RESEARCH PROJECT REPORT

HUMAN MANSONI SCHISTOSOMIASIS IN THE PROXIMITY OF LAKE MUHAZI

By

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A Research Report Submitted in Partial Fulfilment of the Requirements for Master of Science Degree in

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UNIVESITY OF RWANDA

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Kigali, September, 2019

DECLARATION BY THE STUDENT

I do hereby declare that this dissertation submitted in partial fulfillment of the requirement for the Master's degree in EPIDEMIOLOGY, at School of Public Health, College of Medicine and Health Sciences, University of Rwanda is my original work, except where specifically acknowledged and has not previously been submitted elsewhere. It has been passed through anti-plagiarism system and found complaint and this is the final version of the thesis. Also, I do declare that a complete list of references is provided indicating all the sources of information quoted or cited.

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DEDICATION

My research report is dedicated to my relatives, friends, family and my workmates at the department of biomedical laboratory sciences, and the field research team on the schistosomiasis around the lake Muhazi.

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Very grateful to everybody mentioned or not that contributed in any form, encouragement, ideas, facilitation and prayers to the successful completion of my research and research report.

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To everybody mentioned or not once again thanks.

God bless you and all yours.

ABSTRACT

Introduction

The continued treatment with Praziquantel (PZQ) since 2007, of school children without early detection of schistosomiasis do not significantly contribute to continued drop of prevalence. This pre-suggests continual surveys for the stubborn schistosomiasis prevalence and mapping of the high risk villages to inform continual planning of interventions or interruptions for the schistosomiasis transmission. The aim of this study was to explore the extent of the human mansoni schistosomiasis both at the community level and the individual in proximity of Lake Muhazi.

Methodology

This was a cross sectional study using quantitative methods. Both sexes above age five randomly selected from thirteen villages, in proximity of lake Muhazi, were tested for schistosoma ova using morphological methods after concentrating the stool with formol ether concentration techinique.

Results

Proportionally, according to each villages' population, participants mounting to 384 provided their stool samples. Overall schistosomiasis infection across all villlages was 7.03% with village's prevalence ranging from 0% to 23.33%. The difference of the S. mansoni prevalence, between all villages, statistically differ (at P= 0.002),Of the thirteen villages surveyed, the highest risk villages were five: Gasharu Kibara (23.33%), followed by Bwimiyange, Bwingeyo (16.13%), Karambo (10.71%) and Mugorore (9.09%) are the most affected villages since they are above the 5% as set by World Health Organization. Generally, there is low intensity of infection of 1-3 Schistosoma mansoni ova per preparation across most villages.

Conclusion and recommendations

The study revealed continued unacceptable high and persistent prevalence and relatively high intensity of schistosomiasis mansoni in the school aged children and adolescents. For the purpose of effective interruption of transmission, selective PZQ MDA should be extended to all levels of prevalence and all age groups and also complement PZQ to target immature stages of the adult worms.

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ACRONYMS

1. %= Percentage
2. CI= Confidence Interval
3. CMHS= College of Medicine and Health Sciences
4. IRB= Institutional Review Board
5. MDA= Massive Drug Administration
6. OR= Odds Ratio
7. P= Probability Value
8. POC-CCA= Point-of-Care Circulating Cathodic Antigen
9. PZQ= Praziquantel
10. RBC= Rwanda Biomedical Center
11. WHO= World Health Organization

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1.0 INTRODUCTION

1.1 Terms and Definitions

The following key terms and definitions apply in this research report:

Persistent transmission of schistosomiasis is defined as "the continued transmission of schistosomiasis at the community-level for any reason (e.g., residual infection, re-infection) despite multiple rounds of MDA (1)."

Schistosomiasis sometimes called **bilharziasis** or **snail fever,** (2)**,** (3) is a disease that is caused by trematode parasites of the genus *Schistosoma*, of which three major species *Schistosoma mansoni*, *S. japonicum* and *S. haematobium* cause severe and chronic schistosomiasis in humans.

1.2 Background

"Properly, schistosomiasis is the morbidity and disease that are caused by Schistosoma blood fluke infections," (4) and poses a significant public health problem. Schistosomiasis sometimes called **bilharziasis** or **snail fever,** (2)**,** (3) is a disease that is caused by trematode parasites of the genus *Schistosoma*, of which three major species *Schistosoma mansoni*, *S. japonicum* and *S. haematobium* cause severe and chronic schistosomiasis in humans. The species enter humans by attaching to the skin, penetrating it,(5), a situation characteristic of or in limited/ poor/lack/ of water settings with poor sewage disposal and inadequate supply of clean water, (6). Then migrating through the venous system to the portal veins where the parasites produce eggs and eventually, the symptoms of acute or chronic disease, (2) (7)**.** The disease is also responsible for health and socio-economic repercussions as it constitutes an important public health problem in developing countries. The disease affects the poorest of the poor and worsens their poverty,(3), (8), a vicious poverty cycle which is both a result of and contributor to poverty in the endemic communities, (9).

Worldwide there are seventy-eight (78) countries faced with endemic schistosomiasis, (3). In the said 78 countries, about 207 million people have schistosomiasis and about 800 million people are exposed to the infection, (3). Of the estimated 207 million people infected worldwide, 93% are fond in sub Saharan

Africa. Making schistosomiasis ranking second to malaria, (10) in causing serious morbidity in the sub Saharan Africa, (8),(11),(12),(13),(14),(6). *Schistosoma mansoni* alone shares 393 million people at risk of infection and 54 million people already infected, (15). In 2008, 17.5 million were treated for schistosomisis globally, 11.7 million of these were from sub-Saharan Africa only, (5). Of the Estimates of mortality are difficult to calculate, owing to the limited data available (16) but they may be as high as 150,000 per year as a result of non-functioning kidney (Schistosoma haematobium infection) and 130,000 from haematesis (S. mansoni infection) (17). Further more disturbing is that though specific treatment for the schistosomiasis is available at US 50 cents per person including delivery services, only less than 5% of those infected are receiving the coverage, (16). Focal distribution of schistosomiasis is characterized by proximities to water bodies by human kind.

In the great lakes region, parasitological studies were conducted in Uganda, Tanzania, Kenya and Burundi in 28213 children in 404 schools. The overall prevalence for schistosomiasis was 18.1% in locations adjacent to water bodies, (9). Males and older children had a significantly higher prevalence of S. mansoni infections than females and younger children. Locations of high prevalence of S. mansoni infection were clustered in the area of Albert Nile of Uganda, the shores of Lakes Kyoga and Victoria and, in Burundi, the shores of Lake Tanganyika (particularly the northern shore) and Lakes Cohoha and Rweru (in the far northeast of the country, (9).

In 1976, Egypt through its Ministry of Health and population, started its national schistosomiasis control project, including Mass Drug Administration (with praziquantel (PZQ), in most endemic areas in the upper and middle Egypt. Then in 1992 it extended its national schistosomiasis control project in all endemic areas, and in 1997, following World Health Organisation's recommendation, "the Mass Drug Administration was instituted throughout the country. The impact was rapid and substantial with *S. mansoni* prevalence in Middle and Upper Egypt falling from

an average 29.3 % in 1977 to less than 1.5 % average prevalence in the Nile Delta by 2006. However, in spite of the continued control activities this trend was not sustained. Although prevalence rates and intensity of infection remained below the baseline data, an upward trend was observed in high-prevalence villages in 1997," (14). In view of more than 20 years of continuous treatment with PZQ in the upper and middle Egypt, with no continued prevalence drop, pre-suggests continual surveys for the stubborn schistosomiasis prevalence in the high risk villages to inform continual planning of interventions or interruptions for the schistosomiasis transmission.

Trans-versing Tanzania, which ranks second after Nigeria in harboring schistosomiasis, the prevalence estimates range from 12.7% to 87.6%, among the major agents being the *S. mansoni*,(1). On stratification, the most vulnerable age categories that show both high prevalence and intensity levels are adolescents, particularly ten to fourteen years, and then decreases through adulthood, (1).

In Rwanda according to *TRAC-PLUS*−Rwanda, *2008*, intestinal schistosomiasis is known to be prevalent in many areas where fresh water breeds water snails that host the parasites. This disease is mainly prevalent around lakes and swampy areas where rice and sugar cane are grown.

1.3 Problem Statement

Whereas MDA for schistosomiasis continues, since 2008 (18), transmission of schistosomiasis also continues almost uninterrupted, except in some cases when the morbidity and intensity levels drops down but easily or with potential of rising up again due to the residue infection. This is far from the desired situation of zero transmission (elimination) of schistosomiasis or and therefore 0% incidence of schistosomiasis.

Transmission of schistosomiasis continues partially because of: relatively low infection related morbidity, with about 60% of infected patients are symptomatic

and only 10% have serious disease urging them to seek medical service(19). Insufficient and/or Lack of clean water and poor and/or lack of sanitation. Lack of health education on preventive measures of shistosomiasis. Combined with selective mass drug administration of children at school and/or non-compliance of at high risk population in most cases contributes to persistent transmission of schistosomiasis. Worse still, less sensitive, both clinical and laboratory diagnostic methods delays early detection of schistosomiasis infection and further contributes to continued transmission.

Therefore, despite considerable progress made in morbidity control of schistosomiasis in Rwanda facilitated through education and mass drug administration of Praziquantel to school-aged children and other high risk group; the disease continues to increase considerably. This is evidenced by various recent surveys, (11). To demonstrate this world health organization (WHO) reports that there were 400.000 Rwandese infected with schistosomiasis in 2001; 561939 people in 2006; 577397 people in 2007; 594262 people in 2008; 593608 people in 2009 and 610209 people in 2010 meaning an annual increase of 21020.9 population. This has inevitably resulted into clinical social economic consequences. Clinical consequences as allergic swimmers' itching, dermatitis, broncho-pneumonia, hepatomegaly, splenomegaly, anemia, growth stunting and ectopic granuloma formation including death cases. Social economic consequences as low productivity and recreation levels, low school attendance and low mental activity, depletion of scarce resources by payment of hospital bills instead of investing in economically productive activities. Low self-esteem due to low social and economic performances.

Therefore continued treatment with PZQ since 2007, (18), of school aged children with no significant continued prevalence drop, suggests need for continual surveys for the stubborn schistosomiasis prevalence, intensity levels and mapping of the high risk villages(20). The study also intends to detail which sex and age categories;

less than 5 to 17 years, 18 years to 34 years, 35 years to 44 years and above 45 years likely to be more infected.

1.4 Objectives

General objective

To explore the extent of the human mansoni schistosomasis both at the community level and the individual in proximity of Lake Muhazi.

Specific objectives

1. To determine the prevalence and individual intensity levels of schistosomiasis in villages surrounding lake Muhazi.
2. To map the high risk foci around lake Muhazi and therefore the high risk villages.

1.5 Research Questions

What is the overall current mansoni schistosomiasis prevalence and the intensity of infection?

What are the high risk foci of the schistosomisis infection transmission?

1.6 Rational of the Study

The study will inform on: the effectiveness of massive PZQ drug administration and the effectiveness the current strategy for controlling and preventing schistosomiasis in Rwanda. Provide current knowledge on prevalence and intensity levels of schistosomiasis to the environmental and health workers in their catchment areas. And finally provide basis for intervention plan for the highlighted high risk foci around lake Muhazi.

2 .0 LITERATURE REVIEW

2.1 Conceptual Framework

Schistosomiasis is a multifactorial disease, uses environmental, vector, parasitic, behavioral and host factors, (10) to continue its transmission, and therefore its infection, morbidity and prevalence. Figure 1 below summarizes the shistosomiasis transmission risk factors of the various kinds. All conceptualized in interaction between humans, snails, parasite, including complex demographic, biological, environmental, technological, political, social, economic (productivity and income) and cultural process,(21).

Further, the management of schistosomiasis inevitably must be influenced by policies taken and set to develop and control health systems, priorities and resources allocation in the health and other sectors. For example, development for water resources and its subsequent distribution to the end user may not necessarily be targeting those exposed to the water associated health hazards like cercariae (transmission stage for schistosomiasis) contaminated water.

The snail is an important biological vector hosting a complementary life cycle stage of the schistosome parasite. The snails' role in the transmission requires exploring malacology genetics, physiology, ecology and geographical distribution. The resultant knowledge is then based on for interruption of the transmission, for example, by modification of the environment in particular snails' ecosystem.

Equally the schistosome parasite and its rout in man, the definitive host, are important to understand before designing the control and preventive measures of the parasite. Particularly, knowledge in the morphological, immunogenicity, pathological, transmission and infectivity adaptations of the parasite to invade its human host. Also requiring to study is the parasite's response the human host's defense mechanisms including drugs, biological, immunity and styles.

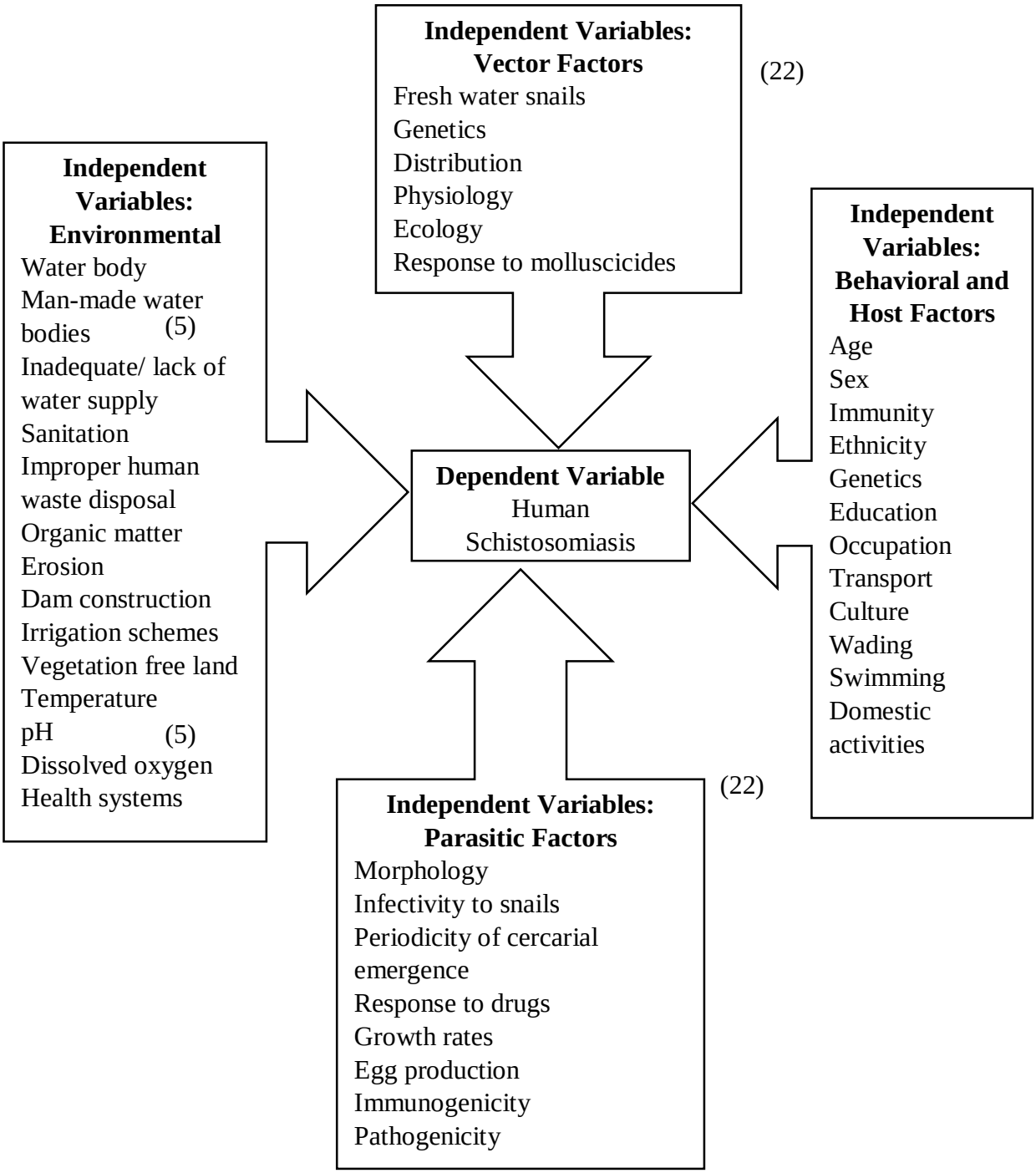

Figure 1: Schistosomiasis conceptual frame work, Source: author

The aim of this study is not to explore each and every aspect of the above factors, rather is to explore the extent of the persistent human mansoni schistosomiasis both at the community level and the individual level.

2.2 Prevalence, Age and Sex

Like elsewhere in the sub Saharan Africa, schistosomiasis mansoni is an important public health problem in Rwanda,(18). Survey results found national overall prevalence rate of 2.7% for schistosomiasis in 2007-2008, with district level prevalence ranging from 0 to 69.5% among school children (18) (10), living in close proximity to lakes Burera, Ruhondo and Muhazi and surrounding swamps (18). Specific details on the prevalence and intensity levels of schistosomiasis mansoni around lake Muhazi could not be found. The WHO sets less than 5% prevalence in all schistosomiasis endemic areas, to achieve control of schistosomiasis morbidity by the year 2020, (23). To achieve elimination of schistosomiasis, the WHO sets less than 1% prevalence of schistosomiasis in the same schistosomiasis endemic locations, by the year 2025 (23). Whereas, to achieve interruption of transmission of schistosomiasis, the WHO sets 0% incidence of schistosomiasis in the schistosomiasis endemic locations (23).

In the high risk communities, numerus influences to Schistosoma infection intensity and prevalence include, but not limited to exposure-related factors such as local environment and behavior, and factors relating to susceptibility to infection such as immunology and genetics. Human populations highly vary according to demographic characteristics, genetic background and behavior linked to exposure to infection, (21). All ages are at risk of infection with travel to endemic areas and contact with contaminated freshwater. Because; swimming, bathing, and wading in contaminated freshwater and other agricultural activities like irrigation and cultivating in swampy areas, (18), cut across the studied age categories and, can result in water body contact and infection (24).

However, studies investigating water contact behaviour in endemic communities have observed that water contacts and therefore, exposure generally decline with age, explaining age-related changes in infection and infection intensity. “Although post-adolescent declines in infection intensities have been observed in populations where adults remain heavily exposed after childhood, this was in an ecological

study where the unit of observation was the population rather than the individual," (25). In schistosomiasis infected communities, characteristic age infection curves demonstrate that infection intensifies in early adolescence and decline thereafter. This suggests that acquired immunity to the infection can develop. Indeed IgE antibody levels to the worm antigens, tend to increase with age, and this have been linked to resistance to reinfection, (25).

Schistosomiasis infection prevalence or intensity varies between sexes, living in the same community, a number studies have documented. Males frequently have heavier infections than females, (25). Sex related variation in the schistosoma infection prevalence and intensity is commonly linked to social-cultural or behavioral attributes and, therefore, to cercarial (the infective stage schistosoma) exposure, rather than the biological differences in susceptibility to infection. However, susceptibility due to the sex differences is not totally ruled out, (21). While sex-related variations in Schistosoma mansoni infection could be explained by differences in exposure, age-related differences appeared linked to the balance of specific IgE and IgG4 antibodies, themselves related to resistance to infection, (25). Lack of clean water and sanitation predisposes both sexes and all age groups to transmission equally, but exposure could differ based on cultural differences and vulnerability differs based on the acquired immunity. This research will narrate the detailed prevalence of the schistosomiasis in villages close to the lake Muhazi. Because, lake Muhazi and its bordering villages potentially and or harbors schistosomiasis.

2.3 Techniques for Laboratory Diagnosis of Schistosomiasis

Laboratory examination of stool for schistosomiasis is composed of; direct and indirect evidence, (3)(8). Indirect like macroscopic or visual examination (26), chemical examination and antibody examination. Direct like microscopic examination, antigen examination, DNA examination. Comparing parasitological methods supported by any concentration method and other available and easy methods, point-of-care circulating cathodic antigen (POC-CCA) test for screening

of schistosomiasis, POC-CCA is preferred due to its sensitivity (27). However, its limited in that it does not differentiate between S. mansoni and S. haematobium and it is short of fixing intensity levels, (26). Further it does not differentiate between active infection and passive infection, (28), Studies have shown that no single technique is 100% sensitive and specific, a combination of techniques should be used, (8).

Despite its known limited sensitivity, microscopic examination after concentration (8), gives good enough sensitivity and perfect specificity and is usually used for schistosomiasis research. Prior concentration is needed because schistosoma shade only a small number of eggs even in moderate and severe infections, (29). Several methods for concentration of the eggs of schistosoma exist, the table 1 below, using such characteristics/attributes as: Range of parasites concentrated; Concentration capacity/sensitivity; Rapidity/Number of samples concentrated in a given time; Quality of fixation; State/type of the stool sample applicable; Health and safety qualities; Applicable in a set laboratory and/or on Field, compares the several methods prior to making choice of one of them.

From the table 1 below, the evaluation, based on the criterion outlined in the first column, showed that the best concentration method for parasites eggs including the schistosoma eggs, in a set/permanent laboratory, was the formol ether concentration technique. It also showed that the best concentration technique for parasites eggs including the schistosoma eggs, on the field, was the Kato Katz concentration method. For this research, therefore, formol ether concentration technique was used since we used a permanent set laboratory.

Table 1: Comparing and selection of the concentration technique for schistosoma eggs, source, author.

METHOD / ATTRIBUTES	SEDIMENTATION TECHNIQUE E.g Formal Ether Technique	FLOATATION TECHNIQUE E.g Saturated Sodium chloride	KATO KATZ TECHNIQUE	SELECTED TECHNIQUE	REFERENCE
Range of parasites concentrated	A wide range of parasites; cysts, eggs (schistosomes inclusive), oocysts, larvae	Suitable is the formol detergent gravitational technique only.	A wide range of parasites are semi-concentrated	Formol ether tech	(29), (15)
Concentration capacity/sensitivity (8)	1g of faeces used	About 0.3g of faeces used	0.1-0.5g faeces used	Formol ether tech	
Rapidity/Number of samples concentrated in a given time	Rapid	Slow	Rapid	Formol ether/Kato katz	
Quality of fixation	Minimal damage to the morphology	Not fixed	Not fixed	Formol ether tech	
Application: State/type of the stool sample	All stool types	All stool types	Formed/soft stool samples	Formol ether tech	
Health and safety qualities	Safe (organisms have been killed) and hygienic	Unsafe and unhygienic alot precautions needed	Unsafe and unhygienic a lot of precautions needed	Formol ether tech	
Location: in set lab and or Field	In set labs not practical in the field	Applicable on field but slow	Applicable on field and rapid	Kato Katz tech	

3.0 METHODOLOGY

3.1 Study Area

The area studied were in Rwamagana, Kayonza, Gatsibo, Gasabo and Gicumbi districts. The villages were Ubwiza, Gasharu-Murambi, Busharu and Babasha villages in Rwamagana district. Butimba village in Kayonza district, Gasave, Umwiga and Buburankwi villages in Gatsibo district all in the Eastern Province. Gasharu-Kibara, Bwingeyo, Bwimiyange villages in Gasabo district in the Kigali City. Mugorore, and Karambo villages in Gicumbi district in the Nothern province, Fig. 2 below shows the locations. The lake Muhazi and swampy areas around it provides the bleeding sites for the snails which are the intermediate hosts of the parasites.

It was in these villages because they were found around and in proximity of lake Muhazi, therefore, raising high risk probability for water body contact and consequently continual transmission of schistosomiais to the villages' inhabitants, if and when the water is infested with cercaria, the infective stage of schistosomiasis. This was the reason and not because of anything else. In these villages, the lake provides fishing occupation and the swamps provides the rice cultivation to the community in the vicinity, both activities increase water body contact and therefore transmission of the parasite. Furthermore, the water is used for recreation (swimming) opportunity, and in most cases it is the only water source for domestic usage. Worth noting also was lack of proper human waste disposal and poor or lack of other sanitary drainage system that result in contamination of the lake.

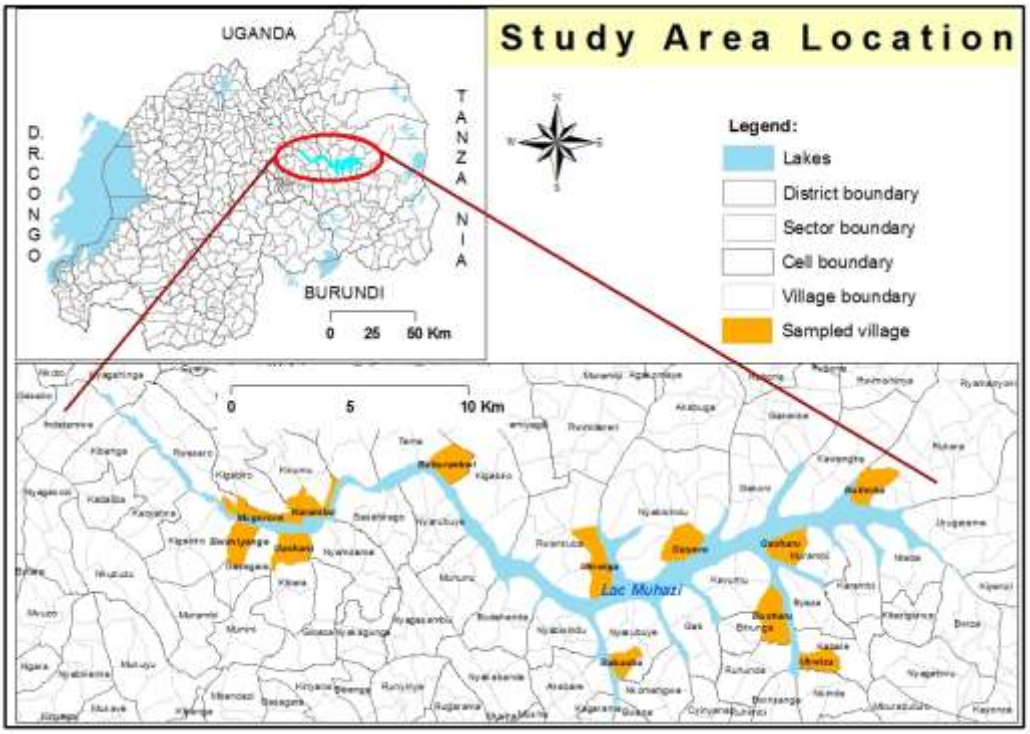

Figure 2: The purposively sampled villages in the studied area

3.2 Study Design

A cross sectional study was used to quantitatively explore the extent of the persistent human mansoni schistosomiasis both at the community level and the individual level.

3.3 Study Population

Both sexes above age five mounting to three hundred eighty-four (384) participants, were drawn from the thirteen villages, close to the lake Muhazi. The age ranged from eight (8) years to eighty (80) years, with the mean age of thirty-one (31) years and standard deviation of 17 years.

Inclusion Criteria

We included both sexes, above age five who permanently live in the purposely selected villages and willing fully consented to participate in the study.

Exclusion Criteria:

We excluded only those who were not showing willingness to participate and those visitors that were not as exposed as the indigenous. Further, children under five years were not required to participate in this study.

3.4 Sampling Strategy

From the study area above, we randomly selected thirteen potentially high risk cells and/or foci, found around and in the proximity of lake Muhazi. From the randomly selected cells, villages touching the lake that were likely to sustain transmission through human-water body contact, were purposively selected for the study participants. Proportionally, according to each villages' population, participants were calculated and drawn randomly from each of the selected thirteen villages.

Table 2 below shows the proportionate sampling. Each participant was asked to provide a random stool sample.

Table 2: The proportionate sample size from each village

#	Province	District	Sector	Cellule	Village	Estimated Population	Sample Size
1	East	Gatsibo	Kiramuruzi	Nyabisindu	Gasave	842	30
2	East	Gatsibo	Gasange	Teme	Buburankwi	783	27
3	East	Gatsibo	Murambi	Rwankuba	Umwiga	1170	41
4	East	Kayonza	Rukara	Rukara	Butimba	1188	42
5	East	Rwamagana	Muhazi	Kabare	Ubwiza	923	32
6	East	Rwamagana	Muhazi	Murambi	Gasharu	581	20
7	East	Rwamagana	Gishali	Binunga	Busharu	860	30
8	East	Rwamagana	Munyiginya	Nyarubuye	Babasha	612	21
9	North	Gicumbi	Bukure	Rwesero	Mugorore	617	22
10	North	Gicumbi	Bukure	Kivumu	Karambo	797	28
11	Kigali City	Gasabo	Gikomero	Kibara	Gasharu	770	27
12	Kigali City	Gasabo	Gikomero	Gasagara	Bwingeyo	896	31
13	Kigali City	Gasabo	Gikomero	Gasagara	Bwingeyo	896	31
	Total					**10935**	**384**

3.5 Data Collection Methods and Procedure

Using a template in the annex 1, we recorded the coordinates of the often visited site of the water body for subsequent mapping of the high risk foci and therefore the villages for that matter. In the same template, we also recorded both demographic data asked from participants and data generated from the laboratory diagnosis of the stool samples. The stool samples collected from the participants and provided in the stool containers were labeled with identification number or code, age, sex, date and village. The standard operating procedure for the formol ether concentration technique and the stool examination was detailed in annex 2.

3.6 Study Sample

The sample size was determined using Taro Yamen method/formula:

$$n = \frac{\mathrm{N}}{1+\mathrm{N}(e)^2}, \quad (30)$$

Where:

N is total number of village (umudugudu) population

E is margin error of sampling= 0.05

n is sample size

According to the national institute of statistics of Rwanda (NISR), as at 2012 census, the total population of the villages, N was 10,935 with 14.6% of the total population being under five years. Therefore, the legible population was 85.4% of the total population.

$$\text{Legible population: } = \frac{85.4}{100} \text{x10,935} = 9{,}338$$

And the sample size, n was not below;

$$n = \frac{9338}{1+10935(0.05)^2} = 384 \text{ participants.}$$

3.7 Laboratory Diagnosis

Laboratory diagnosis was done by microscopic examination after concentration. We collected the samples, fixed them immediately with the formol solution at the place of collection, transported them fixed to the set laboratory, concentrated them with formol ether technique prior to examining them under a microscope for

detection and identification of schistosoma ova or eggs by their characteristic morphology. The slides that were positive were reviewed for correctness of data on the stool container including identification number or code, age, sex, date and village against the same range of data on the collection data sheet. We also controlled the identity of ova seen using the WHO bench aids for the diagnosis of intestinal parasites, (31)

3.8 Data Analysis

The obtained data were analysed by stata version 13 and excel 2016 software to generate histograms. The chi square test was used at the level of significance of 0.05 to compare proportions and odds ratio to determine the strength of association between variables. Spatial data was analysed by the geographical information system Arc Map 10.5.

3.9 Limitations and Problems

3.9.1 Limitations

Insensitiveness of the parasitological or the morphological method this study used to detect the cases of schistosomiasis mansoni. The search for sensitive and specific diagnosis of the schistosomiasis is ongoing, (3). Moreover, the morphological methods for the schistosomiasis detection, were short of detecting early stages of schistosomiasis that is cercarial penetration, schistosomules and adult worms migration to the target sites, until eggs are released in the faeces.

3.9.2 Problems

The 35-45 years' group, is likely to be the daily bread winner and therefore less participation in the study. And there was also scarcity and/or lack of data on schistosomiasis in Rwanda, especially the intensity of schistosomiasis.

3.10 Ethical Consideration

We obtained ethical clearance, a copy of which is herewith attached, from the Institutional Review Board (IRB) of College of Medicine and Health Sciences

(CMHS) and obtained authorization from local authorities before data collection. Adult participants and or guardians consented and appended their signature on the consent form. For those who could not write consented verbally and signed by a finger print. Permission for use of biomedical laboratory sciences, a copy of which is herewith attached, was also got from the school of health sciences college of medicine and health sciences.

4.0 RESULTS

4.1 The Study Sample Distribution

Three hundred eighty-four (384) participants consented to participate and provided stool samples for examination. The participants included both sexes and range from 8 years to 91 years. Table 4 and Fig 3 below shows the overall distribution of the study sample, across age groups and sexes, that participated in the study.

Table 3: The study sample distribution across both sex and age groups

Age Categories	Female	Male	Total
	Number/ %	Number/ %	Number/ %
>5-17 Years	64 52.03	59 47.97	123 32.03
18-34 Years	55 55.00	45 45.00	100 26.04
35-45 Years	34 54.84	28 45.16	62 16.15
>45 Years	51 51.52	48 48.48	99 25.78
Total	204 53.13	180 46.88	384 100.00

P= 0.945

The figure 3 below shows that in the results study sample; both sexes almost equally participated in the study, across all age groups. It also shows that all age groups, rather than the, 35-45 years' group, participated fairly equally. The 35-45 years' group, is likely to be the daily bread winner and therefore less participation. However, the overall distribution of participants across age groups and sex was not statistically significant, P= 0.945.

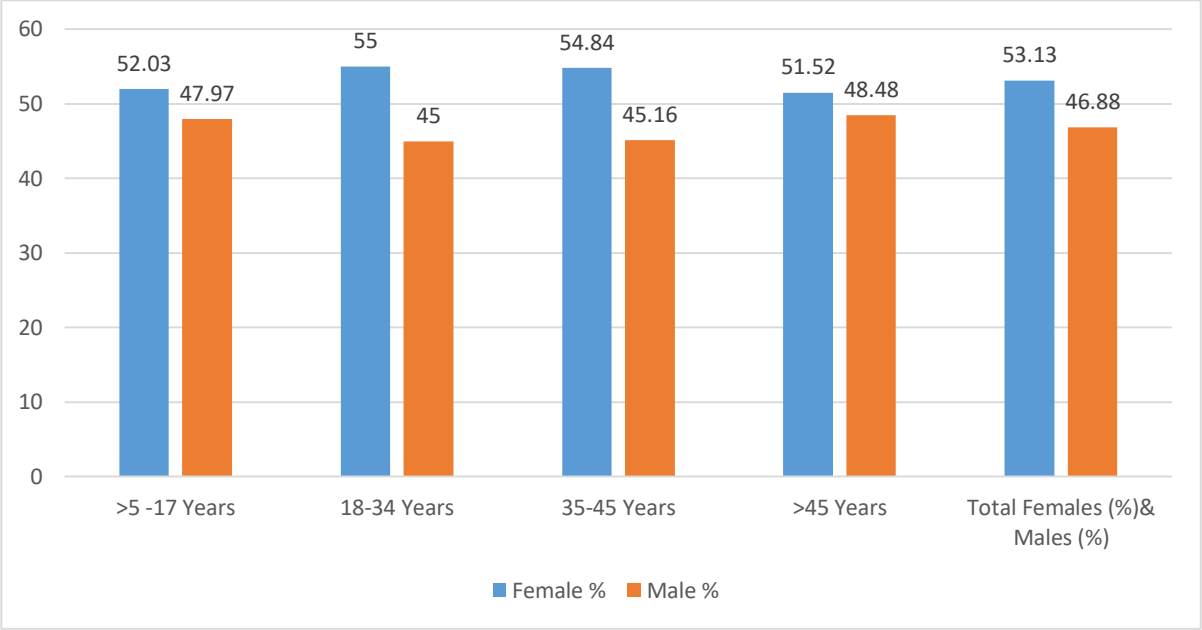

Figure 3: The study sample distribution across both sex and age groups

4.2 General Prevalence of *Schistosoma Mansoni* Infection

Table 4 below shows that the general prevalence of *S. mansoni* infection was 7.03%.

Table 4: General prevalence of *Shistosoma mansoni* infection

Schistosoma mansoni	Frequeny	Percentage
Stool positive	27	7.03
Stool negative	357	92.97
Total	384	100

The subsequent section 4.3 below presents the schistosomiasis infection results across the study area and the study participants at length.

4.3 *Schistosoma Mansoni* Infection prevalence and Intensity

4.3.1 *Schistosoma Mansoni* Infection Prevalence Across Villages

Table 5 and Fig. 4 below shows 7.03% overall prevalence of Schistosoma mansoni infection across the studied villages, with village's prevalence ranging from 0% to 23.33%. The difference of the S. mansoni prevalence, between all villages, statistically differ at P= 0.002. Out of the thirteen villages surveyed, five are high risk villages: Gasharu-Kibara (with prevalence: 23.33%), followed by Bwimiyange and Bwingeyo (with prevalence: 16.13), Karambo (with prevalence: 10.71%) and Mugorore (with prevalence: 9.09). Five of the villages, the prevalence of schistosomiasis, range from 2.44% to 3.7%. Three villages; Butimba, Gasharu_Murambi and Bubasha show no prevalence (0%) of schistosomiasis.

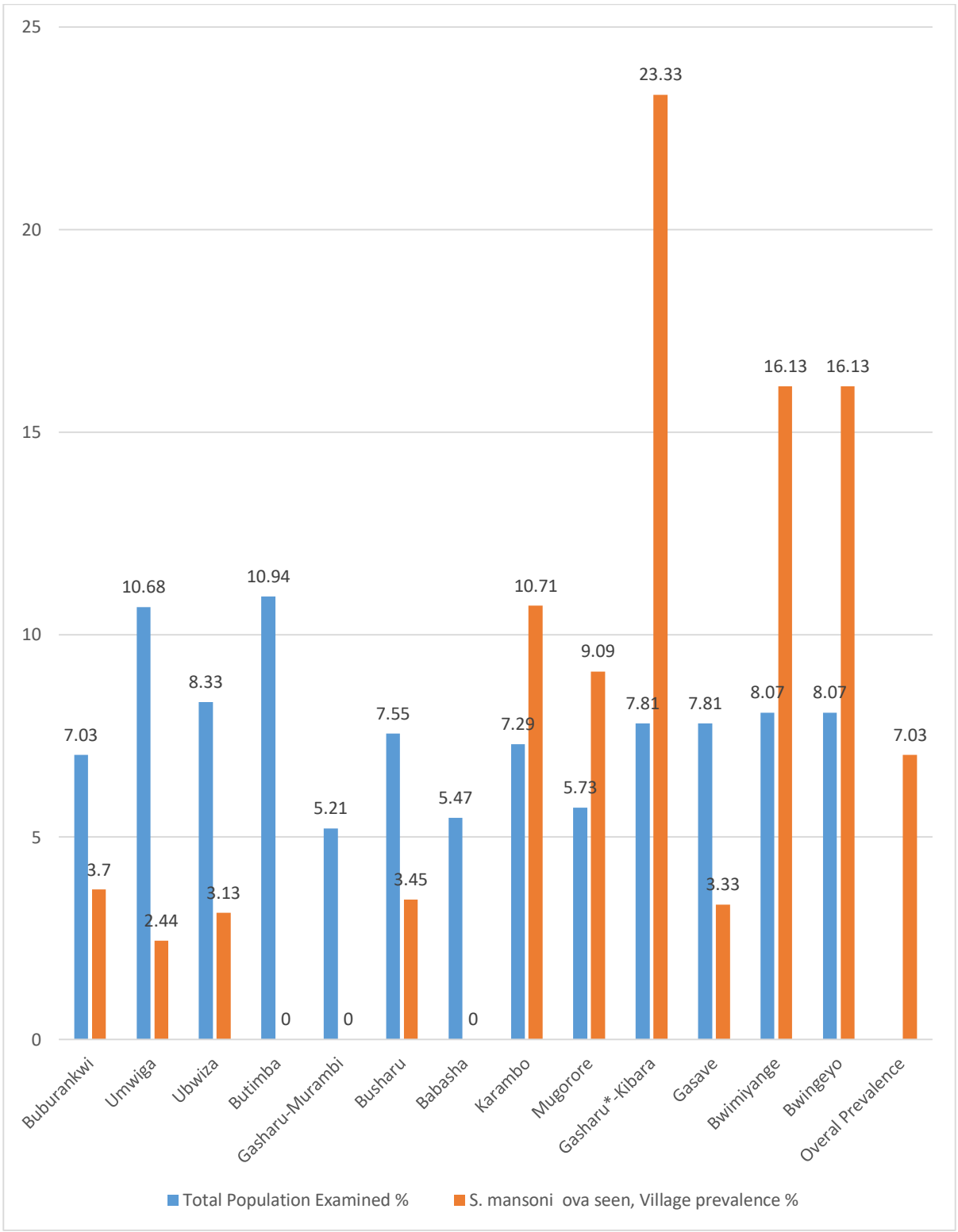

Figure 4: Prevalence of *Schistosoma mansoni* infection across villages

Table 5: *Schistosoma mansoni* infection prevalence and intensity across villages

village	Total Population Examined		*S. mansoni* ova seen, Village prevalence		Intensity/level of *S. mansoni* Infection					
					S. mansoni ova 1-3 seen		*S. mansoni* ova 4-10 seen		*S. mansoni* ova 11-20 seen	
Number/ Percentage	#	%	#	%	#	%	#	%	#	%
Buburankwi	27	7.03	1	3.70	1	3.70	0	0	0	0
Umwiga	41	10.68	1	2.44	1	2.44	0	0	0	0
Ubwiza	32	8.33	1	3.13	1	3.13	0	0	0	0
Butimba	42	10.94	0	0	0	0	0	0	0	0
Gasharu-Murambi	20	5.21	0	0	0	0	0	0	0	0
Busharu	29	7.55	1	3.45	1	3.45	0	0	0	0
Bubasha	21	5.47	0	0	0	0	0	0	0	0
Karambo	28	7.29	3	10.71	3	10.71	0	0	0	0
Mugorore	22	5.73	2	9.09	2	9.09	0	0	0	0
Gasharu*-Kibara	30	7.81	7	23.33	4	13.33	3	10.00	0	0
Gasave	30	7.81	1	3.33	1	3.33	0	0	0	0
Bwimiyange	31	8.07	5	16.13	3	9.68	2	6.45	0	
Bwingeyo	31	8.07	5	16.13	4	12.90	1	3.23	0	0
Overall Prevalence	384	100	27	7.03	21	5.47	2	0.26	5	1.30

P= 0.002 P= 0.014

4.3.2 *Schistosoma Mansoni* Infection Prevalence Across Age Groups

Table 6 and Figure 6 below shows the analysis of cases of schistosomiasis infection across four age categories; 13.01% cases are under 18 years and above five years, about fifteen times greater than the above 45 years' group cases of up to 1.01%, odds ratio (OR) 0.07, confidence interval (CI) 0.01 to 0.52. 35 to 44 years' category has cases of up to 4.84%, with the OR 0.34, CI 0.01 to 1.21, that is about three times less infected than the young age. 18 to 34 years' category has cases of up to 7.00%, with the OR 0.50, CI 0.20 to 1.30, and about two times less infected than the young age. Needless to mention is the marked infection prevalence in the young age group with a steady decreasing prevalence level with the increasing age, statistically significant at P= 0.005.

Table 6: Prevalence Schistosoma mansoni infection across age groups

Age Groups	Total Examined: Number/%	S. mansoni ova seen: Number/ %	Odds ration	95% Confidence Interval
>5 -17	123	16		
Years	32.03	13.01		
18-34	100	7		
Years	26.04	7.00	0.50	0.20 to 1.30
35-45	62	3		
Years	16.15	4.84	0.34	0.01 to 1.21
>45	99	1		
Years	25.78	1.01	0.07	0.01 to 0.52
Total	384	27		
	100.00	7.03		

P= 0.005

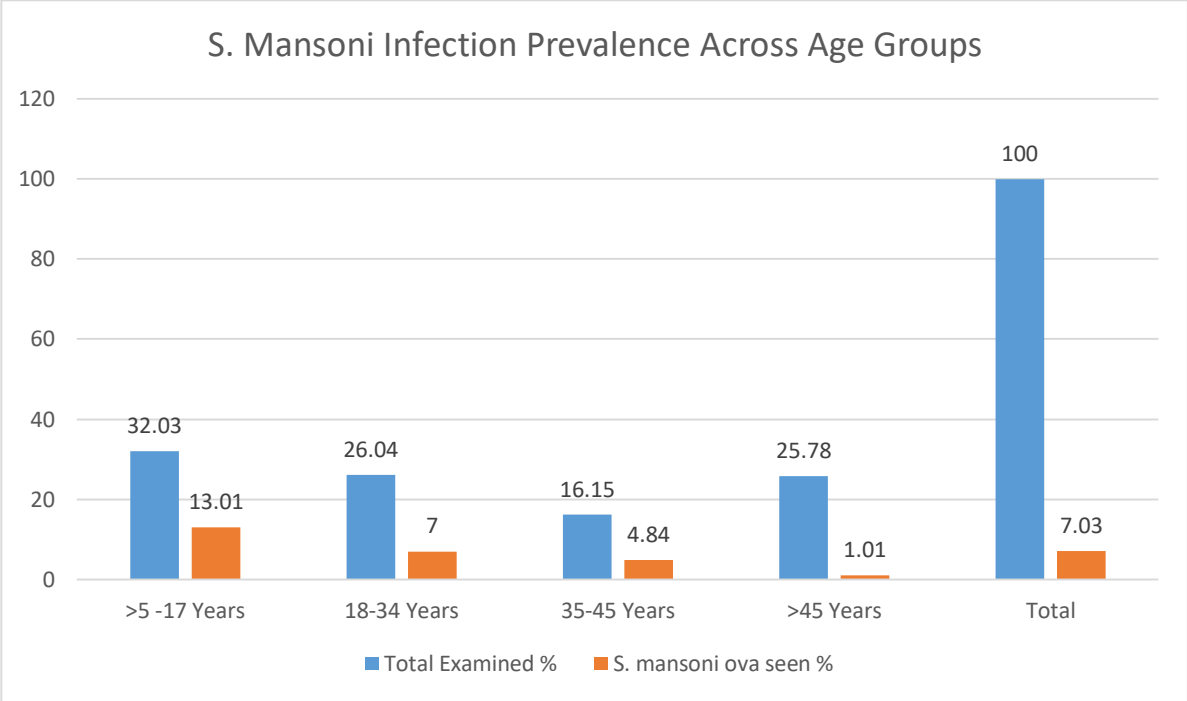

Figure 5: Prevalence of *Schistosoma mansoni* infection across age groups

4.3.3 *Schistosoma Mansoni* Infection Prevalence Across Sex

On the other hand, however, across all the age groups are both sexes; overall schistosomiasis infection prevalence was more in males (8.33%) than in the females (5.88%), table 8 and figure 8 below, OR 1.45 CI (0.66 to 3.20) at 95% confidence level, P= 0.351, well above the critical p=0.05, showing statistically no significant difference in schistosomiasis infection between sexes across all villages.

Table 7: Prevalence of *Schistosoma mansoni* infection across sex

Sex	Total Examined: Number/%	S. mansoni ova seen: Number/ %	Odds Ratio (OR)	95% Confidence Interval
	180	15	1.45	0.66 to 3.20
Male	46.88	8.33		
	204	12		
Female	53.13	5.88		
	384	27		
Total	100	7.03		

P= 0.351

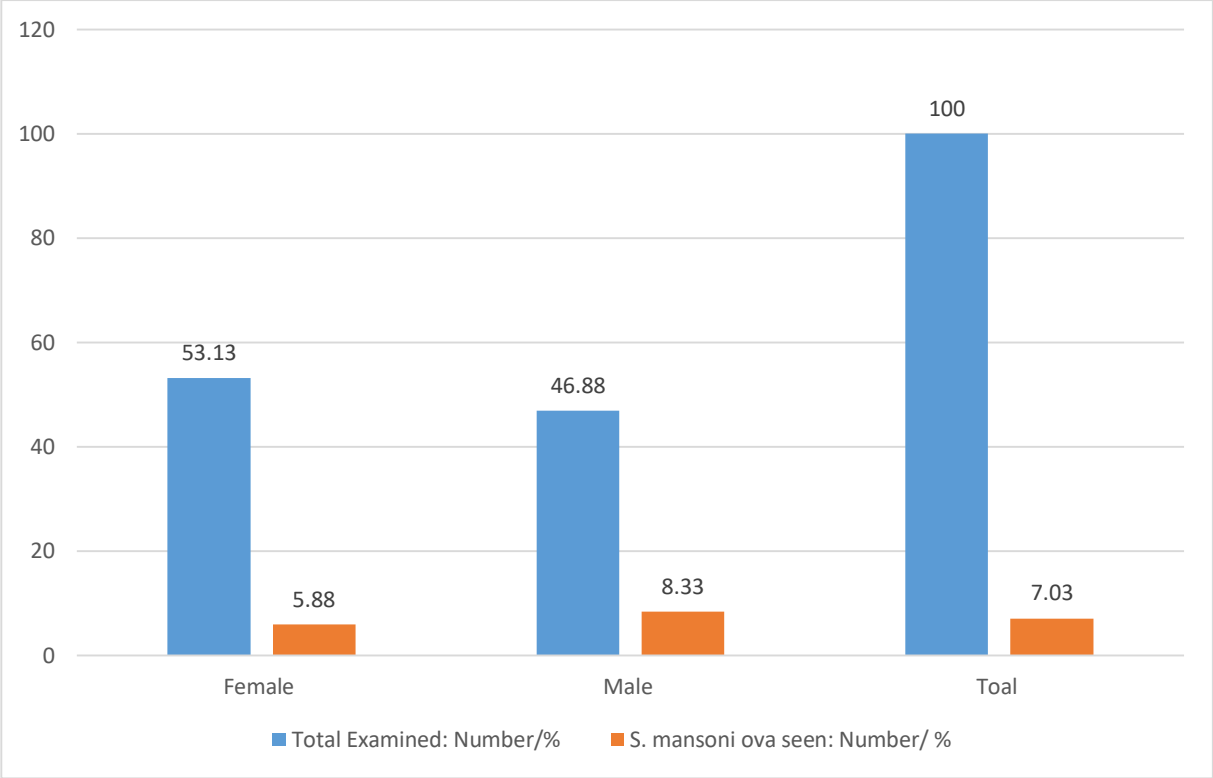

Figure 6: Prevalence of *Schistosoma mansoni* infection across sex

4.3.4 *Schistosoma Mansoni* Infection Intensity Across Age Groups

Table 7 and Figure 7 below shows the intensity of S. mansoni infection across the four age groups. The range of intensities estimated by the test method are 1-3, (the lowest infection intensity), 4-10, 11-20, 21-40, and >41, (the highest infection intensity), ova per preparation. It was shown that there was generally low overall intensity of infection across all age groups, in most cases 1-3 (5.47%), few of 4-11 (0.26%) and of 11-20 (1.3%) ova per preparation. The variation is statistically significant, P= 0.044. No infection level of 21-40 and over 40 ava was found. However, the young age group <5-17 years, had relatively highest intensity of infection (4-10 ova per preparation (5.47%) and 11-20 ova per preparation (2.44%), and a general decrease in the intensity level according to the increasing age.

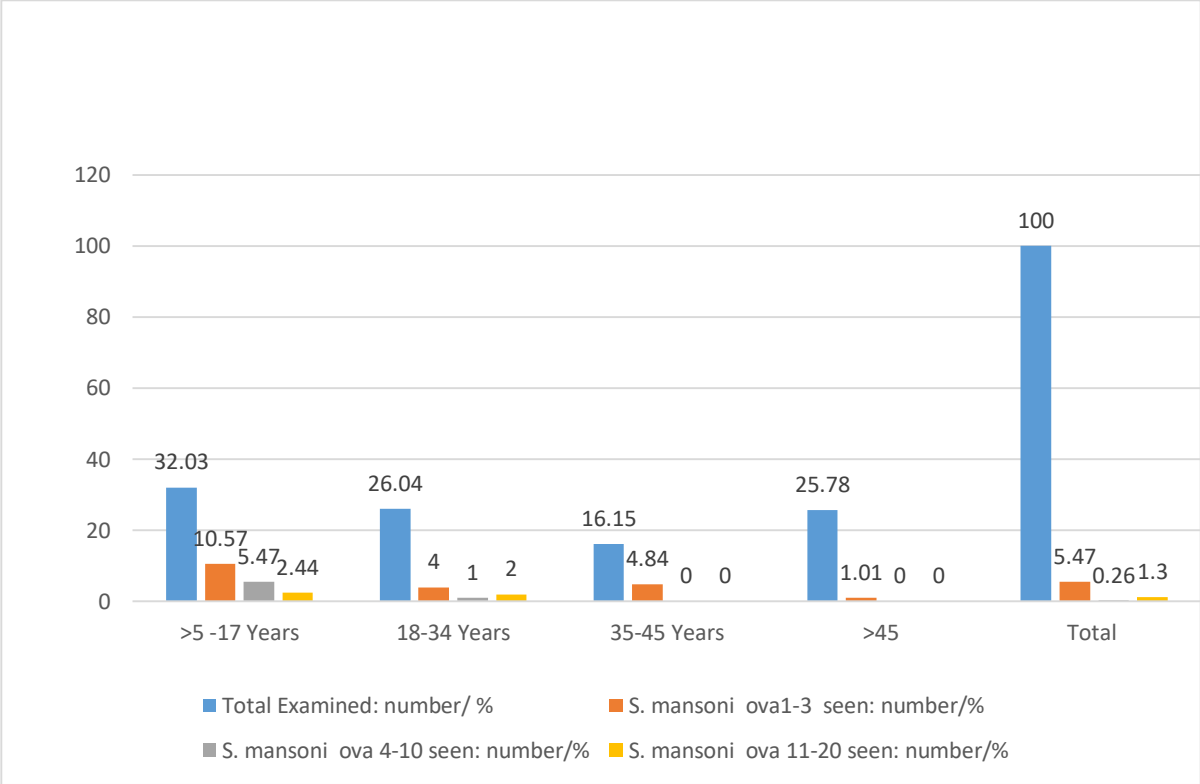

Figure 7: Intensity of *Schistosoma mansoni* infection across age groups

Table 8: Intensity of *Schistosoma mansoni* infection across age groups

Age Groups	Total Examined: Number/ %	*S. mansoni* ova 1-3 seen: number/%	*S. mansoni* ova 4-10 seen: number/%	*S. mansoni* ova 11-20 seen: number/%
>5 -17	123	13	0	3
Years	32.03	10.57	5.47	2.44
18-34	100	4	1	2
Years	26.04	4.00	1.00	2.00
35-45	62	3	0	0
Years	16.15	4.84	0.00	0.00
>45	99	1	0	0
Years	25.78	1.01	0.00	0.00
Total	384	21	1	5
	100.00	5.47	0.26	1.30

P= 0.044

4.4 Mapping the High Risk Villages for Contacting *Schistosomiasis Mansoni*

Fig. 9 below show the geographical distribution of schistosomiasis mansoni. Gasharu-kibara (23.33%), Karambo (10.71%), Bwimiyange (16.13%), Bwingeyo (16.13%), Buburankwi (3.7%), Mugorore (9.09%), Ubwiza (3.13%), Busharu (3.45%), Umwiga (2.44%), Gasave (3.33%), Butimba (O%), Murambi (0%), Bubasha (0%).

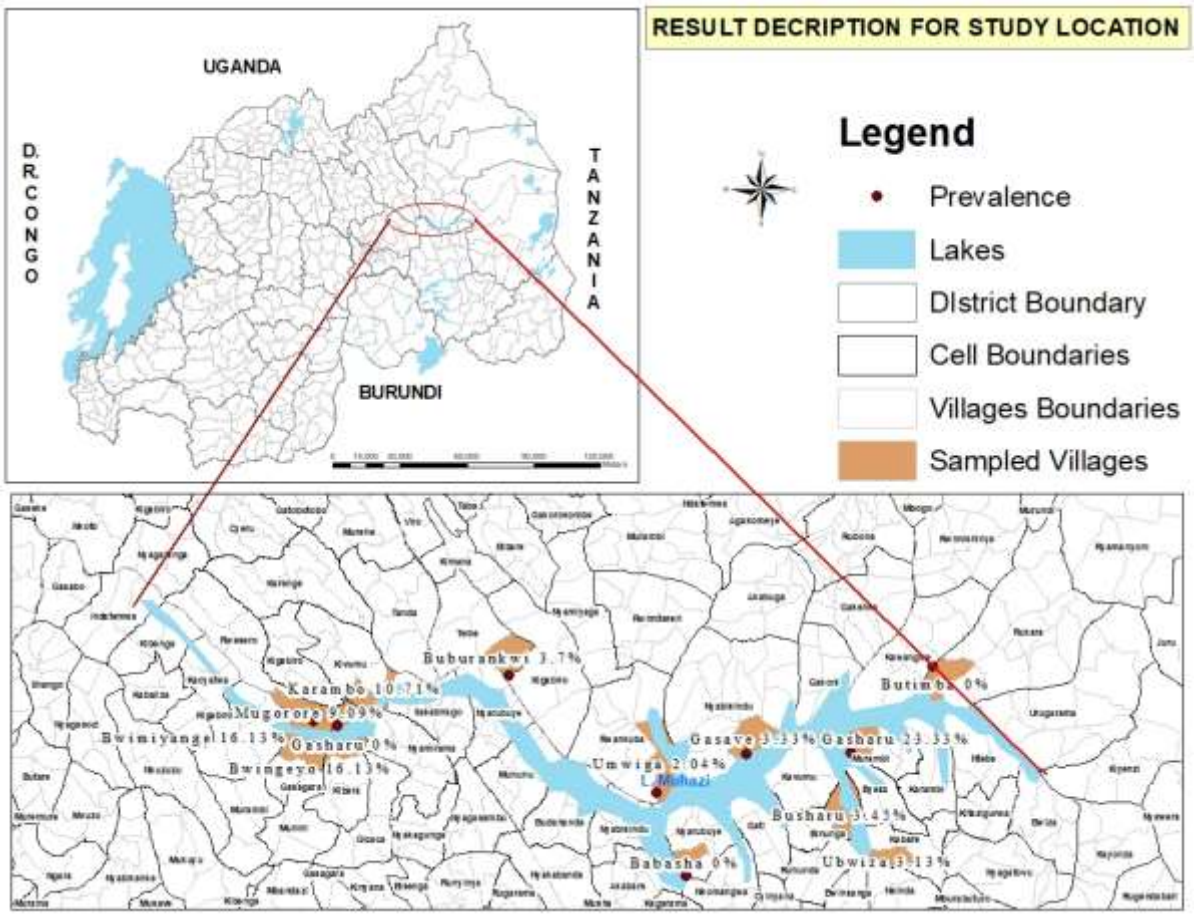

Figure 8: The geographical distribution (mapping) of *Schistosoma mansoni* infection close to lake Muhazi

5.0 DISCUSSION

The general prevalence, 7.03% is above 5%, the critical prevalence set by the WHO to achieve control of schistosomiasis morbidity by the year 2020 (23). Which shows that generally there is a degree of schistosomiasis morbidity among the population living close to the lake Muhazi. Rwanda, through its Ministry of Health and Rwanda Biomedical Centre (RBC), had committed herself to control Schistosomiasis by use of MDA using PZQ in 2008-2010 (11). This has been markedly successful, both with regard to infection intensity/ and or morbidity and to some extent prevalence. However, this is as far as country wide is concerned, there still remains a number of high risk locations being overshadowed by results from other parts of the country without or with negligible prevalence rates. Being a multifactorial, schistosomiasis disease, uses environmental, vector, parasitic, behavioral and host factors, (10) to continue its transmission. And therefore its infection, morbidity and prevalence, despite the continued PZQ massive drug administration (MDA (18), (20) is statically significant at P= 0.002. This could partly explain the presence and/or the persistence of such high mansoni schistosomiasis villages' prevalence, of 9.09% and more. This study revealed Gasharu Kibara, followed by Bwimiyange and Bwingeyo, Karambo and Mugorore, are the high risk villages since they are above the 5%. Same villages also show high intensities of infection, statistically significant at P= 0.014, and accordingly possible high transmission rate too.

The other villages, Buburankwi, Umwiga, Ubwiza, Busharu and Gasave, whose prevalence of schistosomiasis, range from 2.4% to 3.7%, also should attract attention for intervention, because of their residue infections sustaining transmission. the WHO sets less than 1% prevalence of schistosomiasis to achieve elimination of schistosomiasis by the year 2025 (23). Busharu-Murambi, Butimba and Bubasha villages with 0% prevalence also should attract attention to maintain the zero incidences and consideration of measures to prevent them from overshadowing the high risk locations or communities in a given village. The interventions could be like similar and more comprehensive studies to uncover the risk locations and subsequent appropriate

massive chemotherapy administration, that complements PZQ, to target immature stages of the adult schistosome worms.

Stratification of the cases of the schistosomiasis, according to age groups, statistically differ significantly P= 0.005 and, point to the young people, between <5-17 years, as the one that pivoted in the schistosomiasis, 10.57%, about fifteen times greater than the >45 years' s group 1.01%, odds ratio (OR) 0.07. Thanks to the chemotherapy PZQ intervention, the same group has been targeted from 2007 with 69.5% in school children in highest prevalence village/school of the time. However, this decrease especially in the young age group, when compared to the almost 100% intervention coverage of PZQ MDA, (11) in 2008 and beyond, indicates that though the morbidity and infection intensity has been generally managed (we could not find infection intensity data to compare with this study's data however), the transmission remain largely uninterrupted hence the persistence of the schistosomiasis. This could be blamed to the school aged children, but not actually at school and other age categories especially beyond school age children and uncovered risk locations and communities that maintain the transmissions of schistosomiasis.

According to the sex, though more females participated in the study than the males, males indicated higher prevalence of schistosomiasis than females. This could be due to more males-water contacts than it is with females rather than males being more vulnerable than females to cercarial penetration (cercaria is the infective stage of schistosoma) and subsequent infection. Whereas, the general decrease in both the prevalence and intensity of infection with the increasing age, usually with the peak at the adolescent, is characteristic of age-helminths infection curves (schistosoma are helminth). The curves show development of acquired immunity.

For chemotherapeutic intervention comparison, in Egypt, "PZQ MDA is recommended if the prevalence of schistosomiasis exceeds 3 % compared to 20 % according to the WHO recommendations," (14), and 30% for non-school children and 20% for school children in Rwanda. Further, in support of this, is insensitiveness of the parasitological

or the morphological method this study used to detect the cases of schistosomiasis mansoni. The search for sensitive and specific diagnosis of the schistosomiasis is ongoing, (3), (32). Moreover, the morphological methods for the schistosomiasis detection, are short of detecting early stages of schistosomiasis that is cercarial penetration, schistosomules and adult worms migration to the target sites, until eggs are released in the faeces. This suggests that, the actual prevalence and infection intensity could be higher than those found and stated in this study.

6.0 CONCLUSION AND RECOMMENDATIONS

i. Unacceptable high prevalence of schistosomiasis in the four villages and more especially: Gasharu-Kibara, Bwingeyo, Bwimiyange and Karambo. These high risk locations call for better and improved attention in interventions to address not only schistosomiasis morbidity but also interruption of its transmission by more comprehensive strategies. PZQ MDA is known to control morbidity but needs complementary preventive measures to eliminate schistosomiasis like control of snails, improved sanitation and provision of alternative water sources to reduce human- lake Muhazi-water contact.

ii. For the purpose of effective interruption of transmission, selective PZQ MDA should not only be extended to all levels of prevalence and to all age groups rather than the current critical level of prevalence of $\geq 30\%$(11), but also complement PZQ to target immature stages of the adult worms.

iii. Unacceptable high and persistent prevalence and relatively high intensity of infection of schistosomiasis mansoni in the young age groups. This could be due to residue infections that continue to ensure shading of eggs that finally end in the lake and transmission continues. Further, investigations are required to improve on early detection of schistosomiasis in addition to treatment of early stages of schistosomiasis.

7.0 REFERENCES

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ANNEX 1: DATA SHEET FOR HUMAN MANSONI SCHISTOSOMIASIS IN PROXIMITY OF LAKE MUHAZI

Date:

Umudugudud (Village):

Akagari (Cell):

Umurenge (Sector):

Akarere: (District):

Coordinates for Commonly Visited Site on the Lake Muhazi:

Easting	Northing	Elevation

Participant Code	Sur Name	Other Name(s)	Sex/ Age	RESULTS

SOP 5502-1 STOOL EXAMINATION

1.0 TABLE OF CONTENTS

2.0 RECORDS OF CHANGES/REVIEW

NO.	DETAILS OF CHANGE		AUTHORISATION	DATE
	Page	Clause Number and comments	Name & signature	
1				
2				
3				
4				
4				
5				
6				
7				
8				

3.0 PURPOSE

3.1 Stool specimens are used to examine patients or clients for intestinal helminthic and/ or protozoal parasites and also for those helminthic parasites that localize in the biliary tract and discharge their eggs into the intestines.

4.0 SCOPE AND FIELD OF APPLICATION

This SOP is applicable to:

4.1 Correct collection, transport and storage of the stool specimens.

4.2 Stool specimens, both fresh and preserved stools.

4.3 Intestinal parasitic infections and also for those helminthic parasites that localize in the biliary tract and discharge their eggs into the intestine.

4.4 Direct examination of fresh specimen and formalin/ether concentration technique.

This SOP does not allow permanent staining procedures of parasites.

5.0 REFERENCES

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5.2 Monica Cheesbrough, Laboratory Practice in Tropical Countries Part 1, 2nd edition, 2009.

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6.0 ASSOCIATED DOCUMENTS

6.1 QMP 5200-2 Health and Safety of Personnel.

7.0 TERMS AND DEFINITIONS

Not applicable

8.0 SAFETY PRECAUTIONS

Adhere to GOOD AND CAREFUL LABORATORY PRACTICE at all times while carrying out the procedure specified in this standard. For full health and safety regulations code see **QMP 5200-2 Health and Safety of Personnel.**

9.0 INGREDIENTS AND PREPARATION

9.1 Formalin, 10% v/v.

Prepare by mixing 50 ml of strong formaldehyde solution with 450 ml of distilled or filtered rain water.

9.2 Diethyl ether

Use as supplied.

9.3 Iodine (Dobell's) for faecal preparations

9.3.1 Iodine Crystals 2 gm

9.3.2 Potassium iodide 4 gm

9.3.3 Distilled Water 100 ml

9.3.4 Weigh the potassium iodide and dissolve completely in about 50 ml of the water.

9.3.5 Weigh the iodine and add to the potassium iodide solution. Mix well to dissolve.

9.3.6 Caution: Iodine is injurious to health if inhaled or allowed to come in contact with the eyes, therefore handle with care in a well ventilated room.

9.3.7 Add the remainder of the water, and mix. Transfer to a brown bottle.

9.3.8 Label the bottle, and mark it Harmful. Store in the dark at room temperature. The reagent is stable for several months.

9.3.9 For use: Transfer to a small brown bottle with a cap into which a dropper can be inserted.

9.4 Physiological saline, 8.5 g/l, (0.85%)w/v

9.4.1 Sodium chloride ..... 8.5 g

9.4.2 Distilled water ..... to 1 litre

9.4.3 Weigh the sodium chloride, and transfer it to a leak-proof bottle premarked to hold 1 litre.

9.4.4 Add distilled water to the 1 litre mark, and mix until the salt is fully dissolved.

9.4.5 Label the bottle, and store it at room temperature. The reagent is stable for several months. Discard if it becomes contaminated.

9.5 SUPPLIES & EQUIPMENTS

11.1 Glass slides (25 by 75mm).
11.2 Sieve (strainer) with small holes, preferably400–450————————————m in size
11.3 Pasteur pipettes and wood applicator sticks,
11.4 Marker
11.5 Discarding jars,
11.6 Centrifuge
11.7 Vortex mixer
11.8 Clean polypropylene conical tubes with screw cap
11.9 Scissors.
11.10 Funnels of preferably plastic material
11.11 Cover slips
11.12 Timer
11.13 Binocular Microscopes

10 PROCEDURE

10.1 COLLECTION, TRANSPORT, RECEPTION AND STORAGE OF THE STOOL SPECIMENS

10.1.1 Use specimen containers that have wide mouth, are leak-proof, clean, dry, and free from traces of antiseptics and, disinfectants.

10.1.2 Collect the stool with a clean tongue blade or similar object, collect 3 specimens, at 2 to 3 day intervals before a patient is declared free of parasites; unless it is a control specimen.

10.1.3 Tell the patient to collect enough stool sample, at least the size of pigeons egg and not to wrap the sample in any kind of material especially absorbent material.

10.1.4 All samples are accompanied by a requisition form from the physician and /or sample reception giving relevant clinical details and recent travel history.

10.1.5 Samples and forms from patient with a confirmed or suspected diagnosis of certain infectious diseases such as AIDS or hepatitis are clearly labeled with "Biohazard."

10.1.6 Reject the stool contaminated with urine, any dirt; water or other body secretion such as menstrual blood or wrapped in the material and ask the patient to pass another specimen.

10.1.7 Any whole worms or segments passed should be placed in a separate container.

10.1.8 Receive the acceptable specimens and label them carefully with the patient's name and identification number, and also the date and time of collection.

10.1.9 Protect specimens from direct sunlight and heat.

10.1.10 Route the specimens for examination as soon as possible, more especially the blood stained stools, mucus stools, liquid and semiliquid stools must reach testing area within 30 min after passage.

10.1.11 Formed samples can be kept in a refrigerator at 4 C° for a short time, but not in incubator. Add 10% formalin as the suitable fixature or preservative to feces specimen to retain morphology, if time lapse between collection and examination is considerable, i.e. more than 1 day.

10.2 DIRECT EXAMINATION OF FRESH STOOL SPECIMEN

10.2.1 MACROSCOPIC EXAMINATION OF STOOL SAMPLES

10.2.1.1 Put on gloves when carrying out this procedure.

10.2.1.2 Check whether the date and time of collection is provided for each specimen.

10.2.1.3 Describe the specimen as formed, semi-formed, soft, loose, or watery and observe if blood and/or mucus is present.

10.2.1.4 Check for presence of possible adult worms like pin worms, A. lumbricoides, Tape worm proglottids

10.2.1.5 Report and express the results like for example:

Macro: Soft stool sample and mucus present

Ascaris lumbricoides adult worm identified.

10.2.1.6 Other important information derived from macroscopy:

Examine liquid, soft stool samples; and liquid and soft with mucus and/or blood present within 30 min of passage.

10.2.2 MICROSCOPIC EAXAMINATION OF FRESH STOOLS SAMPLES—DIRECT SMEARS

10.2.2.1 Put on gloves when carrying out this procedure.

10.2.2.2 Place one drop 0.85% NaCl on the left side of the slide and one of iodine on the right side of the slide.

10.2.2.3 Take a very small amount of the stool sample (about a head of match stick), and thoroughly emulsify the stool in the saline and iodine preparations (use separate sticks for each).

10.2.2.4 Place coverslip (22 by 22) on each suspension.

10.2.2.5 Systematically scan both suspensions with the 10x objective. Examine the entire coverslip.

10.2.2.6 Use 40x objective to confirm any suspicious finding.

10.2.2.7 Further examine at least a one-third of the coverslip, even if nothing suspicious has been seen, before reporting the specimen.

10.2.2.8 Look for and identify protozoa trophozoites and/or cysts, helminths eggs/ova and larvae.

10.2.2.9 Further look for and semi-quantify WBC. RBC and yeast cells which cannot be seen after formal/ether concentration technique.

10.2.2.10 Report and express the results of examination as:

- **No ova or parasites seen**", by direct smear. If no parasite seen.

  If any parasites are seen, write the scientific name of the parasite with stages, Example:

- ***Giardia lambilia* cyst 1–3 per preparation by direct smear or**
  - 4–10 per preparation by direct smear or

- 11–20 per preparation by direct smear or
- 21–40 per preparation by direct smear or
- over 40 per preparation by direct smear.

10.3 FORMOL ETHER CONTRATION TECHNIQUE

10.3.1 PRINCIPLE

In the Ridley modified method, faeces are emulsified in formol water, the suspension is strained to remove large faecal particles, ether or ethyl acetate is added, and the mixed suspension is centrifuged. Cysts, oocysts, eggs, and larvae are fixed and sedimented and the faecal debris is separated in a layer between the ether and the formol water. Faecal fat is dissolved in the ether.

10.3.2 SCOPE

10.3.2.1 Used to concentrate a wide range of Cysts, oocysts, eggs, and larvae from fresh or preserved stool samples.

10.3.2.2 Eggs that do not concentrate well by this technique are those of Fasciola species and Vampirolepis nana but concentration of these parasites is not usually required.

10.3.2.3 When concentrating the oocysts of coccidia, an additional centrifugation stage is required.

10.3.3 PROCEDURE

10.3.3.1 Using a rod or stick, emulsify an estimated 1 g (pea-size) of faeces in about 7 ml of 10% formol water contained in a screw-cap bottle or tube. Note: Include in the sample, faeces from the surface and several places in the specimen.

10.3.3.2 Mix well by shaking.

10.3.3.3 Sieve the emulsified faeces, collecting the sieved suspension into a 15 ml conical (centrifuge) tube made of strong glass, copolymer, or polypropylene.

10.3.3.4 Add 3–4 ml of diethyl ether.

Caution: 1. Ether is highly flammable, therefore use well away from an open flame.

2. Ether vapour is anaesthetic, therefore make sure the laboratory is well-ventilated.

10.3.3.5 Stopper* the tube and mix for 1 minute. If using a Vortex mixer, leave the tube unstoppered and mix for about 15 seconds (it is best to use a boiling tube).

* Do not use a rubber bung or a cap with a rubber liner because ether attacks rubber.

10.3.3.6 With a tissue or piece of cloth wrapped around the top of the tube, loosen the stopper (considerable pressure will have built up inside the tube).

10.3.3.7 Centrifuge immediately at 750–1 000 g (approx. 3000 rpm) for 1 minute. After centrifuging, the parasites will have sedimented to the bottom of the tube and the faecal debris will have collected in a layer between the ether and formol water

10.3.3.8 Using a stick or the stem of a plastic bulb pipette, loosen the layer of faecal debris from the side of the tube and invert the tube to discard the ether, faecal debris, and formol water. The sediment will remain.

10.3.3.9 Return the tube to its upright position and allow the fluid from the side of the tube to drain to the bottom. Tap the bottom of the tube to resuspend and mix the sediment. Transfer the sediment to a slide, and cover with a cover slip.

10.3.3.10 Examine the preparation microscopically using the 10x objective with the condenser iris closed sufficiently to give good contrast.

10.3.3.11 Use the 40x objective to examine small cysts and eggs. To assist in the identification of cysts, run a small drop of iodine under the cover slip.

10.3.3.12 Report and express the results of examination as shown below, specifying by formol ether concentration method.

- **No ova or parasites seen**", by formol ether concentration technique. If no parasite seen.

  If any parasites are seen, write the scientific name of the parasite with stages, Example:

- *Schistosoma mansoni* ova 1–3 (+) per preparation by formol ether concentration technique or
- *Schistosoma mansoni* ova 4–10 (++) per preparation by formol ether concentration technique or
- *Schistosoma mansoni* ova 11–20 (+++) per preparation by formol ether concentration technique or
- *Schistosoma mansoni* ova 21–40 (++++) per preparation by formol ether concentration technique or
- *Schistosoma mansoni* ova over 40 per preparation by formol ether concentration technique.

CMHS INSTITUTIONAL REVIEW BOARD (IRB)

Kigali, 8th /08/2019

KAYIRANGA Pascal
School of Health Sciences, CMHS, UR

Approval Notice: No 396/CMHS IRB/2019

Your Project Title *" Human Mansoni Schistosomiasis In Proximity Of Lake Muhazi "* has been evaluated by CMHS Institutional Review Board.

		Involved in the decision		
		Yes	No (Reason)	
Name of Members	**Institute**		**Absent**	**Withdrawn from the proceeding**
Prof Kato J. Njunwa	UR-CMHS	X		
Prof Jean Bosco Gahutu	UR-CMHS	X		
Dr Brenda Asiimwe-Kateera	UR-CMHS	X		
Prof Ntaganira Joseph	UR-CMHS	X		
Dr Tumusiime K. David	UR-CMHS	X		
Dr Kayonga N. Egide	UR-CMHS	X		
Mr Kanyoni Maurice	UR-CMHS		X	
Prof Munyanshongore Cyprien	UR-CMHS	X		
Mrs Ruzindana Landrine	Kicukiro district		X	
Dr Gishoma Darius	UR-CMHS	X		
Dr Donatilla Mukamana	UR-CMHS	X		
Prof Kyamanywa Patrick	UR-CMHS		X	
Prof Condo Umutesi Jeannine	UR-CMHS		X	
Dr Nyirazinyoye Laetitia	UR-CMHS	X		
Dr Nkeramihigo Emmanuel	UR-CMHS		X	
Sr Maliboli Marie Josee	CHUK	X		
Dr Mudenge Charles	Centre Psycho-Social	X		

After reviewing your protocol during the IRB meeting of where quorum was met and revisions made on the advice of the CMHS IRB submitted on 8th August 2019, **Approval has been granted to your study.**
Please note that approval of the protocol and consent form is valid for **12 months**.

You are responsible for fulfilling the following requirements:

1. Changes, amendments, and addenda to the protocol or consent form must be submitted to the committee for review and approval, prior to activation of the changes.
2. Only approved consent forms are to be used in the enrolment of participants.
3. All consent forms signed by subjects should be retained on file. The IRB may conduct audits of all study records, and consent documentation may be part of such audits.
4. A continuing review application must be submitted to the IRB in a timely fashion and before expiry of this approval
5. Failure to submit a continuing review application will result in termination of the study
6. Notify the IRB committee once the study is finished

Sincerely,

Date of Approval: The 8th August 2019

Expiration date: The 8th August 2020

Professor GAHUTU Jean Bosco
Chairperson Institutional Review Board,
College of Medicine and Health Sciences, UR

Cc:

- Principal College of Medicine and Health Sciences, UR
- University Director of Research and Postgraduate Studies, UR

Annex 2: Informed consent/Kinyarwanda

Contact information for group member:
Email: kayirangapascal@gmail.com
phone: +250 788570896/0780599715

Nyakubahwa, **.....**
Njyewe, KAYIRANGA Pascal umukozi n' umunyeshuri mu University y'U Rwanda, college of Medicine and Health sciences, School Health Sciences, Biomedical Laboratory Sciences (Staff) School of Public Health, Epidemiology department (Student), Ndimo gukora ubushakashatsi ku nzoka Bilariziyoze hano intara y' iburasirazuba, umujyi wa Kigali, n' intara y'amajyaruguru mu nkengero y'Ikiyaga cya Muhazi n' imidugudu ihaturiye.

Ndabashishikariza kugira uruhare muri ubu ububushakashatsi mutanga umusarani, imyirondoro ku bushake . Aya makuru yose azakirwa n' inzobere muri ubu bushakashatsi mu midugudu mutuyemo hafi y' ikiyaga cya Muhazi. Amakuru yanyu afatwa nk' ingenzi kandi akagirwa ibanga.

Nubwo mutahita mwungukira muri ubu bushakashatsi mugizemo uruhare muraza kuba mutanze umusanzu wanyu ku bijyanye n' amakuru y' indwara Bilariziyoze n' ihererekanywa ryayo ku nkengero z' ikiyaga cya Muhazi. Ibi bizatuma ababakomokaho n' abandi bose babyungukiramo bamenya uko bakwirinda inzoka Bilihoze.

Umukono wawe kuri iyi nyandiko ushatse kuvuga ko wemeye kubushake bwawe kandi wumva uruhare rwawe muri ubu bushakashatsi kandi igihe cyose ubishatse wabivamo nta nkurikizi.

Umukono:.....Telephone: .....

Ugize ikibazo wabaza Prof Kato Njunwa, 0788490522/0783340040 Chairperson, IRB, UR, CMHS.

UNIVERSITY of RWANDA

COLLEGE OF MEDICINE AND HEALTH SCIENCES

REMERA CAMPUS

BIOMEDICAL LABORATORY SCIENCES, SCHOOL OF HEALTHSCIENCES

Kigali, 22nd Jul. 2019

TO WHOM IT MAY CONCERN

I hereby certify that KAYIRANGA Pascal has been granted permission to conduct practical in our Biomedical Laboratory Sciences for his master's thesis proposal titled; "HUMAN MANSONI SCHISTOSOMIASIS IN PROXIMITY OF LAKE MUHAZI".

He is our staff in biomedical laboratory sciences department, school of health science, college of medicine and health sciences as well as a regular student in MSc Epidemiology at SPH/CMHS/UR, with a registration number: 217291120.

Any assistance granted to him is highly recommended.

Yours sincerely,

For Dr. Nadine RUJENI
Dean
SHS

University of Rwanda

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