ASSESSMENT OF EOSINOPHIL CATIONIC PROTEIN LEVELS AS POSSIBLE BIOMARKER FOR ESTIMATING INTENSITY OF SCHISTOSOMIASIS BEFORE AND AFTER PRAZIQUANTEL TREATMENT: A CASE STUDY IN VEA IN THE BONGO DISTRICT OF THE UPPER EAST REGION OF GHANA

THESIS SUBMITTED TO THE DEPARTMENT OF CLINICAL MICROBIOLOGY, KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY.

IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF MASTER OF PHILOSOPHY CLINICAL MICROBIOLOGY DEGREE

BY

EMMANUEL ADONGO

(PG 6067811)

DEPARTMENT OF CLINICAL MICROBIOLOGY, SCHOOL OF MEDICAL SCIENCES, KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

OCTOBER, 2014

DECLARATION

I hereby do declare that with the exception of references to other people's work which I have duly acknowledged and cited, all experimental work described in this thesis was carried out by me. I do further declare that this thesis has not been presented either in part or whole elsewhere for another degree

Emmanuel Adongo — Date

(Student)

Prof. S. C. K. Tay — Date

Department of Clinical Microbiology,
School of Medical Sciences, KNUST

(Supervisor)

Dr. Irene Ayi — Date

Department of Parasitology, Noguchi Memorial Institute for Medical Research, University of Ghana

(Co- Supervisor)

Prof. E.H. Frimpong — Date

Department of Clinical Microbiology,
School of Medical Sciences, KNUST

(Head of Department)

DEDICATION

This work is dedicated to my two lovely sons SHAMMAH and REGINALD. You are the most wonderful thing that ever happened to me

ACKNOWLEDGEMENTS

I wish to express my foremost appreciation and gratitude to Jehovah God Almighty in whose strength I live, move and have my being, whose guidance and making, my research work is a success.

My sincere gratitude also goes to Prof. S. C. K. Tay (Department of Clinical Microbiology, School of Medical Sciences, KNUST) and Dr. Irene Ayi (Department of Parasitology) Noguchi Memorial Institute of Medical Research. Your effortless impact and attention was heart-warming and I am much grateful for that.

To the Head of Department (Department of Clinical Microbiology, School of Medical Sciences, KNUST), Prof. E. H. Frimpong; I say God bless you for your guidance and assistance.

My appreciation also goes to the Director and staff of Noguchi Memorial Institute for Medical Research for opening their doors for me to conduct my research. I also appreciate the efforts of staff of Department of Parasitology, NMIMR, with special mention of Messrs Emmanuel Awusah Blay and Elias Assuming-Brempong for their assistance and guidance during the research period.

To Mr. Yelifari Lawrence (Regional Biologist, Upper East Region) I say a very big thank you for the enormous support and contributions.

I will not forget the entire staff of the Regional Hospital, Bolgatanga, especially Mr. Elvis Acheampong and Mr. Emmauel Kabutey who were with me during the field work.

I also express my sincere thanks to the Headmaster, Teachers and Pupils of Vea Primary School in the Bongo district, Upper Region of Ghana.

I also owe a big gratitude to the Chief and people of the Vea traditional area in the Upper East Region of Ghana.

ABSTRACT

Schistosomiasis is one of the most widespread of all human parasitic diseases, ranking second only to malaria in terms of its socioeconomic and public health importance in tropical and sub-tropical areas. Despite all the efforts by the World Health Organisation and for that matter Ghana Health Service to control morbidity and a possible elimination, the disease persists in endemic areas and new transmission foci are being discovered in different parts of the country over time. This study was conducted in Vea in the Bongo District of the Upper East Region of Ghana from July 2012 to September 2012. The aim was to assess the Eosinophil cationic protein levels as possible biomarker for estimating intensity of infection before and after praziquantel treatment. Participants were examined at baseline and 8 weeks post-treatment with praziquantel. Prevalence and intensity of *S. haematobium* and *S. mansoni* for both baseline and post-treatment urine and stool samples were estimated by microscopy. The filtration method was used for urine samples and the Kato Katz concentration method for stool samples. The formol-ether concentration technique was used to ascertain the veracity of negative stool samples by the Kato Katz concentration method. The factors that contribute to the persistence of schistosomiasis were also assessed by questionnaire interviews. Three hundred and fifteen (315) participants were recruited for the study. However a total of 217 participants gave their consent for the study (Male = 106, Female = 111). Age range for participants was between the ages of 6 – 76 years. 122 (56.2%) of participants were lost to follow-up. Samples were collected from 95 participants. This was made up of 38 (40.0%0 males and 57 (60.0%) females. Microscopy estimated urinary schistosomiasis prevalence of 18.9% (41/217). For intestinal schistosomiasis microscopy estimated a prevalence of 15.7%% (34/217). 1.4 % (3/217) had mixed infection of *S. haematobium* and *S. mansoni*. Prevalence of *S. haematobium* and *S. mansoni* eight (8) weeks after treatment with praziquantel was 4.2% (4/95) and 2.1% (2/95) respectively. The highest *S. haematobium* prevalence of 16.4% was found amongst the 6-15 years age group. The highest *S. mansoni* prevalence of 13.8% occurred amongst the 6-15 years age group. More females, 10.6%, were infected with *S. haematobium* and 8.3% *S. mansoni* than males with 8.3% and 7.4% respectively. Two (2) participants were found to be infected with *H. nana* in the pre treatment samples. Also two individuals each were found to be infected with hookworms and *H. nana* respectively in the post treatment screening. Mean intensity for *S. haematobium* was high among the 6 – 10 year age group (191.4 eggs/10mls of urine) than the 11 – 15 years age group (63.5 eggs/10 ml of urine). For *S. mansoni* intensity was 103.5 epg among the 11 – 15 years age group. Serum ECP levels of infected individuals were very high compared to normal levels (82.9 ng/ ml v 15.6 ng/ ml). There was no significant difference (p= 0.39) in ECP levels between patients infected with *S. mansoni* and *S. haematobium*. There was a significant association {p= 0.01(CI: 95%, α: 0.05)} between the estimation of intensity measured by microscopy and ECP measurement. There was also a significant drop in the ECP levels (p= 0.006) in post treatment samples of schistosomiasis patients as compared to pre-treatment Levels. ECP levels however showed a weak positive correlation(r= 0.19) with increase in intensity by egg count. Knowledge of the disease among participants was quite high, 93.5 % (203/217) but water contact was still high, 99.1 %(215/217). Most respondents could not associate some of the symptoms to the disease, for example passing of bloody stools to *S. mansoni* infection. In conclusion, this study suggests that serum ECP level could be a good biomarker for estimating intensity of schistosomiasis in both pre and post praziquantel

treatment. However, other factors such as allergy and microbial infection could also be responsible for increased ECP levels in the blood. These conditions will affect the validity of the test results.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
CDC	Centers for Disease Control
CNS	Central Nervous System
DALYS	Disability Adjusted Life Years
DDHS	District Director of Health Services
DOT	Directly Observed Therapy
ECP	Eosinophil Cationic Protein
EDN	Eosinophil Derived Neurotoxin
EPC	Egg Per Centiliter
EPG	Egg Per Gram
ELISA	Enzyme Linked Immunosorbent Assay
g	Grams / Gravitational force
GBD	Global Burden of Disease
GHS	Ghana Health Service
GIS	Geographic Information System
HIV	Human Immunodeficiency Virus
IEC	Information Education and Communication
Km^2	Kilometer square
Km^3	Cubic kilometer service
MBL CO LTD.	Medical & Biological Laboratories Company Limited
MDA	Mass Drug Administration
MDGS	Millennium Development Goals
ml	Millilitre
MOH	Ministry of Health
NMIMR	Noguchi Memorial Institute for Medical Research
NTD	Neglected Tropical Disease
^oC	Degree Celsius
PCR	Polymerase Chain Reaction
PCT	Preventive chemotherapy
pH	Hydrogen ion concentration
SHEP	School Health Education Programme
SPSS	Statistical Package for Social Sciences

SSA	Sub-Saharan Africa
STH	Soil Transmitted Helminth
UNICEF	United Nations International Children and Education Fund
WHO	World Health Organization
WWW	World Wide Web
YLD	Years lost with disability
YLL	Years of Life Lost

CHAPTER ONE

INTRODUCTION

1.1 Background

Parasitic infections cause tremendous burden of diseases in both the tropics and sub-tropics as well as in temperate climates. Of all parasitic diseases, malaria causes the most deaths globally. Malaria kills approximately 660,000 people each year, most of them young children in sub-Saharan Africa (http://www.cdc.gov).

The Neglected Tropical Diseases (NTDs) have suffered from a general lack of attention by the public health community and these include parasitic diseases such as schistosomiasis, the second world most common serious infectious infection (http://www.cdc.gov). The NTDs affect more than 1 billion people, one-sixth of the world's population – largely in rural areas of low income countries (http://www.cdc.gov). These diseases are known to cause high morbidities in endemic populations including inability to attend school or work, retardation of growth in children, impairment of cognitive skills and development in young children (http://www.cdc.gov). These factors therefore place a high economic burden on endemic communities (http://www.cdc.gov).

Schistosomiasis which is the second most prevalent tropical disease, considering the number of people infected and persons at risk have been known since the medieval days (Contis *et al*., 1996). According to previous estimates, the disease causes annual loss of between 1.7 and 4.5 million disability adjusted life years (DALYS) worldwide (WHO, 2004; WHO, 2002; Utzinger *et al*., 2004).

Schistosomiasis transmission has been reported in 77 countries. However, 52 countries have their population at higher risk of infection (http://www.emro.who.int). It is estimated that at least 90%

of those requiring treatment for schistosomiasis live in Africa (WHO, Jan. 2012; Fact sheet IF 115).

Schistosomiasis affects at least 240 million people world wide, and more than 700 million people live in endemic areas (WHO, 2012). Schistosomiasis is the most deadly NTD, causing mortality in an estimated 280,000 people in Africa yearly (http://www.cdc.gov). Prevalence rates are high, over 90% in many endemic countries (http://www.medicineonthemove.org).

In Ghana the disease is wide spread and occurs in all the 170 administrative districts with transmission of the disease also occurring in peri-urban localities (www.medicineonthemove.org). It is estimated that about 7 million school-age children in Ghana are at risk of the infection (http://www.medicineonthemove.org).

Schistosomiasis infected people suffer many health conditions including excretion of blood in urine and stool, kidney malfunction, bladder cancer and diseases of the liver and spleen (http://www.medicineonthemove.org).

Recent research in Ghana has also revealed high occurrence of Genital Schistosomiasis with serious implications for reproductive health such as infertility, miscarriage, ectopic pregnancies, erectile dysfunction, and increased risk for acquiring sexually transmitted infections including HIV/AIDS (http://www.medicineonthemove.org).

Schistosomiasis disease burden reduces productivity in adults and compromises child learning abilities (cognitive developments) (http://www.medicineonthemove.org). Furthermore, infection in humans limits growth in agriculture, tourism, water and health sectors, which undermines the attainment of the Millennium Development Goals (MDGs) in Ghana (http://www.ghanaweb.com).

However, efforts at effectively controlling the disease over the decades have achieved limited success. (http://www.ghanaweb.com)

Four trematode species in the genus *Schistosoma*; *S.mansoni, S. haematobium, S. japonicum* and *S. mekongi* are known to cause a series of related disease in human. *S. intercalatum,* a parasite of cattle in West Africa also occasionally causes the disease in humans (http://www.medicalecology.org/pdf).

Except for *S. haematobium* that causes urinary schistosomiasis, the other species known to infect humans cause intestinal schistosomiasis (http://www.medicalecology.org/pdf).

S. haematobium is prevalent in most parts of Africa and in some parts of the Middle East. (http://www.medicalecology.org/pdf).

Its aquatic intermediate host snails are the genus Bulinus (http://www.medicalecology.org/pdf).

There are no important reservoir host for this trematode species, although during an epidemic of the infection in the Omo River Valley of Ethiopia, the origin of the outbreak was traced back to monkeys (Fuller *et al*., 1979). Adult parasites are found in small venules around the bladder and ureter, with the majority of egg deposition in the tissues of these organs, as eggs pass through the bladder wall, to leave the body in the urine (http://www.path.cam.ac.uk/schisto/schistosoma/schisto-distribution.html). The disease is chronic in nature, with the most frequently affected organ being the urinary bladder, where calcification of eggs trapped in the tissues often occurs (www.path.cam.ac.uk/schisto/schistosoma/schisto-distribution.html). Cancer of the bladder is an important complication of infection with *S. haematobium* (http://www.path.cam.ac.uk/schisto/schistosoma/schisto-distribution.html). Eggs may be deposited in the liver, often in high numbers, and granuloma formation may occur, but this is much less severe than with *S. mansoni* (http://www.path.cam.ac.uk/schisto/schistosoma/schisto-distribution.html).

S. mansoni is found throughout most of sub-Saharan Africa, Egypt, Sudan, some parts of the

Middle East and parts of South America and the Caribbean (http://www.medicalecology.org/pdf).

Its aquatic intermediate host snail is the genus *Biomphalaria* (http://www.medicalecology.org/pdf).

Schistosoma japonicum occurs in China, Malaysia, and the Philippines, and to a small extent Indonesia (Minai *et al*., 2003). It has been eradicated from Japan since 1977 (Minai *et al*., 2003). Its intermediate host is in the genus *Oncomelani*a (Minai *et al*., 2003).

The endemicity of bilharzia in the Upper East Region has been an old problem (OMS-WHO Report 1987). Between 1959 and 1961 an exhaustive epidemiological survey conducted in the Upper East Region found prevalence of *S. haematobium* infection to be more than 30 %(OMS-WHO Report 1987). Bongo and Chuchuliga had prevalences more than 70% for *S. haematobium* (OMS-WHO Report 1987).

A study carried out at settlement areas close to the Tono irrigation dam in the Upper East region identified high prevalence and intensities of *S. haematobium* and *S. mansoni* with *Bulinus globussus* and *Biomphalaria pffeiferi* being their aquatic intermediate host respectively (Amankwa *et al*., 1994).

The effective control of parasitic disease requires rapid, reliable and highly sensitive diagnostic tests (Ambrosio and de Waal., 1990). This can also help to monitor the effectiveness and efficiency of therapeutic and prophylactic protocols (Ambrosio and de Waal., 1990). Current methods of diagnosing schistosomiasis include parasitological, immunological and molecular procedures

(http:www.parasitesandvectors.com/content/7/1/138). Microscopy is widely regarded as the "gold standard" while serology and most recently polymerase chain reaction (PCR) – based testing can confirm a diagnosis (Xu *et al*., 2010).

Control efforts in Ghana have been based especially on epidemiological studies involving focal intermediate snail host control. In recent times, microscopic ova identification and haematuria is used as the definitive diagnosis for mass drug administration. However, very little considerations have been given to immunological antibody detection of the disease.

In moderate and low endemic areas, the number of "false negative" microscopic results increase, hence the need for more sensitive methods of diagnosis. Microscopy is the leading method for determining intensity of infection. The relationship between egg count and pathology is not always clear (Cheever *et al*., 1978). Ultrasound scanning remains the gold standard for pathology (Hatz *et al*., 1992). Declining quantitative eosinophil cationic protein (ECP) levels correlate with reduction in severity of the illness (Engels *et al*., 2002). Measurement of ECP levels will therefore be particularly helpful for the determination of intensity of infection.

In controlling and treatment of schistosomiasis in infected persons, the developments of highly efficacious and safe drugs took much longer than molluscicides (Sturrock, 2001)

By the 1970's metrifonate was established for use against *S. haematobium* (Sturrock, 2001). However, the fears for its safety prevented the widespread acceptance of hycanthone, the first single dose drug against *S. mansoni* (Sturrock, 2001). The real turning point was the arrival in the early 1980s of praziquantel, a safe, effective, single dose drug active for all schistosomiasis (Sturrock, 2001).

1.2. Problem Statement

Schistosomiasis is an important public health and socio-economic disease mostly associated with tropical regions of the world. The disease is known to have wide-spread distribution in Ghana (www.ghanahealthservice.org/documents/NTDs).

Preventive chemotherapy (PCT) in Ghana, until recently was focused on trachoma,

Onchocercaisis and soil-transmitted helminths (STH) but not schistosomiasis (http://www.ghanahealthservice.org/documents/NTDs). Health officials in Ghana knew that schistosomiasis was a problem but they lacked the resources to adequately monitor and evaluate the disease burden (http://www.ghanahealthservice.org/documents/NTDs). The large-scale administration of Praziquantel to school-aged children is the main-stay of current programmes focusing on morbidity control (Fenwick *et al*., 2009). Since the Mass Drug Administration (MDA) program focuses mainly on reducing intensity of infection, egg count by microscopy (kato Katz) has been the standard method for measurement but microscopy comes with its limitations. These include difficulty in processing diarrhoeal stools (Siegel *et al*., 1990), lack of sensitivity, if only a single stool sample is examined (Booth *et al*., 2003) and poor reproducibility of results (Kongs *et al*., 2001). Counting of eggs in Kato-Katz smears can be a tedious and time consuming process, and can lead to technical errors (Kato and Miura, 1954; Ebrahim *et al*., 1997).

Eosinophil cationic protein level measurement by ELISA is very sensitive and more stable and may present a better alternative to microscopy.

1.3. Main aim

To assess eosinophil cationic protein levels as possible biomarker for estimation of schistosoma infection intensity before and after praziquantel treatment

1.4 Specific Objectives

1. To determine the prevalence and intensity of *Schistosoma mansoni* and *S. haematobium* and other helminths by microscopy.
2. To estimate the intensity of *S. mansoni* and *S. haematobium* infection by the measurement of eosinophil cationic protein levels using ELISA.

3. To compare intensity of *S. mansoni* and *S. haematobium* infection by egg count and eosinophil cationic protein levels.
4. To assess factors that contribute to the persistence of schistosomiasis by questionnaire interviews
5. To determine the distribution *S. mansoni* and *S. haematobium* infection in various groups and by sex.

1.5. Hypothesis

Measurement of Eosinophil cationic protein levels is an effective marker for estimation of *S. haematobium* and *S. mansoni* infection intensity.

1.6. Justification

Schistosomiasis is recognized by the WHO as one of the Neglected Tropical Diseases. This is because not much attention has been paid to the disease in terms of research for effective control despite the huge socio-economic burden it imposes on populations in endemic areas, especially on women and children. Communities along the Vea dam in the Upper East region of Ghana have high disease burden because of factors such as; lack of access to potable water, poor sanitation and the presence of intermediate snail hosts in the water body(personal observation)..

This necessitated the implementation of an intervention program to control and reduce the prevalence of schistosomiasis in the area. A mass drug administration (MDA) programme was thus implemented by the Ghana Health Service in the area to treat all school age children who were at risk of infection. This study will provide an up-to-date data on the disease intensity in the area. This will be a good opportunity to assess ECP level measurement as a possible biomarker for estimating intensity of infection and also the effectiveness or otherwise of the MDA in the area.

CHAPTER TWO

LITERATURE REVIEW

2.1 Historical Information

Schistosomiasis is an ancient disease of man. Eggs have been recovered from Egyptian and Chinese mummies several thousand years old (Nunn & Tapp, 2000). Scientific studies of the disease did not start until the middle of the 19th century with independent reports, first by Japanese workers, who described the early, acute Katayama syndrome. Fuji (1847) and then Bilharz in 1852 found distome trematodes in the urogenital blood vessels during post mortem examinations of Egyptian corpses (Warren, 1973). Over 60 years later, just before the First World War, Japanese workers Miyairi & Suzuki (1914) finally incriminated amphibious, prosobranch snails of the genus *Oncomelania* as the intermediate host of the Oriental schistosome, *Schistosoma japonicum* and Leiper (1915) showed that aquatic, pulmonate snails of the genera *Bulinus* and *Biomphalaria* transmitted *S. haematobium* and *S. mansoni*, respectively. The taxonomical problems about the genus *Biomphalaria* that have so exercised Dr Lobato Paraense were created by 19th century shell collectors and naturalists ignorant of its medical importance (Lieper, 1915).

Bilharz, in letters to his friend and colleague, von Siebold between 1851 and 1857 described human cases of *S. haematobium*, a parasite of the venous plexus of the bladder and whose eggs possesses a terminal spine.

In 1902 Manson described a case of schistosomiasis in an Englishman who had travelled extensively throughout the Caribbean, and in whose stool, but not urine, he found many eggs with lateral spine (Manson, 1902). Sambon, in 1907 recognised two blood flukes on the bases of morphology and origin of the eggs in stool and urine. In tribute to Manson, Sambon named this new organism after him. Piraja de Silva in1908 also discovered *S. mansoni* in S.

America. By 1918 Leiper had conducted extensive investigations on Schistosomiasis and reported the life cycle *of S. mansoni* in which he described its snail's intermediate host and morphology of the adult worms (Lieper, 1915).

Katsurada, in 1904 described *S. japonicum* adults from infected cats. Coincidentally, Catto, working in Singapore described an identical adult worm in a patient who died of cholera (Catto, 1905). He named it *S. cattoi*, but his publication delayed, and the name *S. japonicum* was accepted instead.

Earlier, in 1888, Majima observed eggs of *S. japonicum* in a liver he examined at autopsy. He was unaware of the adult worms, but correctly ascertained that the eggs were responsible for the cirrhosis (Majima, 1888).

Kawanishi in 1904 made the correlation between the clinical condition, katayama fever (acute schistosomiasis), and the presence *of S. japonicum* adults after finding eggs of this parasite in the stool of patients suffering from the acute phase of the infection (Kawanishi, 1904).

Fujinami and Nakamura in 1909 and Miyagawa in 1912 independently reported on the details of the life cycle (Fujinami and Nakamura, 1909). Miyairi and Suzuki, in 1914 identified *oncomelania sp.* snail as the vectors (Miyairi and Suzuki, 1914). *S. japonicum* infection has had a major impact on the history of modern China. It is believed that Mao's troops were unable to launch an amphibious assault on Taiwan in the late 1940s because they developed katayama fever while encamped along the Yangtze River (Miyairi and Suzuki, 1914). Later on, during the great leap forward, Mao mobilised tens of thousands of peasants to either bury *oncomelania* snail or even to remove them individually by hand (Miyairi and Suzuki, 1914).

Griesinger, in 1854 described in detailed the clinical disease and its pathology. He noted the relation of the infection to the involvement of the bladder and ureters. Lieper, in 1918, described the life cycle of *S. haematobium*, its intermediate host, and its morphology. He also

carried out experimental infections *with S. haematobium* in various indigenous animals in northern Egypt and proved that rats and mice were susceptible (Lieper, 1918).

2.2 Burden of the disease.

There is no doubt schistosomiasis can result in the death of infected persons (WHO, 2002; TRS 912). Historical data from Brazil, for example in areas where there were no antihelminthic intervention, revealed that 1% of infected people died from schistosomiasis (WHO, 2002; TRS 912). Nevertheless, it must be remembered that the major impact of all forms of schistosomiasis worldwide continues to be chronic morbidity (WHO, 2002; TRS
912). The DALY (disability-adjusted life year) is the metric developed to quantify the Global Burden of Disease (GBD) in the early 1990s. It is a summary measure of population health, which combines in a single indicator years of life lost (YLL) from premature death and years of life lived with disability (YLD) (WHO, 2002; TRS 912). One DALY can be thought of as one lost year of 'healthy life'(WHO, 2002; TRS 912). The burden of disease measures the gap between the current health status and an ideal situation where everyone lives into old age free of disease and disability. According to the GBD, schistosomiasis caused the loss of 1.7 million disability-adjusted life years (DALYs) worldwide in 2001, of which 82 percent (1.4 million DALYs) were lost in Sub-Saharan Africa (SSA) alone(WHO, 2002). Schistosomiasis was the third largest cause of tropical disease cluster burden (which does not include malaria) worldwide(WHO, 2002; TRS 912). In Sub-Saharan Africa, schistosomiasis caused one quarter of the tropical disease cluster burden (WHO, 2002).

Schistosomiasis is often examined along with intestinal nematodes in health policy discussions, because both infections are most prevalent among school-age children(WHO, 2002; TRS 912). The two infections often occur simultaneously; causing anaemia, affecting growth and cognitive development; and respond well to simple and affordable drug treatments that can be provided in school based programs. The relative importance of schistosomiasis and intestinal nematode

infections was greater in SSA than it was in other regions. The global schistosomiasis burden was about equal to that due to hookworm disease, whereas in SSA it was double the combined burden of hookworm disease, trichiuriasis and ascariasis(WHO, 2002; TRS 912).

Schistosomiasis burden, nevertheless, represented only a small fraction of the total burden of disease - 0.1 percent of global burden of disease, and 0.4 percent of total burden of disease in sub-Saharan Africa (SSA). This rate is dwarfed by the immense burden due to HIV/AIDS, malaria, childhood diseases, diarrhoeal diseases and tuberculosis(WHO, 2002; TRS 912). According to the WHO information fact sheets, schistosomiasis infects more than 200 million people, and approximately 10 percent of the world's population (over half a billion people) is at risk of infection (WHO, 2002). Given that so many people are infected, the very small share of the estimated schistosomiasis burden is surprising. It was estimated that approximately 40 percent of the 200 million people infected with schistosomiasis worldwide remain asymptomatic (80 million); 60 percent become symptomatic (120 million), and approximately 10 percent develop severe disease (20 million) (Chitsulo *et al*., 2000). Some new disease burden assessments estimate that schistosmiasis accounts for up to 70 million disability-adjusted life years (DALYs) lost annually (King, *et al*., 2008). This global burden estimate exceeds that of malaria or tuberculosis, and is almost equivalent to the DALYs lost from HIV/AIDS (King, *et al*., 2008). Furthermore 300,000 people die annually from schistosomiasis in Africa (van der Werf, 2003), and there is evidence that female genital schistosomiasis caused by *S. haematobium* may significantly increase the likelihood of contracting HIV/AIDS (Kjetland, *et al*., 2006).

Estimates from Chitsulo *et al*., 2000 confirmed the findings from ultrasonography studies, which show that a significant proportion of endemic populations did not develop periportal hepatic fibrosis, and that only a small fraction of the population had severe forms of periportal hepatic fibrosis.

In Ghana, Schistosomiasis is major water borne parasitic disease associated with poverty in most rural settings (http://www.medicineonthemove.org). The disease is widespread and occurs in all 170 administrative districts of Ghana (http://www.medicineonthemove.org). Transmission of the disease also occurs in peri-urban areas (http://www.medicineonthemove.org). Prevalence rates are high, over 90% in many endemic communities (http://www.medicineonthemove.org). It is estimated that about 7million school-age children in Ghana are at risk of infection. (http://www.medicineonthemove.org)

2.3. Epidemiological Pattern

Schistosomiasis occurs in numerous geographic landscapes of varied characteristics, in which specific climatic, physical and human characteristics influence the intensity of transmission (http://www.ncbi.nlm.nih.gov).

The schistosome parasite requires a molluscan intermediate host in which to undergo development, with freshwater snails being an essential component in its lifecycle (Fenwick *et al.*, 2006). This ties transmission to landscapes where people and aquatic snails come together at the same water habitat (Fenwick *et al.*, 2006). Numerous factors act to determine the rate of transmission in a given location. These include biotic and abiotic features, such as climatic, physical and chemical factors that affect the survival and development of schistosome parasites and snail host populations (Sturrock, 1993), as well as socioeconomic and behavioural characteristics of the human community such as water contact behaviour and the adequacy of water and sanitation, which affect the frequency and intensity of exposure to infected water (Bundy and Blumenthal, 1990).

Overall transmission success depends crucially on the establishment, survival and fecundity of adult schistosomes in the human host, and depends less on the survival and fecundity of the two free-living aquatic stages, the miracidia and cercaria, and of the infected snail hosts (Anderson, 1987). This is because the lifespan of adult worms is substantially longer (3—6

years) than those of either infected snail hosts (weeks) or free-living stages (hours). Furthermore, the most significant determinants of the intensity of transmission are changes in water contact patterns through improved water and sanitation and health education, or changes in parasite mortality through the implementation of population-based chemotherapy. However, if these factors remain unchanged, then the rate of parasite establishment and hence the patterns of schistosomiasis are primarily determined by the distribution and abundance of its intermediate hosts, freshwater snails (http://www.ncbi.nlm.nih.gov). The most important determinants of the population dynamics of snails are temperature and rainfall (Reviewed in Sturrock, 1993). The optimal temperature for snail development and survival is about 25ºC. Above 30ºC snail mortality increases, and thermal death occurs at 40 ºC (http://www.ncbi.nlm.nih.gov). However, snails are less sensitive to low temperatures than schistosoma parasites in snails (http://www.ncbi.nlm.nih.gov). Uninfected snails can therefore be found in high altitude areas of endemic countries where low temperatures inhibit larval development in snails. Several studies have demonstrated marked spatial and temporal heterogeneity in snail population dynamics owing to fluctuations in rainfall (Sturrock, 1993). However, it is difficult to quantify precisely the spatial relationships between rainfall and snail population dynamics and schistosome transmission since the effect of rainfall varies according to snail species and geographical location (http://www.ncbi.nlm.nih.gov). Moreover, seasonal fluctuations in snail dynamics are of limited significance to overall parasite transmission since adult schistosomes typically have a longer lifespan relative to such seasonal fluctuations (Anderson, 1987). Delineation of the climatic limits of schistosome transmission at continental scales has been enhanced by the integrated use of GIS and satellite sensor data (Brooker, 2002; Brooker and Michael, 2000; Kabatereine *et al*., 2004; Malone *et al*., 2001; Moodley *et al*., 2003). However, such broad-scale patterns belie the tremendous complexity and variability in transmission between different foci and even within

the same focus. This focal distribution is suggested to reflect the small-scale distribution of habitats suitable for snail species and the multiple factors that determine habitat suitability (Woolhouse *et al*., 1991, 1998). These include physical and chemical factors such as pH, vegetation and water velocity (Sturrock, 1993), and man-made ecological changes such as the construction of large dams and irrigation schemes (Jordan and Webbe, 1993). Genetic differences in interspecific and intraspecific intermediate host—parasite interactions and infectivity may also play a role (Rollinson *et al*., 2001), although this aspect remains poorly understood (http://www.ncbi.nlm.nih.gov). Despite these small-scale heterogeneities and generative mechanisms, it is suggested that large-scale environmental and climatic factors influence the broader-scale patterns of parasite transmission, such that climate-based risk maps can be developed (http://www.ncbi.nlm.nih.gov).

2.4 Clinical indications

As in other helminths infections, clinical diseases resulting from schistosomiasis usually occurs in heavily infected individuals (http://www.medicalecology.org/pdf). The clinical manifestations of acute schistosomiasis occur predominantly in *S. japonicum* and *S. mansoni* infections (www.medicalecology.org/pdf). This is sometimes known as 'Katayama fever' (www.medicalecology.org/pdf). The classical disease attributed to schistosomiasis occurs during chronic infections (http://www.medicalecology.org/pdf). Chronic infections with *S. haematobium* can also lead to squamous cell carcinoma of the bladder

(www.medicalecology.org/pdf).

2.4.1 Acute Schistosomiasis (katayama fever)

The dramatic clinical manifestation of katayama fever occur most commonly in new immigrants who experience intense levels of exposure to either *S. japonicum* or *S. mansoni*

cercariae (www.medicalecology.org/pdf). The name reflects the early descriptions of this syndrome in the katayama valley of Japan (http://www.medicalecology.org/pdf). The symptoms are often dramatic and appear approximately 4 – 8 weeks after initial exposure, when adult worm pairs begin releasing their eggs in the tissue (http://www.medicalecology.org/pdf). Some investigators believe that katayama fever resembles some of the manifestations of serum sickness (http://www.medicalecology.org/pdf). There is also a clinical resemblance to typhoid fever (www.medicalecology.org/pdf). Patients experience hepatosplenomegaly and lymphadenopathy as well as an impressive eosinophil (http://www.medicalecology.org/pdf). The affected individual is frequently febrile and has flu-like symptoms including cough and headache (http://www.medicalecology.org/pdf). At this stage of the illness, schistosome eggs may not yet have appeared in the faeces (http://www.medicalecology.org/pdf).

2.4.2 Chronic Schistosomiasis

This manifestation of infection occurs as a consequence of many years of progressive injury resulting from chronic egg deposition in the tissue and the resulting granuloma formation (http://www.medicalecology.org/pdf). The injury has an immunopathological basis (http://www.medicalecology.org/pdf). In the case of *S. japonicum* and *S. mansoni* infection, the injury occurs when eggs are deposited in the wall of the intestine and in the liver parenchyma (http://www.medicalecology.org/pdf). With *S. haematobium*, injury occurs in the bladder (http://www.medicalecology.org/pdf). The extent of injury depends on chronic worm burden, so chronic schistosomiasis occurs predominantly in individuals who are predisposed to repeated heavy infection (Acosta *et al*; 2004). Generally, heavy infections occur only in less than a quarter of a given population under conditions of heavy exposure to cercariae where up to10% of individuals developed periportal fibrosis (http://www.medicalecology.org).

More severe forms of *S. japonicum* and *S. mansoni* infections can results in chronic intestinal and hepatic dysfunction (http://www.medicalecology.org/pdf). Children with intestinal schistosomiasis develop intermittent abdominal pain, sometimes accompanied with bloody diarrhoea (www.medicalecology.org/pdf).

The blood loss and ulceration due to intestinal schistosomiasis may result in iron deficiency and anaemia (http://www.medicalecology.org/pdf). This may explain why chronic schistosomiasis during childhood can result in physical growth retardation similar to that described for intestinal nematode infection (http://www.medicalecology.org/pdf). Stunting becomes most prominent at the age of peak intensity (McGarvey *et al*., 1992). It is partly reversible by specific anthelmintic therapy (Stephenson, *et al*., 1989).

Hepatomegaly results from portal fibrosis (http://www.medicalecology.org/pdf). Splenomegaly follows, and in advance cases, the spleen may fill much of the left side of the abdomen (http://www.medicalecology.org/pdf). The patient may also develop symptoms of hypersplenism (http://www.medicalecology.org/pdf). Portal obstructive disease due to schistosomiasis is similar to other causes in that it leads to hematemesis from ruptured oesophageal varices (http://www.parasiticdisease.org). As a result of portal hypertension, and the consequent development of a collateral circulation, schistosome eggs are carried to the lungs, where they induce granulomatous inflammation leading to obstructive disease culminating in corpulmonale (http://www.parasiticdisease.org).

As noted above, long standing infections can cause nephritic syndrome, resulting from the deposition of immune complexes onto the glomerular membrane (http://www.medicalecology.org/pdf).

S. haematobium, unlike the other three major schistosome causes involvement of the urinary tract, which is characterised by an inflammation to the eggs as they are deposited in the wall of the bladder (http://www.medicalecology.org/pdf). Patients with chronic *S. haematobium*

infection develop hematuria as well as symptoms that mimic urinary tract infections such as dysuria and increased urine frequency (http://www.medicalecology.org/pdf).

Overtime the inflammatory changes in the bladder can result in fibrosis that can lead to an obstructive uropathy (http://www.medicalecology.org/pdf). This sometimes results in hydronephrosis or hydroureter (http://www.medicalecology.org/pdf). The resulting urinary stasis can sometimes lead to secondary bacterial urinary tract infection that may exacerbate the scarring and fibrosis (http://www.parasiticdisease.org).

2.4.3 Bladder carcinoma

A unique type of bladder carcinoma occurs in regions where *S. haematobium* is endemic (http://www.medicalecology.org/pdf). In contrast to adenocarcinoma, the most common type of bladder cancer in industrialised countries, some patients with chronic *S. haematobium* go on to develop squamous cell carcinoma. Squamous cell carcinoma is the most common type of bladder cancer in parts of Egypt as well as elsewhere in Africa (http://www.medicalecology.org/pdf). Possibly over time the *S. haematobium* eggs function as a human carcinogen that elicits metaplastic changes in the bladder (Hodder, *et al*, 2000).

2.4.4 CNS Schistosomiasis

Rarely, all three schistosomes induce focal inflammatory reactions within the central nervous system caused by deposition of eggs in the spinal cord and the brain (Scrimgeour *et al* 1985).

S. mansoni and *S. haematobium* are more likely to do so in the spinal cord and *S. japonicum* in the brain. Inflammation due to eggs may result in focal transverse myelitis and encephalopathy.

2.5 Geographical Distribution of schistosomiasis

There are three main species of Schistosome that have humans as their definitive hosts, *Schistosoma mansoni*, *Schistosoma haematobium* and *Schistosoma*

japonicum(www.path.cam.ac.uk/-schisto/schistosoma/schisto-distribution.html). There are a number of more minor species affecting man, for example *S. mekongi* as well as some species that may cause accidental infections, *S. Intercalatum* or cercarial dermatitis (http://www.path.cam.ac.uk/-schisto/schistosoma/schisto-distribution.html). **5.1**

Distribution of *S. mansoni*

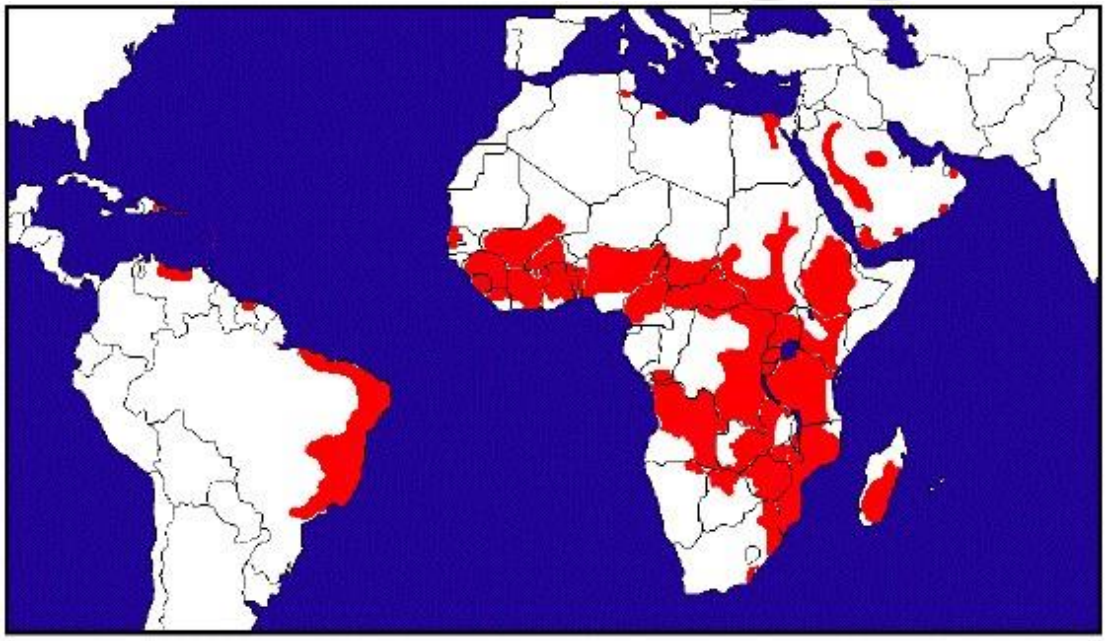

Figure 1: A map showing areas of the world that *S. mansoni* is found (**http://**www.path.cam.ac.uk/schisto/schistosoma/schisto-distribution.html)

Schistosoma mansoni is found in many countries in Africa, South America (Brazil, Surinam and Venezuela), the Caribbean (including Puerto Rico, St Lucia, Guadeloupe, Martinique, Dominican Republic, Antigua and Montserat) and in parts of the Middle East. Reservoir

hosts are not important for this species of schistosome (http://www.path.cam.ac.uk/schisto/schistosoma/schisto-distribution.html).

2.5.2 Distribution of *S. haematobium*

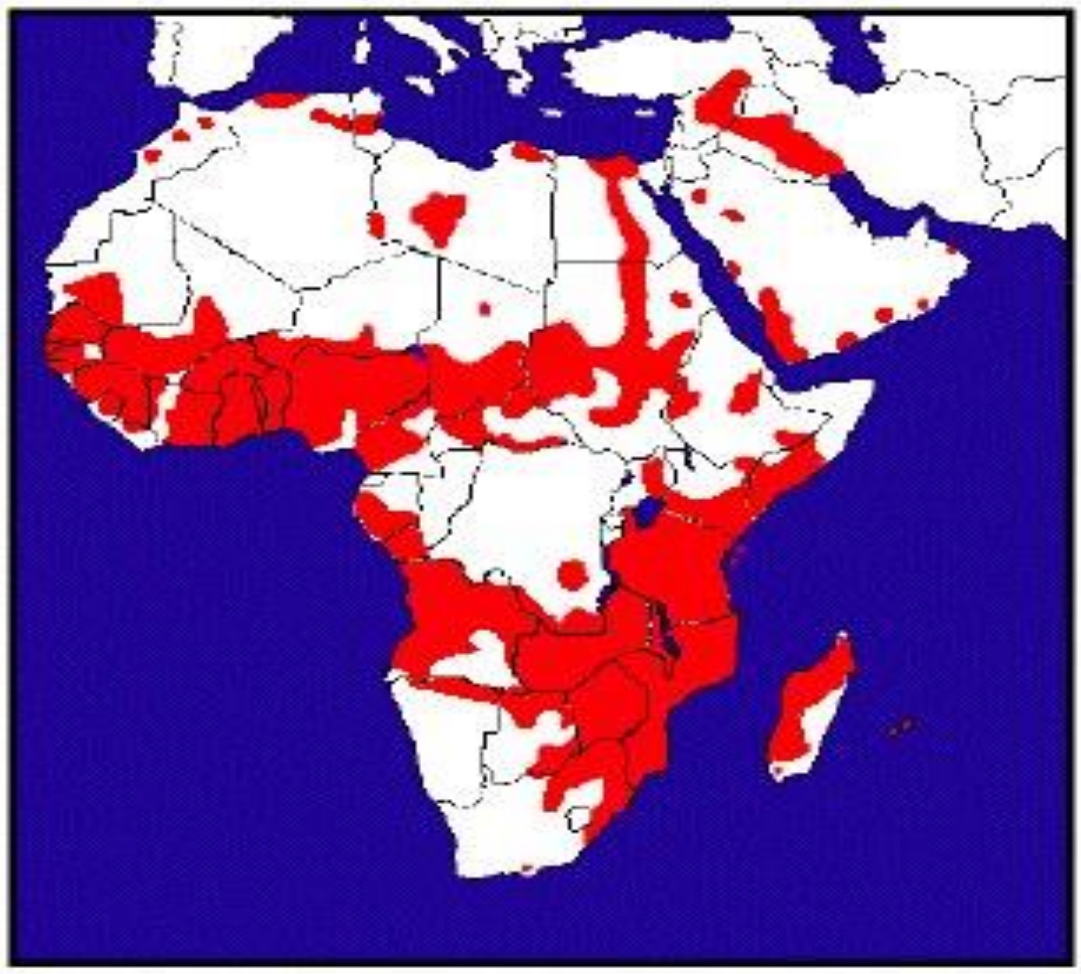

Figure 2 A map showing the distribution of *S. haematobium* in the world.

(http://www.path.cam.ac.uk/schisto/schistosoma/schisto-distribution.html)

S. haematobium is found in large parts of Africa, parts of the Arabia, the Middle East, Khuzestan Province in Iran, Madagascar and Mauritius (http://www.path.cam.ac.uk/schisto/schistosoma/schisto-distribution.html). *S. haematobium* like species has been in the past found in man in a village in Northern India (not shown), but this may now no longer exist (http://www.path.cam.ac.uk/-schisto/schistosoma/schisto-distribution.html). Reservoir hosts are not thought to be of importance for this species (http://www.path.cam.ac.uk/schisto/schistosoma/schisto-distribution.html).

2.5.3 Distribution of *S. japonicum*

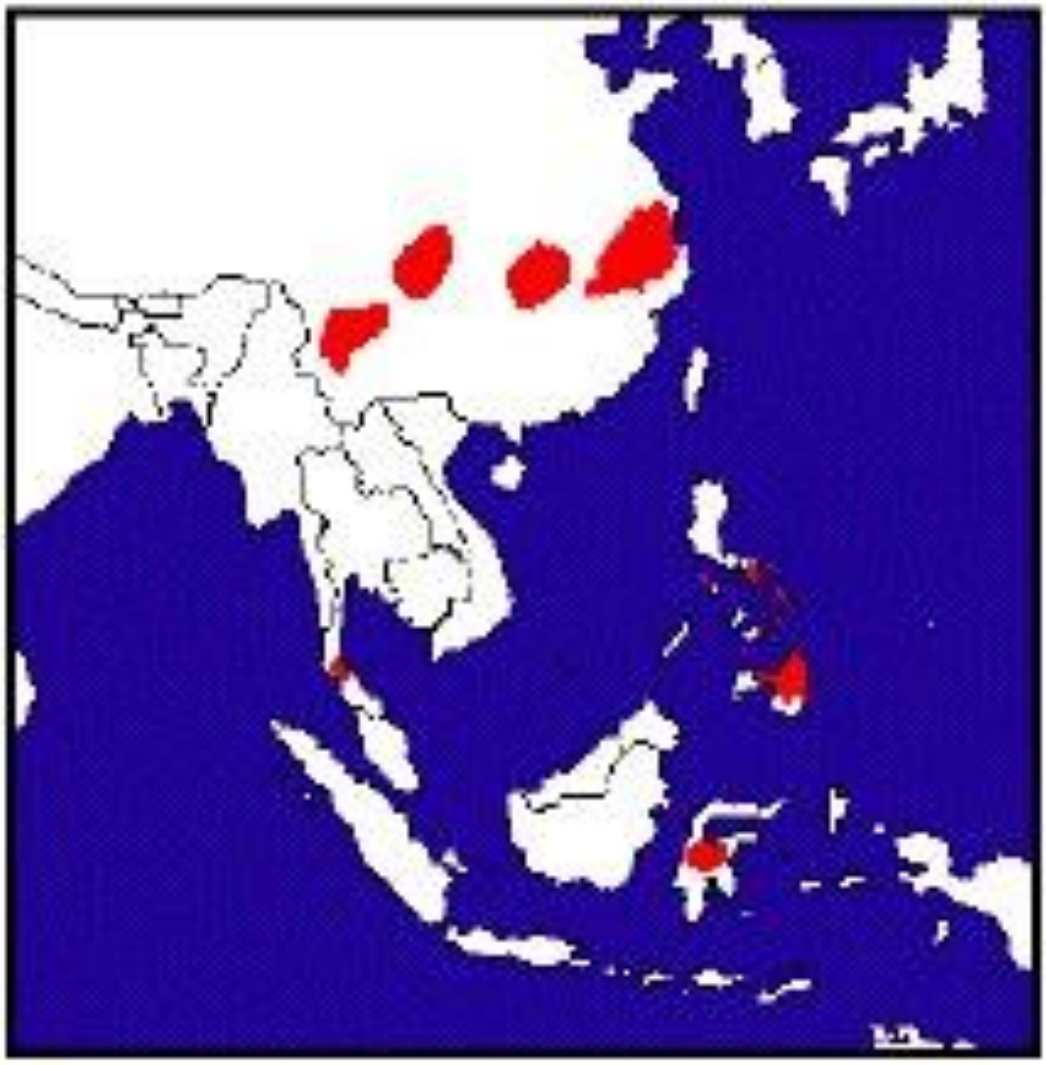

Figure 3 A map showing distribution of *S. japonicum* in the world. (http://www.path.cam.ac.uk/schisto/schistosoma/schisto-distribution.html)

S. japonicum is found in the Far East, particularly China and the Philippines, but not any longer in Japan where successful control programs have been implemented (http://www.path.cam.ac.uk/-schisto/schistosoma/schisto-distribution.html). At one time it was even more widespread in China, but control programs have successfully eradicated it from many areas in Southern China and around Shanghai. In Indonesia it is found in a few

isolated valleys in Central Sulawesi (http://www.path.cam.ac.uk/-schisto/schistosoma/schisto-distribution.html). Control of this parasite is complicated in that many reservoir hosts (such as water buffalo) exist (http://www.path.cam.ac.uk/schisto/schistosoma/schisto-distribution.html). Interestingly there are many strains of *S. japonicum*, which vary considerably in their pathogenicity, drug sensitivity, morphology and infectivity (http://www.path.cam.ac.uk/-schisto/schistosoma/schisto-distribution.html). In Taiwan a strain exists that is not infective to man (http://www.path.cam.ac.uk/schisto/schistosoma/schisto-distribution.html).

2.5.4 Distribution of *S. intercalatum*

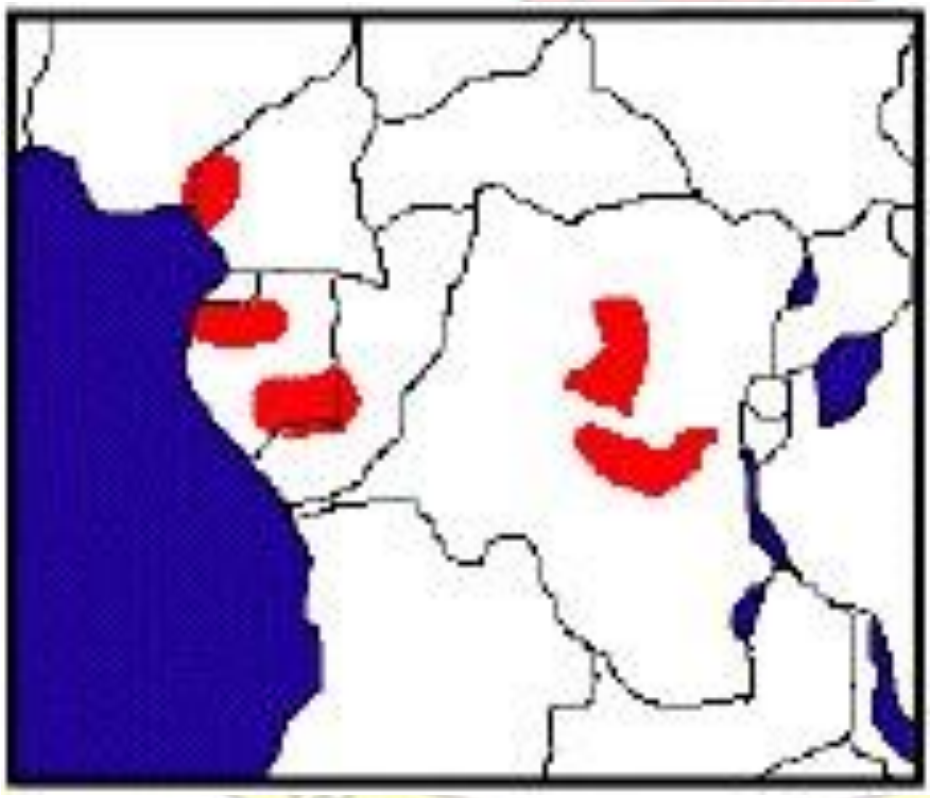

Figure 4 A map showing the distribution of *S. intercalatum* in the world. (http://www.path.cam.ac.uk/schisto/schistosoma/schisto-distribution.html)

S. intercalatum is endemic in parts of Democratic Republic of Congo, Gabon, Cameroon, with other small foci possibly in Central African Republic, Chad, Nigeria, Upper Volta and other

parts of Central Africa (though these have only been reported from isolated cases) (http://www.path.cam.ac.uk/-schisto/schistosoma/schisto-distribution.html). It is possible that zoonotic infections of *S. intercalatum* also occur as it has been demonstrated to infect experimentally primates, sheep, goats and other animals, and has also been found in rats in the field (http://www.path.cam.ac.uk/-schisto/schistosoma/schisto-distribution.html). Snail of the genus *Bulinus* act as intermediate hosts (http://www.path.cam.ac.uk/-schisto/schistosoma/schisto-distribution.html).

2.5.5 Distribution of *S. mekongi*

Figure 5: A map showing the distribution of S. mekongi in the world. (http://www.path.cam.ac.uk/schisto/schistosoma/schisto-distribution.html)

S. mekongi is very similar in terms of its morphology, clinical manifestations and lifecycle to *S. japonicum*, and is found in Laos and Cambodia (http://www.path.cam.ac.uk/schisto/schistosoma/schisto-distribution.html). In Laos dogs have been demonstrated as reservoir hosts. Snails of the genus *Tricula* act as intermediate hosts (www.path.cam.ac.uk/schisto/schistosoma/schisto-distribution.html).

A similar species, *S. malayensis*, has recently been reported in Peninsular Malaysia as occasionally infecting man, and in Thailand other *S. japonicum* - like species may also exist. (www.path.cam.ac.uk/-schisto/schistosoma/schisto-distribution.html)

A number of animal species of schistosome may occasionally accidentally infect man (www.path.cam.ac.uk/-schisto/schistosoma/schisto-distribution.html). Reported species include; *S. bovis* (normally infecting cattle, sheep and goats in Africa, parts of Southern Europe and the Middle East). *S. mattheei* (normally infecting cattle, sheep and goats in Central and Southern Africa) *S. margrebowiei* (normally infecting antelope, buffalo and waterbuck in Southern and Central Africa), *S. curassoni* (normally infecting domestic ruminants in West Africa) has been reported, although this is disputed (http://www.path.cam.ac.uk/-schisto/schistosoma/schisto-distribution.html).

S. rodhaini normally infects rodents and carnivores in parts of Central Africa (www.path.cam.ac.uk/-schisto/schistosoma/schisto-distribution.html). The cercaria of a large number of non-human infecting schistosome may penetrate human skin, but then die (http://www.path.cam.ac.uk/-schisto/schistosoma/schisto-distribution.html). These can give rise to an allergic condition called swimmers itch, or cercarial dermatitis, a reaction caused by release of antigens by the dying parasites in the skin (http://www.path.cam.ac.uk/schisto/schistosoma/schisto-distribution.html). Species that have been implicated in this condition include, *S. Spindale*, a parasite of ruminants, particularly cattle and water buffalo, in South East Asia (http://www.path.cam.ac.uk/-schisto/schistosoma/schisto-distribution.html). *Austrobilharzia variglandis*.- parasitic in water fowl of North America and Hawaii (http://www.path.cam.ac.uk/-schisto/schistosoma/schisto-distribution.html). *Heterobilharzia americanum* - parasitic in racoons and other mammals in Louisiana, U.S.A. (http://www.path.cam.ac.uk/-schisto/schistosoma/schisto-distribution.html) *Microbilharzia*

sp. - parasitic in gulls, ducks and marine wildfowl in East Coast of U.S.A. and Hawaii (www.path.cam.ac.uk/-schisto/schistosoma/schisto-distribution.html). *Trichobilharzia ocellata* - parasitic in ducks of Europe, Asia and North America. *T. physella* - parasitic in ducks of North America and Japan (http://www.path.cam.ac.uk/-schisto/schistosoma/schistodistribution.html). *T. stagnicolae* - parasitic in ducks in the Great Lakes area of America (http://www.path.cam.ac.uk/-schisto/schistosoma/schisto-distribution.html). *Gigantobilharzia sp.* - parasitic in passerine birds.

2.6 The Parasite Life Cycle

Schistosomiasis is caused by infection with flatworms belonging to the genus *schistosoma (*Trematodes, Platyhelminthes) (van der Werf, 2003). The five species that infect man are *S. haematobium,S. mansoni, S. japonicum, S. intercalatum*and *S. mekong*i (van der Werf, 2003). Most African countries and some countries in the Middle East are endemic for *S. haematobium* and *S. mansoni. S. intercalatum*has been reported in ten countries in Africa (Chitsulo *et al*., 2000). Outside Africa, *S. mansoni* is also present in South America, especially in Brazi. *S. mekongi* and *S.japonicum* are confined to the Far East. The five species differ in size and shape of their eggs,egg production (*S. haematobium* and *S. mansoni* 20-300 and *S. japonicum* 3500 eggs per worm per day (Jordan *et al.,* 1993)) and location within the human host and consequently cause different signs and symptoms.

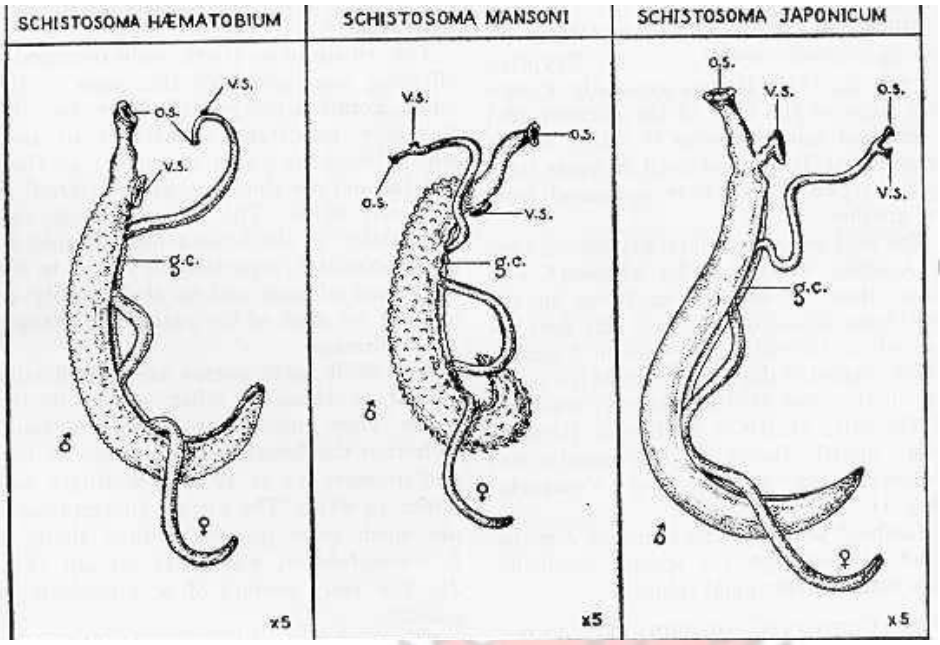

Figure 6 : Sexes of the three main *Schistosoma* species of medical importance

Source: Belding, D.L. Clinical Parasitology, Appleton-Century CO. New York 1942. g.c. = Gynecophoric canal ; v.s. = Ventral sucker and o.s. = Oral sucker

2.6.1 *Schistosoma mansoni* eggs

These eggs are large (length 114 to 180 μm) and have a characteristic shape, with a prominent lateral spine near the posterior end. Anterior end is tapered and slightly curved (http://www.cdfound.to.it/HTML/sch1.htm). When the eggs are excreted, they contain a mature miracidium (http:// www.cdfound.to.it/HTML/sch1.htm)

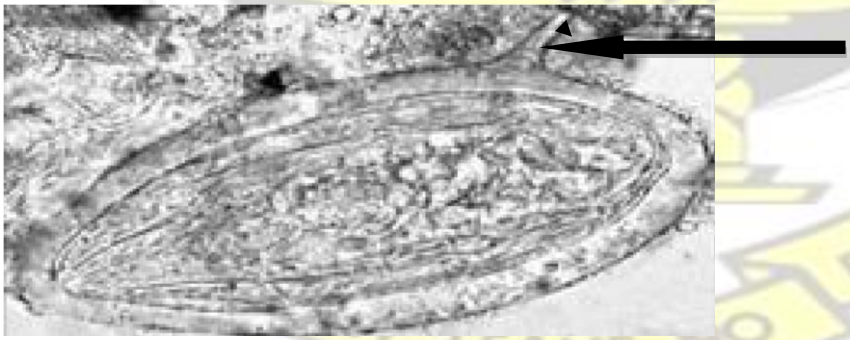

Figure 7: Ovum of *S. mansoni* showing lateral spine (arrowed)

Source: http:// www.cdfound.to.it/HTML/sch1.htm

2.6.2 *S. haematobium* egg:

In this species, the eggs are large and have a prominent terminal spine at the posterior end.

The Length is between 112 to 170 µm (http://www.cdfound.to.it/HTML/sch1.htm).

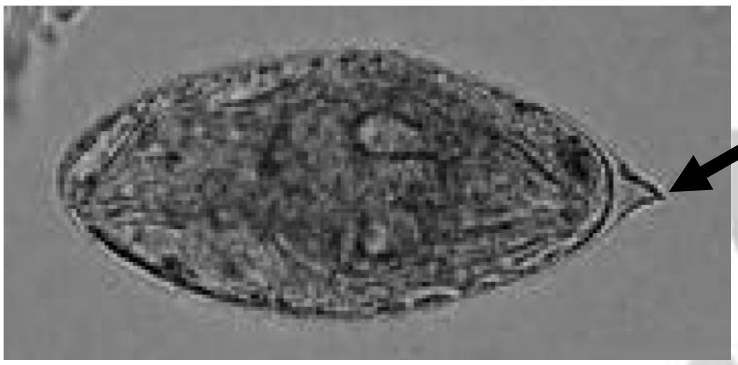

Figure 8 Ovum of *S. haematobium showing* terminal spine (arrowed)

Source: http:// www.cdfound.to.it/HTML/sch1.htm

2.6.3 *S. japonicum* egg

The egg is typically oval and has a vestigial spine. *Schistosoma japonicum* eggs are smaller (68 to 100 µm by 45 to 80 µm) than those of the other species (http://www.cdfound.to.it/HTML/sch1.htm).

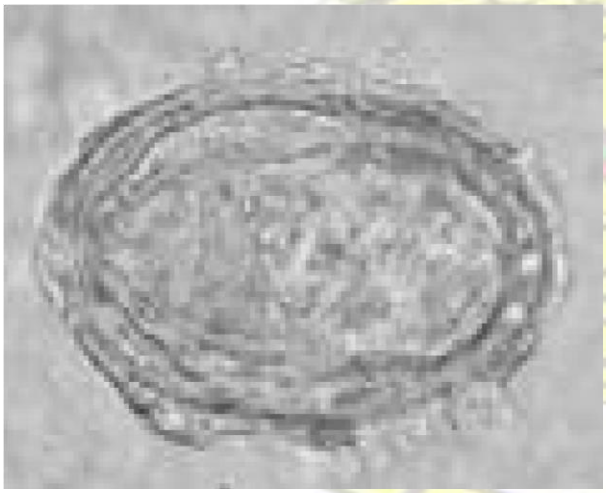

Figure 9 Ovum of *S. Japonicum*

Source: http:// www.cdfound.to.it/HTML/sch1.htm.

2.6.4 Life Cycle of Schistosomes

Humans become infected after contact with surface water in which the intermediate host snails live (van der Werf, 2003). These snails shed cercariae, free living infective *schistosome* larva (van der Werf, 2003). The released of cercariae is most pronounced around noon and starts 3 to 5 hours after the snail are exposed to light (Wolmarans *et al.*, 2002). Cercariae are positively phototropic and therefore congregate towards the surface where possibilities for contact with humans or animals are maximal (McKerrow and Salter, 2002). Upon contact with human skin, they adhere and apply their oral sucker (Jordan *et al.*, 1993). They respond to chemical signals, particularly medium chain fatty acids, as a signal for skin invasion (van der Werf, 2003). By means of both enzymes and vigorous movement the skin is penetrated (van der Werf, 2003). Thereafter, they shed their tail and are transformed to schistosomula, the next larval stage (van der Werf, 2003). These migrate through the venules, right heart chamber and lungs via the mesenteric arteries and portal vein to the liver (van der Werf, 2003). In the liver the schistosomulum starts to grow until it is matured (van der Werf, 2003). The matured male and female worm mate in the liver and migrate to the blood vessels of the intestines (intestinal schistosomiasis caused by *S. mansoni, S. japonicum, S. mekongi* and *S. intercalatum)* or urinary tract (urinary schistosomiasis caused by *S. haematobium*) Where the females live in the gynaecophoric canal of the male worms(van der Werf, 2003). Here, egg laying starts after approximately 30 days (van der Werf, 2003). The lifespan of adult worm ranges from 3 to 38 years (Arnon, 1990, Harris *et al.*, 1984) and thus a large number of eggs will be produced in a lifetime (van der Werf, 2003). Eggs migrate through the wall of the intestines or bladder and are excreted by defecation or urinating respectively. When the excreted eggs come in contact with fresh water they hatch a miracidium which is able to infect the intermediate snail host (van der Werf, 2003). In the snail, new cercariae develop by asexual reproduction (van der Werf, 2003).

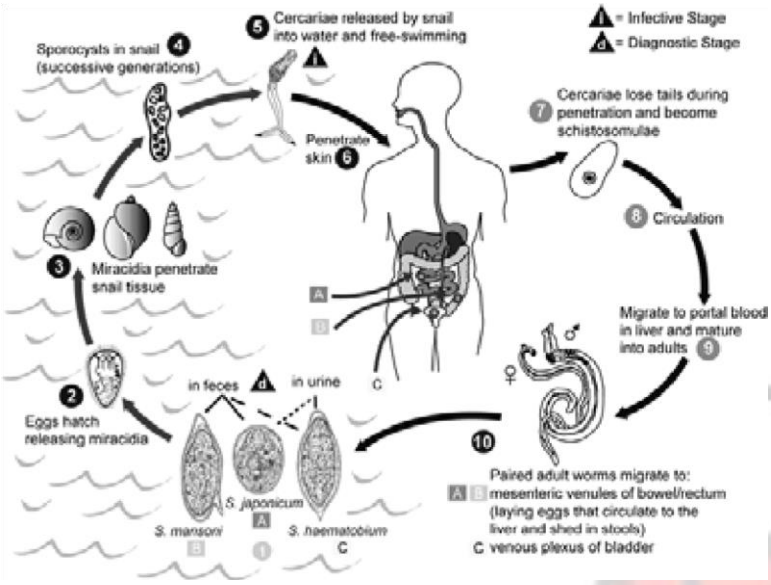

Figure 10: Life cycle *Schistosoma* sp

Source: http://www.dpd.cdc.gov/dpdx

2.6.5 Control of Schistosomiasis

Control measures that interrupt the life-cycle of Schistosomes can be divided into (1) snail control (molluscicides), (2) prevention of water contact by humans (safe water supply and building of bridges), (3) killing of worms in the human host (chemotherapy with antischistosomiasis drugs) and (4) prevention of surface water contamination with urine or stool (sanitation) (WHO, 1993). Health education of population at risk of infection and development of disease can aim at reducing contact of humans with infected water bodies, increasing health care seeking behaviour for schistosomiasis related symptoms and preventing contamination of surface water with human excreta (van der Werf, 2003).

2.7 Laboratory Methods (Procedures) for Diagnosis

2.7.1. Visual Methods

The association of *S. haematobium* with haematuria is well known, especially among children before puberty, although it is not always predictable (Sturrock, 2001). Eggs that become trapped in the bladder wall of an infected person give rise to pathological changes leading to blood in

urine (haematuria), the main symptoms of early *S. haematobium* infection (Mott *et al.*, 1983). In endemic areas, visual inspection of daily urine samples over 3- to 5-days can be a simple, cheap way to detect most infections in schoolchildren and is of use in control programmes, with or without the additional refinement and cost of dipsticks to detect blood and/or protein in the urine (Savioli *et al*., 1990).

In endemic areas, up to 80.0% of infected children have haematuria and those infected with more than 50 eggs/10ml of urine, 98.0-100.0% have haematuria (Cheesbrough, 1998).

Proteinuria is also found to correlate with *S. haematobium* infection (Doehring, 1994). Because of this, a confident diagnosis is made in endemic areas when there are clinical signs especially haematuria (Kumar *et al*., 1994).

After prolonged infection, the urinary system may be seriously affected, resulting in hydroureter, hydronephrosis and kidney failure (Jordan *et al.*, 1993, Chen and Mott, 1989).

Identification of cases or communities for treatment (community diagnosis) is usually based on measuring infection by microscopic detection of eggs in urine (Sturrock, 2001). Haematuria, may be a simpler and cheaper alternative for identifying communities in need of treatment (Lengeler *et al.*, 1991, Guyatt *et al*., 1999). Micro-haematuria can be detected by reagent strip testing (Sturrock, 2001). Macro-haematuria can be detected with the help of a questionnaire (i.e. asking individuals if they have experienced blood in urine in a given period) or by visual examination of urine sample (Feldmeier and Poggensee, 1993). Since 1970, ultrasonography has been applied to visualised lesions in the bladder wall caused by trapped *S. haematobium* eggs (Degremont *et al.*, 1985, Burki *et al.*, 1986, Abdel-Wahab *et al.*, 1992b). This method is not suitable for large scale use in control programmes but is accepted as a relatively simple non-invasive method in hospital or research setting (Hatz *et al.*, 1992).

Helminthic infections can induce digestive abnormalities and influence the nature or consistency of stool produced (Garcia, 1999, 2001).

The macroscopic appearance of stool specimen can give a clue to the type of organisms present (Ash and Orihel, 1991; Goodman *et al*., 2007; Parija and Srinivasa, 1999).

Adult worms of *Ascaris, Enterobius* and tapeworm proglottids may be seen when fresh specimen is visually examined (Garcia, 1999, 2001). Fecal specimens are described as formed, semiformed, soft, loose, or watery and bloody mucoid (Beaver *et al*., 1984).

The association of signs and symptoms is less clear cut for *S. mansoni* and *S.japonicum*: other causes must be ruled out, especially for diarrhoea and bloody diarrhea (Sturrock, 2001). Hepatosplenomegaly and splenomegaly, detected clinically by palpation, often occur in high transmission endemic areas, and indicate significant schistosomal pathology (Sturrock, 2001). Subjective, between-observer variations in these clinical measurements remain a problem despite attempts at standardization using defined axes for reporting results (Doehring-Schwerdtfeger *et al*., 1995).

2.7.2 Parasitological diagnosis

Adult worms lie hidden in the blood system and parasitological diagnosis is therefore indirect evidence of their presence within a patient. Diagnosis is by relying on the detection of eggs in urine or faecal samples. It is though absolutely specific because an egg is direct proof of infection, some caution is needed for atypical eggs, e.g. *S. bovis* eggs in an infected cow liver can pass through the human bowel to give the spurious appearance of infection (Kinoti & Mumo, 1988). The techniques are relatively simple and cheap but technicians require proper training and access to a microscope (Sturrock, 2001). The main limitations are that the techniques are slow, labour intensive and aesthetically unpleasant (Sturrock, 2001). Today, the most widely used methods are the Kato thick smear (Katz *et al*., 1972) and the Nucleopore filtration techniques for faecal and urine examination, respectively (Feldmeier,

1993).

2.7.2.1 Urine Examination

Examination of urine sediment is used mainly for the identification of *Schistosoma* eggs (Peters *et al., 1976)*

Nucleopore filtration is conventionally used to examine 10 ml of urine collected between 11.00 and 14.00 h at the peak of the diurnal egg count cycle.

One aliquot of 10-ml urine sample is filtered through a 13-µm, 12-µm porosity polycarbonate membrane using a 10-ml syringe and the filter placed on a labeled glass slide with identification number. Eggs is counted microscopically to determine intensity of infection and classified into three groups: light (1–49), moderate (50–99) and heavy (100 eggs and more in 10 ml of urine).

2.7.2.2 Stool microscopy

Microscopic or parasitological diagnosis is generally sensitive, simple, and economical (Parija and Srinivasa, 1999). If performed correctly, stool microscopy offers many advantages over other diagnostic methods for detecting intestinal parasites (Watson *et al*., 1988; Bogoch *et al*., 2006). Diagnostic tests involving microscopy include direct wet preparations, concentration methods and the Kato-Katz technique (Markell and Voge, 1976;
Watson *et al*., 1988).

2. 7. 2. 2. 1. Direct Wet Mount Method

Direct wet mount involves microscopic examination of fresh faecal specimens by wet preparations with physiological saline (saline wet mount) or iodine solution (iodine wet mount) or 1% aqueous solution of eosin (eosin wet mount) (Garcia, 1999, 2001; Isenberg, 1998).

The procedure provides rapid diagnosis for intestinal parasites when they are present in sufficient density in the faecal sample (Ukaga *et al*., 2002; Engels *et al*., 1996).

The method is useful for detecting organism motility, including motile larval forms of *Strongyloides stercoralis* and trophozoites of intestinal protozoa (Watson *et al*., 1988). The technique is also useful for diagnosis of parasites that may be lost in concentration techniques (Melvin and Brooke, 1985). It is particularly useful for the observation of motile protozoan trophozoites and the examination of certain diagnostically important objects such as CharcotLeyden crystals and cellular exudates (Parija and Srinivasa, 1999; Garcia, 1999).

The major disadvantage of direct wet mount method is its lack of sensitivity (Estevez and Levine, 1985; Melvin and Brooke, 1985; Engels *et al*., 1996; Pearson, 2002).

Akujobi *et al* (2005) and Bogoch *et al* (2006) have pointed out that infections of low parasite intensities can be missed even by the most experienced microscopist. Ahmadi *et al*. (2007) indicated that even when parasites are detected, other species may be present in a density below the "diagnostic threshold" of the test. Slide preparations from wet mounts dry up easily and motile organisms may not be detected if the preparations are not examined quickly after preparation (WHO, 1991).

2.7.2.2.2. Concentration Methods

Concentration techniques increase sensitivity of stool microscopy to allow the detection of small numbers of organisms that may be missed by using only a direct wet smear (Allen and Ridley, 1970). Basically, concentration techniques operate in two ways, either by sedimentation (Ritchie, 1948) in which the parasite sink to the bottom of the liquid suspension, or by flotation (Truant *et al*., 1981) in which the parasite forms are suspended in a liquid of high specific density to make them buoyant and float to the surface where they are collected for examination (WHO, 1991).

Some parasite stages have been described as “sinkers”, and others are “floaters”, some do both, and some do either (Cox, 1998). Therefore, no ideal method of concentration is capable of detecting all forms of parasites that may be present in stool specimens (Tay *et al.*, 2011).

In general, flotation gives a “cleaner” preparation than sedimentation yet each has a preference over another in certain aspects (Ukaga *et al*., 2002; Truant *et al*., 1981; Cheesbrough, 2005).

Concentration by flotation utilizes a liquid suspending medium heavier than the parasite objects so that they float and can be recovered from the surface film (Tay *et a.,l* 2011).

The floating medium generally employed include brine (i.e., saturated aqueous solution of sodium chloride), and zinc sulfate solution having a specific gravity of approximately 1.20 and 1.18 respectively (Garcia, 1999, 2001).

The procedure is simple and known to be a more sensitive method if protozoan cysts, nematode and tapeworm eggs (with the exception of *Diphyllobotrium* eggs) are sought (Cheesbrough, 2005). However, eggs of common intestinal helminths, *Strongyloides* larvae, and protozoan cysts become badly shrunken; sufficient to render the object undiagnosable (Ukaga *et al*., 2002).

Studies have shown that a sedimentation method recovers the broadest spectrum of parasite species (Truant *et al*., 1981). The formalin-ether concentration procedure as described by Ritchie (1948), and Allen and Ridley (1970) provide the best diagnostic outcome in epidemiological studies (Akujobi, 2005). The technique requires the use of formalin as a fixative and ether (Allen and Ridley, 1970) or ethyl acetate (Young *et al*., 1979) or gasoline (WHO, 1991; Wirkom *et al*., 2007) or hemo-de as a lipid removing agent.

It uses formalin to fix and preserve the faecal specimen and ether or ethyl acetate to extract debris and fat from the faeces, leaving the parasites at the bottom of the suspension (Akujobi *et al*., 2005; Allen and Ridley, 1970). Authors consider the formol-ether concentration as the most effective

technique that recovers the broadest range of organisms, and hence, the "gold standard" method (Wiebe *et al*., 1999) of all parasitological techniques (Melvin and Brooke, 1985; Garcia, 1999, 2001; Cheesbrough, 2005; Markell and Voge, 1976).

The advantages of this method are that it will recover most ova, cysts and larvae and retain their morphology, thereby facilitating identification (Neimeister *et al*., 1987). There is less risk of infection from bacteria and viruses because they may not be able to survive the concentration process involved (Akujobi, 2005).

The concentration technique has additional advantage by allowing for transportation and storage after faeces are preserved in formalin (Oguama and Ekwunife, 2007).

Conversely, it has the disadvantage of destroying trophozoites stages and distorting cellular exudates and liquid stools do not concentrate well (Ash and Orihel, 1991).

Because concentration procedures require a laboratory with trained personnel, centrifuge to separate parasites, electricity to run centrifuges, a well ventilated work space, adequate water supply, a standard light microscope, and consistent availability of regular supply of reagents, it tends to be expensive running the test (Allen and Ridley, 1970).

2.7.2.2.3. Kato-Katz technique

The Kato-Katz technique (Kato & Miura 1954; Katz *et al*. 1972) is useful for the quantitative estimation of worm burdens (Markell *et al*., 1999). It is especially useful for field surveys for helminth infections since it provides estimates of the intensity of infection (Martin and Beaver, 1968).

According to Martin and Beaver (1968), the technique entails the examination of a standard sample (determined by the size of the template) of fresh faeces pressed between a microscope slide and a strip of cellophane that has been soaked in glycerin (Markell and Voge, 1976; Ukaga *et al*., 2002).

The Kato template may be made of stainless steel, plastic, or cardboard, and different sizes have been produced in different countries: a 50 mg template has a hole of 9 mm on a 1 mm thick template; a 41.7 mg template has a hole of 6 mm on a 1.5 mm thick template; and a 20 mg template will have a hole of 6.5 mm on a 0.5 mm thick template (Ebrahim *et al*., 1997).

The cellophane cover slip, 22 x 30 mm, are pre-soaked for at least 24 hours in a glycerinmalachite green solution of 100 ml pure glycerin, 100 ml distilled water and 1 ml of 3% malachite green (Markell and Voge, 1976; Garcia, 1999, 2001).

After the faecal film has cleared, eggs in the entire film are counted, and, the number of eggs of each species reported is multiplied by the appropriate multiplication factor to give the number of eggs per gram (epg) of faeces (Martin and Beaver, 1968).

When using a 50 mg template, the multiplication factors is 20; and for a 20 mg template, the factor is 50 (Katz *et al*. 1972).

The WHO (1998) had recommended the use of a template holding 41.7 mg of faeces, and with a multiplication factor of 24. Siegel *et al*. (1990) pointed out that Kato-Katz technique has limited usefulness in detecting infections in diarrhoeal specimens.

Limitations of this method include difficulty in processing diarrhoeal stools (Siegel *et al*., 1990), lack of sensitivity if only a single stool sample is examined (Booth *et al*., 2003). Counting of eggs in Kato-Katz smears can be a tedious and time consuming process, and can lead to technical errors (Kato and Miura, 1954; Ebrahim *et al*., 1997).

Other drawbacks of the method include high risk of infection for the technicians handling fresh stools (HHS, 1993).

Hookworm eggs clear rapidly, and if slides are not examined within 30-60 minutes, the eggs may no longer be visible (Garcia, 1999). The technique is also known to be unsuitable for detection of cysts, larvae, small fluke eggs or thin-shelled eggs such as *Hymenolepis* species

because eggs disappear during the clearing process in a short time of 30-120 minutes (Knopp *et al*., 2006).

2.7.3. Serological (Immuno-diagnostic) methods

There are increasing availability of non-microscopic methods, such as DNA probes, direct fluorescent antibody methods and enzyme-linked immunosorbent assay- ELISA (Genta, 1988; Char and Farthing 1991; Gasser, 2001). They have proved to be most useful for distinction between acute and chronic *Schistosoma mansoni* infection (Valli *et al*., 1997). Serological methods are sensitive but are expensive for use in the developing world and may show cross reactivity with other helminthic infections (Valli *et al*., 1997). Another disadvantage of serodiagnositic approach is that tests might remain positive even after cure by chemotherapy (Knopp *et al*., 2006).

Immunodiagnosis of schistosomiasis relies mainly on antibodies to detect antigens and *vice versa* (Sturrock, 2001). In some, though not all, instances, their levels in man are more stable than those of egg counts (Sturrock, 2001). Invasive skin tests have been abandoned and most tests are now performed on serum or plasma samples from finger prick or venous blood (Sturrock, 2001). Even these procedures are considered intrusive and attention is turning to other body fluids such as urine, milk, and saliva or other oral exudates (Garcia Santos *et al*., 2000).

Antibody detection tests provide only indirect proof of exposure because they are molecules produced by the host's immune response to the parasite (Sturrock, 2001). Prepatent and early infections may not have stimulated a detectable antibody response, resulting in false negative tests if the wrong antigen is used (Sturrock, 2001). Positive tests do not necessarily denote an active (living) infection (Sturrock, 2001). Antibody tests are also prone to cross reactions with other infections (especially tissue dwelling parasites) which diminishes their sensitivity

(for detecting infected cases) and specificity (for detecting normal, uninfected people) (Sturrock, 2001). However, improved reagents provided by molecular biological techniques have revived interest in antibody detection, particularly for specific isotypes and sub-classes associated with different stages of infection (Sturrock, 2001). Imperfect correlations between antibody levels and egg counts may give some evidence of the intensity of infection in communities. Positive antibody tests are usually, at best, qualitative evidence of past or present exposure to infection (Sturrock, 2001).

Antigen tests, by definition, detect molecules produced by the parasite and are therefore just as much direct proof of infection as finding eggs (Sturrock, 2001).

Antigen levels are often significantly lower than those of antibodies, but, theoretically, are directly related to the number of worms present (Sturrock, 2001). Continuously improving techniques now allow detection of very low antigen levels (Sturrock, 2001). Levels tend to be more stable than egg counts but the relationship between egg counts and antigen levels is not always perfect (Sturrock, 2001). Although antigen tests often confirm suspected light infections with sparse eggs, negative antigen tests still occur in subjects passing eggs (Sturrock, 2001). Despite claims to the contrary, egg counts remain the gold standard for diagnosis (Hagan *et al*., 1998). At first, both antibody and antigen tests required access to dedicated laboratories to process samples (Sturrock, 2001). Recently, simple dip-stick/card tests have been developed for field use (Van Etten *et al*., 1994) but none are available commercially and they will have to be cheap enough for routine use (Sturrock, 2001).

2.7.3.1 Detection of eosinophil cationic protein by an enzyme-linked immunosorbent assay

A newer test for intensity of infection for *S. haematobium* is the eosinophilic cationic protein (ECP) (www.edu/med/epidbio/mphp439/schistosomiasis.htm). Declining quantitative urinary ECP levels correlate with reduction in severity of illness. (Engels *et al.*, 2002) This test is particularly helpful for intestinal schistosomiasis

(www.edu/med/epidbio/mphp439/schistosomiasis.htm).

Eosinophil cationic protein (ECP) is a highly basic and potent cytotoxic single-chain zinccontaining protein present in the granules of eosinophilic granulocytes (Reimert *et al.,* 1991). ECP appears to be involved in defense against parasites and in tissue damage seen in subjects with allergic and inflammatory disease (Reimert *et al.,* 1991). A sensitive and specific enzyme immunoassay has been developed (Reimert *et al.,* 1991). ECP was purified from normal human peripheral blood eosinophils and polyclonal antibodies to ECP were subsequently raised in rabbits (Reimert *et al.,* 1991). The ELISA utilizes the biotin/avidin method and measures ECP within the range 15-1000 ng/l (Reimert *et al.,* 1991).

Eosinophil cationic protein (ECP) is an established marker of *S. haematobium* infection and morbidity (Reimert *et al.,* 1991; Leutscher *et al.,* 2000) This protein has also been evaluated as an infection marker for genital schistosomiasis (Poggensee *et al.,* 1996; Midzi *et al.,* 2003) Preliminary results showed a positive association between egg excretion and increased ECP levels in semen (Midzi *et al.,* 2003). A positive association between *S. haematobium* eggs in cervical biopsy specimens and smears and increased ECP levels in vaginal lavage has also been observed (Leutscher *et al.,* 2000)

2.7.4. Molecular diagnosis

Molecular techniques such as polymerase chain reaction (PCR) using primers derived from different genetic markers are useful diagnostic tools (Michaud *et al*., 2003). PCR based assays that are specific and highly sensitive have been developed for the detection of Schistosome DNA in human excreta, sera or plasma (ten Hove *et al*., 2008); Wichmann *et al*., 2009; Gomes *et al*., 2010; Ibironke *et al.,* 2011). However, they need further validation and standardization before been routinely applied for diagnosis of schistosomiasis. Moreover their application requires expensive high-tech laboratory infrastructure and highly accurate handling of samples, kits and equipment. Hence PCR is a technique which might be useful for individual

patient management but has limited broad-scale field-applicability at the moment (Rollison et al., 2013).

2.8. Control of Schistosomiasis

The life cycle of Schistosomiasis is such that there are four main targets for interventions (Rollison et al., 2013):

The first is to kill the adult worms in man, which is currently achieved through praziquantel-based chemotherapy (Rollison et al., 2013).

The second measure is to kill or replace the snail intermediate host by means of biological control (e.g. competitor snails and snail-eating fish), chemical control (i.e. molluscicide) and environment management (Rollison et al., 2013);

Another intervention measure is to prevent the snail from getting infected, hence preventing contamination of water by infected individuals, using information, education and communication (IEC), sanitation and behaviour changes (Rollison et al., 2013).

The last is to stop humans from getting infected by preventing contact with water containing infected snails or cercariae (achieved through IEC and safe water supplies) (Rollison et al., 2013).

Simple control measures can ameliorate the Schistosomiasis burden in high prevalence areas, and can be implemented in all circumstances (WHO, 2002).The following control strategies are recommended in a recent WHO report: preventive chemotherapy intensified case management, vector control, and provision of safe water and environmental sanitation (WHO,2010)

2.8.1. Preventive chemotherapy

Praziquantel is the sole drug for treatment and morbidity control of Schistosomiasis in Sub

Saharan Africa (Ferwick *et al*., 2003; Doenhoff *et al*., 2009; Utizinger *et al*., 2011). Praziquantel is safe, cheap and effective against adult worms. Cure rates of up to 85-90% have been achieved but complete cures (100%) have seldom, if ever been recorded in endemic areas (Doenhoff *et al*., 2009; Olliaro *et al*., 2011)

The large-scale administration of Praziquantel to school-aged children is the mainstay of current programmes focusing on morbidity control (Fenwick *et al*., 2009). Preventive chemotherapy administered via the school route has a big impact on helminth infections as it targets those at highest risk, and takes advantage of the educational infrastructure and resource already in place (Miguel and Krener, 2004). The disadvantage of this delivery strategy are that there are inherent age and sex inequalities in children attending school and that as many as 40% of children in Sub Saharan Africa may not be enrolled in school (Rollison et al., 2013).

Treatment coverage is an important factor determining the effectiveness of control programmes emphasising preventive chemotherapy (Rollison et al., 2013). Coverage can be improved by IEC campaigns, mobilisation and community participation (Smuts, 2009), For example, Tallo *et al*, (2008) investigated the effects of community wide treatment in the Philippians and found that participation in treatment campaigns is determined by individual factors, such as age, gender and knowledge.

The problem of treatment adherence may arise as people may alternate between or combine treatments obtained through formal healthcare providers and informal sources, such as traditional herbalists (Rollison et al., 2013). This is a function of the level of knowledge or education of the patients, their knowledge of infection status, logistics of transport and their anticipation of the quality and benefits of treatment (Aagaard-Hansen *et al*., 2009). A more fundamental challenge that needs to be addressed at the individual and community level is an understanding especially in relatively low prevalence areas, that there is a problem, i.e. that Schistosomiasis exist in the community and is a health problem. In many settings especially in

regard to *S.mansoni* infection, the level of the health problem is not appreciated by those who are infected or those around them (Rollison et al., 2013). Very often people appreciated that they feel better upon treatment but did not realise prior to treatment that they were ill (Rollison et al., 2013). Even if full coverage was possible, chemotherapy will reduce pathology, but may not adequately reduce transmission without additional control measures (lardans and Dissous, 1998; Urbani *et al*., 2002). A single round of treatment in a highly endemic area can result in an extended period of low transmission, but prevalence may increase after initial success (Smits, 2009). In some endemic areas, once preventive chemotherapy is ceases, prevalence can return to baseline levels within 18 months to 2 years (Gray *et al*., 2010)

King (2009) refers to large scale treatment campaign as a "stop gap measures". Praziquantel does not prevent reinfection hence control programmes based solely on monitory control will be neither completely effective nor sustainable. Moreover, some people infected with immature worms will be receiving the drugs, but will not have parasite clearance, as praziquantel is not effective against schistosomula (Sabah *et al*., 1986)

Therefore, it is of particular importance that treatment campaigns are timed appropriately in the low transmission areas where transmission is seasonal (el malataw *et al*., 1992; Augusto *et al*., 2009) and that the frequency of treatment is in line with WHO recommendations according to infection prevalence and intensity (WHO, 2006). Re-treating people with praziquantel 2-8 weeks after the first dose can increase cure and egg reduction rates in infected populations and thus add a benefit to the health and quality of life of the people (King *et al*., 2011).

Another confounding factor impeding the success of preventive chemotherapy are so called "super spreaders", people who are heavily infected, unreceptive to health education messages, who do not comply with treatment and who contaminate water bodies continually, therefore perpetuating transmission even in an area of high treatment compliance (Rollison et al., 2013).

Environmental, culture and social determinants of health are all important in schistosomiasis transmission (Rollison et al., 2013). Although large-scale distribution of praziquantel has achieved significantly reduced egg excretion in some areas (Barakat *et al*., 1995; Zhang *et al*., 2007; Toure *et al*., 2008) it has failed to break the transmission cycle in other high endemic communities (curtale *et al*., 2010). Without modification of transmission factors the best that can be achieved with preventive chemotherapy alone is low equilibrium of transmission and low level infection for an indefinite period of time. Without changes in transmission potential, adequate Schistosomiasis control will not be achieved, l*et al*one the disease being eliminated (King *et al*., 2006). However, the example of *S mekongi* in Cambodia demonstrate that 8 years of annual treatment with praziquantel with coverage between 62% and 86% had a dramatic impact on the disease prevalence with reduction to just three cases in 2005 and no case of severe morbidity (Sinuon *et al*., 2007)

At present, WHO guidelines recommend to treat children once every second year when prevalence is moderate (between 10% and 50%); at low prevalence (<10%) the recommendation is to treat children twice during primary school i.e. ideally upon entry and again before children leaves school (WHO, 2002)

2.8.2. Snail Control

Snail control and /or changes in water use have been shown to interrupt transmission in highrisk communities (King *et al*., 2006). The application of molluscicides may, however, be beyond the scope or financial means of current control initiatives (Rollison et al., 2013). That said, snail control measures become highly important in settings where low transmission intensity has been achieved, but where infected people from other endemic areas might immigrate into and which is hence prone to disease resurgence (Fenwick *et al*., 2006). In addition, intermediate host Snail Control is useful to enhance the impact and performance of preventive chemotherapy and case management (WHO,2010) It has been shown that the optimum time

for preventive chemotherapy is when there is no risk of reinfection with schistosomes, i.e. when the snails have been reduced and transmission is halted (Rollison et al., 2013). Hence, it is sensible to reduce snail population before undertaking a treatment during the low-transmission season (Augusto *et al*., 2009)

The molluscicide niclosamide has been found to be highly active against all stages of the snail life cycle, as well as schistosome larvae (McCullough *et al*., 1980) on the other hand; it is toxic to fish and thus has a negative impact on the environment and biodiversity (Rollison et al., 2013).

Moreover, niclosamide is expensive partly due to its limited use, and does not prevent snails from recolonising their origin habitats (Rollison et al., 2013). However, if used in a highly focused manner, ecological modelling indicates that niclosomates can be beneficial (woolhouse *et al*., 1998). Indeed, repeated molluscіding can be effective in the long-term management of snail populations (Sturrock, 1995: Lardans and Dissoud, 1998; Fenwick *et al.*, 2006) and hence has played a key role in the elimination of schistosomiasis in Morocco and Japan and in control programmes in Egypt and P.R China.

Alternatives to synthetic chemical molluscicides for snail control include plant – based derivatives (e.g. endod), environmental management to eliminate snail habitats, and biological control with fish, ducks, crayfish, dominant trematodes and snail Competitors (displacement, miracidal sponges) (Rollison et al., 2013). Such integrated snail control gives leverage to further accelerate control programmes towards elimination (Rollison et al., 2013).

2.8.3. Water, sanitation and Hygiene

Water, sanitation and hygiene have recently been phrased “the forgotten foundation of health” (Bartram and Cairncross, 2010). Indeed, access to and use of clean water and improved sanitation are essential in preventing re-emergence of helminthic disease after successful

treatment campaigns (Asalou and Ofozie, 2003; Utzinger *et al*., 2003; Singer and Castro, 2007; Smits, 2009; Ziegebauer *et al*., 2012) and would also aid in numerous other pathogens that are transmitted by the faecal – oral route (Rollison et al., 2013). A literature review published in the early 1990s documented a substantial reduction of schistosomiasis (77%) and a particular notable impact on egg counts and thus disease severity impact due to improved water supply and sanitation facilities (Esrey *et al*., 1991). Improvements in water and sanitation often go hand in hand with a general increase in economic development (Bergquist, 2001; Knopp *et al*., 2012). Although improvements of access to safe water is imperative, many authorities only pay lip service to that effect, and such improvement is still hard to achieve in many parts of sub-Sahara Africa (Rollison et al., 2013). Indeed, the WHO targets for safe water, sanitation and hygiene are far from being met, and without access to clean water, elimination of Schistosomiasis will remain a distant goal (WHO, 2010)

It must be noted, however, that better access to safe water and sanitation does not necessarily impact favourably on Schistosomiasis transmission, since many factors are involved in people's choice of water source for different purposes (Rollison et al., 2013). For example, provision of latrines does not mean that they will be used as intended. People may fear them, or not use them if they are not convenient (Rollison et al., 2013). Latrines may also be poorly maintained and so people will be less likely to visit them (Aagaard-Hansen *et al.*, 2009).

While improved sanitation is central to sustainable control, if water contact remains high, transmission is likely to persist even if latrines are available (Rollison, 2009). In relation to Schistosomiasis elimination, it is crucial to monitor and encourage improvements in the provision of water and sanitation, as changes in use of water may have a considerable impact on transmission at the local level (Rollison et al., 2013). Intersectoral collaboration and community participation are essential for the design, implementation and long-term monitoring of the impact and cost-effectiveness of hygiene, and a host of other diseases

(Holveck *et al*., 2007; Wang *et al.*, 2009b)

2.8.4. Health education and behavioural change

Open water contact in most rural settings is inevitable; therefore a change in behaviour of the population is necessary to stop the contamination of open water bodies with excreta (Rollison et al., 2013). In theory, the non-contamination of open water bodies is an easy and simple way to stop transmission, but in practice it is very difficult to achieve, even in the face of health education and access to safe water and sanitation (Jordan, 1985; Fenwick *et al.*, 2006). Children will always play in water and urinate, releasing *S. haematobium* eggs into the environment (Rollison et al., 2013). Similarly, despite sanitation and hygiene measures eggs of *S. mansoni* may remain on the perianal folds, so providing a transmission risk (Rollison et al., 2013). There is a need for greater and more comprehensive health education for both children and adults to guide behavioural change, especially in relation to reduction of water contact, to minimize the risk of Schistosomiasis transmission (Stothard *et al*., 2006; 2009b). Sanitation hinges on health education, with active teaching methods focusing on personal hygiene (Lansdown *et al*., 2002; Nock *et al*., 2006). Education has been found to impact health-seeking behaviour which may have an effect on prevalence of infection (Lansdown *et al*., 2002; Aagaard-Hansen *et al*., 2009).It also provides the impetus behind the success of deworming programmes preventing the contamination of the environment, and hence transmission(Nock *et al*., 2006).

However, human behaviour is resistant to change and behavioural modification will be achieved only with the increase in the knowledge and understanding of Schistosomiasis transmission in conjunction with an increase in standards of living (Fenwick *et al*., 2006; Rollinson, 2009). Thus, although education has been successful in promoting behavioural change, it may have a limited impact on the prevalence or intensity of infection without access to appropriate infrastructure (Rollison et al., 2013). Health education should be relevant to local

knowledge and practices, and should be ‘’health communication’’ dialogue, as opposed to “health instruction’’ which is one directional (Aagaard-Hansen *et al.,* 2009).

2.8.5. Vaccine Development

Generating immunity through the use of vaccines is complex (http://www.cwru.edu/med/epidbio/mphp439/schistosomiasis.htm). In the presence of high prevalence, vaccine would not be given to naïve patients (http://www.cwru.edu/med/epidbio/mphp439/schistosomiasis.htm). Rather, those receiving the vaccine can be expected to have already been exposed, and to experience repeated exposure to schistosomiasis after getting the vaccine (http://www.cwru.edu/med/epidbio/mphp439/schistosomiasis.htm). It is precisely the host immune response that gives rise to the granulomas responsible for the morbidity of schistosomiasis (http://www.cwru.edu/med/epidbio/mphp439/schistosomiasis.htm).

Potentially, by triggering the production of immunity to various schistosomiasis antigens, the vaccine could promote the production of granuloma formation (http://www.cwru.edu/med/epidbio/mphp439/schistosomiasis.htm). In fact, however, progress is being made in Phase 1 and 2 clinical trials of an *S japonicum* vaccine (http://www.cwru.edu/med/epidbio/mphp439/schistosomiasis.htm).

In much of Asia, where *S. japonicum* is a zoonotic disease, successful vaccination of water buffalo promises to interrupt the life cycle of the schistosome (http://www.cwru.edu/med/epidbio/mphp439/schistosomiasis.htm). The development of a successful veterinary vaccine is also a promising step on the way to the introduction of a human vaccine (http://www.cwru.edu/med/epidbio/mphp439/schistosomiasis.htm). Human trials using a few different vaccines are underway, with encouraging results (http://www.cwru.edu/med/epidbio/mphp439/schistosomiasis.htm).

Even without eradication of schistosomes from the environment, the vaccine appears to reduce susceptibility to re-infection (www.cwru.edu/med/epidbio/mphp439/schistosomiasis.htm). It is postulated that the vaccine's artificially-induced immunity is boosted by re-exposure to the not-yet-eradicated schistosomes (http://www.cwru.edu/med/epidbio/mphp439/schistosomiasis.htm). This suggests that immunogenicity may need to be assessed if and when schistosomes are eliminated (http://www.cwru.edu/med/epidbio/mphp439/schistosomiasis.htm).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study design

A follow-up study on schistosomiasis was conducted among inhabitants of Vea in the Bongo District of the Upper-East Region of Ghana. Individuals who gave their consent after explanation of the study were recruited and registered. Information on knowledge, and perceptions associated with the disease was collected from participants by a structured and guided questionnaire. Willing participants aged 6 years and above and resident in the community were required to provide stool, urine and blood samples.

All participants provided at least 50 ml of urine, 10 g of stool and 5 ml of blood samples and children of school age were treated with praziquantel by the Ghana Health Service. Posttreatment urine, stool and blood samples were collected from recruited participants 8 weeks after treatment.

3.2 Bongo District

The Bongo District within which the Vea irrigation Dam is located has a land mass of 459.5 square kilometres and lies between longitudes 0.45° West and latitudes 10.50° North. Bongo

District shares boundaries to the north with Burkina Faso, Kassena Nankani East District to the West and Bolgatanga municipality and Talensi Districts to the south (Bongo District Assembly, 2013)

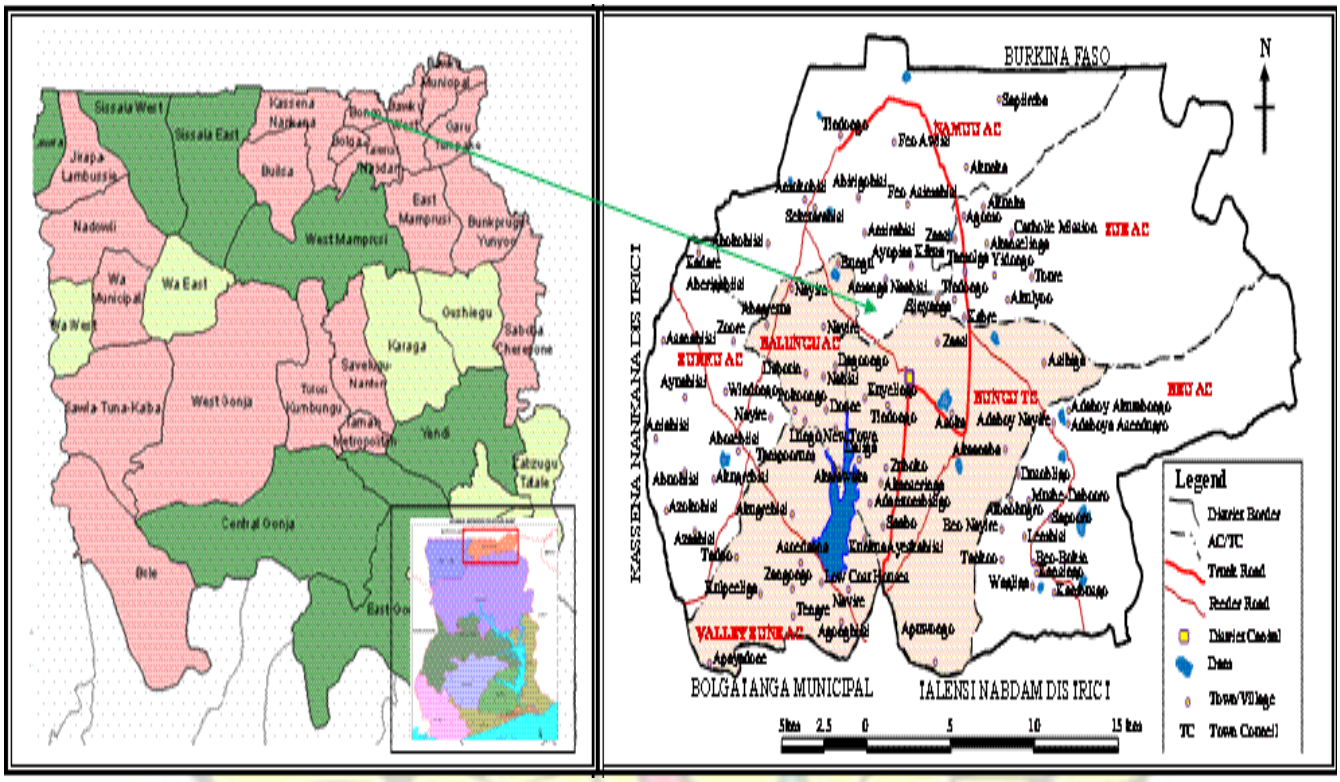

Figure 11: A map showing the location of the Bongo District

Source: Bongo District Assembly, April 2013.

With the exception of an inselberg near Bongo (which rises to a height between 92 and 122 meters above the surrounding area) the District is a low-lying with undulating areas and isolated hills rising up to 300 meters above sea level. The main water bodies comprise the Atankuidi, Yaragatan-Atanure streams and the Red volta river. The vegetation of the District is classified as a Guinea Savannah Woodland with a single rainy season with maximum rainfall ranging between 600mm and1400mm and a maximum and minimum temperature of 40°c and 12°c respectively (Authors construct, April 2010).

The 2010 population and housing census gave the population of Bongo District to be 84,545. A projection of this gave the District population 187,625 in 2013(Ghana Statistical Service,

2012).

3.3 Study Site

The Vea community is one of the communities that border the Vea irrigation dam. The Vea project was started in 1960 but became fully operational in 1980 (Gordon, 2006). The reservoir area is about 40.5 km^3 with a catchment area of 136 km^2 (Gordon, 2006). The study community comprises; Gonga, Zangongo and Vea Central with a total population of about 3,139 (District Directorate Health Service (DDHS), Bongo).The main economic activity of the people is agriculture (crop production, animal rearing, fishing and agro-forestry). Along the shores of the dam are aquatic plants (mostly water hyacinth) which harbour schistosome host snail (*Bulinus globusus* and *Biomphalaria pfeifferi*) making the lake the main source of infection. Inhabitants use water from the dam mainly for fishing, bathing, swimming and other recreational activities. There are basic schools and a Health centre in the community. The Health centre is government-owned and manned by Medical Assistants. Treatment for schistosomiasis is based on signs and symptoms and laboratory diagnosis (microscopy). The community is some few kilometres from the Regional hospital, Bolgatanga and the Bongo District hospital. Because of the high numbers of positive cases of schistosomiasis, the community was chosen for Mass Drug Administration in the region.

3.4 Ethical Issues

The study protocol was approved by the Committee on Human Research, Publications and Ethics, School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi. Permission was also granted by the DDHS, Bongo and the District School Health Education Programme (SHEP) co-ordinator of the Ghana Education Service (G.E.S.). Prior to the onset of the study, meetings were held with the staff and parents of the schools in the study area. At these meetings informed consent was obtained from the parents.

Informed assent for the children was obtained directly from their parents and teachers before specimen were collected.

3.5 Study population

The study population included all inhabitants who were aged six years and above, resident in the study area and who volunteered to participate in the study.

3.6 Sample Size Determination

The required number of persons to be sampled was estimated using the formula;

$N = (Z / \Delta)^2 P (1- P)$ where

N = sample size

Z = Confidence Level 95% (1.96)

P = prevalence. (From pilot study, P = 25%)

Δ = margin of error 5% (0.05)

From the formula, 288 participants were estimated and about10% percentage allowance of participants was added to become 315 to cater for any sample loss or any other such eventualities. However a total of 217 participants gave their consent and were recruited for the study (Male = 106, Female = 111). Participants ranged between the ages of 6 – 76 years. 122 (56.2%) of participants were lost to follow-up. Samples were collected from 95 participants. This was made up of 38 (40.0%0 males and 57 (60.0%) females.

3.7. Sampling criteria

3.7.1. Inclusion criteria

All residents above six years who had stayed in the community for 3 months or more in a year and willing to consent to the study were eligible to participate unless they fell within the exclusion criteria or willingly withdrew from the study.

3.7.2. Exclusion criteria

1. All inhabitants who were diagnosed to have the infection and have been treated for schistosomiasis in the past three months.
2. Individuals who were visibly ill.
3. Individuals who were anaemic.
4. Pregnant women.

3.8 Data collection

3.8.1. Participants information

Personal data, information on knowledge, and perceptions associated with the disease were collected from participants by a questionnaire (Appendix 3).

3.8.2 Sample Collection

Consented subjects were provided with two (2) labelled, clean, dry, leak-proof, and widemouthed plastic specimen containers. Participants were given instructions on how to collect the stool and urine samples without contamination. About 10 grams of stool was collected into one container and about 50 ml of urine in the other and delivered within 2 hours after collection (Booth *et al*., 2003). About 5 ml of venous blood samples was collected into serum separator tubes before Mass Drug Administration with praziquantel. The blood sample was collected by qualified medical personnel using sterile disposable hypodermal syringes and needles, one per each person. Sera

were extracted by centrifugation and stored at -20^{o}C until assayed for ECP levels by ELISA at the Noguchi Memorial Institute for Medical Research
(NMIMR).

Urine, stool and blood samples were collected again 8 weeks after treatment.

3.9 Drug administration

A Mass Drug Administration campaign is designed each year by the GHS and MOH with health personnel from regional, district and community health centres mobilized to offer treatment. Ghana national schistosomiasis control program adopts the preventive chemotherapy strategies recommended by the WHO (WHO, 2002; 2006). Drugs were administered by school teachers who had been trained to do so. Ivermectin and praziquantel dosages were determined using height (WHO dose poles). The two dose poles were harmonised on a single-dose pole by having the lines and corresponding number of tablets on opposite sides of the pole for the different parasites. This eliminated the problem of having to carry two poles about. Drug administration was by directly observed therapy (DOT) and all treatments were taken concurrently. The height–dosage relationship for praziquantel was 94– 109 cm for 1 tablet, 110–124 cm for 1½ tablets, 125–137 cm for 2 tablets, 138–149 cm for
2½ tablets, 150–159 cm for 3 tablets, 160–177 cm for 4 tablets and ≥178 cm for 5 tablets) and that for ivermectin was 90–119 cm for 1 tablet, 120–140 cm for 2 tablets, 141–158 cm for 3 tablets and ≥159 cm for 4 tablets). The inclusion ⁄ exclusion criteria were the same as in the National Control Programme (Gyapong *et al*. 2001), which exclude pregnant women, children 4 years and younger and persons seriously ill. Pregnant women were not given praziquantel though it is now permissible (WHO, 2002).

3.10 Parasitological methods for urine analysis

3.10.1 Dip-stick Method

Samples were tested for micro-hematuria and proteinuria using urine reagent strips (Uripath, Plasmatec Laboratory, UK) and results were scored as negative, +, ++ or +++ as per manufacturer's recommendations.

3.10.2 Filtration Method

Urine samples collected in the field were transported to Bolgatanga Regional Hospital laboratory, processed and examined. All urine samples were processed on the same day. One aliquot of 10-ml urine sample was filtered through a 13-µm, 12-µm porosity polycarbonate membrane (Millipore, Company, through UNICEF Supply Division, Copenhagen) using a 10-ml syringe and the filter placed on a single slide labeled with the identification number of the participant and date of collection. Eggs were counted microscopically to determine intensity of infection and classified into three groups: light (1–49), moderate (50–99) and heavy (100 eggs and more in 10 ml of urine).

3.11. Parasitological methods for Stool analysis

3.11.1 Kato Katz

The Kato-Katz technique (cellophane faecal thick smear) was the method used for the stool sample examination. *Schistosoma mansoni* infection was determined by microscopically examining 41.7 mg of faecal material and systematically counting the eggs in the faecal specimens.

The Kato template (with hole) was placed in the centre of a microscope slide. With a wooden applicator stick and nylon mesh a small mount of faecal material was sieved by pressing the mesh on top of a small amount of faecal material placed on a newspaper.

A spatula was used to scrape across the upper surface of the screen so that sieved faeces accumulate on the spatula.

The collected sieved faecal specimen was added in the hole of the template so that it was completely filled. The side of the spatula was used to pass over the template to remove excess faeces from the edge of the hole.

The template was carefully removed from the slide so that a cylinder of faeces was left on the slide. The faecal material was covered with a pre-soaked hydrophilic cellophane strip in 3% methylene blue-glycerol. The microscope slide was then inverted and the faecal sample was firmly pressed against the hydrophilic cellophane strip on a smooth hard surface (a glazed tile), to spread faecal material evenly.

The slide was carefully removed by gently sliding it sideways to avoid separating the cellophane strip or lifting it off. The slide is then placed on the bench with the cellophane upwards.

After 30 minutes incubation at room temperature (allowing water to evaporate and glycerol to clear the faecal material), the Kato thick smear is microscopically examined, with the X10 objective, in a systematic manner so that the entire coverslip area is observed, and the number of eggs of each species is reported. Having used the WHO recommended 41.7 mg template, the number of eggs per gram (epg) of faeces was obtained by multiplying the number of eggs found by a factor of 24.

To increase the visibility of the parasite eggs, the cellophane was soaked in a 3% methylene blue, glycerol/water solution for 24 h. The intensity of infection was expressed as eggs per gram (epg) of faeces. The intensity of *S. mansoni* infection was classified into three groups: light (1–99), moderate (100–399) and heavy (400 eggs and over per gram of faeces) (WHO, 1998). Quality

control was done by an independent Biomedical Scientist on a 10% random selection of all slides (both positive and negative) collected.

3.11.2 Formol-ether concentration method

All stool samples that were negative for ova by the kato katz method were also processed by the formalin-ether concentration method. The test was duplicated. This was to ensure that the samples were truly negative samples. The formol-ether concentration procedure as described by Ritchie (1948), and Allen and Ridley (1970) provide the best diagnostic outcome in epidemiological studies (Akujobi, 2005). The technique requires the use of formalin as a fixative and ether (Allen and Ridley, 1970) or ethyl acetate (Young *et al*., 1979) or gasoline (WHO, 1991; Wirkom *et al*., 2007) or hemo-de as a lipid removing agent. It uses formalin to fix and preserve the faecal specimen and ether or ethyl acetate to extract debris and fat from the faeces, leaving the parasites at the bottom of the suspension (Akujobi *et al*., 2005; Allen and Ridley, 1970). Authors consider the formol-ether concentration as the most effective technique that recovers the broadest range of organisms, and hence, the "gold standard" method (Wiebe *et al*., 1999) of all parasitological techniques (Melvin and Brooke, 1985; Garcia, 1999, 2001; Cheesbrough, 2005; Markell and Voge, 1976).

With a wooden applicator stick, 1 gram of stool specimen was added to 10 ml of 10% formalin in a small beaker and thoroughly emulsified, and brought into suspension.

The suspension was strained through a double layer of wet gauze directly into a 15 ml screw cupped centrifuge tube. The gauze was discarded, and more 10% formalin added to the suspension in the tube to bring the total volume to 10 ml.

3 ml of ether was added to the suspension in the 15m tube, cupped tightly and shaked vigorously for 10 seconds. The mixture was then spun at 1200× g for 5 minutes.

With an applicator stick the plug of debris was loosened by a spiral movement and the supernatant (comprising the top 3 layers) was decanted, in a single movement, into a bowl containing disinfectant; allowing the last few drops of residual fluid to flow back onto the sediment.

The sediment was re-suspended with a disposable Pasteur pipette. Sometimes it was necessary to add a drop of physiological saline to have sufficient fluid to re-suspend the sediment. A few drops of the suspension was transferred onto a microscope slide and covered with a coverslip.

The preparation was scanned using the low power (X10) objective, and in a systematic manner as to observe the entire coverslip area. If an organism or suspicious objects are seen, the higher magnification of X 40 objective was used to observe its detailed morphology.

3.12. The ELISA procedure

The non-competitive heterogeneous ELISA method was used to detect eosinophil cationic protein levels in individual serum samples.

3.12.1. ECP level measurements (ELISA)

Levels of ECP in serum were measured by enzyme-linked immunosorbent assay (ELISA) technique using MESACUP ECP TEST KIT (MBL CO., LTD.) and following the manufacturer's instructions. The assay is based on a polyclonal sandwich-type ELISA with a biotin-avidin-peroxidase amplification step. This ELISA detects human ECP with a minimum detection limit of 0.125 ng/ ml and does not cross-react with Eosinophil Derived Neurotoxin (EDN). The wells are pre-coated with anti-human ECP monoclonal antibody.

A volume of 100 µl of pre-diluted sample (1:5), standard, positive and negative controls are added to the antibody coated microwell plate and incubated at room temperature (20-25ºc) for 60 minutes. The microwell plate was washed four times and the wash solution was decanted. 100µl of conjugate was added. The plates were then incubated at room temperature for 60

minutes. After incubation, a second washing (4×) was done and 100 µl of substrate added after wash solution has been decanted. This was then incubated for 10 minutes at room temperature. The reaction was stopped by adding 100 µl of stopping solution. The absorbance of each well was read at 450nm in an ELISA plate reader. Standard curves were drawn and these were used to calculate sample ECP concentrations.

3.12.2. Performance characteristics of ELISA kit

Serum sample from healthy blood donors were assayed by the MESACUP ECP TEST. 148 healthy donor sera were measured. After removing those samples measuring over the mean + 3SD, the new mean + 3SD was determined to be: mean + 3SD = 15.6 ng /ml

Sample n =148 mean = 4.06 ng/ ml, SD =3.86; mean + 3SD =15.6 ng /ml

As with other diagnostic test procedures, the results obtained with the MESACUP ECP TEST serve only as an aid in the diagnosis and should not be interpreted as diagnostics in themselves.

When measuring control samples of known concentrations, each value shall be within ±20% of known concentration (specificity).

When measuring 3 control samples of known concentrations eightfold simultaneously, CV% of each sample shall be within 15%.

Bilirubin F (up to 18.3 mg/ dl), Bilirubin C (up to 19.0 mg/ dl), Chyle (up to 1,390 units as Formazine) and /or Rheumatoid factor (up to 500 IU/ ml) are not affective on the assay result, but avoid using highly lipemic samples. Hemoglobin does affect the assay results

Hemolysed samples should not be used. No effect was found to assay values in adding up to 500 ng/ mL of EDN.

The assay range of this kit is from 0.125 ng/ ml to 40 ng/ ml.

3.13. Data Analysis

Data was entered into Microsoft Excel and the SPSS version 16.0. Descriptive analyses for the study were mainly done using the Microsoft Excel software. Frequencies distribution was the main tool for descriptive statistics The SPSS version 16.0 software, 2 x 2 cross tabulation tool, chi square and the Pearson Correlation Coefficient were used for inferential statistics with a set Confidence Interval of 95%.

CHAPTER FOUR

RESULTS OF STUDY

4.1 Characteristics of the participants

The demographic characteristics of study participants included the age and sex. Results obtained are presented in Table 1. The mean age of the participants was 19.2± 1.049 with a range of 6-76 years.

Table 1: Demographic characteristics of participants at pre-treatment

	Characteristics	Number Involved	Percentages
Ages	6-10 years	51	23.5
	11-15 years	103	47.5
	16-25 years	16	7.4
	26-35 years	20	9.2
	36-45 years	10	4.6
	46-55 years	4	1.8
	Above 55 years	13	6.0
	Total	217	100.0
Sex	Male	106	48.8
	Female	111	51.2
	Total	217	100

4.2 Results of Parasitological screening at pre-treatment

Overall schistosomes infection status of the study participants are as presented in Table 2.

In the pre-treatment survey, 18.9% of the study population were infected with *S. haematobium* with geometric mean egg counts of 99.1 egg/ 10ml (EPC), 50 more than the WHO threshold for heavy intensity infections. *Schistosoma mansoni* had a prevalence of

15.7% with a mean geometric egg count of 102.0 egg/ gram (EPG).

Table 2: General infection status of respondents by microscopy (Pre-treatment)

Indicator	*Male*	*Female (%)*	*Total (%)*
Mean Age years(SD)	*21.4*	*17.1*	*19.2 (±1.049)*
Prevalence of S. haematobium infection only	*18/217(8.3)*	*23/217(10.6)*	*41/217(18.9%)*
Prevalence of S. mansoni infection only	*16/217(7.4)*	*18/21798.3)*	*34/217(5.7)*
Prevalence of both S. haematobium and S. mansoni	*2/217(1.4)*	*0/217(0.0)*	*2/217(1.4)*
Geometric mean egg density of S. haematobium	*93.1*	*104.0*	*99.1*
Geometric mean egg density of S. mansoni	*96.0*	*108.9*	*102.0*
Geometric mean egg density of S. haematobium & S. mansoni	*24.3*	*0.0*	*24.3*
Prevalence of other helminths	*1/217(0.46)*	1/217(*0.46)*	*2/217(0.92)*
Geometric mean egg density of other helminths	*1200*	*22*	*611*

Figure 12: Prevalence of Schistosomiasis by microscopy as categorized by age groups (pre-treatment)

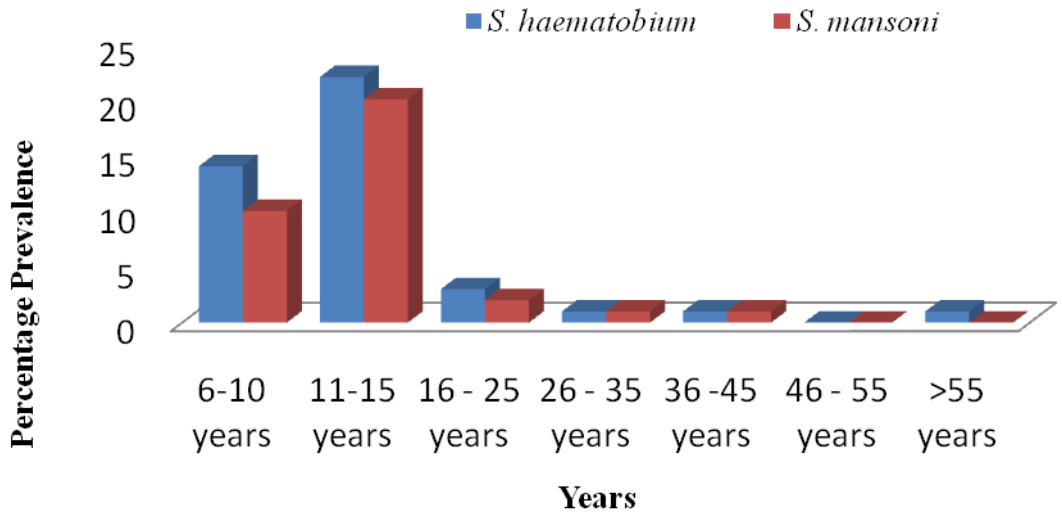

Figure 2 shows the 11 – 15 years as the most infected group in both infections. The 46 – 55 years group are the least infected.

4.2.1 Intensity of infection by microscopy pre-treatment.

The intensity of infection for *S. mansoni* was measured on the basis of egg counts, expressed as eggs per gram (epg) of stool counted by the Kato-Katz method. Intensity for *S. haematobium* was determined by eggs counted in 10 ml of urine by filtration. Infection intensities were classified as light, moderate and heavy in accordance with the criteria by the WHO (2002).

Table 3: Percentage of intensity of schistosomiasis by microscopy at (pre-treatment)

Infection intensity	*S. haematobium (%)*	*S. mansoni (%)*	*Mixed (%)*

Light infection	*31/217(14.1)*	*22/217(10.1)*	*3/217(1.4)*
Moderate infection	*2/217(1.2)*	*11/217(5.1)*	*0/217(0.0)*
Heavy infection	*8/217(3.6)*	*1/217(0.5)*	*0/217(0.0)*
Total	***18.9***	***15.7***	***0.0***

Table 4: Sex distribution of intensity of urinary schistosomiasis by microscopy at (Pretreatment)

	Male (%)	**Female (%)**	**Total (%)**
Prevalence	18/217(8.3)	23/217(10.6)	41/217(18.9)
Light intensity	13/217(5.9)	18/217(8.2)	31/217(14.1)
Moderate intensity	1/217(0.9)	1/217(0.3)	2/217(1.2)
Heavy infection	4/217(1.8)	4/217(1.8)	8/217(3.6)

Table 5: Sex distribution of intensity of intestinal schistosomiasis by microscopy at (Pre-treatment)

	Male (%)	**Female (%)**	**Total (%)**
Prevalence	16/217(7.4)	18/217(8.3)	34/217(15.7)
Light intensity	12/217(5.5)	10/217(4.6)	22/217(10.1)
Moderate intensity	4/217(1.9)	7/217(3.2)	11/217(5.1)

Heavy infection	0/217(0.0)	1/217(0.5)	1/217(0.5)

4.2.2 General infection status of respondents by microscopy at (Post-treatment).

Table 6 shows the general infection status of respondents by microscopy (Post-treatment)

Indicator	***Male (%)***	***Female (%)***	***Total (%)***
Mean Age years(SD)	*12.7*	*11.2*	*11.8(4.29)*
Prevalence of S. haematobium	*1/95(2.1)*	*1/95(2.1)*	*4/95(4.2)*
Prevalence of S. mansoni	*2/95(2.1)*	*0/95(0.0)*	*2/95(2.1)*
Prevalence of S. haematobium & S. mansoni	*1/95(1.1)*	*0/95(0.0)*	*1/95(1.1)*
Geometric mean egg density of S. haematobium	*84*	*30*	*57*
Geometric mean egg density of S. mansoni	*12.5*	*0.0*	*12.5*
Geometric mean egg density of S. haematobium & S.mansoni	*48.5*	*0.0*	*48.5*
Prevalence of other helminths	*1/95(1.0)*	*3/95(3.2)*	*4/95(4.2)*
Geometric mean egg density of other helminths	*2.0*	*12.0*	*9.5*

Table 7: Percentage of intensity of schistosomiasis by microscopy (post-treatment)

Infection intensity	***S. haematobium (%)***	***S. mansoni (%)***	***Mixed (%)***
Light infection	*2/95(2.1)*	*2/95(2.1)*	*0/95(0.0)*
Moderate infection	*2/95(2.1)*	*0/95(0.0)*	*1/95(1.1)*
Heavy infection	*0/95(0.0)*	*0/95(0.0)*	*0/95(0.0)*
Total	*4/95(4.2)*	*2/95(2.1)*	*1/95(1.1)*

4.3 Eosinophil Cationic Protein Levels in Participants

Table 8: ECP levels of persons infected with urinary schistosomiasis pre and post treatment

		***S. haematobium* Pre-treatment**		***S. haematobium* Post-treatment**	
		Positive	Negative	Positive	Negative
ECP RESULTS	Positive	37	25	2	32
	Negative	3	6	0	2
	Unknown	1	7	2	6
Total		**41**	**38**	**4**	**40**

Table 9: ECP levels of persons infected with intestinal schistosomiasis pre and post treatment

		S. mansoni Pre-treatment		***S. mansoni Post-treatment***	
		Positive	*Negative*	*Positive*	*Negative*
ECP RESULTS	*Positive*	*24*	*38*	*1*	*33*
	Negative	*4*	*5*	*0*	*2*
	Unknown	*0*	*8*	*0*	*8*
	Total	***28***	***51***	***1***	***43***

ECP levels in the pre and post sera of both infected and non infected participants were assessed. Most participants who were egg negative had their ECP levels higher than normal levels in both infections.

4.3.1 Comparison of Schistosomiasis intensity by microscopy and ECP levels

Table 10: Intensity of schistosomiasis infection as measured by microscopy and ECP levels in sera

	Microscopy(mean egg count)		***ECP Levels(mean ECP level)***	
	S. haematobium (egg/10mls)	*S. mansoni (epg)*	*S. haematobium (ng/ml)*	*S. mansoni (ng/ml)*
Light intensity	*15.67*	*27.77*	*77.62*	*78.19*
Moderate intensity	*70.4*	*163.2*	*77.27*	*104.32*
Heavy intensity	*502.86*	*480*	*90.06*	*94.000*

The intensity levels of schistosomiasis were assessed by using microscopy and ECP levels in the sera of participants. The results obtained indicate that there was a considerable comparison between the microscopic readings and the ECP levels. There was a significant association {p= 0.01(CI: 95%, α: 0.05)} between the estimation of intensity measured by microscopy and ECP measurement. There was a positive by rather weak correlation (r = 0.19) between intensity by egg count and ECP levels. There was no significant difference (p= 0.39) in ECP levels between patients infected with *S. mansoni* and *S. haematobium*. There was also a significant drop in the ECP levels (p= 0.006) in post treatment samples of schistosomiasis patients as compared to pre-treatment Levels.

4.4 Economic and Educational Status of Participants

The economic activities of the respondents were obtained through questionnaire to know persons who were employed and not employed. Table 11 gives an illustration of this. A total of 77% (167/217) of the respondents were unemployed and 21.7% (47/217) were employed. However, further probing of the nature of their employment showed that their employment was not water-related.

Table 11: Occupational Status of Respondents

Response	Number Involved	Percent (%)
Employed but not water related	47	21.7
Not employed	167	77.0
Unknown	3	1.4
Total	217	100.0

4.4.1 Education Level

Respondents were asked about their education to find out those who were currently in school. Majority of the respondents (168/217) representing 77.4% were schooling. However the educational status of 35/217 representing 16.1% of the respondents could not be determined.

Table 12: Educational status of respondents (Currently Schooling)

	Number of participants	**Percent**
Yes	168	77.4
No	14	6.5
Unknown	35	16.1

Total	217	100.0

This was done to obtain the class distribution of the pupils. All the pupils were in the primary school and were in classes two to six. A graphical representation of this is in Fig 14.

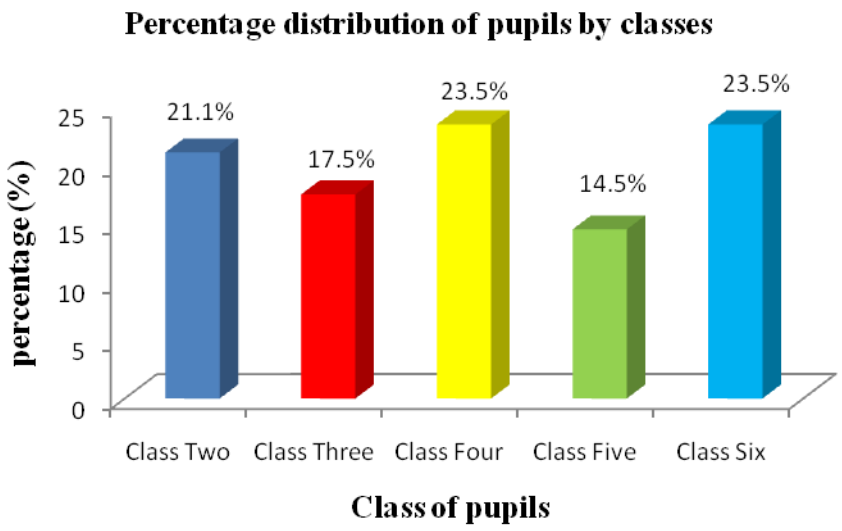

Figure 13: Percentage distribution of pupils by class

4.5 Knowledge and Practices

4.5.1 Knowledge on Schistosomiasis

Knowledge of schistosomiasis is one of the critical factors for the control of the disease. Results from this study (as indicated in Table 13) shows that most of the respondents, 203/217 representing 93.5% had knowledge of schistosomiasis as a disease, with only a few, 6.5 %(14/217) not knowing what it was.

Table 13: Knowledge of the disease by the respondents

	Frequency	Percent
Yes	203	93.5
No	14	6.5

Total	217	100.0

4.5

.2 General belief of causes of schistosomiasis

Table 14: Respondents perception about Schistosomiasis

	Frequency	Percent (%)
Sign of productivity	3	1.4
A disease	201	92.6
Have no idea	1	.5
Other	1	.5
Did not answer	11	5.1
Total	217	100.0

4.5.3 Knowledge of symptoms of Schistosomiasis

Respondents were asked to associate four (4) clinical indications to schistosomiasis as part of assessing their knowledge of the disease. Results obtained are represented in Table 15 which shows that 12/217 representing 5.5% could not associate any of the symptoms to the disease. It is interesting to note that 93.5% (203/217) could associate passing of bloody urine and painful urination to schistosomiasis but only 1% (2/217) could associate swollen stomach and passing of bloody stool to schistosomiasis.

Table 15: Association of symptoms to Schistosomiasis

	Frequency	Percent (%)
Swollen stomach	1	0.5
Passing bloody urine	137	63.1
Feeling pains	66	30.4
Passing bloody stool	1	0.5

4.5

No idea	12	5.5
Total	217	100.0

.4 Visitation to water contact sites

Contact with open fresh water source which harbours the intermediate hosts and cercariae is the way by which schistosomiasis can be transmitted. Table: 7 represent respondents who had water contact. Almost all 215/217 representing 99.1% of respondents come into contact with a water body source.

Table 16: Water contact by respondents

Response	Frequency	Percent (%)
Yes	215	99.1
No	2	0.9
Total	217	100.0

4.5.5 Rate of visitation to water contact sites

The rate of visitation to the water site was also recorded since a major predisposing factor to schistosomiasis infection is the access to infected water bodies. The results obtained are indicated in Table 17 below.

Table 17: Frequency of visit by participants to water contact site

Frequency	No. of participants	Percent (%)
Daily	101	46.5
One to three times a week	88	40.6
Four to seven times a month	11	5.1
Once a month	14	6.5

4.5

Once a year	1	0.5
No idea	2	0.9
Total	217	100.0

.6 Activities at water contact sites

Table: 18 show the main activity that respondents go to do at water contact sites. 50.8 %(121/217) use the water for recreation. 10.1 %(22/217) go there fishing whiles 30.0 %(65/217) go to fetch water.

Table 18: Water contact by participants due to economic activities

Factor/ Activity	No. of participants	Percent (%)
Swimming and Wading	121	55.8
Fetch water	65	30.0
Fishing	22	10.1
Sand winning	6	2.8
Other activities	1	.5
No idea	2	.9
Total	217	100.0

4.6 General Health status of respondents

Symptoms play important parts in the diagnosis of diseases. Certain symptoms are associated with schistosomiasis. Table 19 represent responses to whether respondents have ever had any of 3 symptoms of schistosomiasis. 71.9 %(156/217) had had blood in urine, 71.0 %(154/217) had had painful urination and 49.3 %(107/217) had ever passed blood in stool.

Table 19: Knowledge of symptoms of Schistosomiasis

Factor	Response	Number Involved	Percent
Have you ever passed blood in your urine	Yes	156	71.9
	No	57	26.3
	Don't know	4	2.1
	Total	217	100.0
Do you feel pains when you were urinating	Yes	154	71.0
	No	38	17.5
	No idea	25	11.5
	Total	217	100.0
Have you ever passed blood in your stool	Yes	107	49.3
	No	99	45.6
	No Idea	11	5.1
	Total	217	100.0

4.7 Mass Drug Administration Participation

Because of the endemicity of schistosomiasis, children of school going age in the study site received Mass praziquantel treatment in 2011. Respondents were asked whether they have had praziquantel treatment within the last three months.

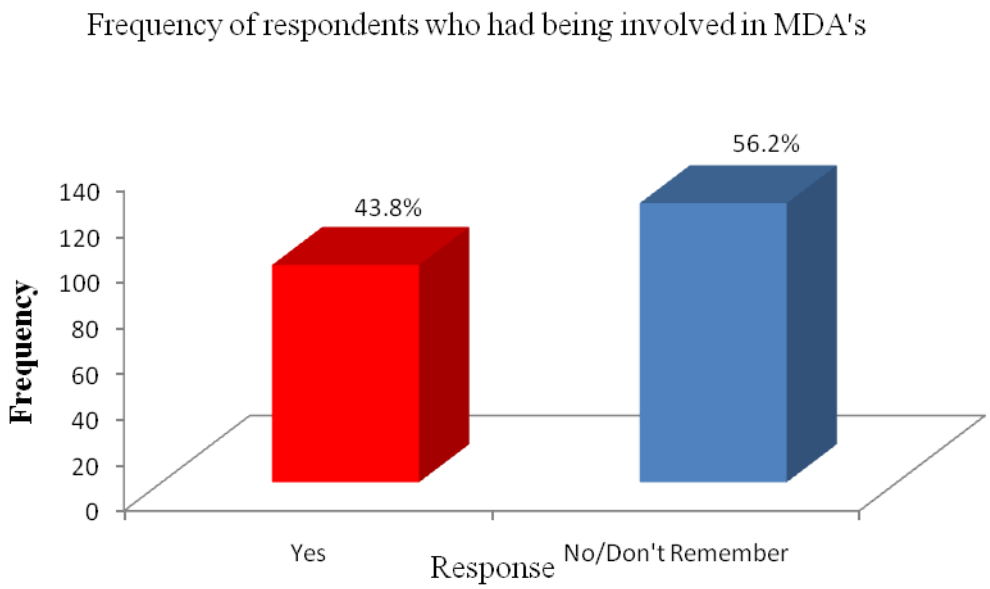

Figure 14: A graph showing percentage of participants who had taken MDA in the past 3 months prior to pre-treatment sample collection

The study area is one of the sites for the national schistosomiasis MDA program. It is very important to know respondents who received treatment for the past 12 months. From figure 15 it is shown that 56.2% had not received praziquantel in the past 12 months.

4.7.1 Health seeking behaviour of persons who reported symptoms of schistosomiasis

Morbidity control of schistosomiasis through integration within existing health care delivery systems is considered a potentially sustainable and cost-effective approach. The study tried to found out whether infected individuals self-reported to health centres or clinics.

Table 20: Actions taken by respondents having symptoms of Schistosomiasis

	Frequency	Percentage
Visited hospital/health centre	52	28.3
Administered Herbal Medicine	14	7.6
Both Herbal medicine and orthodox medicine	15	8.2
Received Praziquantel from MDA programmes	10	5.4
Did nothing	93	50.5
Total	184	100.0

4.8 History of Malaria prophylaxis

Anti-malaria drugs especially the arthemisinin combination therapy is known to have antischistosome activity. Table 21 represents who have received anti-malaria in the past 6 months. 83.9% of respondents have not received anti-malaria for the past 6 months.

Table 21: Use of anti-malaria by participants in the past 6 months proir to sampling

	Response	Frequency	Percent
When was the last time you had malaria treatment	Less than 2 weeks	7	3.2
	2 weeks-1 month	16	7.4
	3 months-6months	12	5.5
	More than 6 months	61	28.1
	Don't Remember	121	55.8
	Total	**217**	**100.0**
Type of Anti-Malaria Taken	Arthemisinin Combination Therapy	4	1.8
	Don't Know	212	97.7
	Paracetamol	1	.5
	Total	**217**	**100.0**

CHAPTER 5

5.1 Discussion

Schistosomiasis or bilharzias is caused by infection with *Schistosoma sp*, is one of the major neglected tropical diseases (NTD) (Landoure *et al*., 2012). It remains a major cause of morbidity in developing countries, especially in sub-Saharan Africa (Landoure *et al*., 2012).

The endemicity of schistosomiasis in the Upper East region has been an old problem (Amankwa *et al*., 2003). Between 1959 and 1961 an exhaustive epidemiological survey conducted in the then Upper Region found prevalence of *S. haematobium* infection to be more than 30% in the

eastern part, now Upper East region (Amankwa *et al*., 2003). Bongo and Chuchuliga had prevalence more than 70% for *S. haematobium* (OMS – WHO Report 1987).

A Schistosomiasis survey conducted in the Kassena Nankana district of the Upper East region gave prevalences of 68.7% and 67.7% respectively for *S. mansoni* and *S. haematobium* infections (Amankwa et al., 1994).

A study supported by IFAD in 1995 (unpublished) showed a mean prevalence of 33.7% and 9.8% for *S. haematobium* and *S. mansoni* respectively (MOH/ IFAD Report 1995).

A study conducted in the Upper East region in 2003 by the Ghana Health Service and the Ghana Education Service gave prevalences of 63.6% and 65.2% respectively for *S. haematobium* and *S. mansoni* in Gowrie in the Vea dam area (Amankwa *et al*., 2003).

The mainstay of current control strategy recommended by the World Health Organisation (WHO) against schistosomiasis is preventive chemotherapy (PCT) with praziquantel (PZQ) (WHO 2002).

It is against this background that the Ghana Health Service started the implementation of PCT against schistosomiasis targeting school age children in 2011 in three (3) districts, Kassena Nankana, Kassena Nankana West and Bongo in the Upper East region. These exercise recorded 93.7% coverage in 2011 and 85.3% in 2012 in the Bongo District (Disease Control Unit, GHS, Upper East).

This study was conducted to evaluate the impact of the MDA exercise in the Vea dam area and also access the possibility of using serum ECP levels to measure the intensity of schistosomiasis.

The distribution of respondents by sex was 46.8% males and 51.2% females, exactly the same as the national distribution (Ghana Statistical Service, 2012). Most of the respondents (66.9%) were between the ages of 6-15 years with those above 55 years accounting for 5.8%.

Again, this is identical to the national distribution (Ghana Statistical Service, 2012).

The overall prevalence for *S. haematobium* was 18.9% and that for *S. mansoni* recorded 15.7%. Mixed infection for *S. haematobium* and *S. mansoni* was1.4%. Out of 30 people who had haematuria, 24 had *S. haematobium* eggs in their urine. 41 people had proteinuria.

This prevalence is a significant reduction to the study in 2003 by the GHS and GES in Gowrie. This is consistent with the work done by Kabore *et al*., 2013, which puts the prevalence in the area to be between 10 – 50%.

Although, there was no significant difference in infection status between females and males, females were more infected with *S. haematobium* 10.6% and *S. mansoni* 8.3% than males 8.3% and 7.4% respectively. This is quite similar to some studies where girls were more infected with Schistosomiasis than boys (McCullough et al., 1965). This difference in infection status can be the result of different water usage. This can be explained by the fact that 30% of respondents go to water contact site to fetch water. In such communities it is the girls who usually fetch water to the households and washing of clothes along the banks of the dam.

Age dependent pattern of prevalence of schistosomiasis is highest among children of school going age with age range of 5 – 15 years old (Guyatt *et al*., 1999). This is comparable to this study where the age range 6 – 15 years accounted for 16.4% of the 18.9% prevalence of *S. haematobium* and 13.8% of the 15.7% prevalence of *S. mansoni*. The age range 11 – 15 years recorded the highest prevalence of 10.0% and 9.2% of *S. haematobium* and *S. mansoni* respectively. This is similar to the study by the GHS and the GES in 2003 in Gowrie.

In spite of the high prevalence of schistosomiasis among the 11 – 15 year age group mean intensity for *S. haematobium* was rather high among the 6 – 10 year age group (191.4

eggs/10mls of urine) than the 11 – 15 years age group (63.5 eggs/10 mls of urine). For *S. mansoni* intensity was 103.5 epg among the 11 – 15 years age group.

Intensity of infections was high in very few individuals and very low in many of the infected people. Worm burden is neither uniformly nor randomly distributed amongst individuals but is highly overdispersed (Bundy et al., 1998). Very few individuals normally shed large amounts of eggs and therefore contamination of the environment is by a few in the community.

Prevalence of *S. haematobium* and *S. mansoni* eight (8) weeks after treatment with praziquantel was 5.1% and 1.1% respectively. This is a significant reduction of prevalence after two rounds of MDA. Intensity for *S. haematobium* had also reduced to 55.5 eggs/ 10mls of urine and 12.5 epg for *S. mansoni*.

Parasitic infections caused by *Schistosoma haematobium* (the aetiological agent of urinary schistosomiasis) and hookworm (a soil-transmitted helminth; STH) are widely endemic among human populations in sub-Saharan Africa (SSA) (Hotez *et al*., 2007; Hotez, 2008).

Morbidity, including iron-deficiency anaemia, reduced growth and impaired cognition, is exacerbated by multiple species infections (co-infection).

Infection with other helminths was rather low (0.92%) than the situation previously in a larger study in northern Ghana (Yelfari *et al*., 1999). Only two people were found to be infected with *H. nana* in the pre survey. Two individuals each were found to be infected with Hookworm and *H. nana* respectively in their post treatment samples. This may be due to the mass use of ivermectin and albendazole in the Lymphatic filariasis control programme and the indiscreminate use of antihelminthes

In this study, the level of ECP in sera of schistosomiasis patients as measured by ELISA was significantly higher than normal levels (82.9 vs 15.6 ng/ ml). This is similar to a study in Egypt by Hassan et al., 2002.

Unlike, in the study by Hassan et al., 2002, where ECP levels in S. haematobium infected people was significantly higher than S. mansoni infected people, there was no significant difference (p= 0.39) in ECP levels between patients infected with *S. mansoni* and *S. haematobium*.

There was a significant association {p= 0.01(CI: 95%, α: 0.05)} between the estimation of intensity measured by microscopy and ECP measurement. There was also a significant drop in the ECP levels (p= 0.006) in post treatment samples of schistosomiasis patients as compared to pre-treatment Levels. This indicates that there is a level of association between ECP levels and infection status

ECP levels however did not show a consistent increase with increase in intensity by egg count(r= 0.19).

The knowledge of respondents on schistosomiasis was quite high. This is similar to other study areas (Sow *et al*., 2003: Mekheima and Talaat, 2005). Respondents could however not associate some of the symptoms to the disease, for example passing of bloody stools to *S. mansoni*. This corroborates previous findings from Tanzania (Guyatt *et al*., 1999; Mwanga *et al.*, 2004).

Almost all respondents have water contact with about 90% getting contact at least three (3) times a week. Over 70% had or had ever had symptoms of urinary schistosomiasis as compared to 49% of intestinal schistosomiasis. Surprisingly, over 50% of these people responded they did nothing to get treatment.

5.2 Conclusion

MDA with PZQ has been conducted in Vea since 2010 and two rounds of treatment have been delivered. This study showed significant reduction in intensity of infection on both infections. Most importantly, proportion of moderate and heavy infections was reduced in school-age children.

Serum ECP levels of infected individuals are very high compared to normal levels. ECP levels were significantly associated with the estimation of intensity measured by microscopy. ECP levels showed a positive but rather weak correlation with intensity by microscopy.

This study showed that ECP could be a good biomarker for estimating the intensity of schistosomiasis in both pre and post praziquantel treatments.

5.3 Limitations

1. Only one urine and stool samples were collected from each respondent. This decreases the chances of detecting ova especially those with low intensity of infection.
2. A lot of respondents (56.7%) were lost to follow-up
3. ECP is not only involved in defence against parasites but also in the tissue damage seen in subjects with allergic and inflammatory disease as such other confounders could be responsible for ECP titre values.

5.4 Recommendation

1. There is the need to incorporate practical public health education in the school curriculum for the purpose of increasing knowledge and promoting behavioural changes in school children to improve disease control.
2. MDA exercise should be sustained and intensified to cover almost all areas in the district.
3. Praziquantel should always be available at the health center so that anybody who present with symptoms of schistosomiasis could be treated.
4. New diagnostic tools that are more sensitive than microscopy should be developed and used as point of care diagnostic means in effectively diagnosing schistosomiasis and evealuating the intensity of the infection
5. Many school going children should be recruited for the MDA programme.

REFERENCES

Aagard-Hansen J, Mwanga JR, Bruun B (2009). Social Science Perspectives on Schistosomiasis Control in Africa; past trends and future directions. Parasitology 136: 1743-1758.

Abdel-Wahab MF, Ramzy I, Esmat G, el Kafass H, and Strickland GT (1992b). Ultrasound for detecting *Schistosoma haematobium* urinary tract complications: Comparison with radiograhic procedures. *J. urol* 148:346-50.

Acosta LP, McManus DP, Aligui GD, Olveda RM, Tui WU (2004). Antigen-specific antibody isotope patterns to *Schistosoma japonicum* recombinant and native antigens in a defined population in Leyte, the Phillipines. Am J Trop Med Hyg 70: 549-55

Ahmadi NA, Gachkar L, Pakdad K, Ahmadi O (2007). Potency of wet mount, formalinacetone and formalin-ether methods in detection of intestinal parasitic infections. Iranian J Infect Dis Trop Med 12:43-47.

Akujobi CN, Iregbu KC and Odugbemi TO (2005). Comparative evaluation of direct stool smear and formol-ether concentration methods in the identification of *Cryptosporidium* species. Nigerian J Heal Biomed Sc. 4(1): 5-7.

Allen AVH and Ridley OS (1970). Further observations on the formol-ether concentration technique for faecal parasites. J. Clin. Path. 23: 343-352.

Amankwa JA, Bloch P, Meyer-Lassen J, Olsen A, Christensen NO (1994). Urinary and intestinal schistomiasis in the Tono irrigation Scheme, Kassena/Nankana District, Upper East Region Ghana. Trop. Med. Parasitol. 45: 319-323

Amankwa JA, Agana PN, Yelfari L, Samari S (2003). Report on prevalence studies on Schistosomiasis and Soil Transmitted Helminths in the Upper East Region. pp 15 - 18 Anderson RM (1987). Determinants of infection in human schistosomiasis. Bailli`eres Clin.

Trop. Med. Comm. Dis. 2: 279—300.

Ambrosio RE and de Waal DT (1990). Diagnosis of parasitic disease. *Rev Sci Tech* 9: 759778.

Arnon R (1990). Life span of parasite in Schistosomiasis patients. Israelian j Med Sc. 26: 40405

Asalou SO, Ofozie IE (2003). The role of health education and sanitation in the control of helminth infections. Acta Tropica 86: 283-294.

Ash LR, Orihel TC (1991). Parasites: A Guide to Laboratory Procedures and Identification. Chicago: Am Soc of Clin Pathol.

Augusto G, Magnussen P, Kristensen TK, Appleton CC, Vennervald BJ (2009).The influence of transmission season on parasitological cure rates and intensity of infection after praziquantel treatment of Schistosoma haematobium infected schoolchildren in Mozambique. Parasitology136: 1771-1779.

Barakat R, el Masry AG, Farghaly A, el Morshidy HN, el Sayed, MK., Husein MH, Miller FD (1995). Impact of population-based selective chemotherapy on prevalence and intensity of *schistosoma mansoni* infections in the Nile Delta: Kafr El Sheikh. Tropical and Geographical Medicine 47: 266-270.

Bartram J, Cairncross S, (2010). Hygiene, Sanitation and water: forgotten foundations of health. PLoS Medicine 7: e1000367.

Beaver PC, Jung RC, Cupp EW (1984). Examination of specimens for parasites, Clinical parasitology, 9th ed. Lea & Febiger Philadelphia pp 432.

Berquist R, (2001). Strategies for control of infection and diseases: Current Practice and future potential. In: Mahmoud AAF (Ed). Schistosomiasis, Imperial College Press, London, pp, 413-469.

Bogoch II, Raso G, N'Goran EK, Marti HP, Utzinger J (2006). Differences in microscopic diagnosis of helminths and intestinal protozoa among diagnostic centres. Eur J Clin Microbiol Infect Dis 25: 344–347.

Bongo District Assembly (2013). Department of Town and Country Planning, Bongo Upper East Region.

Booth M, Vounatsou P, N'Goran EK, Tanner M and Utzinger J (2003). The influence of sampling effort and the performance of the Kato–Katz technique in diagnosing *Schistosoma mansoni* and hookworm co-infections in rural Côte d'Ivoire, Parasitology 127: 525–531.

Brooker S and Michael E (2000). The potential of geographical information systems and remote sensing in the epidemiology and control of human helminth infections. Adv. Parasitol. 47: 245—288

Brooker S (2002). Schistosomes, snails and satellites. Acta Trop. 82: 209—216.

Bundy DAP, Blumenthal U (1990). Human behaviour and the epidemiology of helminth infection, in: Barnard C, Behnke JM (Eds). Human behaviour and the epidemiology of helminths infection. Parasitism and Host Behaviour. Taylor and Francis, London, pp. 264—289.

Bundy DA, Silva NR(1998). Can we deworm this wormy world? British Medical Bulletin 54 (2) : 421 – 432.

Burki A, Tanner M, Burnier E, Schweizer W, Meudt R, and Dergremont A (1986) Comparison of ultrasonography, intravenous pyelography and cystocopy in detection of urinary tract lesions due to *S. haematobium.* Acta Tropica, 43: 139-51.

Catto I (1905). *Schistosoma cattoi*, a new blood fluke of man. BMJ 1:11-13.

Char S, Farthing MJG (1991). DNA probes for diagnosis of intestinal infection. Gut. 32

(1):1-3

Cheesbrough M. (1998). District Laboratory Practice in Tropical Countries, Part 1, 216: 236-239.

Cheesbrough M (2005) ed., Parasitological tests, in: District Laboratory Practice in Tropical Countries, part 1, Tropical Health Technologies, Cambridge pp. 178–306.

Cheever AW, Kamel IA, Elwi AM, Mosimann JE, Danner R and Sippel JE (1978) Schistosoma mansoni and S. haematobium infections in Egypt. III. Extrahepatic pathology. *Am J Trop Med Hyg* 27, 55-75.

Chen MG and Mott KE (1989). Progress in assessment of morbidity due to *S. haematobium.* A review of recent literature. Tropical Disease Bulletin 86: R1-R36.

Chitsulo L, Engels D, Montessor A, Savioli L (2000). The global status of schistosomiasis control and its control. Acta Trop. 77:41-51.

Contis D, David AR (1996). The epidemiology of bilharzias in ancient Egypt: 5000 years of schistosomiasis. Parasitol Today. 12:256-55

Cox FEG (1998). History of human parasitology, pp. 3-18. Cox FEG, Kreier JP and Wakelin D. (ed.), Topley and Wilson's microbiology and microbial infections, 9th ed., vol. 5. Parasitology. Arnold, London, U.K.

Curtale D, Mohammed MY, Youssef ZN (2010). Comprehensive primary healthcare, a viable strategy for the elimination of schistosomiasis. Trans R Soc of Trop Med & Hyg 104: 70-72.

de Clercq D, Sacko M, Vercruysse J, Diarra A, Landoure A, van den Bussche V, Gryssels B, Deelder A, (1995). Comparison of the circulating anodic antigen detection assay and

urine filtration to diagnose Schistosoma haematobium infections in Mali. Trans R Soc Trop Med & Hyg 89: 395–397.

Degremont A, Burki A, Burnier E, Schweizer W, Meudt R and Tanner M (1985). Value of ultrasonography in investigating morbidity due to *S. haematobium* infection. The Lancet, 1: 662-5

District Directorate of Health Services, Bongo Upper East Region.

Doehring E, Ehrich JH, Vester U, Feldmeier H, Poggensee U and Brodehl J (1994). Proteinuria, haematuria and leukocyturia in Children with mixed urinary and intestinal schistosomiasis. Kidney Int.

Doehring-Schwerdtfeger E, Kardoff R (1995). Ultrasonography in schistosomiasis in Africa. Mem Inst Oswaldo Cruz 90: 141-145.

Doenhoff MJ, Hagan P, Cioli D, Southgate V, Pica-Mattocia I, Botros S, Coles G, Tchuem Tuente LA, Mbaye A, Engels D (2009). Praziquantel: Its use in control of schistosomiasis in sub-Saharan Africa and current research needs. Parasitology 136: 1825-1835.

Ebrahim A, El-Morshedy H, Omer E, El-Daly S, Barakat R (1997). Evaluation of the Kato Katz thick smear and formol ether sedimentation techniques for quantitative diagnosis of *Schistosoma mansoni* infection. Am J Trop Med & Hyg 57: 706–708.

Engels D, Nahimana S, Gryseels B (1996). Comparison of the direct faecal smear and two thick smear techniques for the diagnosis of intestinal parasitic infections. Trans R Soc Trop Med Hyg 90: 523-25

Engels, D., Chitsulo, L., Montresor, A., & Savioli, L. (2002). The global epidemiological situation of schistosomiasis and new approaches to control and research. Acta tropica, *82*: 139-146

Esrey SA, Potash JB, Roberts L, Shiff C (1991). Effects of improved water Supply and sanitation on ascariasis, diarrhoea, dracunculiasis, hookworm infection, schistosomiasis and trachoma. Bulletin of the World Health Organisation 69:609-621.

Estevez EG, Levine JA (1985). Examination of preserved stool specimens for parasites: lack of value of the direct wet mount. J Clin Microbiol 22: 666–667.

Feldmeier H, Poggensee G (1993). Diagnostic Techniques in Schistosomiasis control. A review. Acta Tropica, 5: 205-20.

Feldmeier H (1996). Diagnosis of female genital schistosomiasis by indirect disease markers: determination of eosinophil cationic protein, neopterin and IgA in vaginal fluid and swab eluates. Acta Trop 62: 269–280.

Fenwick A, Savioli L, Engels D, Bergquist NR. Todd MH, (2003). Drug for the control of parasitic disease: current status and development in schistosomiasis, Trends in Parasitology 19: 509-515.

Fenwick A. and Webster JP. (2006) Schistosomiasis: challenges for control, treatment and drug resistance. *Curr Opin Infect Dis* 19:577-582.

Fenwick A, Rollinson D, Southgate V (2006). Implementation of human schistoso-miasis control: challenges and prospects. Advances in Parasitology 61,567–622

Fenwick A, Webster JP, Bosque-Oliva E, Blair L, Fleming, FM., Zhang Y, Garba A, Stothard, JR, Gabriell AF, Clements ACA, Kabatereine NB, Toure S, Dembele R, Nyandindi N, Nwansa J, Koukounari A, (2009). The Schistosomiasis control initiative (SCI): rationale, development and implementation from 2002-2008, parasitology 136: 1719-1730.

Fuji, I. (1847). Katayama disease. Katayamaki: Chugai Iji Shimpo, 691:55-56.

Fujinami K, Nakamura H (1909). Katayama disease in Hiroshima prefecture: route of infection, development of the worm in the host and animals in katayama disease in Hiroshima prefecture (Japanese blood sucking worm disease-*Schistosoma japonica*). Kyoto lg za 6:224-252.

Fuller GK. Lemma A. Trinidad H (1979). Schistosomiasis in Omo National Park of southwest Ethiopia. Am J Trop Med Hyg 28:467-471.

Garcia LS (1999). Practical Guide to Diagnostic Parasitology. ASM Press, Washington, D.C.

Garcia Santos MMA, Garcia TC, Orsini M, Disch J, Katz N, Rabello A (2000). Oral fluids for the immunodiagnosis of *Schistosoma mansoni* infections. AmJ Trop Med Hyg 94: 289-292.

Garcia LS (2001). Diagnostic Medical Parasitology, 4th ed. ASM Press, Washington, D.C.

Gasser RB (2001). Identification of parasitic nematodes and study of genetic variability using PCR approaches. In: Parasitic Nematodes: Molecular Biology, Biochemistry and Immunology (eds MW Kennedy & W Harnett), CABI Publishing, Oxon and New York, pp. 53–82.

Genta RM (1988). Predictive value of an Enzyme-Linked Immunosorbent Assay (ELISA) for the serodiagnosis of strongyloidiasis. Am J Clin. Pathol. 3:391-4.

Ghana Statistical Service (2012). 2010 population and Housing Census, Ghana Statistical Service, Accra.

Gomes LI, Dos Santos Marques LH, Enk MJ de Oliveira M C, Coelho PM, Rabello A (2010). Development and evaluation of a sensitive PCR-ELISA systems for detection of schistosoma infection in faeces. PloS Neg Trop Dis 4: e664

Goodman D, Haji HJ, Bickle QD, Stoltzfus RJ, Tielsch JM, Ramsan M, Savioli L, and

Albonico M (2007). A comparison of methods for detecting the eggs of *Ascaris*, *Trichuris*, and hookworm in infant stool, and the epidemiology of infection in Zanzibari infants, Am. J. Trop. Med. Hyg. 76: 725–731.

Gordon, C. (2006). The Multi-stakeholder Consultation Process for Dams Development in Ghana. Volta Basin Research Project, University of Ghana, Accra.

Gray DJ, Macmanus DP, Li Y, Williams GM, Bergquist R, Ross AG (2010). Schistosomiasis eliminated: lessons from the past guide the future. Lancet inf Dis 10: 733-736.

Griesinger W (1854). Klinische und anatomische Beobachtungen uber die krankherten von Aegypten. Arch physiol Helik 13:528-575.

Guyatt H, Brooker S, Lwambo NJ, Siza JE, and Bundy DAP (1999). The perfomance of school-based questionnaires of reported blood in urine in diagnosing *S. haematobium* infection: Patterns by age and sex. Trop Med Interl Hea 4:751-7.

Gyapong M, Gyapong JO & Owusu-Banahene G (2001). Community-directed treatment: the way forward to eliminating lymphatic filariasis as a public-health problem in Ghana. Annals of Tropical Medicine and Parasitology 95: 77–86.

Hagan P, El Meleigy M, Traore M (1998). Schistosomiasis research: the end of the beginning. Parasitol Today *14*: 392-394.

Harris AR, Russell RJ and Charters AD (1984). A review of schistosomiasis in immigrants in Western Australia, demonstrating the unusual longevity of *schistosoma mansoni*. Trans R soc Trop Med Hyg. 78: 385-8

Hassan MM, el-Motaim MH, Mattar MA (2002). Assessing the morbidity of schistosomiasis by measuring eosinophil cationic protein in serum. J Egypt Soc Parasitol 32: 517-24.

Hatz C, Jenkins JM, Meudt R, Abdel-Wahab MF and Tanner M (1992). A review of the literature on the use of ultrasonography in Schistosomiasis with special reference to its use in field studies: 1. *S. haematobium.* Acta Tropical, 51: 1-14.

HHS (Health and Human Services, 1993). Biosafety in Microbiological and Biomedical Laboratories, U.S. Department of Health and Human Services publication no. (CDC) 93-8395.

Hodder SL, Mahmoud AA et al., (2000). Predisposition to urinary tract epithelial metaplasia in *schistosoma haematobium* infection. Am J Trop Med Hyg 63:133-8,

Holveck JC, Ehrenberg JP, Ault SK, Rojas R, Vasquez J, Cerqueira MT, Ippolito-Shepherd J, Genovese MA, Periago MR (2007). Prevention, control and elimination of neglected disease in the Americas; pathways to intergrated inter-programmatic, inter-sectoral action for health and development. BMC Public Health 7, 6.

Hotez PJ, Molyneux DH, Fenwick A, Kumaresan J, Sachs SE, Sachs JD, Lorenzo-Savioli(2007). Control of neglected tropical diseases. N Engl J Med 357: 1018–1027.

Hotez P (2008) Hookworm and poverty. Ann N Y Acad Sci 1136: 38–44

http://www.cdc.gov/parasite/about.html Nov 2, 2010, accessed on 23/02/2014. http:// www.cdfound.to.it/HTML/sch1.htm, accessed on 04/12/2012.

http//:www.cru.edu/med/epidbio/mphp439/schistosomiasis.htm, accessed on 10/03/2014.

http://www.fic.nih.gov/dcpp/wps, accessed on 23/07/2012.

http//:www.ghanahealthservice.org/documents/NTD, accessed on 15/11/2012.

http://www. ghanaweb.com/GhanaHomePage/NewsArchive/artikel,php?ID=234554, accessed on 02/03/2014.

http://www.medicalecology.org/water/schistosomiasis/schistosomiasis.htm, accessed on

10/03/2014. http://medicineonthemove,org/index.php/insci/what-is-schistosomiasis, accessed on

02/03/2014.

http://modernghana.com./news/383780/1/vra-holds-national-forum-on-schistosomiasiscontrol.html Revised 15/03/2012.

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1975763/, accessed on 10/03/2014

http//:wwwparasiteandvectors.com/content/7/1/138, accessed on 23/02/2014.

http://www.parasitesinhumans.org/schistosoma-blood-flukes.html, accessed on 10/03/2014.

http://www.path.cam.ac.uk/~schisto/schistosoma/schisto_pathology.html, accessed on 10/04/2014. http://www.who.int/irq/programmes/schistosomiasis.html, accessed on 23/02/2014.

Ibironke OA, Phillips AE, Garba A, Lamine SM, Shiff C (2011). Diagnosis of *Schistosoma haematobium* by detection of specific DNA fragments from filtered urine samples, Am j Trop Med Hyg 84: 998-1001.

Isenberg HD (ed.) (1998). Essential Procedures for Clinical Microbiology. (1st ed.). ASM Press, Washington, D.C.

Jordan P (1985). Schistosomiasis: The St. Lucia Project, Cambridge University Press, UK.

Jordan P, Webbe G (1993). Epidemiology, in: Jordan P, Webbe G, Sturrock RF. (Eds),Epidemiology. Human Schistosomiasis. CAB International, Wallingford, pp. 87—158.

Jordan P, Webbe G and Sturrock RF (1993). Human schistosomiasis. CAB International, Cambridge.

Kabatereine NB, Brooker S, Tukahebwa EM, Kazibwe F, Onapa A (2004). Epidemiology and geography of *Schistosoma mansoni* in Uganda: implications for planning control. Trop. Med. Int. Health 9: 372—380.

Kabore A, Biritwum N-K, Downs PW, Soares Magalhaes RJ, Zhang Y(2013). Predictive vs Empiric Assessment of Schistosomiasis: Implications for treatment projections in Ghana. PLoS Negl Trop Dis 7(3): e2051. Doi:10. 1371/journal.pntd.0002051

Kato K, Miura M (1954). Comparative examinations of faecal thick smear techniques with cellophane paper covers. Japanese J Parasitol 3: 35–37.

Katsurada F(1904). The etiology of parasitic diseases. Iji Shimbun 669:1325-1332.

Katz N, Chaves A, Pellegrino J (1972). A simple device for quantitative stool thick-smear technique in *schistosoma mansoni*. Revista do Inst Med Trop de Sao Paulo 14: 397–400.

Kawanishi K (1904) . A report on a study of the 'katayama disease' in Hugo-No-Kuni. Tokyo lg za 18:31-48,

King CH, Dangerfield-Cha M (2008).The unacknowledged impact of chronic schistosomiasis. Chronic Illn 4: 65–79.

King CH, Olbrych SK, Soon M, Singer ME, Carter J, Colley DG (2011). Utility of repeated praziquantel dosing in the treatment of schistosomiasis in high risk Communities in Africa: a systematic review. PLos Neg Trop Dis 5: e1321.

King CH, Strurock RF, Kariuki HC, Hamburger J, (2006). Transmission control of schistosomiasis – why it matters now. Trends in parasitology 22: 575-582.

King M, Smith A, Gracey M (2009). Indigenous health part 2: the underlying causes of the health gap. The Lancet, 374(9683), 76-85.

Kinoti GK, Mumo JM (1988). Spurious human infection with *Schistosoma bovis*. *Trans R Soc Trop Med Hyg 82*: 589-590.

Kjetland EF, Ndhlovu PD, Gorno E, Mduluza T, Midzi N, et al., (2006) Association between genital schistosomiasis and HIV in rural Zimbabwean women. AIDS 20: 593–600.

Knopp S, Mgeni AF, Khamis IS, Steinmann P, Stothard JR, Rollinson D, Marti H and Utzinger J (2006). Diagnosis of Soil-Transmitted Helminths in the Era of Preventive Chemotherapy: Effect of Multiple Stool Sampling and Use of Different Diagnostic Techniques. PLoS Negl Trop Dis 2: 331.

Knopp S, Stothard JR, Rollinson D, Mohammed KA, Khamis IS, Marti H, Utzinger J (2012). From morbidity control to transmission control: time to change tactics against helminthes in Unguja, Tanzania. Acta Tropica.

Kongs SA, Marks G, Verle P, van der Stuyft P (2001). The unreliability of the Kato Katz method for evaluating *S. mansoni* infection. Trop Med Int Health 6: 163-69

Kumar P, Clark M, (1994). Clinical Medicine. A Textbook for Medical Students and Doctors. 82-84.

Landoure A, Dembele R. Goita S, Kane M, Tuinsma M, Sacko M, Toubali E, French MD, Keita AD, Fenwick A, Traore MS and Zhang Y. (2012) Significantly reduced intensity of infection but persistent prevalence of schistosomiasis in a highly endemic region in Mali after repeated treatment. *PLoS Negl Trop Dis* 6, e1774.

Lansdown R, Ledward A, Hall A, Issae W, Yona E, Matulu J, Mweta M, Kihanua C, Nyandindi U, Bundy D (2002). Schistosomiasis, helminth infection and health education in Tanzania: achieving behavior change in our primary schools. Heal Edu R

17: 425-433

Lardens V, Dissous C (1998). Snail Control Strategies for reduction of schistosomiasis transmission, Parasitol Today 14: 413-417.

Leiper, R T (1915). Report on the Results of the Bilharzia Mission in Egypt. Journal of the Royal Army Medical Corps, 25(1; 2).

Leiper RT (1918). Researches on Egypt Bilharziosis. John Bale sons and Danielsson. London.

Lengeler C, de Savigny D, Mshinda H, Mayombana C, Tayari S, Hatz C, Degremont A and Tanner M (1991). Community-base questionnaires and health statistics as tools for the cost-effective identification of communities at risk of urinary schistosomiasis. Int j epidermiol 20: 796-807.

Leutscher P, Ramarokoto CE, Reimert CM, Feldmeier H, Esterre P, Vennervald BJ (2000). Community-based study of genital schistosomiasis in men from Madagascar. Lancet 355: 117–118.

Majima T (1888). A strong case of liver cirrhosis caused by parasitic ova. Tokyo lg za 2:898-901.

Malatawy A, el Habashy A, Lechine N, Dixon H, Davis A, Mott KE (1992). Selective Population Chemotherapy among schoolchildren in Beheira governorate: the UNICEF (Arab Republic of Egypt/WHO Schistosomiasis Control Project). Bulletin of World Health Organisation 69: 609-621.

Malone JB, Yilma JM, McCarroll JC, Erko B, Mukaratirwa S, Zhou X (2001). Satellite climatology and the environmental risk of *Schistosoma mansoni* in Ethiopia and East Africa. Acta Trop. 79: 59—72.

Manson P (1902. Report of the case of bilharzias from the West Indies. BMJ 2:1894-1895.

Markell EK and Voge M (1976). Diagnostic Medical Parasitology. 4th ed. W. B. Saunders,

Philadelphia.

Markell EK, John DT and Krotoski WA (1999). Markell and Voge's Medical Parasitology, 8th ed. W. B. Saunders Co., Philadelphia, Pa.

Martin LK and Beaver PC (1968). Evaluation of Kato thick-smear technique for quantitative diagnosis of helminth infections. Amer. J. Trop. Med. & Hyg. 17: 382-389.

McCullough FS and Ali YM. (1965). The distribution and prevalence of *Schistosoma haematobium* and *Schistosoma mansoni* in Ghana. Rural Health Services, Ministry of Health, Ghana.

McCullough FS, Gagral P, Duncun J, Christie JD (1980). Molluscicides in schistosomiasis control. Bulletin of the World Health Organisation 58: 681-689.

McGarvey ST, Aligui G, et al (1992). Child growth and *Schistosomiasis japonica* in northeastern Leyte. Philipines. I. Cross sectional results. Am J Trop Med Hyg 46:571-581.

McKerrow JH and Salter J (2002). Invasion of skin by *schistosoma* cercariae. Trends in parasitology. 18: 193-5.

Mekheimar SI and Talaat M. (2005). Schistosomiasis knowledge, attitudes and practices among school children. *El-Fayoum, Egypt. Eastern Medit Health J*; 11: 101-20.

Melvin DM and Brooke MM (1985). Laboratory Procedures for the Diagnosis of Intestinal Parasites, p. 163–189. U.S. Department of Health, Education, and Welfare publication no. (CDC) 85-8282. U.S. Government Printing Office, Washington, D.C.

Michaud CM, Gordon WS, Reich MR (2003). The Global Burden of Disease due to Schistosomiasis. Disease Control Priorities Project Working Paper 19.

Midzi N, Ndhlovu PD, Nyanga L, Kjetland EF, Reimert CM, Vennervald BJ, Gomo E, Mudenge G, Friis H, Gundersen SG, Mduluza T (2003). Assessment of eosinophil

cationic protein as a possible diagnostic marker for female genital schistosomiasis in women living in a *Schistosoma haematobium* endemic area. Parasite Immunol 25: 581–588.

Miguel E and Kremer M (2004). Worms: indentifying imparts on education and health in the presence of treatment externalities. Econometrica 72: 159-217.

Minai M, Hosaka Y, Ohta N (2003). Historical view of schistosomiasis Japonica in Japan: implementation and evaluation of disease control strategies in Yamanashi Prefecture. Parasitol Int. 52:321-6.

Miyagawa Y (1912). Uber den Wanderungswey des *Schistosomum japonicum* van der Haut bis zum pfortadersystem und uber die korperkonstitution der jungsten Wurmer zur zeit der Hautinvasion.Zentralbi Bacteriol parasit Lnfekt 66:406-417.

Miyairi K, Suzuki M: Der zwischenwirt der *Schistosoma japonicum* katsurada. Mitt Med Fakultat Kaiserlichen Univ Kyushu 1:187-197

Moodley I, Kleinschmidt I, Sharp B, Craig M, Appleton C (2003). Temperature-suitability maps for schistosomiasis in South Africa. Ann. Trop. Med. Parasitol. 97: 617—627.

Mott KE, Dixon H, Osei-Tutu E, England EC, Davis A (1983). Relation between intensity of *S. haematobium* infection and clinical haematuria and proteinuria. The Lancet, 1: 1005-8

Mwanga JR, Magnussen P, Mugashe CL, Gabone RM, Aagaard-Hansen J. (2004) Schistosomiasis-related perceptions, attitudes and treatment-seeking practices in Magu district, Tanzania: Public health implications. *J Biosocl Sc*; 36: 63-81.

Neimeister R, Logan AL, Gerber B, Egleton JH, Kleger B (1987). Hemo-De as substitute for ethyl acetate in formalin-ethyl acetate concentration technique. J Clin Microbiol. 25: 425-426.

Nock IH, Aken 'Ova T, Galadima M (2006). Deworming: Adding Public Health Education to the equation. Trends in parasitology 22: 7-8.

Nunn JF, Tapp E (2000). Tropical diseases in ancient Egypt. Trans R Soc Trop Med Hyg 94: 147-153.

Oguama VM, Ekwunife CA (2007). The need for a better method: comparison of direct smear and formol-ether concentration techniques in diagnosis of intestinal parasites. The internet j Trop Med. Vol. 3 no. 2.

Olliaro PL, Vaillant MT, Belizaro VJ, Lwambo NJ, Ouldabdallahi M, Pieri OS, Amarillo ML, Kaatano GM., Diaw M, Domingues AC, Favre TC, Lapujade O, Alves F, Chitsulo L (2011). A multicenter randomized controlled trial of the efficacy and saftey of single-dose praziguantel of 40mg/kg vs 60mg/kg for treating intestinal schistosomiasis in the Philippines, Mauritania, Tanzania and Brazil. PLoS NegTrop Dis 5: e1165.

OMS – WHO. (1987) Atlas of the Global distribution of Schistosomiasis. pp115 – 122.

Parija SC and Srinivasa H (1999). Viewpoint: The neglect of stool microscopy for intestinal parasites and possible solutions. Trop Med & Int Heal. 4: 522-4.

Pearson RD (2002). An Update on the Geohelminths: Ascaris lumbricoides, Hookworms, Trichuris trichiura, and Strongyloides stercoralis. Current Infectious Disease Reports 4: 59–64.

Peters PA, Mahmoud AA, Warren KS, Ouma JH, Soingok TK (1976). Field studies of a rapid accurate means of quantifying *Schistosoma haematobium* eggs in urine samples. Bull. WHO, 54: 159-162.

Piraja de Silva MA (1908). Contribucao para o estuda da schistosomiasena Bahia. Brazil Med 2:281-283.

Poggensee G, Reimert CM, Nilson LA, Jamaly S, Sjastad A, Roald B, Kjetland EF, HellingGiese G, Richter J, Chitsulo L, Kumwenda N, Gundersen SG, Krantz I, Reimert CM,

Ouma JH, Mwanje MT, Magak P, Poulsen LK, Vennervald BJ, Christensen NO, Kharazmi A, Bendtzen K (1993). Indirect assessment of eosinophiluria in urinary schistosomiasis using eosinophil cationic protein (ECP) and eosinophil protein X (EPX). Acta Trop 54: 1–12.

Reimert CM, Venge P, Kharazmi A, Bendtzen K (1991). Detection of eosinophil cationic protein (ECP) by an enzyme-linked immunosorbent assay. J Immunol Methods 138: 285–290.

Ritchie LS (1948). An ether sedimentation technique for routine stool examination. Bull U S Army Med Dept. 8:326.

Rollinson D, Stothard JR., Southgate VR (2001). Interactions between intermediate snail hosts of the genus *Bulinus* and schistosomes of the *Schistosoma haematobium* group. Parasitology 123 (Suppl.), S245—S260.

Rollinson D (2009). A wake up call for urinary schistosomiasis: reconciling research efforts with public health importance. Parasitology 136: 1593-1610.

Rollinson D, Knopp S, Levitz S, Stothard JR, Tchuem Tchuente LA, Garba A, Mohammed K.A, Schur N, Person B, Colley DG and Utzinger J. (2013) Time to set the agenda for schistosomiasis elimination. *Acta Trop* 128, 423-440.

Sabah AA, Fletcher C, Webbe G, Doenhoff MJ (1986). *Schistosoma mansoni*; chemotherapy of infections of different ages, Experimental Parasitology 61:294-303.

Sambon LW (1907). New or little known African entozoan. J. Trop Med Hyg 10:117.

Savioli S, Hatz C, Dixon H, Kisumku UM, Mott KE (1990). Control of morbidity due to S*chistosoma haematobium* on Pemba Island: egg excretion and haematuria as indicators of infection. Am J Trop Med Hyg 43: 289-295.

Scrimgeour, EM and Gaidusek, CD (1985). Involvement of the central nervous system in *schistosoma mansoni* and *schistsosoma haematobium* infections. Brain 108: 10231038.

Siegel DL, Edelstein PH and Nachamkin I (1990). Inappropriate testing for diarrheal diseases in the hospital. JAMA 263**:**979–982.

Singer BH and Castro MC (2007). Bridges to sustainable tropical health, Proceedings of the National academy of Sciences of the United States of America 104: 16038-16043.

Sinuon M, Tsuyouka R, Socheat D, Odermatt P, Ohmae H, Matsuda H, Montresor A, Palmer K (2007). Control of *schistosoma mekongi* in Cambodia: results of eight years control activities in the two endemic provinces. Transact R Soc Trop Med & Hyg 101: 34-39.

Smits HL (2009). Prospects for the control of neglected tropical diseas by mass drug administration. Expert review of Anti-Infective Therapy 7: 37-56.

Smuts H, van der Merwe A, Loock M, Kotzé, P (2009, October). A framework and methodology for knowledge management system implementation. In Proceedings of the 2009 Annual Research Conference of the South African Institute of Computer Scientists and Information Technologists (pp. 70-79). ACM.

Sow S, de Vlas SJ, Mbaye, A, Polman K, Gryseels B. (2003). Low awareness of intestinal schistosomiasis in northern Senegal after 7 years of health education as part of intense control and research activities. *Trop Med Int Health*; 8: 744-9.

Stephenson LS, Lathan MC, et al., (1989). Single dose metrifonate or praziquantel treatment in Kenyan children. II. Effects on growth in relation to schistosoma haematobium and hookworm counts. Am J Trop Med Hyg 41: 445-453.

Stothard JR, French MD, Khanus IS, Basafiez MG, Rollinson D (2009). The epidermiology of urinary schistosomiasis and soil transmitted helminthiasis in school children on Unguja Island, Zanzibar. Transac R Soc Trop Med & Hyg 103:1031-1044.

Stothard JR, Mook P, Mgeni AF, Khanus IS, Khanus AN, Rollinson D (2006). Control of urinary schistosomiasis on Zanzibar (Unguja Island): a pilot evaluation of the educational impact of the juma na Kichocho health booklet within primary schools. Memorias do Instituto Oswaldo Cruz 101 (Suppl. 1), 119-124.

Strurrock RF (1995). Current Concepts of Snail Control Memorias do Instituto Oswaldo Cruz 90:241-248.

Sturrock RF (1993). The intermediate hosts and host—parasite relationships, in: Jordan, P., Webbe, G., Sturrock, R.F. (Eds), The intermediate hosts and host—parasite relationships. Human Schistosomiasis. CAB International, Wallingford, pp. 33—85.

Sturrock, RF (2001). Schistosomiasis epidemiology and control: how did we get here and where should we go? Memórias do Instituto Oswaldo Cruz, 96:17-27.

Tallo VL, Carabin H, Alday PP, Balolong Jr E, Olveda R M, McGarvey ST (2008). Is mass treatment the appropriate schistosomiasis elimination strategy? Bulletin of the World Health Organization, 86(10), 765-771.

Tay SCK, Gbedema SY and Gyampomah TK. (2011) Accuracy of diagnosis of intestinal helminth parasites in a reference diagnostic laboratory in the Ashanti region of Ghana. *International Journal of Parasitology Research* 3:12-16.

ten Hove RJ, Verweij JJ, Vereecken K, Polman K, Diege L, van Lieshout L (2008). Multiplex Real-time PCR for the detection and quantification of schistosoma mansoni and *S. haematobium* infection in stool samples collected in northern Senegal, Trans R Soc Trop Med & Hyg 102: 179-185

Toure S, Zhang Y, Bosque-Olivia E, Ky C, Ovedraogo A, Koukounari A, Gabrielli A, F, Bertrand S, Webster JP, Fenwick A (2008). Two-year impact of single praziquantel treatment on infection in the national control programme on schistosomiasis in Burkina Faso. Bulletin of World Health organization 86: 780-787.

Truant AL, Elliott SH, Kelly MT, Smith JH (1981). Comparison of formalin-ethyl ether sedimentation, formalin-ethyl acetate sedimentation, and zinc sulfate floatation techniques for detection of intestinal parasites. J Clin Microbiol 13: 882.

Ubanic C, Sinuon M, Socheat D, Pholsena K, Strandgaard H, Ordermatt P, Hatz C (2002). Epidermiology and control of mekongi schistosomiasis. Acta Trop 82:157-168.

Ukaga CN, Onyeka PI, Nwoke EB (2002). Practical medical Parasitology. 1st edition. Avan Global publications, p. 18-26.

Utzinger J, Bergquist R, Xiao SH, Singer BH, Tanner M (2003). Sustainable schistosomiasis Control: the way forward. Lancet 362: 1932-1934.

Utzinger J, Keiser J (2004). Schistosomiasis and soil-transmitted helminthiasis: common drugs for treatment and control. Expert Opin pharmacother. 5:263-85

Utzinger J, N Goran EK, Caffrey CR, Keiser J (2011). From innovation to application: Social-ecological Context, diagnostics, drugs and integrated control of schistosomiasis. Accta Tropica 120 (suppl. 1), S121-S137.

Valli LCP, Kanamura HY, Silva RM, Silva MIPG, Vellosa SAG, Garcia ET (1997). Efficacy of an enzyme-linked immunosorbent assay in the diagnosis of and serologic distinction

between acute and chronic *Schistosoma mansoni* infection. Am J Trop Med Hyg 57: 358-362.

van der Werf MJ, de Vlas SJ, Brooker S, Looman CW, Nagelkerke NJ, et al., (2003) Quantification of clinical morbidity associated with schistosome infection in subSaharan Africa. Acta Trop 86: 125–139.

Van Etten L, Folman CC, Eggelte TA, Kremsner PG, Deelder AM (1994). Rapid diagnosis of schistosomiasis by antigen detection in urine with a reagent strip. J Clin Microbiol 32: 2404-2406.

Wang LD, Guo JG, Wu XH, Chen HG, Wang TP, Zhu SP, Zhang ZH, Steinmann P, Yang GJ, Wang SP, Wu ZD, Wang LY, Hao Y, Bergquist R, Utzinger J, Zhou XN(2009b). China's new strategy to block Schistosoma Japonicum transmission: experiences and impact beyond schistosomiasis. Tropical Medicine and International Health14:1475–1483

Warren, KS. (1973). Schistosomiasis. The evolution of a medical literature. Selected abstracts and citations, 1852-1972.

Watson B, Blitzer M, Rubin H, Nachamkin I (1988). Direct wet mount versus concentration for routine parasitological examination: are both necessary? Am J Clin Path 89: 389–391.

WHO (1993). The control of Schistosomiasis. Geneva: World Health Organisation, Technical Report Series, No 830.

WHO (2002). Prevention and control of schistosomiasis and soil-transmitted helminthiasis: report of the WHO expert committee. WHO Tech Ser 2002; 912:1-57

WHO (2002). Report of the WHO Informal Consultation on the use of Praziquantel during pregnancy/lactation and Albendazole/Mebendazole in Children under 24 months.

Geneva, 8–9 April 2002.

WHO (2004). World Health report. Changing history. Geneva: WHO, 2004

WHO (2006). Preventive chemotherapy in human helminthiasis: coordinated use of anthelminthic drugs in control interventions. Geneva: World Health Organisation.

WHO (2010). First WHO Report on Neglected Reported Diseases 2010: working to overcome the global Impact of Neglected Diseases. World Health Organisation, Geneva.

Wichmann D, Panning M, Quack T, Kramme S, Burchard GD, Grevelding C, Drosten C (2009). Diagnosing schistosomiasis by detection of cell-free parasite DNA in human plasma. PloS Neg Trop Dis 3: e422

Wiebe, Janyce M, Rebecca F, Bruce, Thomas P O'Hara (1999). Development and use of a gold-standard data set for subjectivity classifications. In ACL99, Proceedings of the 37th Annual Meeting of the Association for Computational Linguistics. College Park, MD.

Wirkom V, Tata R, Agba M, Nwobu G, Ndze R, Onoja O, Utien G, Bongkisheri L, Nsadzetreno V, Banseka E (2007). Formol-petrol stool concentration method (Wirkom-Tata's stool concentration method): A Cheap Novel Technique For Detecting Intestinal Parasites In Resource-Limited Countries . The Internet J Trop Med. Vol. 5 No.1:1-8.

Wolmarans CT, de Koch KN, Strauss HD, Bornman M (2002). Daily emergence of *schistosoma mansoni* and *S. haematobium* cercariae from naturally infected snail under field conditions. *Journal of Helminthology,* 76:273-7

Woolhouse ME, Etard JF, Dietz K, Ndhlovu PD, Chandiwana SK (1998). Heterogeneities in schistosome transmission dynamics and control. Parasitology 117: 475—482.

Woolhouse MEJ, Watts CH, Chandiwana SK (1991). Heterogeneities in transmission rates and the epidemiology of schistosome infection. Proc. Biol. Sci. 245:109—114.

World Health Organisation (1991). Basic Laboratory Methods in Medical Parasitology. Geneva: World Health Organization. Geneva.

World Health Organisation (2012). The control of schistosomiasis. World Health Organisation fact sheet IF 115

Xu, J., Rong, R., Zhang, H. Q., Shi, C. J., Zhu, X. Q., & Xia, C. M. (2010). Sensitive and rapid detection of *Schistosoma japonicum* DNA by loop-mediated isothermal amplification (LAMP). International journal for parasitology, 40(3), 327-331.

Yelifari L, van Lieshout L, Dery G. Anemana S, Agongo E, Bloch P, Magnussen P, Polderman T (1999). Distribution of human Oesophagotomum bifrcum, hookworm and Strogyloides infections in Northern Ghana.

Young KH, Bullock SL, Melvin DM, Spruill CL (1979). Ethyl acetate as a substitute for diethyl ether in the formalin-ether sedimentation technique. J Clin Microbiol. 106:852–853.

Zhang Y, Koukounari A, Kabatereine N, Fleming F, Kazibwe F, Tukahebwa E, Stothard JR, Webster JP, Fenwick A (2007). Parasitological impart of 2-year preventive chemotherapy on schistosomiasis and soil transmitted helminthiasis in Uganda, BMC Medicine 5: 27.

Ziegelbauer K, Speech B, Mausezahl D, Bos R, Keiser J, Utzinger J (2012). Effects of sanitation on soil transmitted helminth infections: systematic review and metaanalysis. PLoS Med 9: e1001162.

APPENDICES

Appendix 1: Reagents

PHYSIOLOGICAL SALINE		10% FORMALIN	
Sodium chloride	8.9g	Formalin	10 ml

Distilled water	100 ml	Distilled water	70 ml

GLYCEROL-MALACHITE

50% Glycerol 200ml

3% Malachite Green 1ml

DIETHYL-ETHER GREEN SOLUTION

MICRO-PLATE

Microwell strips are coated with Ready to use anti-human ECP antibody

ECP STANDARDS AND CONTROLS

WASH SOLUTION

Wash concentrate 100ml Distilled water 900ml

CONJUGATE REAGENT

Horseradish Peroxidase conjugated Anti-human ECP polyclonal antibody; Ready to use.

STOP SOLUTION

0.5 mol/L H_2SO_4 ; ready to use

SUBSTRATE BUFFER

TMB/H_2O_2 ; ready to use

ASSAY DILUENT

Ready to use

Appendix 2: Ethical Approval

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY
COLLEGE OF HEALTH SCIENCES
SCHOOL OF MEDICAL SCIENCES / KOMFO ANOKYE TEACHING HOSPITAL
COMMITTEE ON HUMAN RESEARCH, PUBLICATION AND ETHICS

Our Ref: CHRPE/AP/209/13

27th September, 2013.

Mr. Emmanuel Adongo
Department of Clinical Microbiology
School of Medical Sciences
KNUST-KUMASI.

Dear Sir,

LETTER OF APPROVAL

Protocol Title *"Epidemiology of Schistomsomiasis in Anendemic Area: Comparing Intensity of Infection by Egg Count and Eosinophil' Cationic Protein Level Measurement Before and After Praziquante Treatment."*

Proposed Site: *Vea in the Bongo District of the Upper East Region.*

Sponsor: *Principal Investigator.*

Your submission to the Committee on Human Research, Publications and Ethics on the above named protocol refers.

The Committee reviewed the following documents:

- A notification letter of 22nd March, 2013 from the Bongo District Education Office (study site) indicating approval for the conduct of the study in the District.
- A completed CHRPE Application Form.
- Participant Information Leaflet and Consent Form.
- Research Proposal.
- Questionnaire.

The Committee has considered the ethical merit of your submission and approved the protocol. The approval is for a fixed period of one year, renewable annually thereafter. The Committee may however, suspend or withdraw ethical approval at anytime if your study is found to contravene the approved protocol.

Data gathered for the study should be used for the approved purposes only. Permission should be sought from the Committee if any amendment to the protocol or use, other than submitted, is made of your research data.

The Committee should be notified of the actual start date of the project and would expect a report on your study, annually or at close of the project, whichever one comes first. It should also be informed of any publication arising from the study.

Thank you Sir, for your application.

Yours faithfully,

Osomfuor Prof. Sir J. W. Acheampong MD, FWACP
Chairman

Room 7 Block J, School of Medical Sciences, KNUST, University Post Office, Kumasi, Ghana
Phone: +233 3220 63248 Mobile: +233 20 5453785 Email: chrpe.knust.kath@gmail.com / chrpe@knust.edu.gh

Appendix 3: Consent Form

Consent form for the study entitled:"ASSESSMENT OF EOSINOPHIL CATIONIC PROTEIN LEVELS AS POSSIBLE BIOMARKER FOR ESTIMATING INTENSITY OF SCHISTOSOMIASIS BEFORE AND AFTER PRAZIQUANTEL TREATMENT''

Information: To be read or translated to adult participants, parents/guardians of child participants in their own mother tongue.

Dear Sir/Madam,

We kindly request you/your child to enrol into this study, which we describe below. We will like to emphasize that this study is strictly voluntary. Should you decide not to participate; it will have no consequences for you. If at any point in time during the study you take the decision not to participate any further, you are free to do so immediately without any further discussion, and again it will have no consequences for you whatsoever.

Background: It is widely established that people living near infested fresh water bodies are the most affected by schistosomiasis. This is because of their constant contact with infested water as a result of farming, swimming and doing other activities like household chores. The Vea dam is believed to be infested with schistosomes because of the presence of the intermediate host snail and positive cases have been reported at the Bongo District Hospital and the Bolgatanga Regional Hospital which serve communities that access the dam. Due to the high nature of infection levels the site was chosen for the Mass Drug administration exercise.

Purpose: This research is to compare the intensity of schistosomiasis infection by microscopy and ECP levels before and after praziquantel treatment. This study will be very important in assessing the effectiveness of the deworming exercise and also show whether ECP levels measurement is a better indicator than microscopy.

What we require from you/your child: We will take urine, stool and blood samples from you. A trained person will take about a quarter of a teaspoon of blood sample using a sterile disposable syringe and needle. Taking of the blood sample may cause a little pain or discomfort. The three samples will be tested to find out if you have the *Schistosoma* infection.

Privacy and confidentiality: We will ask you/your child a few questions. The information you give us will be used only for the study and will not be used in anyway that will harm you. Your participation and test results will remain confidential. Your samples will be labelled with a code number and not your name. Your name will also not be identified in reporting the study results. You are at liberty to withdraw unconditionally from this research after enrolment and this will not affect you in any way.

Benefits: You will be screened free of charge and the results of the test will be given to you so that you can have treatment in case you are infected. Also you will be educated on how to reduce exposure to risk factors to avoid possible infection.

Contacts: If you have any question about the research study, you may contact any of the following people: Prof. S.C.K. Tay (School of Medical Sciences, KNUST, 0245670710) Dr. Irene Ayi (Noguchi Memorial Institute of Medical Research, Tel 0243670493).

DECLARATION

I, the undersigned, voluntarily agree to participate in the study by providing urine, stool and blood samples. The risk and benefits have been explained to me and I am aware every effort will be made to ensure my confidentiality.

……,… …… ……

Name of participant Signature/ Thumbprint Date

…… ……

Name of Investigator Date

Appendix 4: Questionnaire

Questionnaire on Personal and Bilharzia (Schistosomiasis)-related
Information for study participants

Kindly provide the necessary information by filling the blank spaces or ticking [✓] the appropriate response(s). Thank you.

PART A: Personal Information

Name: …… Age: ……. Sex: M [ ] F [ ]

Town/Village: …….. House No.: …….

Occupation: ……..

Other [ ] (specify) …….

(For school-age children)

Name of parent / guardian: ……..

Are you in school? Yes [ ] No [ ]

If yes, Name of school: …… Class: …..……..

PART B: Bilharzia (Schistosomiasis)-related information

1. For how long have you lived here? ……..
2. How long do you stay in this community within a year?
    a. Up to 5 months [ ]
    b. 6 – 8 months [ ]
    c. 9 – 12 months [ ]
3. Do you come into contact with any water body in your community Yes [ ] No [ ]
4. What is the name of this water body? …….
5. What do you do when you visit this water body? (Tick the most appropriate; multiple choices allowed)

a. bath, play games etc [ ]

b. fetch water (for drinking, washing, farming etc) [ ]

c. catch fish from it [ ]

d. sand winning [ ]

e. other (specify) ……

6. How often do you come into contact with this water body?

a. daily [ ]

b. one to three times per week [ ]

c. four to seven times per week [ ]

d. once a month [ ]

e. once a year [ ]

f. never [ ]

7. Do you accompany any of your parents to fish in a river/pond? **(For children only)**

Yes [ ] No [ ]

8. Do you accompany any of your parents to the farm/the garden? **(For children only)**

Yes [ ] No [ ]

If yes to question 8 above, ask question 9

9. Do you cross a pond/river Yes [ ] No [ ]

10. Do you know Bilharzia? Yes [ ] No [ ]

If yes to question 10 above, ask question 11

11. What do you think it is?

a. sign of reproductive maturity [ ]

b. a disease [ ]

c. punishment from gods/ancestors [ ]

d. don't know [ ]

e. Other (please specify) ……

12. Which of the following is true about Bilharzia? (Multiple responses are allowed) a. chills/shivering [ ]

b. swollen stomach [ ]

c. passing blood in urine [ ]

d. feeling pains when urinating [ ]

e. passing blood in stool [ ]

13. Have you ever passed blood in your urine? Yes [ ] No [ ] Don't know [ ]

If yes to question 13 above, ask question 14

14. Did you feel pains when you were urinating? Yes [ ] No [ ]

15. Have you ever passed blood in your stool? Yes [ ] No [ ] Don't know [ ]

16. If yes to any of questions 13, 14, or 15, what was done about it?

a. I visited a hospital/health centre [ ]

b. I / (or my parents) used herbal medicine [ ]

c. I (or my parents) bought medicine from a drug store [ ]

d. I received medicine from some health workers who came to our community [ ]

e. I did not do anything about it [ ]

f. Other (specify)…….

If (a) or (c) in question 16 is ticked ask question 17

17. When was the last time you took medicine?

a. up to 6 months ago [ ]

b. up to one year ago [ ]

c. up to two years ago [ ]

d. more than two years ago [ ]

18. What kind of drug did you take?

19. When was the last time you treated malaria?

a. 1 day – 2 weeks [ ]

b. 2 weeks – 1 month [ ]

c. 3 months – 6 months [ ]

d. More than 6 months [ ]

20. What kind of antimalaria did you take?

Appendix 5: Pictures from the field

Plate 1: Registration of participants

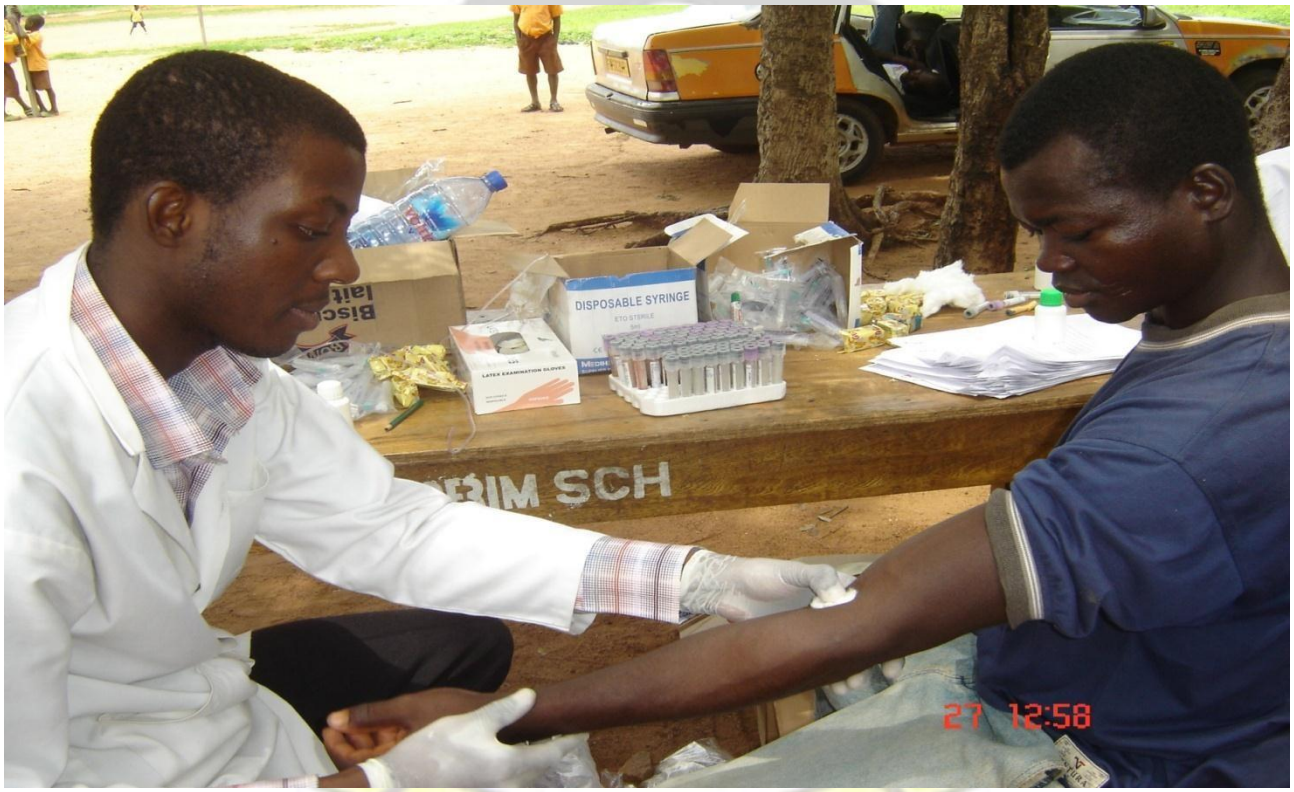

Plate 2: Blood sample collection from general public

Plate 3: Blood sample collection from school children

Appendix 6: Pictures in the Laboratory

Plate 4: Running ECP ELISA

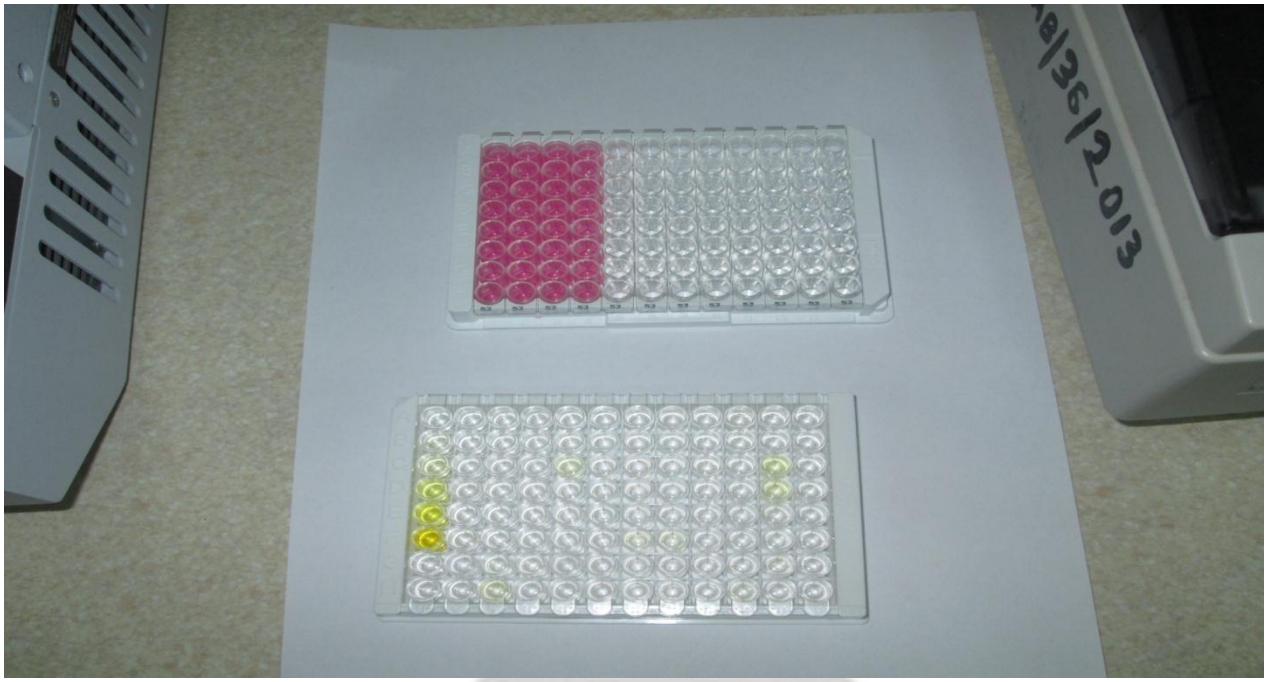

Plate 5: ELISA Plates

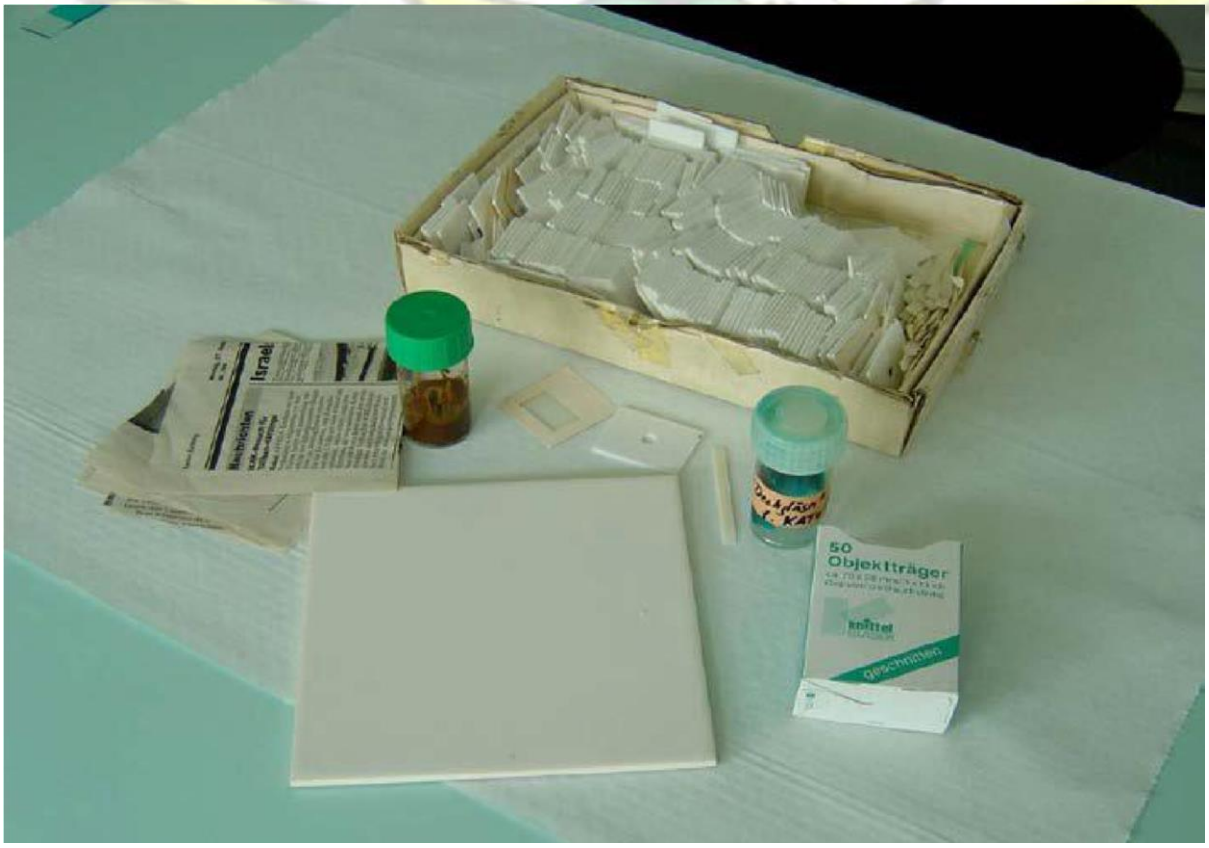

Plate 6: Photograph of Kato Katz materials

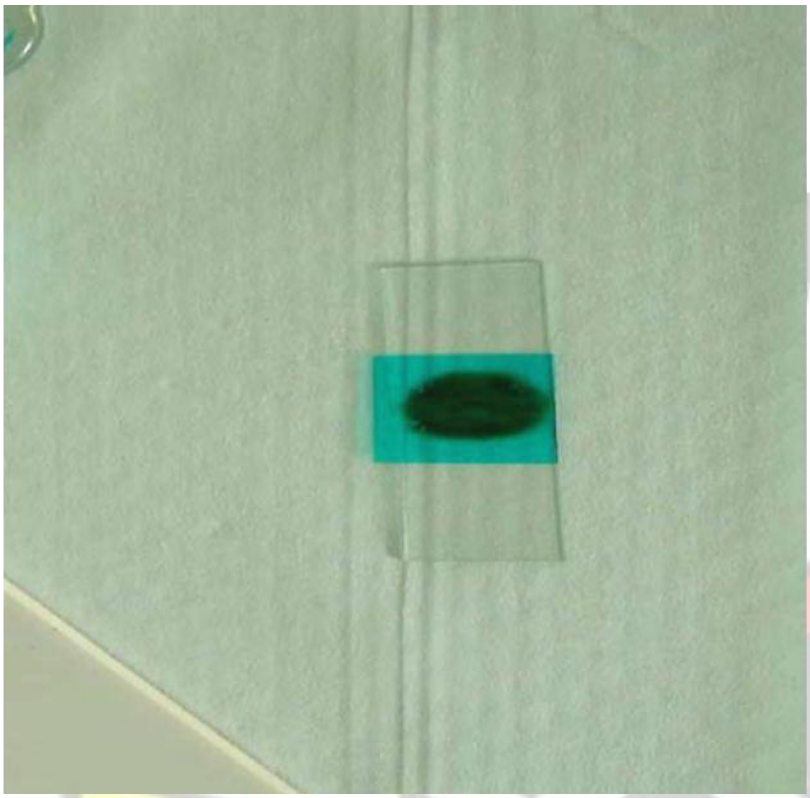

Plate 7: Photograph of Kato Katz thick smear