DETECTION OF *SCHISTOSOMA* SPECIES IN CENTRAL RIVER REGION OF THE GAMBIA

BY

ALPHONSE MENDY

DEPARTMENT OF BIOCHEMISTRY,
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA

APRIL, 2018

DETECTION OF *SCHISTOSOMA* SPECIES IN CENTRAL RIVER REGION OF THE GAMBIA

BY

ALPHONSE MENDY

BSc. Biomedical Science (University of Hull) 2009

(P15SCBC8072)

A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY IN PARTIAL FULFILLMENT FOR THE AWARD OF MASTER OF SCIENCE IN BIOTECHNOLOGY

DEPARTMENT OF BIOCHEMISTRY,

FACULTY OF LIFE SCIENCES,

AHMADU BELLO UNIVERSITY,

ZARIA NIGERIA

APRIL, 2018

DETECTION OF *SCHISTOSOMA* SPECIES IN CENTRAL RIVER REGION OF THE GAMBIA

BY

ALPHONSE MENDY

DEPARTMENT OF BIOCHEMISTRY,

AHMADU BELLO UNIVERSITY,

ZARIA. NIGERIA

A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY IN PARTIAL FULFILLMENT FOR THE AWARD OF M.Sc. DEGREE IN BIOTECHNOLOGY

APRIL, 2018

DECLARATION

I declare that the work in this dissertation entitled ‘‘Detection of *Schistosoma* species in Central River Region of The Gambia” has been carried out by me in the Department of Biochemistry under the supervision of Dr. T.T. Gbem and Professor Y.K.E Ibrahim. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

Alphonse Mendy

……. …….. ……

Name of Student Signature Date

CERTIFICATION

This dissertation entitled ‘‘Detection of *Schistosoma* species in Central River Region in The Gambia’’ by Alphonse Mendy meets the regulations governing the award of the degree of M.Sc Biotechnology of the Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

Dr. T.T. Gbem

…….. ……. …….

Chairman, Supervisory Committee Signature Date

Prof. Y.K.E. Ibrahim

…… ……. ……

Member, Supervisory Committee Signature Date

Prof. M.N.Shuaibu

……. ……. ……..

Head of Department Signature Date

Prof. S.Z. Abubakar

…….. …… …….

Dean, Postgraduate school Signature Date

DEDICATION

This dissertation work is dedicated to my family and all the people of Central River Region of The Gambia

ACKNOWLEDGEMENT

In the name of God, the Beneficent, the Merciful, I wish to express my profound gratitude to my humble and tireless supervisors Dr. T.T.Gbem andProf. Y.K.E. Ibrahim for their advice, assistance and immense contributions to the success of this work. I also acknowledge with gratitude the teamof African Centre of Excellence on Neglected Tropical Disease, Ahmadu Bello University, the African Centre of Excellence team of The Gambia, the National Public Health Laboratories of The Gambia and The Gambia Government for their financial and logistics support. ToDr. E. O. Balogun, Dr. I. A.Aimola, Dr. G.Chechet and all other lecturers and staff of Department of Biochemistry, Ahmadu Bello University, Zaria I thank you for your intellectual guidance and support.

My sincere gratitude and appreciations also goes to the Director and Staff of the Centre for Biotechnology Research and Training, the Director and Staff of Veterinary Teaching Hospital Ahmadu Bello University for allowing me to use their laboratory space.

To all the Biotechnology students, Department of Biochemistry, Ahmadu Bello University most especially Entonu Moses Edache and Joseph Yahaya for their continued support and assistance throughout the research work.

To all the people of the Central River Region whose urine and blood samples were used for this research, I remain grateful to them. To the entire public health workers at Brikama Ba Health Centre, ModouLaminSanneh; Laboratory staff of Brikama Ba Health Centre and FatouMballow of Kuntaur Health Centre for their help in the sample collection. To Joseph Mendy and Musa Ceesay for their help in the sample collection and the microscopic analysis of the samples.

To my parents, Dominic Mendy and Marie Mendy, I say thank you for your prayers towards my success and I pray that God will grant them long life. I wish to sincerely and whole heartedly say thank you to my beloved and dear wife Margo AroJattaMendy who steadfastly and religiously stood by me and encouraged me towards the completion of this work. To my brothers and sisters, I say thank you all for the prayers and moral assistance. I am indebted to my beloved son Pierre Boli Mendy for bearing with my frequent absence from home during the period of the research, I pray to God for his guidance and protection.

Above all, I thank God the Father Almighty who spared my life to this time in good health, and for making everything possible in His infinite will and Mercies.

ABSTRACT

A study on schistosomiasis infection was carried out among students, farmers and fishers (fishermen/women) from four villages in the Central River Region of The Gambia between April and May 2017. Sample collection form were used to obtain data such as age, gender, prior schistosomiasis infection and treatment. One hundred and ninety-five (195) blood and 192 urine samples collected from 117 females and 78 males were examined usingmicroscopy, ELISA and PCR techniques to detect and identifyschistosome isolates from the biological samples. Incidence of *S. haematobium* was 28.7% with 41.0% in males and 23.9% in females. The highest incidence among the village was in Brikama Ba with 53.1% while the age group 6-15 years had the highest incidence of 50.0%. *Schistosoma mansoni* was only detected in Jahallyvillage with 1.5 % incidence. Schistosomiasis detection was highest with ELISA (40%) and lowest with Microscopy (24.5%). Molecular technique using Nested PCR gave 28.7% incidence. Incidence of schistosomiasis infection was highest among the people that has never received treatment for schistosomiasis. The high incidence of schistosomiasis indicates that the disease is still a serious public health problem in the study area.

LIST OF ABBREVATION

NTD - Neglected Tropical Diseases

WHO - World Health Organization

GI – Gastrointestinal

ELISA – Enzyme-Linked Immunosorbent Assay

GIT – Gastrointestinal tract

CRR – Central River Region

MDA - Mass Drug Administration

PZQ - Praziquantel

PCR – Polymerase Chain reaction

DNA – Deoxyribo Nucleic Acid

dATP – deoxy Adenine Triphoshate

dTTP – deoxy Thymine Triohoshate

dGTP - deoxy Guanine Triohoshate

dCTP - deoxy Cytosine Triohoshate

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Schistosomiasis also known as bilharziasis, or snail fever is an acute and chronic parasitic tropical disease caused by eggs of the blood flukes (trematode worms) of the genus *Schistosoma* that inhabits the circulatory system of their vertebrate hosts (Steinauer*et al*., 2008).The name bilharzia was coined from the name of Theodor Bilharz, a German surgeonwho was the first to identify the etiological agent *Schistosoma haematobium* in 1851 while working in Cairo, Egypt (Nour, 2010). Schistosomiasis is one of the neglected tropical diseases (NTDs) in Africa.

Neglected tropical diseases (NTDs) are hidden epidemics of enormous health and economic consequence for African countries. They are hidden because many African countries are unable to establish and address the associated health issues due partly to inadequate resources for proper understanding of the biological and social characteristics of these diseases and, also due to the fact that they have been largely wiped out in the developed parts of the World. Schistosomiasis is the second most prevalent tropical parasitic disease after malaria, and is a leading cause of morbidity and mortality for developing countries especially in Africa. At least 206.5 million people required treatment for schistosomiasis in 2016, at least 88 million are reported to be treated. 90% of those that required treatment live in Africa. Of the more than 700 million people that live in endemic areas, children aged 5–15 years have the highest prevalence and parasite load. The infection is prevalent in tropical and sub-tropical areas particularly in poor communities without potable water and adequate sanitation (WHO, 2017). It is one of the World's most common infections of people living in poverty (Hotez and

Kamath, 2009). The disease is prevalent in most African countries and in limited areas of South America, the Caribbean, the Middle East, and Asia (Corachan, 2002). In children, schistosomiasis can cause anaemia, stunting of growth and a reduced ability to learn, although the effects are usually reversible with treatment. Chronic schistosomiasis may affect people's ability to work and in some cases can result to death. In sub-Saharan Africa, more than 200,000 deaths per year are due to schistosomiasis (WHO, 2017). Schistosomiasis has profound negative effects on child development, outcome of pregnancy, and agricultural productivity (Hotez and Kamath, 2009).

Adult schistosomes are white or greyish worms of 7–20 mm in length with a cylindrical body that features two terminal suckers, a complex tegument, a blind digestive tract, and reproductive organs (Gryseels*et al*., 2006). Infection is acquired by exposure to fresh water that contains cercariae (the parasitic stage that is infective for humans) released by infected snails (the intermediate host) (Corachan, 2002).The transmission cycle requires contamination of surface water by excreta, specific freshwater snails as intermediate hosts, and human water contact (Gryseels*et al*., 2006). There are several species of *Schistosoma* that are pathogenic parasites of humans: *Schistosoma haematobium*, which is responsible for urinary schistosomiasis; *Schistosoma mansoni*, *Schistosoma japonicum*, *Schistosomamekongi*, and *Schistosomamalayi*, which are responsible for gastrointestinal (GI) and hepatosplenic schistosomiasis; and *Schistosomaintercalatum*, which affects the GI tract but is associated with lower morbidity. *Schistosomamatheei*and *Schistosomabovis*are occasional parasites of human and *Schistosomaincognitum*may also prove infective to humans (Almeda*et al*., 1994; Corachan, 2002). The species differ in their predilection site in the human host, the species of

the intermediate snail host, the pathology they induce, and the number, size and shape of the eggs produced (IARC, 2012).

1.2 Statement of Research Problem

Schistosomiasis is mostly common in poor rural communities where fishing and agricultural activities are dominant. Domestic and recreational activities such as washing, water fetching for domestic use and swimming exposes women and children to infection (WHO, 2017). The river Gambia is at the centre of activities for people living in Central River Region (CRR). People living in these communities wash their clothes and take bath in the river. This region has several fresh water bodies and harbours the major irrigated rice fields of the country of which the river is one of them hence the high prevalence of *Schistosoma*in the region (Gambia NTD Mapping Report, 2015). The Gambian Government depends on the region to attain its goal of rice self-sufficient production among other agricultural development goals. The high prevalence of schistosomiasis hinders the attainment of these goals. Most of the children in these regions help their parents in farming, when they are sick, the parents will have to stop farming and look after the children which will be a double loss.

Laboratories in The Gambia detect *S. mansoni*in stools, which is sometimes difficult to obtain as most people are reluctant to take stool to the laboratories for testing thereby leading to latedetection and subsequently resulting to severe chronic phase of schistosomiasis. This can lead to bladder cancer, damaged of organs and developmental problems in children (WHO, 2017).

Previous investigations on the prevalence of schistosomiasis in humans were done using only microscopy which has low sensitivity compare to molecular techniques, it is unspecific as eggs of different species may be difficult to differentiate. To the best of our knowledge, no

investigation/research has been conducted using moleculartechniques or immunological tests in The Gambia. Molecular tools which are the most sensitivity and specific tools will be used to detect S. *mansoni* in blood and urine.

1.3 Justification

Children are at risk of the disease due to close proximity of irrigation farms and rivers to their homes and their personal outdoor activities. Since it is not practical to stop people using the river, effective measures should be in place to control schistosomiasis.

Molecular approaches of detection would enhance reliability in prevalence studies by early detection and hence early treatment. *Schistosoma* has been successfully eradicated in several countries such as Japan, Tunisia, whilst Morocco, Brazil, China, Egypt and some Caribbean Islandshave made significant progress in control and management of the disease. (Utzinger*et al*., 2009; Adenowo*et al*., 2015). A more accurate, sensitive and reliable diagnosis tool will facilitateand enhance the control and management of this disease in The Gambia.

1.4 Aim

To evaluate the incidence and distribution of *Schistosoma* species towards effective control of schistosomiasis in The Gambia.

1.5 Objectives

- **i.** To identify the most at risk population and respond to treatment by collecting data from participants.
- **ii.** To identify *Schistosoma*speciesin urine samples collected from children, farmers and fishers (fishermen/women) by microscopy.
- **iii.** To detect the presence of *Schistosoma* in urine and blood samples from farmers, fishers and school children in Central River Region using ELISA technique.

iv. To identify the*Schistosoma*speciesin the study population in Central River Region of The Gambia by molecular method using primers.

1.6 Null Hypothesis

Schistosoma haematobium and *mansoni*are not prevalent inpeople living inthe Central River Region of The Gambia.

1.7 Limitations and Constraints

Limitations

The study is limited to four villages in the Central River Region of The Gambia, namely Brikama Ba, Jahally, Kuntaur and WaliKunda

Constraints

i. Low response in some villages which results to the uneven number of participants from the various villages.

ii. Samples were only collected between 10:00 am and 2:00pm and during this time some people were not accessible.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Historical Development of Schistosomiasis

Characteristic symptoms of schistosomiasis are described in early Egyptian papyri and immunological analysis revealed its presence in ancient mummies. *Schistosomahaematobium* eggs from two mummies were discovered using ELISA and are believed to be more than 5000 years, and thus the oldest infection known to date (Deelder*et al*., 1990).

Theodor Bilharz a German physician was the first to identified adult male and female *S. haematobium* during post-mortem examination whilst working in Cairo, Egypt in 1851.*S. mansoni* was named in honour of Sir Patrick Manson who was the first to reportthe difference in egg morphology (terminal versus lateral spine) and manner of excretion (faecal versus urinary) of African schistosomes was due to the existence of two separate species (*S. haematobium* and *S. mansoni*) in 1907.*S. japonicum* was discovered in 1904, but the clinical symptoms had been described as a syndrome for more than half a Century before its discovery.

Chronic parasitic worm infection with *Schistosoma haematobium* primarily affects the genitourinary tract (i.e., urogenital schistosomiasis) and can result in terminal haematuria, genital sores increasing risk to HIV, bladder and ureteral fibrosis, and bladder cancer. A correlation between urinary schistosomiasis and bladder carcinoma was first suspected byGoebel in 1905. In 1911, Professor Ferguson at the Faculty of Medicine in Cairo reported a likely association of bladder carcinoma with granulomas caused by Urinary Schistosomiasis (Berry *et al*., 2017). The International Agency for Cancer Research in association with the

World Health Organization categorized *S. haematobium* infection as carcinogenic. *S. mansoni*and *S. japonicum* are associated with cervical, liver, as well as colorectal carcinomas (Samaras *et al*., 2010)

2.2 Species of*Schistosoma*

Schistosomiasis is a disease caused by one of the five species of the parasitic trematodehelminths of the genus *Schistosoma*. The species of widespread importance are *S. haematobium*, *S. japonicum* and *S. mansoni*. The two lesser species of *Schistosoma* that parasitize humans are *S. intercalatum*, and *S. mekongi* (Mahmoud, 2001).

Classification

Kingdom - Animalia

Phylum - Platyhelminthes

Class - Trematoda

Sub-class – Digenea

Order – Strigeidida

Family - Schistosomatidae

Genus – *Schistosoma*

2.2.1 *Schistosoma haematobium*

Schistosoma haematobium is the only species that affects the human urinary system. They are found in areas where their intermediate hosts such as *Bulinus* and *Physopsis*reside (Roberts and Janovy, 2000), mostly in Africa, Middle East and Southern Europe (WHO, 2017). Human infection occurs when the definitive host comes into contact with water containing cercariae released from snails infected with *S. haematobium*. The cercariae then burrow into the skin,

spread, grow, and reproduce. Over the next few days, the eggs are passed through the urine of the host (Basch, 1991).

Schistosoma haematobium is pathogenic to humans and causes blood in the urine and sometimes in the stool. Persons infected by *S. haematobium* may also develop cough, fever, skin inflammation, and tenderness of the liver because the spined eggs attach to vital organs and causes tissue degeneration. Later stages of the disease may be characterized by the swelling and damage of the bladder, liver, and other organs. The eggs of *S. haematobium* (Fig. 2.1) can clog the bladder neck and cause infection. *S. haematobium* is distinct from many trematodes in that the sexes are separate. Adult males are around 10 mm and females are 15 mm in length. Both sexes have a strong oral sucker and a smaller posterior ventral sucker (Agnew *et al*., 1988; Basch, 1991). Common clinical presentation of *S. haematobium* infection includes haematuria, proteinuria, pollakiuria, and leukocyturia accompanied by such symptoms as dysuria and nocturia(Gryseels, 1989; Gryseels*et al*., 2006). The parasite causes lesions in the female lower genital tract (ie, cervix, valva, and vagina). Female genital schistosomiasis has been identified as a major social and medical problem that may facilitate the spread of some sexually transmitted diseases, such as human immunodeficiency virus and human papilloma virus (Mosunjac*et al*., 2003).

Figure 2.1 Egg of *S. haematobium* in a wet mount of urine concentrate showing the characteristic terminal spine
(https://www.cdc.gov/dpdx/schistosomiasis/gallery.html)

2.2.2 *Schistosoma mansoni*

Schistosoma mansoni is a parasitic flatworm that has intermediate and a definitive host. The intermediate host is a freshwater snail of the genus *Biomphlaria*, and the definitive host is the human. Most commonly, these parasites infect a human host by piercing the skin and moving into the blood stream. *S. mansoni* adults are parasitic to humans and commonly reside in mesenteric venules and in the large intestine (Bogitsh*et al.,* 2005). They communicate through chemical means because they have several receptors that respond to chemical changes in the internal environment of their host. Once inside their host, intermediate or definitive, they are not known to make many changes to its chemistry, but they are thought to protect themselves from localized immune system signals and enzymatic activity by releasing their own signals that lessen or weaken the signals of the host (Berriman and El-Sayed, 2009). The eggs of *S. mansoni* (Fig. 2.2)can also be found in primates and rodents but the main hosts are human beings (Adenowo*et al*., 2015).

Figure 2.2 Egg of *S. mansoni* in an unstained wet mount
(https://www.cdc.gov/dpdx/ schistosomiasis/gallery.html)

2.2.3 *Schistosoma japonicum*

It causes schistosomiasis and it is mostly found in China, Taiwan, the Philipines and Southeast Asia (WHO, 2017). Schistosomes alternate between a mammalian host and a snail host through the medium of fresh water during their life cycle. After burrowing out of the snail host, free-swimming cercariae penetrate the skin of the mammalian host, travel through the blood to the liver via the lungs, and transform into schistosomula. These mature in the hepatic portal vein, mate and migrate to their final destination in the mesenteric venous plexus (Zhou *et al*., 2009).

Figure 2.3 Egg of *S. japonicum* in an unstained wet mount
(https://www.cdc.gov/dpdx/ schistosomiasis/ gallery.html)

2.2.4 *Schistosomaintercalatum*

*Schistosoma. intercalatum*is restricted to the rain forest area of Central Africa. The eggs are similar to *S. haematobium* in general shape and in possessing a terminal spine, but are usually longer, often have an equatorial (central) bulge and are shed in stool unlike *S. haematobium* which is found in urine (WHO, 2107).

Figure 2.4 Egg of *S. intercalatum* in an unstained wet mount
(https://www.cdc.gov/dpdx/ schistosomiasis/gallery.html)

2.3 LIFE CYCLE OF SCHISTOSOMA

The life cycle of *Schistosoma*species is characterized by alteration of generation; asexual reproduction occurs in the snail intermediate host while sexual reproduction occurs in humans (Fig.2.5). The pathology of schistosomiasis is due to egg-mediated immune response in the form of granuloma formation followed by fibrosis which manifest as obstructions in the gastrointestinal tract (GIT) in case of intestinal schistosomiasis and in the urinary tract in the case of *Schistosoma haematobium* (Wilson *et al*., 2007; Barakat*et al*., 2014).The eggs are highly antigenic and can induce an intense granulomatous response, migrate through the bowel or bladder wall and shed in faeces or urine. During this time (approximately 10 days), the organisms begin to mature into miracidia. The free-swimming miracidia that are shed into fresh water survive 1-3 weeks, during which time they must infect a susceptible snail (intermediate host) to complete the life cycle. Within the infected snail, 2 generations of sporocysts multiply and mature into free-swimming cercariae before exiting the snail to seek a human host and begin a new cycle. As the eggs enter the urinary system, they can find their way to the female genital region, fallopian tube, and ovaries.

In the case of *S japonicum*, which is probably the species with the highest risk of complications, the life cycle may include domesticated animals (including rodents) and wild animals. Eggs that are not shed successfully may remain in the tissues and can end up in the skin, brain, muscle, adrenal glands, and eyes (Corachan, 2002; Houston *et al*., 2004).

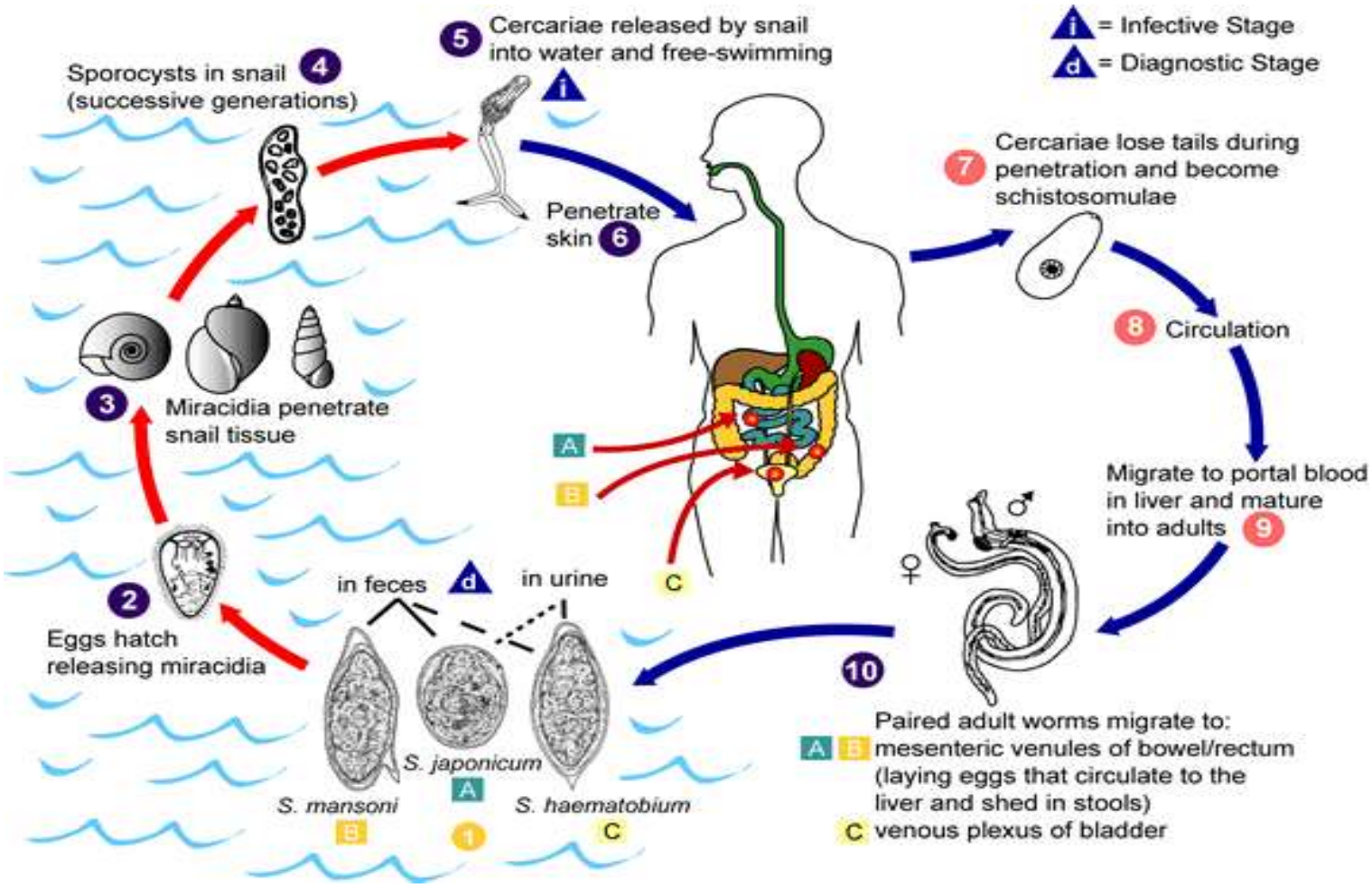

Figure 2.5 Life cycle of *Schistosoma*species

(http://www.cdc.gov/parasites/schistosomiasis/biology. html)

KEY:

1. Eggs are eliminated with faeces or urine.
2. Under optimal conditions the eggs hatch and release miracidia.
3. The miracida swim and penetrate specific snail intermediate hosts.
4. The stages in the snail include 2 generations of sporocysts and the production of cercariae.
5. Upon release from the snail, the infective cercariae swim.

6. The cercariae penetrate the skin of the human host.
7. The cercariae shed their forked tail.
8. The cercariae become schistosomulae.
9. The schistosomulae migrate through to their residence in the veins.
10. Adult worms in humans reside in the mesenteric venules in various locations, which at times seem to be specific for each species. For instance, *S. japonicum* is more frequently found in the superior mesenteric veins draining the small intestine A, and *S. mansoni* occurs more often in the superior mesenteric veins draining the large intestine B. However, both species can occupy either location, and they are capable of moving between sites. *S. haematobium* most often occurs in the venous plexus of bladder C, but can also be found in the rectal venules.

2.4 EPIDEMIOLOGY AND IMPACT OF SCHISTOSOMIASIS

Schistosomiasis is prevalent in tropical and subtropical areas, especially in poor communities without access to safe drinking water and adequate sanitation and access to rivers and streams (WHO, 2017). Schistosomiasis has profound negative effects on child development, outcome of pregnancy, and agricultural productivity (Adenowo*et al*., 2015)**.** It is estimated that at least 92% of the 206.5 million people that required preventive treatment in 2016 live in Africa. Schistosomiasis transmission has been reported from 78 countries. However, preventive chemotherapy for schistosomiasis, where people and communities are targeted for large-scale treatment (mass drug administration), is only required in 52 endemic countries with moderate-to-high transmission (WHO, 2017).

The significance of morbidity and mortality from schistosomiasis is believed to be grossly underestimated in most developing countries (Adenowo*et al*., 2015) Studies of *Schistosoma-*

infected children reported that schistosomiasis can cause growth retardation, increased risk of anaemia, exhaustion, loss of memory and cognitive reasoning which leads to poor academic performance, thus limiting the potential of infected children most especially in schools. These negative outcomes in children add to the socioeconomic problem of the society (Contehet *al*., 2011)

The prevalence of schistosomiasis in The Gambia is put at 4.3%, with a higher prevalence in Central River Region (CRR) at 14.2% followed by Upper River Region at 9.4%. The River Gambia remains fresh throughout the year at these two regions. The two regions also have several fresh water bodies and are the major irrigated rice fields in the country. However, all other regions apart from North Bank East Region (NBER) are also endemic for schistosomiasis (Gambia NTD Mapping Report, 2015).

In Senegal the closest neighbour of The Gambia, reported that urinary schistosomiasis is present in all regions of the country with a mean prevalence of 25% as at 2003 (Briand *et al*., 2005). A more recent study in the District of Niakhar, Region of Fatick reported an endemicity for urinary schistosomiasis, with a high intensity of infection and prevalence of 57.6% among school going children (age 7-15 years) (Senghor *et al*., 2014).

The highest occurrence of schistosomiasis in Africa is seen in Nigeria (29 million), which is closely followed by United Republic of Tanzania (19 million), Ghana, and Democratic Republic of Congo (15 million) making up the top five countries in Africa with *Schistosoma*l infection. However, it is believed the true prevalence of schistosomiasis is greatly underestimated in most African countries (King, 2010; Adenowo*et al*., 2015; WHO, 2017). There is a relatively low mortality rate but very high morbidity rate causing severe devastating illness in millions of people. It is often associated with development projects, such as dam and

irrigation schemes where the snail breed and people use the water for swimming, washing, bathing and fishing (Usman *et al*., 2016).

2.5 GEOGRAPHICALDISTRIBUTION OF SCHISTOSOMA

Schistosoma mansoni is widespread in Africa, the Eastern – Mediterranean, the Caribbean and South America and can only infect humans and rodents as shown in Table 2.1. *Schistosomamekongi*is prevalent only in the Mekong river basin in Asia. *Schistosoma japonicum* is limited to China and the Philippines and can infect mammals, such as pigs, dogs and water buffalos, in addition to humans. As a result, it can be more difficult to control disease caused by this species. *Schistosomaintercalatum*is found in Central Africa while *Schistosoma haematobium* occurs predominantly in Africa and the eastern Mediterranean (WHO, 2017). Three out of these species are of vast medical importance; *S. haematobium* that causes urinary schistosomiasis, *S. mansoni* and*S. japonicum* which cause intestinal schistosomiasis.

Table 2.1 Different Species of *Schistosoma* and their Geographical Distribution

Types	Species	Geographical distribution
Intestinal schistosomiasis	*Schistosoma mansoni*	Africa, the Middle East, the Caribbean, Brazil, Venezuela
	Schistosoma japonicum	China, Indonesia, the Philippines
	Schistosomamekongi	Several districts of Cambodia and the Lao People's Democratic Republic
	Schistosomaguineensis and *Schistosomaintercalatum*	Rain forest areas of central Africa
Urogenital schistosomiasis	*Schistosoma haematobium*	Africa, the Middle East

(http://www.who.int/schistosomiasis/epidemiology/table3/en/)

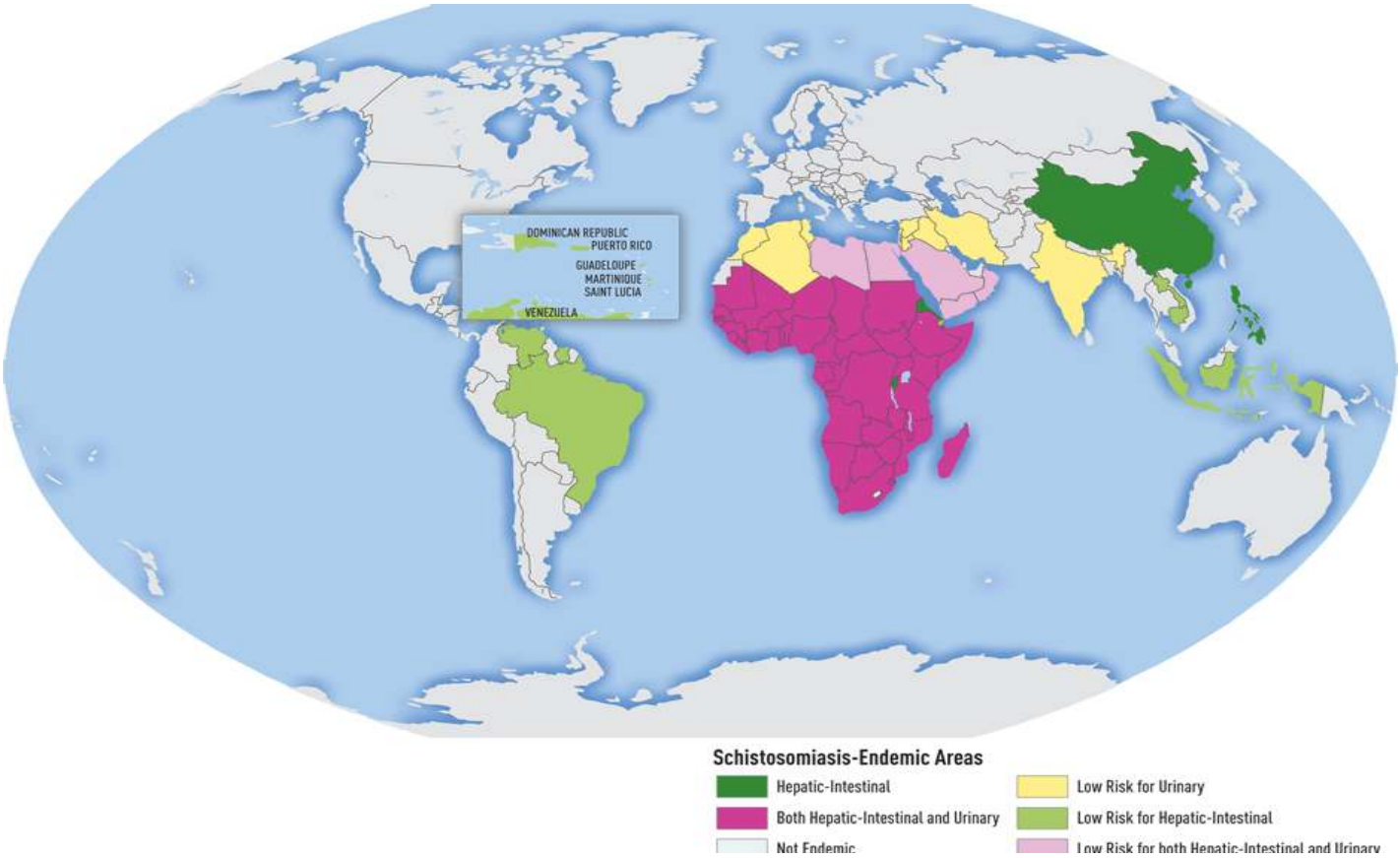

Figure 2.6 Geographic distribution of schistosomiasis (www.cdc.gov/parasites/ schistosomiasis)

2.6 INTERMEDIATE HOST OF SCHISTOSOMES

Snails are the intermediate hosts for schistosomiasis, because humans harbour the sexual stages of the parasites and the snails harbour the asexual stages. Humans also serve as vectors by contaminating the environment. Therefore, schistosomiasis is prevalent in areas where the snails breed in waters contaminated by faeces or urine of infected persons. Most intermediate hosts of human *Schistosoma* parasites belong to three genera, *Biomphalaria, Bulinus*and *Oncomelania*. The species involved can be identified by the shape of the outer shell. The snails can be divided into two main groups: aquatic snails that live under water and cannot usually survive anywhere else (*Biomphalaria*, *Bulinus*), and amphibious snails adapted for living in and out of water (*Oncomelania*). Snails of the genus *Bulinus* serve as the intermediate hosts of *S. haematobium*in, which is the major cause of urinary schistosomiasis in Africa and in the Arab world. Snails of the genus *Biomphalaria* serve as intermediate hosts of *S. mansoni*as and of *S. intercalatum.* (Gryseeles*et al*., 2006; Adenowo*et al*., 2015). *Oncomelania*serve as the intermediate host of *S. japonicum* and it is responsible for intestinal and hepatosplenicSchistosomal infections in Indonesia, Peoples Republic of China, and the Phillipines. It is a zoonotic parasite infecting animals including pigs, dogs, cattle, and rodents (Adenowo*et al*., 2015; WHO, 2017). The *Biomphalaria* snails comprise many species including *B. alexandrina, B. sudanica, B. pfeifferi*, and *B. hoanomphala*, while the genus *Bulinus* comprises the following species; *B. tropicus, B. globosus, B. truncatus, B. forskalli*, and *B. africanus*(Ekpo *et al.,* 2012).

2.7 DRUG MANAGEMENT OFSCHISTOSOMIASIS

Several drugs such as praziquantel, oxamniquine and metrifonate have been developed and have been shown to be safe and effective against schistosomiasis. These drugs are known to

reduce the in situ worm's activity. The drugs however cannot prevent recontamination. Praziquantel is effective against all forms of schistosomiasis and has few side effects. This drug is given in either two or three doses over the course of a single day. Oxamniquine is typically used in Africa and South America to treat intestinal schistosomiasis. Metrifonate has been found to be safe and effective in the treatment of urinary schistosomiasis. Patients are typically checked for the presence of living eggs at three and six months after treatment. If the number of eggs excreted has not significantly decreased, the patient may require another course of medication (King, 2009).

2.7.1 Praziquantel (PZQ)

PZQ is a 2-cyclohexycarbonyl 1,2,3,6,7,11b-hexahydro-4H-pyrazino (2,1-a Isoquinolin-4one) broad spectrum anti-*Schistosoma*l compound (Rokni, 2012). PZQ is principally active against the adult stage of all the Schistosome species infective to man.It is effective, safe and low-cost(WHO, 2017), with reported side effects such as abdominal discomfort, nausea, headache, dizziness, drowsiness and pyrexia especially in people with high egg counts.PZQ is the recommended drug of choice for the treatment of schistosomiasis, at a dose of 40 mg/kg body weight. It is available as a 600mg tablet (Rokni, 2012). It has cure rate of 65-90% after a single treatment (one tablet). In individuals not cured, PZQ reduced egg excretion by 90%; it paralyses the worm by affecting the membrane permeability which causes vacuolation of the tegument thus exposing it to attack by the host immune system (Fenwick *et al*., 2006). Even though re-infection may occur after treatment, the risk of developing severe disease is diminished and even reversed when treatment is initiated and repeated in childhood (WHO, 2017). PZQ can safely be given to pregnant and lactating women; it decreases the disease burden and improves pregnancy and foetal outcomes (Savioli*et al*., 2003).

2.7.2 Oxamniquine

Oxamniquine is a tetrahydroquinoline derivative only effective against *S.mansoni* (Ali, 2011). Oxamniquine resistant strains of *S.mansoni* have been reported in South America but they have been effectively treated with PZQ. The effective dose by mouth in West Africa, South America, Caribbean islands is 15 mg/kg as a single dose for adults and in children under 30 kg, it is 20 mg/kg divided in 2 doses. Oxamniquine has more side-effects and is more expensive than praziquantel, at least in Africa. Common side effects include dizziness, drowsiness, headache, nausea, vomiting, diarrhoea and reddish discoloration of urine. Patients suffering from epilepsy should be closely observed as treatment may precipitate seizures. It is not recommended for pregnant and lactating women due to the lack of information on whether oxamniquine is excreted in breast milk (WHO Model Formulary, 2008; Ali, 2011).

2.7.3 Metrifonate

Metrifonate is an organophosphorus compound originally used as an insecticide. It is only effective in the treatment of *S. haematobium* and must be given in 3 doses of 10 mg/kg two weeks apart (Danso-Appiah and De-Vlas, 2002). Side effects include abdominal pain, nausea, vomiting, diarrhoea, headache and vertigo (Ali, 2011).

2.8 CONTROL OF SCHISTOSOMIASIS

There is still no vaccine to prevent schistosomiasis and only one drug has been effectively used for *Schistosoma* species affecting humans. This gives the need to develop control strategies based on eliminating schistosomes at the snail-stage (Knight *et al*., 2014). Freshwater snails are the intermediate hosts for the completion of the life cycle of schistosome parasite. A combination of mass drug administration with praziquantel and molluscicides to eliminate the snail from fresh water bodies, such as lakes and rivers, has reduced the

prevalence of schistosomiasis in several endemic countries (Olveda*et al*., 2014). The frequent use of molluscicides has a negative impact on the environment and also damage delicate ecosystems and therefore its use has been greatly discouraged (Knight *et al*., 2014).

An alternative method, the use of biological control, has been adopted in some countries by using incompatible snails to replace resident susceptible ones in endemic foci. In Brazil, the introduction of parasite resistant strains of *B. tenagophila* into an endemic site was found to reduce transmission as cross hybridization between resident susceptible and introduced snails increased over time. This method focused on reducing schistosomiasis by blocking the snail stage of the parasite life cycle (De Almeida Marques *et al*., 2014)

Other countries that biological control as an alternative method was studied are Senegal and Kenya. Prawn stocking experiment in Senegal showed that river prawns can control snail populations while Kenyan field experiments showed that crayfish reduce snail populations and human re-infection (Sokolow*et al*., 2015).

In Kenya, crayfish were introduced into aquatic habitats harbouring *Bulinus* snails (the intermediate host for *S. haematobium*). At the end of the study, a reduction in the number of snails was observed (Mkoji*et al.*, 1999).

In Senegal, a dam was built at the Senegal River Basin, which caused a massive outbreak and persistent epidemic of schistosomiasis. The dam blocked the annual migration of native river prawns (*Macrobrachiumvollenhoveni*) that are voracious predators of the snail intermediate hosts for schistosomiasis. Before the construction of the dam, when river prawns were more common, human schistosomiasis prevalence was low (Sokolow*et al*., 2015). The extinction of prawns upriver of the dam was concurrent with a dramatic increase in the prevalence and intensity of human schistosomiasis in the Senegal River Basin (Sow *et al*., 2002). Stocking

thereafter with prawns was associated with a subsequent decline in snail densities and reduced schistosomiasis transmission. The study reported that the restoring of *M. vollenhovenii* prawns to the Senegal River system could benefit villagers who are subjected to chronic schistosomiasis. The study indicates that health benefits would increase with prawn stocking density and that the high stocking density has the potential to achieve local disease elimination (Sokolow*et al*., 2015).

2.8.1 Complementary Approaches

Complementary approaches with drug therapy can bring a sustained education on schistosomiasis infection level in the long term. The major route through which parasitic infection spreads is the faecal-oral route either directly by hand to mouth or indirectly through food and water(Mascarini-Serra, 2011). Preventive strategy should be aimed at reducing re-infection and transmission. Sanitation and personal hygiene is a definitive preventive intervention that can eliminate infection. This can be achieved by providing safe supply of drinking water, improvement of environmental sanitation, and encouraging good hygiene practices such as hand washing with soap, and washing and cooking vegetables properly (Albonico*et al*., 2006).

One of the primary vectors for transporting parasites is the human hands, washing of hands with soap and water prevent the spread of parasitic infections. Dirty and untrimmed nails are associated with an increase in susceptibility towards acquiring parasitic infections. Therefore, nails should be regularly trim and the skin below the nails should always be keep clean (Mahmud *et al*., 2015).

Niclosamide lotion, used on the skin prior to getting in contact with infected waters has been shown to be helpful (John and William, 2006).

2.9 DIAGNOSIS OF SCHISTOSOMIASIS

The gold standard for diagnosis of schistosomiasis is through the detection ofparasite eggs in urine or faeces microscopically(Ibironke*et al*., 2011). A simple and cheaper alternative to microscope is the detection of haematuria by reagent strip for urinary schistosomiasis due to *S. haematobium* infection (Lengeler*et al.,* 1991). Macrohaematuria can be detected by asking individuals if they pass blood in urine or by visual examination of urine samples (Friedman *et al.,* 2007). Current diagnosis of schistosomiasis is based mainly on clinical symptoms, therefore low-level and chronic or asymptomatic infections are often missed. Inadequate diagnosis is potentially a serious problem, particularly in cases of chronic infection with *S. haematobium* that has been associated with damage to the urinary tract and eventual development of squamous cell carcinoma of the bladder (Mostafa*et al.,* 1999). Poor diagnosis in adults with chronic urinary schistosomiasis is thought to be a result of long-term infection, where the passage of eggs through the bladder causes local inflammation and development of lesions and fibrous tissues that trap eggs (Ibironke*et al*., 2011).

2.9.1 Microscopical Tests

Parasitological diagnosis of *Schistosoma haematobium* infection depends on the identification of the eggs which is readily undertaken by microscopy after urine filtration or centrifugation. Urine specimens should be ideally collected between 10am and 2pm because the excretion of eggs occurs with a circadian rhythm and peaks about mid-day (McCarthy *et al*., 2012). Diagnosis of intestinal schistosomiasis (*S. mansoni* and *S. japonicum*) is generally made by examination of stool specimens. Stool specimen may be examined in a thick smear using methylene blue-stained cellophane soaked in glycerine or glass slides (Kato-Katz technique) or by formol-ether concentration technique. The Kato-Katz technique is the gold standard

methodand it is recommended by the World Health Organization (WHO) for both qualitative and quantitative diagnosis of intestinal schistosomiasis but it is limited in the diagnosis of low-grade infections and in evaluating drug therapeutic effects (McCarthy *et al*., 2012, WHO, 2017). Its main advantage is that it is highly specific, relatively simple and inexpensive, even under field conditions. Furthermore, it produces semi-quantitative egg counts that can be used as surrogates of infection intensity. Quantitative evaluation allows assessment of the degree of infection and treatment response. Hatching assays may be performed on fresh stool specimens to distinguish active from treated infection because dead eggs may be shed for up to 1 year after treatment (He *et al*., 2016).

2.9.2 Immunological Tests

The enzyme-linked immunosorbent assay (ELISA) technique using soluble egg antigen is used to detect *Schistosoma* antibodies. The test is specific and sensitive and requires the services of highly trained personnel. However, its application is limited as these antibodies will only appear after 4–6 weeks in response to the laying of eggs. The test is most useful for patients with signs and symptoms of disease and a history of likely exposure (Corcoran and da Silva, 2014). The test cannot distinguish between old and new infections.

The ELISA plate is coated with *Schistosoma* antigens, the antibodies in the patient's sample (blood) bind to the antigens in the test well during the first incubation. The unbound antigen antibodies are wash off during the first wash. The next incubation allows the enzyme complex to bind to the antigen-antibody complex. The second wash removes the unbound enzymes. A substrate is added that develops a blue colour in the presence of the enzyme complex and peroxide. The stop solution ends the reaction and turns the blue assay colour to yellow(AccuDiag™ *Schistosoma*IgG ELISA Kit pamphlet).

2.9.3 Molecular Test

Polymerase chain reaction (PCR) is a molecular tool for DNA replication that amplifies a "target" DNA sequence using thermal cycling. Minute amount of DNA are amplified to millions of copies in just a few hours. The technique consists of cycles of repeated heating and cooling of the reaction for DNA denaturation and enzymatic replication of the DNA. Primers (short DNA fragments) containing sequences complementary to the target region along with a DNA polymerase are key components to enable selective and repeated amplification. As PCR progresses, the DNA generated is itself used as a template for replication, setting in motion a chain reaction in which the DNA template is exponentially amplified.

Basic PCR reaction requires the following components:

- DNA Template: The double stranded DNA of interest
- DNA Polymerase: an enzyme that does not rapidly denature at high temperatures
- Oligonucleotide primers: Short pieces of single stranded DNA (often 20-30 base pairs) which are complementary to the 3' ends of the sense and anti-sense strands of the target sequence
- Deoxynucleotidetriphosphates: Single units of the bases A, T, G, and C (dATP, dTTP, dGTP, dCTP) provide the energy for polymerization and the building blocks for DNA synthesis
- PCR Buffer often includes magnesium and potassium to provide the optimal conditions for DNA denaturation and renaturation; also important for polymerase activity and stability

PCR is a very specific and highly sensitive tool for the detection of *Schistosoma* DNA in urine, stool, blood and serum. Its main advantage is being able to diagnose schistosomiasis in all stages of infection. Detection of *S. haematobium* and *S. mansoni* in urine or stoolusing PCR has been shown to be a very sensitive and specific for urinary and intestinal tract schistosomiasis. PCRtoolon serum has shown to be a very sensitive for acute schistosomiasis with sensitivity of more than 90% when compared to antibody tests and microscopy(Corcoran and da Silva, 2014).

Real-time PCR has been shown to be highly sensitive in determining the prevalence and intensity of *S. haematobium* infection (Obeng*et al*., 2008) and Genus-specific real-time PCR was found to be highly sensitive in the diagnosis of imported schistosomiasis among international travellers and migrants (Cnops*et al*., 2012). It has the potential to serve as a gold standard (Obeng*et al*., 2008). However, many real-time PCR methods use SYBR green dye (non-saturating dye) which may inhibit DNA polymerase when used at high concentrations (Sady*et al*, 2015).

Multiplex real-time PCR method has been evaluated for the detection and quantification of *S. mansoni* and *S. haematobium* infections in an endemic setting and has shown to be more sensitive than microscopy (Ten Hove*et al*., 2008).

Nested PCR is a modification of PCR that is designed to improve sensitivity and specificity. It involves the use of two primer sets and two successive PCR reactions. The first set of primers are designed to anneal to sequences upstream from the second set of primers and are used in an initial PCR reaction. Amplicons resulting from the first PCR reaction are used as template for a second set of primers and a second amplification step. Sensitivity and specificity of DNA amplification is significantly enhanced with this technique. However, the

potential for carryover contamination of the reaction is typically also increased due to additional manipulation of amplicon products. To minimize carryover, different parts of the process should be physically separated from one another (Carr *et al*., 2010).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Equipment and reagents

3.1.1 Equipment

The following pieces of equipment were used.

- Microscope
- Thermocycler(Eppendorf)
- ELISA Plate Reader
- Centrifuge
- Water Bath
- Vortex
- NanoDropSpectrophometer
- Gel Electrophoretic apparatus
- Gel documentation Unit

3.1.2 Chemical reagents

- Phosphate Buffer Saline with 0.05% Tween 20(VWR Life Science)
- *Schistosoma*IgG ELISA test kit (Diagnostic Automation/Cortez Diagnostics, Inc)
- Quick DNA Miniprep Plus Kit (ZYMO RESEARCH)
- Outer forward primer Sh1 (5′-CGTATTTTAGGTTTATGG-3′)
- Outer reverse primer, Sh2 (5′-CGAACTACACTTCCTAAGCA-3′)
- Inner forward primer Sh3 (5′- CGTGGTTTCATTAGATGTTTA-3′)

- Inner reverse primer Sh4 (5′- CGACAAATCAATCCATAATAC-3′)
- Outer forward primer Sm1 (5`- CGTTGATTAAGAAGATTATGA -`3
- Outer reverse primer Sm2 (5`-CGTGAAATTGACAGATCCA-`3),
- Inner forward primer Sm3 (5`-ATGTTACGATGTCTGTTCGGT-3`)
- Inner reverse primer Sm4 (5`- CGATAAAGGAGGATATAGAGTTC-3`).
- Nuclease free water
- 10X Standard Taq buffer with 25mM $MgCl_2$ (NEW ENGLAND BioLabsInc)
- dNTPs (Sigma-Aldrich)
- Taq polymerase (NEW ENGLAND BioLabsInc)
- Ethidium bromide

The primers were obtained from Inqaba Biotechnical Industries (pty) Ltd.

3.2 STUDY AREA

The Gambia is the smallest country in mainland Africa, it is located in West Africa surrounded by Senegal on all three sides (East, South and North) and the Atlantic Ocean on the western side. The Gambia is situated on either side of The Gambia River from where it got its name. The river flows through the centre of The Gambia and empties into the Atlantic Ocean. The Gambia has a landmass of about 10,689 square kilometers with a population of 1,833,460. The Gambia is divided into five regions West coast Region (1 and 2), North Bank Region (East and West), Lower River Region, Central River region and Upper River Region. CRR (Fig. 3.1) is divided into two parts north and south separated by the river, it has a population of 226,018 (GBOS, 2013).

Four villages were selected at random from both parts of the region; Jahally(13^{o}33` 40.09``N 14$^{o}$58'19.53``W), WaliKunda(13^{o}34` 0``N 14$^{o}$53` 0``W), Brikama Ba (13^{o}32` 11.96``N 14$^{o}$55 53.78`W), Kuntaur (13$^{o}$40` 14.74``N 14^{o}53` 23.90``W).

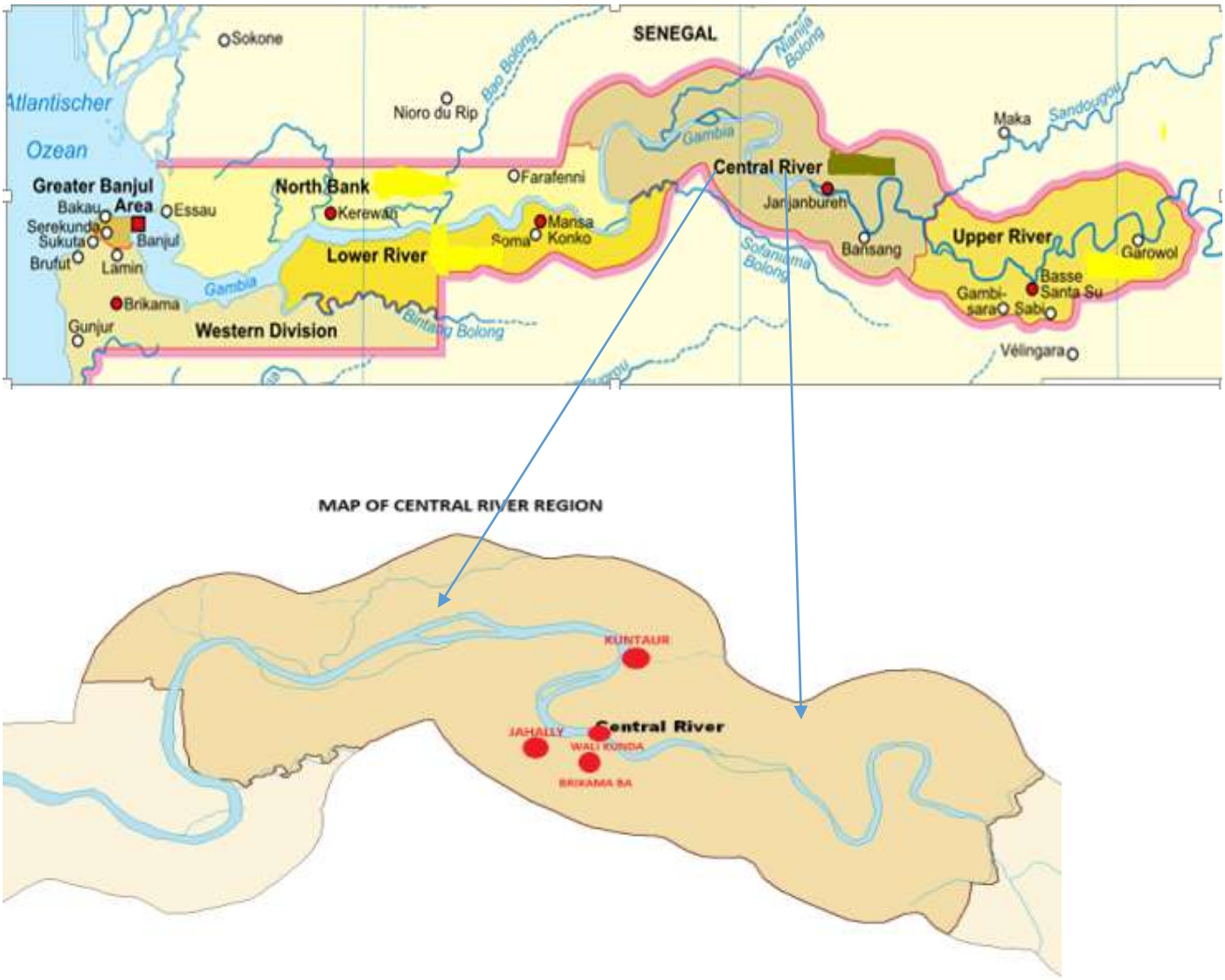

Figure 3.1: Map of Central River Region of The Gambia showing the different study villages

(www. google.com)

3.3 SAMPLING

Children of both sexes from the ages of 6-15 were randomly selected. Farmers and Fishers were also selected using simple random sampling.

The sample size was determined using the formula described byThrusfield (2007). The prevalence of *Schistosoma* used in CRR is14.2% (Gambia NTD Mapping Report, 2015)

$$n = \frac{Z^2 pq}{d^2}$$

where

n = minimum sample size

Z = appropriate value for the standard normal deviation for the desired confidence interval (1.96)

p = prevalence 14.2% (CRR)

q = complementary probability (1-p)

d = level of significance 5% (0.05)

Therefore, $n = \frac{1.96^2 * 0.142(1 - 0.142)}{0.05^2}$

$= 187.6 \approx 188$

3.4 ETHICAL CLEARANCE.

Ethical clearance was obtained from the Joint Scientific Coordinating Committee of The Gambia government and Medical Research Council. Before commencing the sample collection, permission was obtained from the Ministry of Basic and Secondary Education. The study was explained to each participant for their understanding and cooperation. Additionally,

an informed written consent form was obtained from each study participant, and for the children, consent was obtained from their parents/guardians.

3.5 SAMPLE COLLECTION FORM

Sample collection form were used to record data of study participant before sample collection. Data such as age of participant, gender, occupation and prior schistosomiasis infection and antibiotic treatment were collected and recorded.

3.6 SAMPLE COLLECTION

3.6.1 Urine collection

Urine samples were collected in 50ml conical tubes between the hours of 10.00 and 14.00 when schistosome eggs excretion is known to be highest (Obeng*et al.*, 2008). The samples were kept at ambient temperature in a cooler with ice packs between collection site and the laboratory.

3.6.2 Blood sample collection

Blood samples were collected by the finger prick method. The finger was cleaned with alcohol wipes and left for about three seconds to dry. Then using a pricker, the finger was pricked and gently squeezed to drop blood on the spots created on the filter paper. (The first drop of blood was wiped out because it might contain excess tissues fluids). The spotted papers were left to dry away from direct sunlight packed in sealable bag with desiccants kept at ambient temperature and transported to Department of Biochemistry at Ahmadu Bello University Zaria in Nigeria for analysis.

3.7 SAMPLE PROCESSING

3.7.1 Macroscopic examination

All the urine samples were examined and recorded as either clear, amber and clear, cloudy or bloody before centrifugation for microscopy.

3.7.2 Microscopy examination

Urine samples were examined by centrifugation technique (Ukaga*et al.,* 2002). Ten millilitre (ml) of each urine sample was centrifuged at 1,500 revolutions per minute (rpm) for five minutes. The supernatant was decanted and the sediment examined under the microscope. Pasture pipette was used to add two drops of the sediment to a frosted microscope slide and gently covered with a cover slip without the formation of air bubbles. The slide was examined under the microscope at low magnification (X10 and X40 objective lenses) for the presence of eggs of *S. haematobium* and *S. mansoni.* For quality control, duplicate slides were prepared for all samples. All the positive slides and 10% of the negative were re-read by an experiencedmicroscopist.

3.7.3 Enzyme Linked Immunosorbent Assay (ELISA)

A regular paper puncher was used to cut 6 mm size paper discs from eachfilter paper and placed in a well of the ELISA plate. To each sample in a well, 300ul of phosphate buffer saline (PBS) containing 0.05% Tween 20 was addedand incubated overnight in a refrigerator at 4^{o}C(Grune*et al*. 2015).

Fifty micro-litre (50ul)of the elute was used to test for IgG antibody response directed against schistosome egg antigen using*Schistosoma*IgG ELISA Kit. The ELISA plates are pre-coated with *Schistosoma*antigens. On addition of the elute to the plate,antibodies in the samples will bind to the antigen in the test well during the first incubation. After washing, enzyme

conjugate was added and incubated at room temperature to allow the enzyme complex to bind to the antigen-antibody complex. After a few washings to remove unbound enzymes, a substrate was added that developed a blue colour in the presence of the enzyme complex and peroxide. The plate was read on the ELISA plate reader at 450nm with a reference filter at 620 nm (AccuDiag™ *Schistosoma*IgG ELISA Kit pamphlet).

3.7.4 Elution of Blood and DNA Extraction

DNA was extracted from the dried blood spot on the filter paper as follows:

A regular paper puncher was used to cut 6 mm discs from each filter paper and put in a labelled 1.5ml Eppendorf tube. Between the cuttings, the paper puncher was cleaned with 70% ethanoland allowed to dry to avoid contamination. 500µl of phosphate buffered saline was added to the tubes and incubated (Lodh*et al*., 2013). The DNA was extracted using Quick-DNA Miniprep Plus Kit (ZYMO RESEARCH) according to the manufacturer's protocol. The DNA concentration was measured using the NanoDropspectrophotometer. Extracted DNA samples were stored at−20°C until required.

3.7.5 Molecular Identification of Schistosome Isolates

Nested schistosome-specific PCR was performed using the DNA extracted from each sample. Each of the sample was tested for both *S. mansoni* and *S. haematobium* using specific designed primers to amplify variable regions 600bp and 770bp within cox1mitochondrial DNA (mtDNA) of *S. mansoni* and *S. haematobium*respectively.For the *S. haematobium*,the schistosome cox1 mitochondrial DNA (mtDNA) region was amplified using the following primers;

Outer forward primer Sh1 (5′-CGTATTTTAGGTTTATGG-3′) with

Outer reverse primer, Sh2 (5′-CGAACTACACTTCCTAAGCA-3′) and

Inner forward primer Sh3 (5′- CGTGGTTTCATTAGATGTTTA-3′) with

Inner reverse primer Sh4 (5′- CGACAAATCAATCCATAATAC-3′).

For *S. manson*, the PCR was carried out by using the following primers:

Outer forward primer Sm1 (5`-CGTTGATTAAGAAGATTATGA-`3) with

Outer reverse primer Sm2 (5`-CGTGAAATTGACAGATCCA-`3), and

Inner forward primer Sm3 (5`-ATGTTACGATGTCTGTTCGGT-3`) with

Inner reverse primer Sm4 (5`- CGATAAAGGAGGATATAGAGTTC-3`).

The cox1 mitochondria DNA gene was used as a DNA barcode to identify animal species because its mutation rate is often fast enough to distinguish between closely related species. It is highly abundant in the cell and has highly conserved regions and structures among con-specifics (Sady*et al*, 2015).

PCR amplification was performed in 25µl reaction mixture and consisted of 14.75µl of nuclease free water, 2.5µl of PCR buffer containing 25mM $MgCl_2$, 2.5µl of dNTPs,and 0.5µl of each of the primers, 0.25µl of Taq polymerase and 4µl of DNA. The outer primers were used for the first round of the nested PCR with the following PCR cycling conditions:

- initial denaturing step of 95°C for 5 min
- followed by 30 cycles of 95°C for 60 seconds
- annealing temperature of 60°C for 30 seconds
- elongation at 72°C for 45 seconds and
- final extension at 72°C for 4 minutes, for the first round of the nested.

The second round in which 4µl of the products of the first round was used as DNA, with the inner primers and the same volume of the other components (nuclease free water, PCR buffer dNTPs and Taq polymerase with the following PCR conditions:

- initial denaturing step of 95°C for 5 min
- followed by 30 cycles of 95°C for 60 seconds
- annealing temperature of 55°C for 30 seconds
- elongation at 72°C for 45 seconds
- final extension at 72°C for 4 minutes

The difference in annealing temperature is due to the expected amplicon size; for the outer primers it is expected to be around 770bp whilst for the inner, it is around 600bp.

Amplicons were visualized on a 1% agarose gel stained with ethidium bromide using 100bp ladder to estimate band sizes.

3.7.6 Statistical Analysis

The prevalence of schistosomiasis in the study area was calculated. The Chi-square test was used to determine the statistically significant difference in incidence of infection between males and females and different age groups. Odds ratio (OR) was determined to show association between the factor and incidence rate. Overall, age and sex specific incidence of the disease was calculated and expressed as percentage (%).

CHAPTER FOUR

4.0 RESULTS

4.1 Age and Demographic Distribution of Participants

Age distribution of the study participant (Figure 4.1) shows that adults of the age bracket 16-30 years constituted almost half (47.2%) of the participants from which blood and urine sample were obtained. Children of school age (6-15) were about one-quarter (25.6%) while those above 30years constituted the remaining (28.3%). Analysis of the occupational status of the participants also showed that farmers formed approximately half of the participants (50.3%), with students (25.60%)and fishers (24.10%) each accounting forabout one quarter (Figure 4.2). A large proportion (80%) of the population had never received any drug treatment for schistosomiasis in the past.

4.2 Infection load of *schistosoma* eggs from urine by physical examination and microscopy

Physical examination of urine showed that Amber clear urine (44.8%) was more frequent than cloudy urine (39.9%) or bloody (10.9%) urine with clear urine (4.7%) being less frequent.

Urine microscopy revealed an overall incidence of 24.5% (47 of 192) of*S. haematobium* infection. Among the four villages, Brikama Ba village had the highest incidence of 44.8% (13/29), followed by Jahally 35.3% (24/68); WaliKunda village had 14.7% (5/34) the least incidence was recorded in Kuntaur village 8.2% (5/61) individuals were infected as shown in

Figure 4.4. The incidence was higher in males (33.3%) than in females (18.8%). The age specific distribution of the infection revealed that school children from the ages of 6-15years have the highest incidence with 43.8% (21 of 48) followed by age group 16-30 years with 21.1% (19 of 90). The oldest age groups 61- 75 years had an incidence of 20% (1 of 5) and 46-60 years recorded 15% (3 of 20). The lowest incidence occurred in the age group 31-45 years with 10.3% (3 of 29) (Figure 4.3). In terms of occupation (figure 4.5), fishers had the lowest incidence 15.9% (7 of 44). Students had the highest incidence with 41.2% (21 of 51) whilst farmers record an incidence of 19.6% (19 of 97). 28.8% (44 of 153) of those that are positive have never received drug (praziquantel) for treatment of schistosomiasis while 7.7% (3 of 49) have recently been treated with Praziquantel.

Statistical analysis (Table 4.1) showed that there is statistically significant difference in incidence between males and females and between different age groups ($P<0.05$).

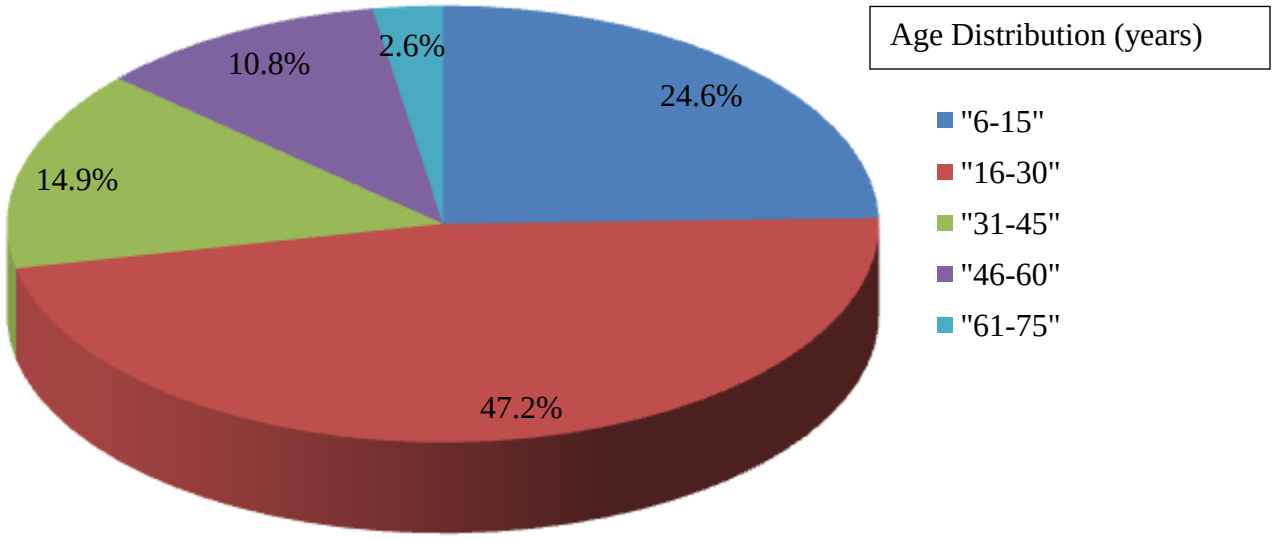

Figure 4.1 Age Distribution of participants

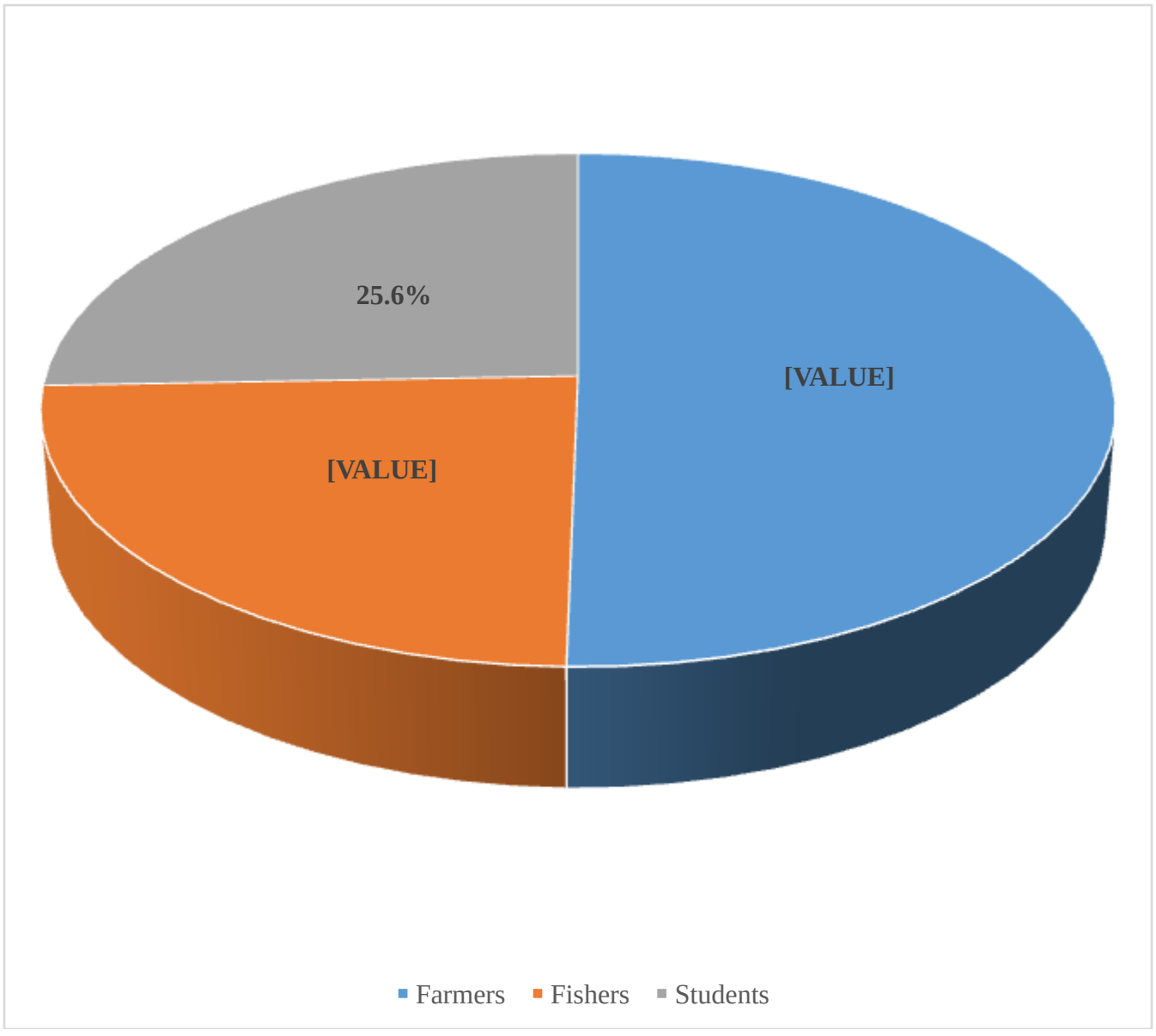

Figure 4.2 Occupational distribution of participants

Table 4.1 Statistical analysis for Urine Microscopy showing the different susceptibility to infection and their prevalence

Susceptibility to infection	Number analysed	Positive	Prevalence (%)	P value
AGE				
6-15	48	17	35.4	0.001*
16-30	90	21	23.3	
31-45	29	4	13.8	
46-60	20	4	20	
61-75	5	1	20	
Total	192	47	24.5	
SEX				
FEMALE	117	18	15.4	0.001*
MALE	75	29	38.7	
DRUG TREATMENT				
NO	153	44	28.8	0.001*
YES	39	3	7.7	
OCCUPATION				
FARMERS	97	19	19.6	
FISHERS	44	7	15.9	0.001*
STUDENTS	51	21	41.2	

Note: * means it is statistically significant (P value < 0.05)

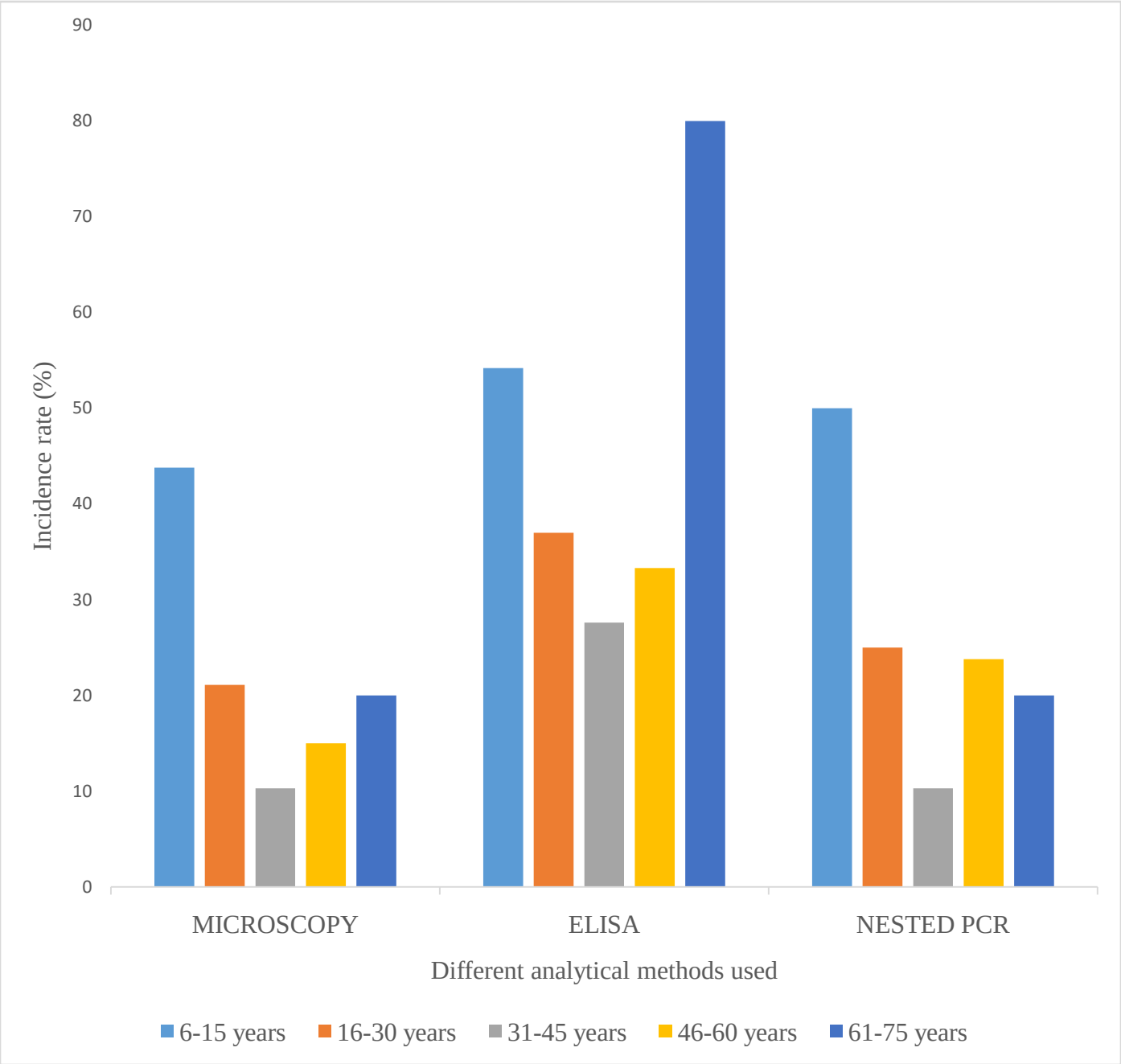

Figure 4.3 Percentage positive incidence rates of the different analytical method for the different age groups

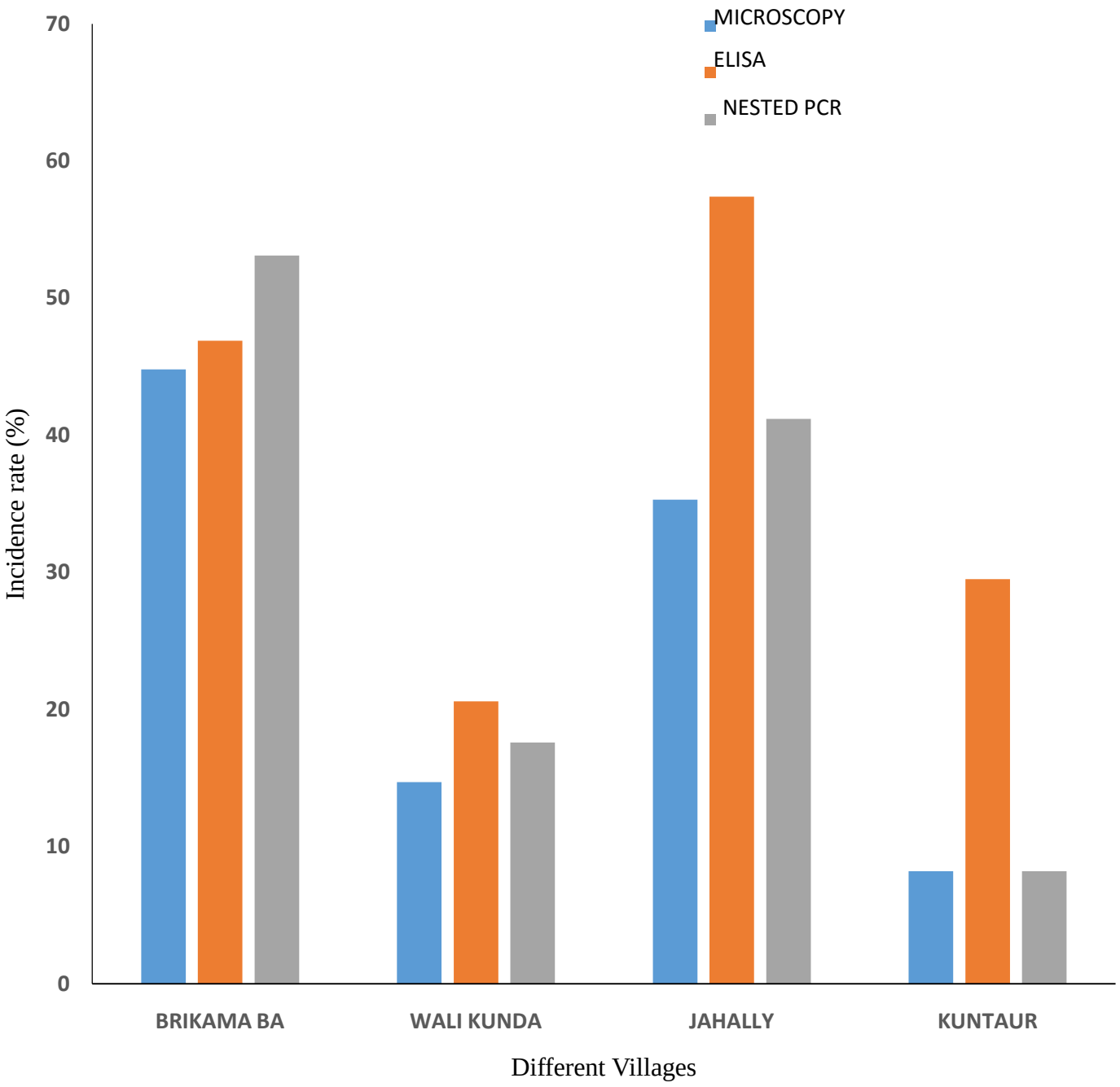

Figure 4.4 Percentage positive incidence rates of the different tests for the different villages

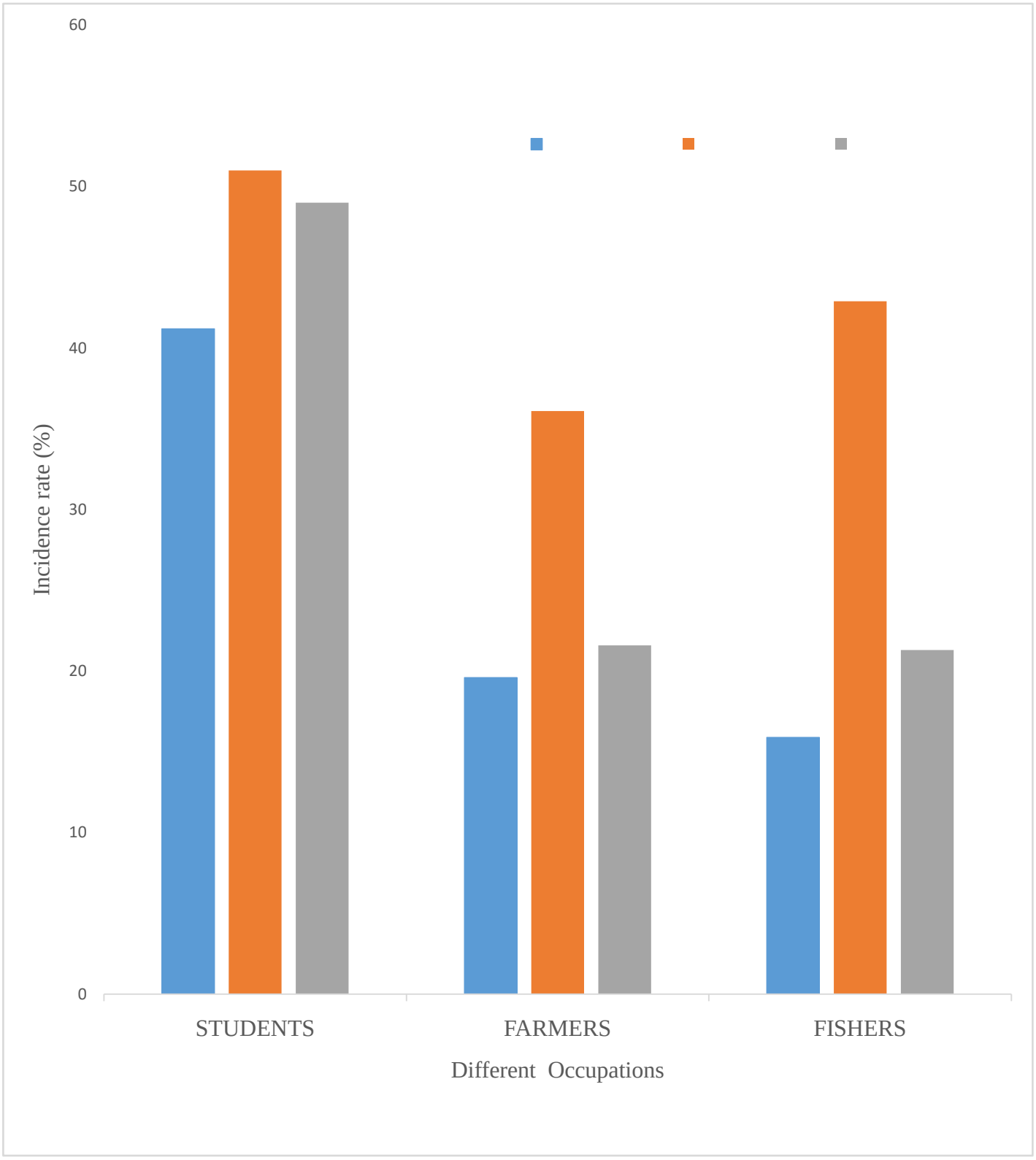

Figure 4.5 Percentage positive incidence rates of the different tests for the different occupation

4.3 Detection of the incidence of schistosomiasis from urine using Nested PCR

There is an incidence of 28.7% (56 of 195) based on the PCR results for *S. haematobium.* Brikama Ba had the highest incidence among the four villages with 53.1% (17 of 32) and Kuntaur with the lowest incidence 8.2% (5 of 61). Jahally has an incidence of 41.2% (28/68) whilstWaliKundahas an incidence of 17.6% (6 of 34). Plate 4.1 shows some of the bands at about 700bp for *S. haematobium*for blood samples while Plate 4.2 shows the bands for *S. mansoni* in urine samples

The age specific incidence of infection shows that school children from the ages of 6-15 years had the highest incidence50.0% (24 of 48) followed byage group 16-30 years with 25.0% (23 of 92). Age group 46-60years had an incidence of 23.8% (5 of 21). The oldest age group 61-75 years had an incidence of 20% (1 of 5) while the age group 31-45 years had the lowest incidence with 10.3% (3 of 29) (Table 4.2). In terms of occupation, fishers have the lowest incidence with 21.3% (10 of 47). Students have the highest incidence with 49.0% (25 of 51) and farmers with an incidence of21.6% (21 of 97). 34.0% (53 of 156) of those that are positive have never received any drug (praziquantel) for treatment of schistosomiasis whilst 7.7% (3 of 39) have recently received praziquantel. Generally, a higher infection was recorded among males 41.0% (32/78) than in females 23.9% (28 of 117)

There was statistically significant difference (Table 4.2) in incidence between males and females ($P<0.05$) as well as in all other factors (age, drug received and occupation).

Table 4.3shows the distribution of the s*chistosoma* species detected in the urine samples.*S. mansoni* was detected only in Jahallywhilst *S. haematobium* was identified in all four villages.

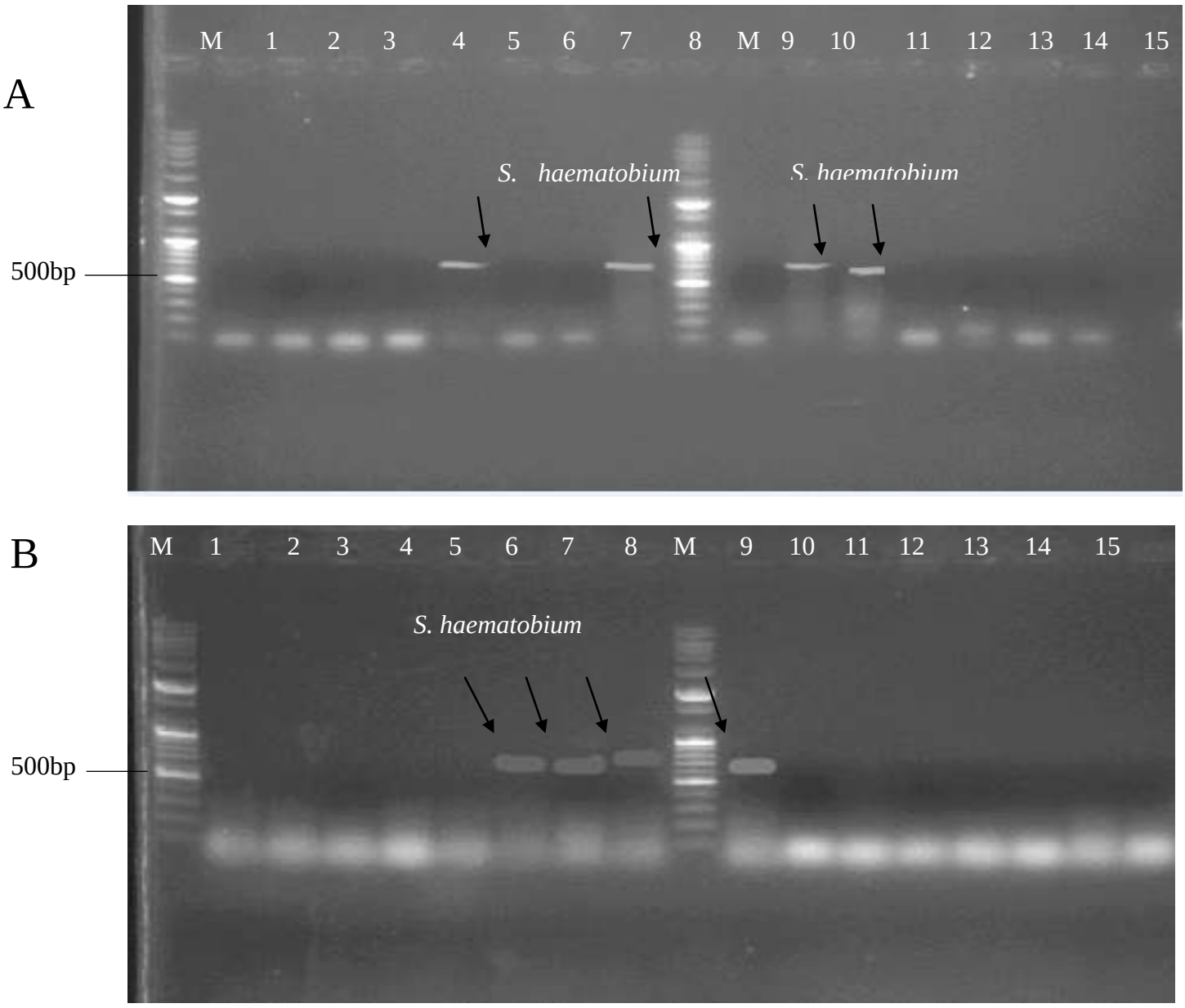

Plate 4.1 Electrophoresis showing bands of *S. haematobium* in urine samples after nested PCR

KEY:

M - **Molecular weight marker**
Gel A: **Lanes 5,8, 10, 11 (*S. haematobium)***

Gel B: **Lanes 6,7,8, 9 *(S. haematobium)***

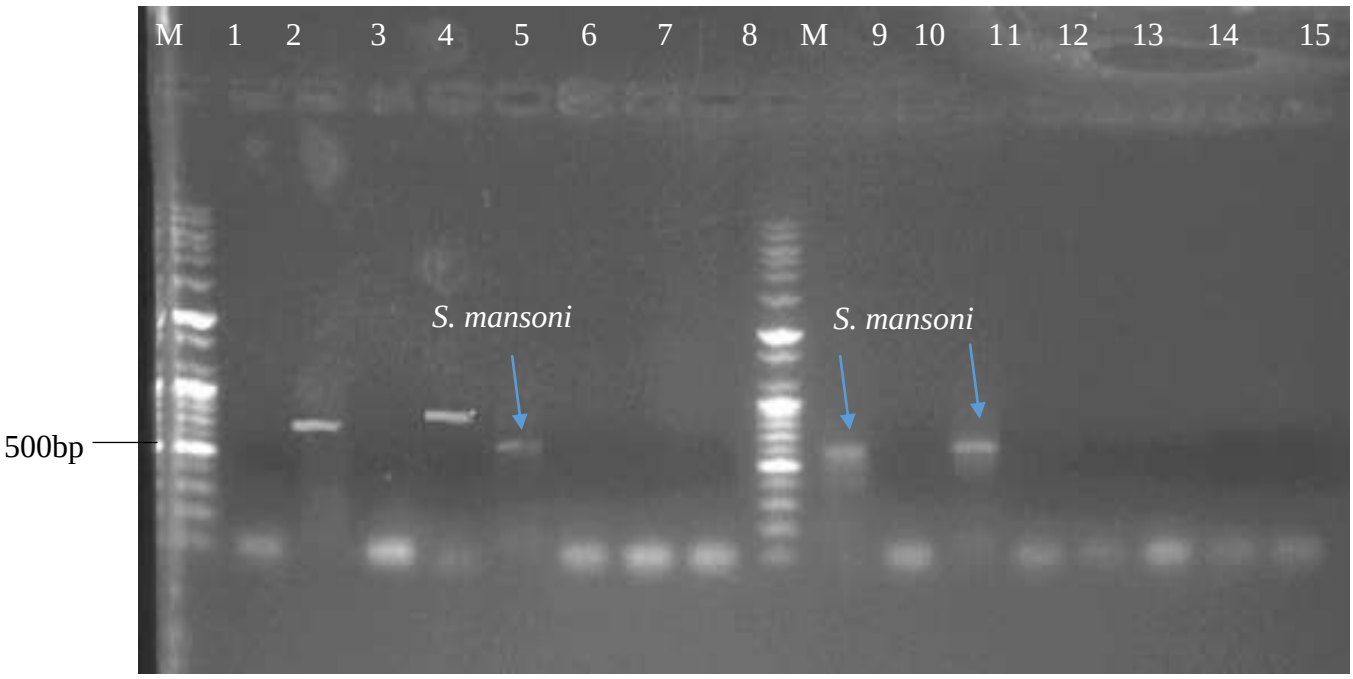

Plate 4.2 Gel electrophoresis showing bands of *S .mansoni*in in urine sample after Nested PCR.

KEY

M -	**Molecular weight marker**
LANE 5, 9 and 11-	***S. mansoni***
LANE 2, 4 –	***S. haematobium***

Table 4.2Statistical analysis for susceptibility to infection based on age interval on urine samples tested usingNested PCR

Susceptibility to infection	Number analysed	Number positive	Number negative	Incidence (%)	Odd ratio	Confidence interval	P value
Age							
6-15	48	24	24	50.0	1		
16-30	92	23	69	25.0	0.18	0.03-0.70	
31-45	29	3	26	10.3	2.95	0.26-66.05	0.016*
46-60	21	5	16	23.8	8.03	2.02-54.03	
61-75	5	1	4	20.0	0.12	0.02-0.49	

Note: * means it is statistically significant (P value < 0.05)

Table 4.3 Distribution of Different Species of Schistosomes in the sample areas using Nested PCR

Sample area	Frequency (%)	
	S. haematobium	*S. mansoni*
Brikama Ba (n=32)	17(53.1%)	0
Walikunda (n=34)	6 (17.6%)	0
Jahally (n=68)	28 (41.2%)	3 (4.4%)
Kuntaur (n=61)	5 (8.2%)	0
Total (n=195)	**56 (28.7%)**	**3 (1.5%)**

*n = number of sample analysed

4.4 Detection of the incidence of schistosomiasis from blood using ELISA

ELISA results revealed an overall incidence of 40.5% (79 of 195) for schistosomiasis. The highest incidence was recorded in Jahally with 57.4% (39 of 68) followed by Brikama Ba 46.9% (15 of 32), Kuntaur had 29.5% (18 of 61) and the lowest incidence 20.6% (7 of 34) was recorded in WaliKunda.

The incidence was higher in males (51.3%) than in females (33.3%). The age specific distribution of the infection revealed that the oldest age group 61- 75 years had the highest incidence of 80% (4 of 5) followed by school children from the ages of 6-15 years with an incidence of 54.2% (26 of 48). Age group 16-30 years had an incidence of 37.0% (34 of 92) and 46-60 years recorded 33.3% (7 of 21). The lowest incidence occurred in the age group 31-45 years with 27.6% (8 of 29) (Table 4.4).

In terms of occupation, farmers had the lowest incidence 37.1% (36 of 97). Students had the highest incidence with49.0% (25 of 51) whilst fishers record an incidence of 38.3% (18 of 47).There was statistically significant difference in incidence between the males and females ($P<0.05$)and no significant difference in incidence between the different age groups ($P>0.05$).

Table 4.4Statistical analysis for susceptibility to infection based on age interval and sex on blood samples tested usingELISA

Susceptibility to infection	Number analysed	Number positive	Number negative	Incidence (%)	Odd ratio	Confidence interval	P value
Age							
6-15	48	26	22	54.2	1		
16-30	92	34	58	37.0	0.73	0.26-1.91	
31-45	29	8	21	27.6	1.37	0.41-4.50	0.091
46-60	21	7	14	33.3	1.16	0.40-3.42	
61-75	5	4	1	80.0	0.86	0.29-2.44	
Sex							
Female	117	39	78	33.3	1	1.170–3.787	0.022*
Male	78	40	38	51.3	2.11		

CHAPTER FIVE

5.0 DISCUSSION

This study revealed that *Schistosoma haematobium*is endemic in Central River Region (CRR)of the Gambia with anincidence of 28.7% among school children, farmers and fishers. This is in agreement with findings from previous study that revealed high transmission rate of 14.2% for *S.haematobium* infection in CRR (Gambia NTD Mapping Report, 2015).The higher incidence obtained in this study could be due to the fact the PCR used in this study is more sensitive and can detect *Schistosoma*species in all stages of infection (Corcoran and da Silva, 2014) compared with microscopy that was employed in the diagnosis and characterisation of the *schistosoma* species in previous studies.

The lowest incidenceof schistosomiasis recorded in Kuntaur may be attributed to mass administration of anti-schistosomial drug carried out in early 2017, few months before commencement of sample collection in that village. Questionnaire survey attested to the fact, 60.7% of people in that village benefited from the mass drug administration held in early 2017, the other villages were not part of the mass drug administration.

The study also revealed a higher incidence in males compared to their female counterpart. Statistically significant difference in incidence in males and females.This result is similar to the NTD mapping study carried out in The Gambia which showed a higher prevalence in male than in female (Gambia NTD Mapping Report, 2015).A similar study in Senegal also revealed a higher prevalence in boys than girls (Senghor *et al*. 2014). This could be due to the fact that males (especially the students) have more frequent contacts with water than females because in traditional Africansettings, young females are more associated with indoor activities than

their male counterpart.In the study population, 73.9% of the positive cases that falls between 6-15 years age group are boys and they are fond of going to the streams and ponds to swim, wash the domestic animals, bath etc.Generally, prolonged and more frequent contacts with water causes more exposure to the snail intermediate hosts in the water bodies this gives more chances to cercarial penetration through the skin. During the act of swimming and fishing the whole body remain in contact with water providing more surface area for penetration cercariae through skin(Singh *et al*., 2016).Since the villages have pipe-borne water, the females usually stay at home and generally use tap water for their house chores, thus reducing their contacts with other water sources. This differences in the exposure of male and female to water bodies where the snail intermediate host in habit might explain the differences in the infection rates rather than their gender differences.

The incidence of infection among different age groups shows that overall there is a significant association between the age and incidence of the disease. This is recorded in most of the age groups but in the age group 61 – 75 years there was no association with schistosomiasis.

The association between praziquantel administration and incidence of schistosomalinfection isaptly demonstrated in the study. Communities who had received mass drug administration against schistosomal infection recorded much lower incidence as compared to those that did not. This shows that the drug (praziquantel)protects against schistosomiasis in the region although there is still an appreciable level of infection persistence.The cure rate was not associated with age, occupation or gender.

Students has the highest incidencein terms of occupation- this correlates with the age group 6-15 years as almost all the students are within this age group. There is a significant association

between the occupation and incidence of the disease. The higher incidence in fishers (though not significant) as against farmers may be attributed to longer contacts with waters.

The higher detection rate usingELISA technique ascompared with PCR and Microscopy might be due to the fact that ELISA detects antibodies to *Schistosoma* and cannot distinguish between active and past infection, with parasite-specific antibodies remaining in the system long after beingcured (Doenhoff *et al*. 2004). ELISA test kit used cannot distinguish between the different s*chistosoma*species. It detects antibodies of all *schistosoma* species hence the incidence recorded in this study is for both *S. mansoni* and *S. haematobium*and possible other types of s*chistosoma*species even though there has been no report of any other type apart from *mansoni and haematobium*in the region or within the country.There is a possibility of false positives due to cross reaction. The manual washing carried out during the ELISA test because of lack of automated ELISA plate washer may have caused split over from one well to another thereby causing false positives which may also have contributed to the observed higher incidence.

The low incidence of*S. mansoni*reported for CRR in this study agrees with previously reported low prevalence for *S. mansoni* in the country (NTD Mapping Report, 2015). The NTD mapping study reported a prevalence of 0.4% for *S. mansoni* in CRR. The detection of *S. mansoni* in blood using nested PCR is revealing as this organism (*S. mansoni*) is usually detected in stool samples of infected patients.

CHAPTER SIX

6.0SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 SUMMARY

An overall incidence of *Schistosoma haematobium*in the Central River Region of The Gambiawas 28.7% out of 195 people examined. The results varied within the different villages ranging from Brikama Ba with the highest to Kuntaurwith the lowest incidence.

The incidence of the disease by age showed that the highest incidence was recorded in the age group of 6 – 15 years while lowest incidence was recorded in 31-45 years age group. Males had a higher incidence than females.

Students recorded the highest prevalence followed by farmers then fishers recorded the lowest incidence.An incidence rate of 34.0% was recorded among people who have never received any kind of treatment for schistosomiasis compared to 7.7% of those who have received treatment.

Schistosoma mansoni was detected in 1.5% of the sampled population and was only recorded in one village among the four villages tested.

6.2 CONCLUSION

The study showed that incidence of schistosomiasis infection is highest among students but variesamongthe different age groups and occupation in the Central River Region of The Gambia which was largely dictated by level of contact with water bodies and previous drug treatment with praziquantel. *Schistosoma haematobium* and *mansoni* were both identified in urine samples by microscopy. ELISA technique detects schistosoma species in blood samples

but could not distinguish between active and past infections. Nested PCR showed that*S. haematobium* is the major causative nematode of bilharzias in the Gambia. The study also showed that*Schistosoma mansoni* can be detected in blood and urine.

6.3 RECOMMENDATIONS

I. In order to reduce the prevalence of schistosomiasis in The Gambia, mass drug administrationusing praziquantel should be carried out in the villages that have not benefited from it yet.

II. A community health eradication campaign and adequate health education should be promoted on the control of the disease.

III. Since the infection of children can be avoided, the community should be educated on the mode of transmission of the disease and the pathology of the disease and therefore encourage them to adopt control measures. Since there is availability of running water, children should be discouraged from swimming and washing in the river.

REFERENCES

Adenowo, A.F., Oyinloye, B.E., Ogunyinka, B.I. andKappo, A.P., (2015). Impact of human schistosomiasis in sub-Saharan Africa. *The Brazilian Journal of Infectious Diseases*, 19 (2).

Agnew, A., Lucas, S. and Doenhoff, M., (1988). The host-parasite relationship of *Schistosomahaematobium* in CBA mice. *London School of Hygiene and Tropical Medicine*, **3**: 403-424.

Albonico, M., Montresor, A., Crompton, D.W.T. and Savioli, L., (2006). Intervention for the Control of Soil-Transmitted Helminthiasis in the Community. Advances in Parasitology, Academic Press. Volume 61, Pages 311-348

Ali, S.A., (2011). Natural products as therapeutic agents for schistosomiasis. *Research Journal* of *Medicinal Plant*, **5**: 1-20.

Almeda, J., Corachan, M., Sousa, A., Ascaso, C., Carvalho, J.M., Rollinson, D. and Southgate, V.R., (1994). Schistosomiasis in the Republic of Sao Tome´ and Principe: human studies. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 88:406–9.

Barakat, R., Morshedy, H.E. andFarghaly, A., (2014).Human Schistosomiasis in the Middle East and North Africa Region. *Neglected Tropical Diseases*, DOI 10.1007/978-3-7091-1613-5_2.

Basch, P., (1991). *Schistosomes.* New York: Oxford University Press.

Berriman, M. and El-Sayed, N., (2009). The genome of the blood fluke *Schistosomamansoni*. *Nature Genetics*, 400: 352-358.

Berry, A., Iriart, X., Fillaux, J. andMagnaval, J.F. (2017). Urinary schistosomiasis and cancer. *Bulletin de la Société. PathologieExotigue*, 110: 68. (https://doi.org/10.1007/s13149-017-0547-4)

Bogitsh, B., Carter, C. and Oeltmann, T., (2005). *Human Parasitology*. Burlington: Elsevier Academic Press.

Briand, V., Watier, L., Le Hesran, J.Y., Garcia, A. and Cot, M., (2005). Co-infection with *Plasmodium falciparum* and Schistosoma haematobium: protective effect of schistosomiasis on malaria in Senegalese children? *American Journal of Tropical Medicine* and *Hygiene*, 72:702–707.

Carr, J., Williams, D.G., Hayden, R.T., (2010).Molecular Diagnostics. *Techniques and Applications for the Clinical Laboratory*. Elsevier Inc. Amsterdam, Netherlands, pp. 289-300

Conteh, L., Engels, T. andMolyneux, D., (2011). Socioeconomic aspect of neglected tropical diseases. *Lancet*, 2010; 375:239–47.12

Corachan, M., (2002). Schistosomiasis and International Travel. *Clinical Infectious Disease.* 35 (4): 446-450.

Corcoran, C. and da Silva, M., (2014). *Diagnosing Schistosomiasis*,Pathchat Edition No. 11. https://www.ampath.co.za/wp-content/newupload//2014/.../pathchat_11-Bilharzia

Cnops, L., Tannich, E., Polman, K., Clerinx, J. and van Esbroeck, M., (2012). *Schistosoma* real-time PCR as diagnostic tool for international travellers and migrants. *Tropical Medicine & International Health*, 17:1208–1216

Danso-Appiah, A. and De Vlas, S.J., (2002). Interpreting low praziquantel cure rates of *Schistosoma mansoni* infections in Senegal. *Trends in Parasitology*, 18 (3):125 – 129.

Deelder, A.M., Miller, R.L., de Jonge, N. and Krijger, F.W., (1990). "Detection of schistosome antigen in mummies". *The Lancet*,335 (8691): 724–725.

De Almeida Marques, D.P., Rosa, F.M., Maciel, E., Negrao-Correa, D., Teles, H.M.S. andCaldeira, R.L., (2014). Redueced susceptibility of a

*Biomphalaria*tenagophilaPopulation to *Schistosoma mansoni* after introducing the Taim/RS strain of B. tenagophila into Herivelton Martins stream. *PLoS ONE* 9:e99573. doi: 10.1371/journal.pone.0099573

de Jonge, N., Kremsner, P.G., Krijger, F.W., Schommer, G., Fillié, Y.E., Kornelis, D., van Zeyl, R.J., van Dam, G.J., Feldmeier, H. and Deelder, A.M., (1990). Detection of the schistosome circulating cathodic antigen by enzyme immunoassay using biotinylated monoclonal antibodies.*Transactions of the Royal Society of TropicalMedcine andHygine*, 84(6):815-8.

Doenhoff, M.J., Chiodini, P.L. and Hamilton, J.V., (2004) Specific and sensitive diagnosis of schistosome infection: can it be done with antibodies? *Trends in Parasitology*, 20: 35-39

Ekpo, U.F., Oluwole, A.S., Abe, E.M., Etta, H.E., Olamiju, F. andMafiana, C.F., (2012). Schistosomiasis in infants and pre-school children in sub-Saharan Africa: implication for control. *Parasitology*, pp. 835–841

Fenwick, A., Rollinson, D. and Southgate, V., (2006). Implementation of human schistosomiasis control: Challenges and prospects. *Advances in Parasitology*, 61:567-622

Friedman, J.F., Mital, P., Kanzaria, H.K., Old, G.R, and Kurtis, J.D. (2007). Schistosomiasis and Pregnancy. *Trends in Parasitology*, 23 (4):159-164.

Gambia Bureau of Statistics (2013) Gambia Population and Housing Census Preliminary Results. Available at: www.gbos.gov.gm/2013.php

Gambia Neglected Tropical Disease (Schistosomiasis and Soil Transmitted Helminths) Mapping Report (2015). Unpublished manuscript

Gruner, N., Stambouli, O. and Ross, R.S., (2015). Dried Blood Spots - Preparing and processing for use in Immunoassays and in Molecular Techniques. *Journal of Visualized Experiments*, (97), e52619, DOI: 10.3791/52619.

Gryseels, B., (1989). The relevance of schistosomiasis for public health. *Tropical Medicine and Parasitology*; 40:134–42

Gryseels, B., Polman, K., Clerinx, J. and Kestens, L., (2006). Human schistosomiasis. *Lancet*, 368: 1106–18

He, P., Song, L., Xie, H., Liang, J., Yuan, D., Wu, Z. and Lv, Z., (2016). Nucleic acid detection in the diagnosis and prevention of schistosomiasis. *Infectious Diseases of Poverty* (2016) 5:25

Hotez, P.J. and Kamath, A., (2009). Neglected tropical diseases in sub-Saharan Africa: review of their prevalence, distribution, and disease burden. *PLoSNeglected Tropical Disease*, 3: e412.

Houston, S., Kowalewska-Grochowska, K., Naik, S., McKean, J., Johnson, E.S. and Warren, K., (2004). First Report of *Schistosoma*mekongi Infection with Brain Involvement. *Clinical Infectious Diseases*, 38:e1-6.

Ibironke, O.A., Phillips, A.E., Garba, A., Lamine, and S.M., Shiff, C., (2011). Diagnosis of *Schistosoma haematobium* by detection of specific DNA fragments from filtered urine samples. *American Journal of Tropical Medicine and Hygiene*,84:998–1001

International Agency for Research on Cancer (IARC); (2012). Monographs on the Evaluation of Carcinogenic Risks to Humans, No. 100B. *Schistosoma haematobium*. Available from: https://www.ncbi.nlm.nih.gov/books/NBK304343/

John, D.T., and William A.P., (2006). *Markell and Voge's Medical Parasitology*. 9th ed. United States: Saunders Elsevier, 181-196

Kang Y and Wedekin L*(2013)* Dynamics of anintraguild predation model with generalist or specialist predator. *Journal of Mathematical Biology*, 67(5):1227–1259

King, C.H. (2010). Parasites and poverty: the case of schistosomiasis. *ActaTropica*, 113

King, C.H. (2009). Toward the elimination of Schistosomiasis, *England Journal of Medicine*, 8, 260(2): 106-109.

Knight, M., African-Goktas, H.D., Lttiprasert, W., Odoemelam, E.C., Miller, A.N. and Bridger, J.M., (2014). Gene function in schistosomes: recent: advances towards a cure. *Frontiers in Genetics*. https://doi.org/10.3389/fgene.2014.00230

Lengeler, C., De Savigny, D.O., Mshinda, H., Mayombana, C., Tayari, S., Hatz, C., Degremont, A. and Tanner, M. (1991). Community based questionnaires and Health statistics as tools for the cost efficient identification of communities at risk of urinary schistosomiasis. *International Journal of Epidemiology*, 20(3): 796– 807.

Lodh, N., Mwansa, J.C.L., Mutengo, M.M. and Shiff, C.J., (2013). Diagnosis of *Schistosoma mansoni* without the Stool: Comparison of Three Diagnostic Tests to Detect *Schiostosoma mansoni* Infection from Filtered Urine in Zambia. *American Journal of Tropical Medicine and Hygiene*, 89(1): 46–50

Mahmoud, A. F. (2001). ED. *Schistosomiasis*. *Tropical Medicine Science and Practice*, Vol. 3. Imperial College Press, Singapore.

Mahmud, M.A., Spigt, M., Bezabih, A.M., Pavon,I.L., Dinant,G-J. andVelasco, R.B., (2015). Efficacy of Handwashing with Soap and Nail Clipping on Intestinal Parasitic Infections in School-Aged Children: A Factorial Cluster Randomized Controlled Trial. *PLoSMedciene*.https://doi.org/10.1371/journal.pmed.1001837

Mascarini-Serra, L. (*2011*) Prevention of Soil Transmitted Helminth Infections. *Journal of Global Infectious Diseases*, 3: 175-182.

McCarthy, J.S., Lustigman, S., Yang, G-J., Barakat, R.M.,García, H.H., Sripa, B. and Willingham, A.L., (2012). A Research Agenda for Helminth Diseases of Humans: Diagnostics for Control and Elimination Programmes. *PLoS Neglected TropicalDisease*,6 (4): e1601. https://doi.org/10.1371

McManus, D.P and Loukas, A., (2008). Current status of vaccine for schistosomiasis. *ClinicalMicrobiologyReviews*, 21:225–242.

Mkoji,G.M., Hofkin, B.V., Kuris, A.M., Stewart-Oaten,A., Mungai,B.N., Mungai, F.,LokerE S., (1999). Impact of the crayfish Procambarusclarkii on *Schistosomahaematobium* transmission in Kenya. *The American Journal of Tropical Medicine and Hygiene*, 61 (5):751 - 759

Mosunjac, M.B., Tadros, T., Beach, R., Majmudar, B., (2003). Cervical schistosomiasis, human papilloma virus (HPV), and human immunodeficiency virus (HIV): a dangerous coexistence or coincidence?*Gynecologic Oncology*,90(1):211-4

Mostafa, M.H., Sheweita, S.A. and O'Connor, P.J. (1999). Relationship between schistosomiasis and bladder cancer. *Clinical MicrobiologyReview*,12:97 – 111.

Nour, N. M. (2010). Schistosomiasis: Health Effects on Women. *Reviews in Obstetrics and Gynecology*, *3*(1), 28–32.

Obeng, B.B., Aryeetey, Y.A., de Dood, C.J., Amoah, A.S., Larbi, I.A., Deelder,A.M., …Yazdanbakhsh, M. (2008). Application of a circulating-cathodic-antigen (CCA) strip test and real-time PCR, in comparison with microscopy, for the detection of *Schistosoma haematobium*in urine samples from Ghana. *Annals of Tropical Medicine and Parasitology*, 102:625–633

Olveda, D.U., Li, Y., Olveda, R.M., Lam, A.K., Mcmanus, D.P., Chau, T.N., ...Ross, A.G. (2014). Bilharzia in the Philippines: past, present, and future. *International Journal of infectiousDiseases*,18, 52–56. doi: 10.1016/j.ijid.2013.09.011

Rokni, M. B. (Ed.). (2012). Control of Schistosomiasis, Schistosomiasis. Croatia: InTech

Roberts, L. and Janovy, J., (2000). *Foundations of Parasitology*, Sixth Edition. Boston: McGraw Hill Companies Inc.

Sady, H., Al-Mekhlafi, H.M., Ngui, R., Atroosh, W.M., Al-Delaimy, A.K., Nasr, N.A., …Dawaki, S. (2015). Detection of *Schistosomamansoni* and *Schistosomahaematobium* by Real-Time PCR with High Resolution Melting Analysis. *International Journal of Molecular Sciences*,16: 16085-16103; doi: 10.3390/ijms160716085

Savioli, L., Crompton, D.W. and Neira, M., (2003). Use of anthelminthic drugs during pregnancy. *American Journal of Obstetrics and Gynecol*ogy, 188(1):5-6.

Senghor, B., Diallo, A., Sylla, S. N., Doucouré, S., Ndiath, M.O., Gaayeb, L., ...Djuikwo- Teukeng, F.F. (2014). Prevalence and intensity of urinary schistosomiasis among school children in the district of Niakhar, region of Fatick, Senegal. *Parasites & Vectors*, 7:5

Samaras, V., Rafailidis, P.I.Mourtzoukou, E.G., Peppas, G. and Falagas, M.E., (2010).Chronic bacterial and parasitic infections and cancer: a review. *Journal of Infection in Developing Countries*, pp. 267-281

Singh, K., Muddasiru, D. and Singh, J., (2016). Current status of schistosomiasis in Sokoto, Nigeria. *Parasite Epidemiology and Control*, 1(3).

Sokolow, S.H., Huttinger, E., Jouanard, N., Hsieh, M.H., Lafferty, K.D., Kuris, A.D., ... De Leo, G.A. (2015). Reduced transmission of human schistosomiasis after restoration of a native river prawn that preys on the snail intermediate host Proceedings of the National Academy of Sciences112 (31)9650-9655

Sow, S., de Vlas, S.J., Engels, D. and Gryseels, B., (2002) Water-related disease patterns before and after the construction of the Diama dam in northern Senegal. *Annals of Tropical Medicine and Parasitology*,(6):575–586.

Steinauer, M.L., Agola, L.E., Mwangi, N.I., Mkoji, G.M. and Loker, E.S., (2008). Molecular Epidemiology of *Schistosomamansoni*. *Infection, Genetics and Evolution,*. 8(1): 68–73.

Ten Hove, R.J., Verweij, J.J., Vereecken, K., Polman, K., Dieye, L. and van Lieshout, L., (2008) Multiplex real-time PCR for the detection and quantification of *Schistosoma mansoni* and *Schistosoma haematobium* infection in stool samples collected in northern Senegal. *Transactions of theRoyal Society of Tropical Medicine Hygiene*, *102,* 179–185

Thrusfield, M., (2007). *Veterinary Epidemiology*, Blackwell, USA.

Ukaga, F.N., Onyeka, P.I.K. and Nwoke, B.E.B. (2002). *Practical medical parasitology for biological and medical students,*Avan Global Publication. Owerri, Nigeria. 341pp

Usman, A.M., Malann, Y.D. and Babeker, E.A., (2016). Prevalence of *Schistosoma* haematobium among School Children in Bauchi State, Nigeria. *International Journal of Innovation and Scientific Research*, ISSN 2351-8014 Vol. 26, pp. 453-458

Utzinger, J., Raso, G. and Brooker S., (2009). Schistosomiasis and neglected tropical diseases: towards integrated and sustainable control and a word of caution. *Parasitology*, 136, pp. 1859–1874.

Wilson, M.S., Mentink-Kane, M.M., Pesce J.T., Ramalingam, T.R., Thompson, R. and Wynn, T.A. (2007) Immunopathology of schistosomiasis. *Immunology Cell Biology*, 85:148–54

World Health Organization (2008). *Model Formulary*,

World Health Organization (2017). *Schistosomiasis*, *Fact Shee*t, No. 115. Available at: http://www.who.int/mediacentre/factsheets/fs115/en/.

Zhou,Y., Zheng, H., Chen, Y., Zhang, L., Wang, K. and Guo, J. (2009). The *Schistosomajaponicum* genome reveals features of host–parasite interplay. *Nature,*460**:** 345-351

APPENDICES

Appendix 1 Child Consent / Assent Form

Participant's Name _____

Participant's Identification Number: |__|__|__|__|__|__|__|__|__|__|__|__|

_____**OR** _____

(Printed name of parent) (Printed name of guardian)

☐ I have read the written information **OR**

☐ I have had the information explained to me by study personnel in a language that I understand,

and I

- confirm that my choice to let my child participate is entirely voluntarily,
- confirm that I have had the opportunity to ask questions about this study and I am satisfied with the answers and explanations that have been provided,
- understand that I grant access to data about my child to authorised persons described in the information sheet,
- have received sufficient time to consider to let my child take part in this study,
- agree to allow my child take part in this study.

Tick as appropriate

I agree for my child's samples to be shipped outside the Gambia

Yes ☐ No ☐

I agree to further research on my child's samples as described in the information sheet

Yes ☐ No ☐

Participant's signature/ thumbprint* for **assent** (child aged 12-17 years)

Date (dd/mmm/yyyy) Time (24hr)

Participant's parent/guardian signature/thumbprint*

Date (dd/mmm/yyyy) Time (24hr)

Printed name of witness*

Printed Name of Person obtaining consent

I attest that I have explained the study information accurately in _____ to, and was understood to the best of my knowledge by, the participant/parent/guardian. He/she has freely given consent to participate *in the presence of the above named witness (where applicable).

Signature of Person obtaining consent

Date (dd/mmm/yyyy) Time (24hr)

** Only required if the participant is unable to read or write.*

Appendix 2 Adult Consent Form

Participant Identification Number: |__|__|__|__|__|__|__|__|__|__|__|__|__|__|

_____

(Printed name of participant)

☐ I have read the written information **OR**

☐ I have had the information explained to me by study personnel in a language that I understand,

and I

- confirm that my choice to participate is entirely voluntarily,
- confirm that I have had the opportunity to ask questions about this study and I am satisfied with the answers and explanations that have been provided,
- understand that I grant access to data about me to authorised persons described in the information sheet,
- have received sufficient time to consider to take part in this study,
- agree to take part in this study.

Tick as appropriate

I agree for my samples to be shipped outside of The Gambia

Yes ☐ No ☐

I agree to further research on my samples as described in the information sheet

Yes ☐ No ☐

Participant's signature/ thumbprint*

Date (dd/mmm/yyyy) Time (24hr)

Printed name of witness*

Printed name of person obtaining consent

I attest that I have explained the study information accurately in _____ to, and was understood to the best of my knowledge by, the participant. He/she has freely given consent to participate *in the presence of the above named witness (where applicable).

Signature of person obtaining consent

Date (dd/mmm/yyyy) Time (24hr)

** Only required if the participant is unable to read or write.*

Appendix 3 Ethical Clearance

The Gambia Government/MRC Joint
ETHICS COMMITTEE

C/o MRC Unit: The Gambia, Fajara
P.O. Box 273, Banjul
The Gambia, West Africa
Fax: +220 – 4495919 or 4496513
Tel: +220 – 4495442-6 Ext. 2308
Email: ethics@mrc.gm

16 October 2017

Mr Alphonse Mendy
National Public Health Laboratories.
Bertil Herding Highway, Kotu

Dear Mr Mendy

SCC 1541v1.2, Biochemical and molecular characterzation of *schistosoma* species in central and upper river regions of the Gambia

Thank you for submitting your updated proposal and response letter addressing the issues raised by The Gambia Government/MRC Joint Ethics Committee at its meeting held on 28 April 2017.

I have looked at your response – these are quite satisfactory. This project has now received full ethics committee approval and may proceed.

With best wishes

Yours sincerely

Mr Malamin Sonko
Chairman, Gambia Government/MRC Joint Ethics Committee

Documents submitted for review:

- Response letters – 31 May 2017; 11 August 2017
- SCC application form, version 1.2 – 31 May 2017
- Sample collection form, version 1.0 – 20 March 2017
- Revised proposal – January 2017
- Revised ICDs (child and adult),version 1.2 – 16 October 2017
- CV: Alphonse Mendy
- Budget

Appendix 4 Sample collection Form

S/N	NAME	AGE	SEX	ADDRESS	RECEIVED DRUG (PRAZIQUANTEL)	OCCUPATION	SAMPLE COLLECTED	
							BLOOD	URINE