyi	12	11	23	**9.2**
Total	**121(48.2%)**	**130(51.8%)**	**251**	**100.0**

4.1.2 Socio-demographic characteristics of the parents of pre-school aged children

As shown in the table 4.3, the parents of surveyed pre-school aged children by social demographics characteristics indicate that 87.6% of parents/guardians were married, 24.7% of parents had no formal education, 46.2% of parents had not complete

primary education, 28.3% of parents/guardians had completed primary education and none had studied at university. The most percentage (92.8%) of parents/guardians were famers, 5.2% are fishermen, by age group the parents/guardians were grouped in 5 groups where 48.2% were in 30-39 year group, followed by 20-29 year with 38.6%. Among the parents/guardians of PSAC 68.1% were female.

Table 4.3: The parents of pre-school aged children by age, sex, marital status, education and occupation

Social demographic characteristics		**N^{o}.**	**%**
Marital status of child's parent/guardian	single	11	4.4
	married	220	87.6
	divorced	15	6.0
	widowed	5	2.0
Education of child's parent	No formal education	62	24.7
	incomplete primary	116	46.2
	complete primary	71	28.3
	secondary	2	0.8
	university	0	0.0
Occupation of child's parent	farmer	233	92.8
	fishing	13	5.2
	fish trader	0	0.0
	public servant	1	0.4
	potter	4	1.6
Age group of child's parents	20-29	97	38.6
	30-39	121	48.2
	40-49	22	8.8
	50-59	9	3.6
	60-69	2	0.8
Gender of parents	male	80	31.9
	female	171	68.1
Total		**251**	**100**

4.2 Prevalence of *Schistosoma Mansoni* and STH

4.2.1 Prevalence of *Schistosoma mansoni* and STH based on KK

In a total of 251 pre-school aged children examined, none was found to be positive for *Schistosome mansoni* based on Kato-Katz. The most prevalent parasitic infection was ascariasis (*A. lumbricoides*) 32 (12.7%), followed by *Trichuris trichiura* 14 (5.6%), Hookworm 6 (2.3%), and others 2 (0.8%). Of the positive cases, 56.1% were females and 43.9% were males, the difference was not statistical significant (P > 0.05), as it is shown in table 4.4.

Table 4.4: The prevalence of *S. mansoni* and soil transmitted helminthes on Kato-Katz thick smears

	Male	Female	Total	X^2	P-value
Parasite	N=130	N=121	N=251		
S. mansoni	0 (0)	0 (0)	0(0)	N/A	N/A
Hookworm species	1 (16.7)	5 (88.3)	6 (2.3)	3.037	0.08
A. lumbricoides	14 (43.7)	18 (56.3)	32 (12.7)	0.950	0.329
T. trichiura	6 (42.8)	8 (57.2)	14 (5.6)	0.474	0.491
Others	2 (100)	0 (0)	2 (0.8)	1.864	0.1707
Total	18 (43.9)	23 (56.1)	41 (16.3)	7.398	0.116

Number in brackets ( ) indicate percentage

4.2.2 Prevalence of *Schistosoma mansoni* based on CCA cassette

As shown in figure 4.1, the prevalence of *Schistosome mansoni* based on CCA cassette test considering trace as positive (CCA t+), 42/248, 16.9% (95% CI: 12.6-21.8) of participants were infected with *Schistosome mansoni*. According to the sex, the prevalence in male children was 19.4% and in the female children the prevalence was 14.3%, this difference was not statistically significance (X2=1.14, P=0.28). According to age group the prevalence of *S. mansoni* was 19.4%, 19.7%, 13%, and 15.7% for 12-24, 25-36, 37-48, and 49-59 months respectively, the difference was not statistically significant (Fisher's exact test=1.45, P= 0.72) as it is indicated in table 4.5.

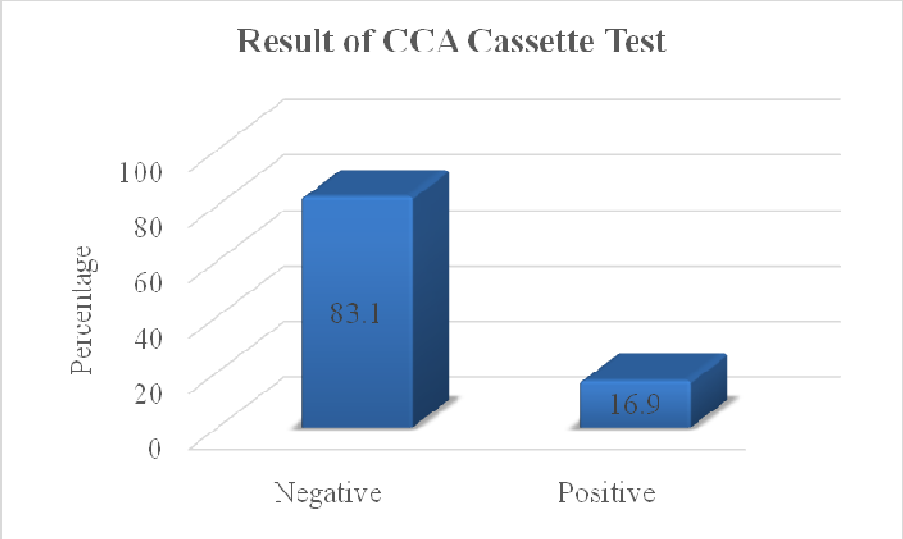

Figure 4.1: Prevalence of *S. mansoni* based on CCA cassette test in PSAC

Table 4.5: Prevalence of S. mansoni based on CCA cassette test according to sex and age group of PSAC

	CCA test results			X^2	*P*-value
	Negative	Positive	Total		
Sex					
Male	104 (80.4%)	25 (19.4%)	129	1.14	0.28
Female	102 (85.7%)	17 (14.3%)	119		
Age group					
12-24	50 (80.6%)	12 (19.4%)	62	1.45	0.72
25-36	53 (80.3%)	13 (19.7%)	66		
37-48	60 (87%)	9 (13%)	69		
49-59	43 (84.3%)	8 (15.7%)	51		

4.3 Intensity of *Schistosoma Mansoni* and STH

As shown in table 4.6, the intensity of infection was predominantly light with 41 individuals out of the 52 (78.7%) having a light infection intensity. Moderate infection intensity accounted for 10 cases (19.2%) while heavy infection intensity was relatively rare with only 1 case (1.9 %).

Table 4.6: The intensity of *S. mansoni* and STH by Kato-Katz technique

Light	**Moderate**	**Heavy (high)**		
Parasite		**N (%)**	**N (%)**	**N (%)**
S. mansoni		0 (0)	0 (0)	0 (0)
Hookworm species		6 (11.5)	0 (0)	0 (0)
A. lumbricoides		23 (44.2)	8 (15.4)	1(1.9)
T. trichiura		12 (23)	2 (3.8)	0 (0)
Total		**41 (78.7)**	**10 (19.2)**	**1 (1.9)**

4.4 Poly-Parasitism

Most of the infected PSAC (73.1%) harbored only one parasite, whereas 24.3% harbored two parasites and only 2.4% of PSAC harbored more than two parasites.

The combination of parasites in one pre-school aged child was *Ascaris lumbricoides* and *Trichuris trichiura*, and few cases with combination of *Ascaris lumbricoides* and Hookworm species as indicated in table 4.7.

Table 4.7: Pre-school aged children with 2 or more infections (poly-parasitism) by age in month

parasites	Only one parasite	two parasites	more than two
Age group	**N (%)**		**N (%)**
N (%)			
12-24	3 (7.3)		0 (0)
0 (0)			
25-36	8 (19.5)		2 (4.9)
1 (2.4)			
37-48	13 (31.7)		2 (4.9)
0 (0)			
49-59	6 (14.6)		6 (14.6)
0 (0)			
Total	**30 (73.1)**		**10 (24.3)**
1 (2.4)			

4.5 Factors Associated with *S. Mansoni* Infection in PSAC

4.5.1 Sanitation and lake's water contact habit

As shown in table 4.8, 94.8% of surveyed children were living in household with the presence of toilet. 62.5% of children were defecating in the toilet while 35.1% were defecating around the house. 88.4% of the children had parents/guardian who use water from the lake as first choice of water use at home while only 4.4% use tap water, 81.3% of the study children use water from lake as first choice of water bathing. Approximately, 97% of the parents/guardians of PSAC reported that their children got frequently into contact with lake water. 79% of children were in contact

with water from lake when bathing while 13.5% had contact when accompanying adults.

Table 4.8: Sanitation and lake's water contact habit of studied PSAC

	Frequency	95% CI
Presence of toilet		
Yes	238 (94.8%)	91.6-97.5
No	13 (5.2%)	2.5-8.4
Areas where the child defecates		
Toilet	157 (62.5%)	56.7-68.8
Around house	88 (35.1%)	29.2-41.0
In bushes	6 (2.4%)	0.4-4.8
First choice of the source of water used at home		
Tap water	11 (4.4%)	2.1-7.2
Stream	10 (4%)	2.0-6.8
Pond	8 (3.2%)	1.2-5.6
Lake	222 (88.4)	84.2-92.0
First choice of the source of water used to bath child		
Tap water	15 (6%)	3.2-9.0
Stream	21 (8.4%)	4.8-12.0
Pond	11 (4.4%)	2.0-7.0
Lake	204 (81.3%)	76.9-85.7
Contact with lake water		
Yes	244(97.2%)	95.2-98.8
No	7 (2.8%)	1.2-4.8
How		
At home (bathing with lake water)	195 (79.9%)	74.6-84.7
Accompany parent/older children to the lake	33 (13.5%)	9.4-18.6
Goes him/herself to lake	16 (6.6%)	3.7-10.1

4.5.2 Factors associated with *S. mansoni* infection in PSAC

Although not significant (p> 0.05), children living in household without toilet had roughly 2 times the odd to have *Schistosome mansoni* infection compared to children who had toilet (OR 2.16(0.46-10.12). Even though not significant (p> 0.05), children defecating around the house had 1.5 odds (OR 1.59(0.69-3.61) of having *Schistosome mansoni* infection compare to those defecating in the toilet (p>0.05).Although not significant (p> 0.05), children who had contact with lake had nearly 5 times the odds (OR 4.8 (0.87-27.16)) to have *Schistosome mansoni* infection compare to those who did not have contact with lake (p>0.05). Accompanying

parents and older children by the PSAC to the lake was significantly associated with *Schistosome mansoni* infection with 10 times the odd than other children ($P<0.000$), as indicated in table 4.9.

Table 4.9: The factors associated with the intestinal schistosomiasis in PSAC

Characteristic	CCA test result			Univariate
Negative	**positive**	**Total**	**OR(95%CI)**	***P*-value**
Toilet ownership?				
Yes	199(84.7)	36(15.3)	235	0.328
No	7(53.8)	6(46.2)	13	2.16(0.46-10.12)
Child defecates				
In toilet	134(85.9)	22(14.1)	156	0.467
Around the house	67(77.9)	19(22.1)	86	1.59(0.69-3.61)
In the bushes	5(83.3)	1(16.7)	6	0.66(0.03-11.5)
Home using water				
Tap water	9(81.8)	2(18.2)	11	0.616
Stream	7(70)	3(30)	10	6.06(0.29-122.4)
Pond	5(62.5)	3(37.5)	8	7.24(0.35-148.5)
Lake	185(84.5)	34(15.5)	219	2.46(0.17-33.9)
Water used to bath child				
Tap water	10(66.7)	5(33.3)	15	0.604
Stream	20(95.2)	1(4.8)	21	0.13(0.009-1.81)
Pond	9(81.8)	2(18.2)	11	0.89(0.09-8.18)
Lake	167(83.1)	34(16.9)	201	0.67(0.14-3.15)
Child contact with lake's water				
Yes	202(84.6)	39(15.4)	241	0.065
No	4(57.1)	3(42.9)	7	4.86(0.87-27.16)
How				
Accompany parent/ older children to the lake	15(45.5)	18(54.5)	33	0.000
Use lake's water to bath children	172(89.6)	20(10.4)	192	0.11(0.04-0.27)
Child goes to the lake her/himself	15(93.8)	1(6.2)	16	0.06(0.007-0.56)
Total	**207(83.1)**	**42(16.9)**	**248(100)**	

CHAPTER FIVE

DISCUSSION

5.1 Discussion

The aim of this study was to determine the prevalence and intensity of *Schistosome mansoni* infection in pre-school aged children (PSAC) in an area located on the shores of Lake Rweru in Rwanda eastern province, and considered endemic for intestinal schistosomiasis, and where infection intensity is categorized as moderate, and determine the factors associated with infection transmission among the PSAC.

Intestinal *schistosomiasis* caused by *Schistosome mansoni* is endemic in Rwanda, especially around the lakes Ruhondo, Burera, Rweru, Kivu and on Nkombo Island (Hanotier and Gigase 1981; Mupfasoni *et al*., 2009; Ruberanziza *et al*., 2010; Ruberanziza *et al*., 2015), with data being available for school age children. Until now, virtually no information was available on the prevalence of *Schistosome mansoni* among the PSAC. The present study conducted around Lake Rweru located in Rwanda eastern province was the first to obtain data on *Schistosome mansoni* prevalence for this age group in Rwanda.

The fact that in the present study no *Schistosome mansoni* infection was detected among pre-school aged children in villages surrounding Lake Rweru in Bugesera district, Rwanda using the Kato-Katz procedure, and the fact that only about 17% of the PSAC tested positive with the urine CCA test when trace results were considered as positive, it does suggest that the prevalence of *Schistosome mansoni* among this age group, in this particular area, may be too low to be of public health significance.

Even though the Kato-Katz technique has been the backbone of intestinal schistosomiasis (and soil-transmitted helminthiasis) diagnosis in epidemiological studies for decades. Daily fluctuations in egg excretion and heterogeneous distribution of eggs within the stool have both been demonstrated to contribute to a reduced sensitivity of Kato–Katz thick smears compared to true infection prevalence (Kongs *et al*., 2001; Booth *et al*., 2003). While increasing sampling effort, particularly by collecting stool samples over consecutive days, can greatly improve sensitivity of Kato–Katz in detecting low-intensity *Schistosome mansoni* infections (Kongs *et al*., 2001; Booth *et al*., 2003), this has considerable cost implications. Recent studies have shown that a commercially available, urine-based POC-CCA cassette test is a promising method for the diagnosis of *Schistosome mansoni* in preschoolers and school-aged children (Shane *et al* 2011; Coulibaly *et al*., 2011; Verani *et al*., 2011).

Between the microscopy-based Kato-Katz procedure and the immune-diagnostic-based urine circulating cathodic antigen (POC- CCA) assay, it appears the latter is a more sensitive test, in terms of being able to detect even low level *Schistosome mansoni* infection. With respect to sensitivity of the tests, the results from the present study are similar to those obtained from other studies done elsewhere, in which the two tests were compared (Adriko *et al*., 2014; Colley *et al*., 2013; Degarege *et al*., 2014; Mwinzi *et al*., 2015), and they do suggest the value of the urine CCA test in epidemiological studies of *Schistosome mansoni*, particularly where infection prevalence is relatively low.

Based on the information above, the prevalence of *Schistosome mansoni* may has been underestimated in the current study by examination of duplicate Kato–Katz

thick smears from a single stool per child. It is therefore possible that the positive CCA results were actually due to light *Schistosome mansoni* infections in PSAC. Our results corroborate recent findings from Kenya and Uganda, where CCA tests detected *Schistosome mansoni* infections in preschool-aged children considerable earlier and at higher frequency than the Kato-Katz technique (Verani *et al*., 2011; Sousa-Figueiredo *et al*., 2013).

In present study the observed difference between the prevalence of *Schistosome mansoni* based on Kato-Katz and CCA cassette test, was also demonstrated in a systemic review, where in the areas with POC-CCA prevalence below 30%, it appears that the Kato-Katz prevalence could be less than 10% or even zero (Kittur *et al*., 2016).

In addition, the CCA-positive results where Kato-Katz results were *Schistosome mansoni* egg-negative among the PSAC, could also, suggest the presence of pre-patent immature infections (where the parasites are not at the stage of laying eggs). Alternatively, the eggs laid by the *Schistosomes* in the infected children were below the detection levels of the Kato-Katz procedure (Adriko *et al*., 2014; Polman *et al*., 2000). It is also, possible that we were dealing with residual infections after chemotherapeutic interventions, which normally reduce the prevalence and increase in the number of infections of low intensity (Coulibaly *et al*., 2012; Knopp *et al*., 2011). Schistosomiasis control programs have been undertaken in Rweru area since 2010 (Rwanda Ministry of Health, 2010).

The low prevalence of *Schistosome mansoni* infection observed in the Rweru area, Rwanda among the PSAC in the current study differs from the results of similar

studies conducted elsewhere, within the sub-Saharan region, and where prevalence with the Kato-Katz procedure was in the range 7-44% and with the CCA test, was 40-80% (Coulibaly *et al*., 2013; Odogwu *et al*., 2006; Ruganuza *et al*., 2015).

The results of the present study also show that there was no significant difference in intensity of infection by sex. This is likely due to a similarity of PSAC's water contact behaviour and parents' or guardians' behaviour towards their children's water contact between the two sexes. Similar findings were reported in Ivory Coast (Coulibaly *et al*., 2013)

In this study no a statistically significant association was observed between prevalence and intensity of *Schistosome mansoni* and age of PSAC. This is in contrast with other studies conducted else way, like in Tanzania, stratified by age, the prevalence of *Schistosome mansoni* (CCAt+) was 69.3, 64.5, 76.1, 93.0, 87.0 and 96.0 % for age groups 1- < 2, 2- < 3, 3- < 4, 4- < 5, 5- < 6, 6- < 7 years respectively (Ruganuza *et al*., 2015), showing statistically significant association between *Schistosome mansoni* and age of PSAC.

The overall prevalence of STH in the present study was 16.3%. According to WHO, this prevalence in this area should be classified as low transmission area (WHO, 2006). The prevalence of STH in the present study is lower than the findings of other studies (Nkengazong et al., 2010; Sowemimo & Asaolu, 2011).This low prevalence may be attributed to many factors such as population heterogeneity, genetics, time of study, parasitological technique used, personal hygiene practices, soil type, climate, the presence of control activity, and so on (Mokua *et al*., 2014; Shumbej *et al*., 2015).

In the present study, *A. lumbricoides* was the most common species of STH recovered from the children. This is consistent with the findings of similar studies conducted elsewhere (Kirwan *et al.*, 2009; Sowemimo & Asaolu, 2011).This may be due to the same environmental factors.

Among infected pre-school aged children, 11 (26.7%) had two or more parasites in their stool. The combination of parasites in one pre-school aged child was *A. lumbricoides* and *T. trichiura*, and few cases with combination of *A. lumbricoides* and Hookworm species. *A. lumbricoides* was observed to couple with the other two helminthes because it had the highest frequency in this study. Many researchers have reported polyparasitism in endemic areas (Mokua *et al.*, 2014).

Multiple infections occurred in 11 individuals making 26.7% of those who had intestinal parasites. The level of double infections with intestinal parasites determined in present study (24.3%) was lower than what reported from southwest Ethiopia portraying a double infection of 35.8% (Mengistu *et al*., 2007), higher than what observed in highland and lowland dwellers in Gamo area, South Ethiopia where the level of double infections was 6.2% (Wegayehu *et al*., 2013). The possible difference in the socio-demographic condition of the study population and the environmental condition might explain the observed difference in double infection in these different areas.

Parasite intensity was analyzed in this study and most infected pre-school aged children had light infection 78.7%. *A. lumbricoides* was the only one observed with heavy infection. *T. trichiura* infection was light and moderate but the hookworm

species infection was light. The similar results have been seen in many others studies (Kirwan *et al*., 2009; Mokua *et al.*, 2014; Shumbej *et al.*, 2015).

Our study also examined the factors associated with S. mansoni infection in PSAC, whereby the results show a high prevalence of schistosomiasis, in the PSAC living in the household without a functioning toilet compare to the household with toilet even though was not statistical significant and this was in accordance with previous studies (WHO, 2002, Ugbomoiko *et al*., 2012). Although not significant (p> 0.05), children who had contact with lake had nearly 5 times the odds (OR 4.8 (0.87-27.16)) to have Schistosome mansoni infection compare to those who did not have contact with lake (p>0.05), this high prevalence in PSAC who had a contact with lake's water was documented in other studies (Sady *et al*., 2013).

It was noted that majority of the study children in Rweru did not have direct contact with lake water, but contacted lake water at home during bathing. *Schistosome* cercariae present in the water are known to lose their infectivity with time, (Adenowo *et al*., 2015).Interestingly, there was a significant association between infection with *Schistosome mansoni* and going to the lake by the PSAC especially when the children accompany their parents/guardians or older children to the lake. The similar findings have been reported in western Kenya and in Tanzania (Handzel *et al*., 2003; Ruganuza *et al*., 2015).It is conceivable that most of the infections in pre-school–aged children occur at times during which pre-school–aged children are taken along to the lake by their parents/guardians and/or elder siblings for swimming, bathing, washing, or fishing activities, the lake's water remains essential for the household many needs due to a lack of alternative water sources (for washing, bathing and drinking purposes).

5.2 Limitations of the Study

The present findings have a geographic limitation, cannot be generalized to the all endemic area in the country. This study did not provide the malacology information in the area because of study design. This study also has a limitation on time; as it was conducted in November-December raining season. In this period the frequency of going to the lake to fetch water is decreased as they use rain water.

5.3 Conclusion

In conclusion the findings of this study have demonstrated that the prevalence of intestinal schistosomiasis in pre-school aged children in village surrounding lake Rweru on Kato- Katz thick smears was 0%, but 16.9% on the Point Of Care-Circulating Cathodic Antigen (POC-CCA) test, which indicates some level of the risk of *S. mansoni* infection among this age group. Concerning the intensity of infection, the Kato-Katz technique which is used to determine the intensity of intestinal schistosomiasis was negative, so the intensity of Schistosomemansoni based on Kato-Katz was not determined, but based on CCA cassette the infection was light as all positive results were trace and 1+. The results showed a significant association between infection with *Schistosome mansoni* and going to the lake by the PSAC which indicates that the water may be contaminated with cercariae.

5.4 Recommendations

As there is insufficient information on *Schistosome mansoni* infection among pre-school aged children in other endemic areas of Rwanda, further studies are needed to determine the public health significance of schistosomiasis in these areas. Pre-school aged children should be included in schistosomiasis research and control

programmes since results from this study showed them (children <5 years old) to be harboring infections.

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APPEENDICES

Appendix 1: Informed Consent Documents (English)

TITLE: "**Intestinal schistosomiasis infections and the associated transmission factors in pre-school aged children in villages surrounding Lake Rweru in Bugesera district, RWANDA**."

Investigators: The study will be led by Elias NIYITUMA, Institute of Tropical Medicine and Infectious Diseases/ Jomo Kenyatta University of Agriculture and Technology. Phone number: +250788677624/+254700740267; and will be supervised by Dr. Gerald M. MKOJI, CBRD-KEMRI; Prof. Kato J. NJUNWA, CMHS/UR and Dr. Amos K. MBUGUA, laboratory department-JKUAT.

Introduction: I am doing a study on intestinal schistosomiasis in under school age children. This study will help to know at which level the under school age children are affected by this worm so that implementation of control actions in this group can be justified. Control interventions on schistosomiasis are frequently directed towards school-aged children by using regularly treatment by Praziquantel, and under school age children have often been ignored in disease control and prevention programs because of little information on the extent of the problem. The study will be carried out on children aged of 12 months to 59 months and their parents or guardians. So we will need permission from their parents/guardians to include their children into the study. As parent/guardian we request you to participate in this study and your permission for your child to take part in this study. Being in this study is voluntary and you and your child may decide to withdraw from the study at any time, without

suffering any penalty or losing any benefits available for him/her through this study. This form tells you about the study, read it carefully and feel free to ask any questions you have at any time.

Purpose of the study: To get information about how common the infection is in the children aged of 12 months to 59 months, and to determine the factors that lead to the children becoming exposed to infection.

Procedures: If you accept and allow your child to participate in this study, you will be requested to collect early morning stool sample and urine sample of your child. The stool sample will be processed using Kato-Katz technique and examined under microscope to look for *S. mansoni* eggs. The urine sample will be examined using CCA cassette to detect the presence of *S. mansoni*. A structured questionnaire will be administered to you to capture factors associated with infection.

Benefits: Your child will be tested for schistosomiasis infection and soil transmitted helminthiases free of charge, and if we find that your child is infected, he/she will get appropriate drugs according to the national guidelines. Data from this study may help schistosomiasis control program in the future.

Risks: The study has no risks to the participants, because the samples that we will use are stool and urine, which are obtained in normal life processes. And information obtained on the questionnaire will be confidential and it is not easily to be traced back to the participant.

Confidentiality: The samples (stool and urine) of your child will be assigned a code number and the key to the code will be maintained by the principal investigator. Data will be help in folders, which will be locked in cabinets for storage throughout the

study period. Computer documents will have passwords only accessible to the researcher. All the information gathered by the researcher will be used in confidence for the sole purpose of this research only.

Costs and Compensation: Your participation in the study will not take long time. You will not be paid for the study procedure. Your child will not be compensated for the stool and urine specimen.

New findings: Results will be disseminated to the Ministries of Health before being published in scientific journal.

Right to refuse or withdraw: You may withdraw your consent for you and for your child at any time and discontinue him/her from participation without penalty.

Questions: If you have questions, please ask. If you have any additional question later, please contact the researcher Elias NIYITUMA on +250788677624/+254700740267, or on e-mail *niyoelias@yahoo.fr*

If you have any questions about the rights of you and/or your child as a research participant you may contact the chairperson of Rwanda National Ethics Committee (RNEC), Dr. Jean Baptiste MAZARATI Telephone: (+250)788309807, or the secretary of RNEC +250788592004P.O.Box 84 Kigali, Email: *rnec@moh.gov.rw*.

INFORMED CONSENT AGREEMENT TO REQUEST PERMISSION OF PARENT/GUARDIAN'S CHILD

I have read this consent form and I have been given the opportunity to ask questions and all my questions have been responded to my satisfaction. I have received a copy of this consent form.

I

…….

. (Name of the parent/guardian) being 21 years or older and a parent/guardian of

……

…….. I give permission to Mr. Elias NIYITUMA for my child to participate in this study.

Signature (or thumb print) of participant ……..

Date ……..

Signature of researcher: ……..

Date ……..

Name of witness and signature:……

Date ……

INFORMED CONSENT FOR PARENT/GUARDIAN TO PARTICIPATE IN THIS STUDY

I have read this consent form and I have been given the opportunity to ask questions and all my questions have been responded to my satisfaction. I have received a copy of this consent form.

I……being 21 years or older, I accept to participate in this study.

Signature (or thumb print) of participant ……. …..

Date ……..

Signature of researcher: ……..

Date ……..

Name of witness and signature:……

Date ……

Appendix 2: Questionnaire (English)

Tittle of the study: "Intestinal schistosomiasis and the associated transmission factors in pre-school aged children in villages surrounding Lake Rweru in Bugesera district, RWANDA."

Part I: General information

1. Address

Village name:_____

Cellule name: _____

2. Demographic data

1) Subject ID number (child):
2) Age:_____
3) Sex: ☐ Male ☐ Female
4) Marital status of child's parent/guardian:

☐ Single ☐ Married ☐ Divorced ☐ Widowed

5) Education level of child's parent/guardian:

☐ Uneducated ☐ Incomplete primary education ☐ Complete primary education

☐ Secondary education ☐ University

6) Occupation of child's parent/guardian:

☐ Agricultural ☐ Fisherman ☐ Fish trader ☐ Public servant

Other (specify)……

Part II. Hygiene and sanitation

7) Do you have a toilet/pit latrine:

☐ Yes ☐ No

8) Where your child defecates mostly:

☐ In toilet ☐ Around the house ☐ In bushes

9) Order in descending way from 1 to 3 the source of water that you use at home:

1st choice		**2nd choice**		**3rd choice**	
Tap water		Tap water		Tap water	
Stream		Stream		Stream	
Pond		Pond		Pond	
Lake		Lake		Lake	

10) Order in descending way from 1 to 3 the source of water that you use to bath your child:

1st choice		**2nd choice**		**3rd choice**	
Tap water		Tap water		Tap water	
Stream		Stream		Stream	
Pond		Pond		Pond	
Lake		Lake		Lake	

Part III. Water contact habit of child

11) Does the child come into contact with Lake water?

☐ Yes ☐ No

12) If Yes how?

☐ Accompany you or older children to the lake ☐ Use lake water to bath him/her

☐ Child goes to the lake him/herself (fetch water or swim)

Appendix 3: Sample Analysis Form

Subject ID number: …….

Date of birth …….. (dd-mm-yyyy), estimated age in months ……

Sex: ☐ Male ☐ Female

Village name…….

STOOL ANALYSIS

Sample appearance:

Parasites examined	**Eggs/Kato thick smear**		**Eggs /Gram of feaces**	**Intensity of infection**		
	1st smear	**2nd smear**		**light**	**Moderate**	**High**
S. mansoni						
Hookworm species						
A. lumbricoides						
T. trichiura						
Others: specify **1.** **2.** **4.** **5.**						

URINE ANALYSIS

Test	**Result**				
	Negative	**Positive**			
		Trace	**1+**	**2+**	**3+**
CCA cassette test					

Done by …….

Date: ……/……../…….