UNIVERSITY OF CAPE COAST

IMMUNO-EPIDEMIOLOGY OF URINARY SCHISTOSOMIASIS INFECTION AMONG INDIVIDUALS IN ADANSI SOUTH DISTRICT, GHANA

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BY

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Thesis submitted to the Department of Microbiology and Immunology of the School of Medical Sciences of the College of Health and Allied Sciences of the University of Cape Coast in partial fulfilment of the requirements for the award of a Master of Philosophy degree in Infection and Immunity

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DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my original research and that no part of it has been presented for another degree at this university or elsewhere.

Candidate's Signature: …… Date…….

Name: Enoch Ameshia

Supervisors' Declaration

We hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

Supervisor's Signature…… Date……..

Name: Rev. Dr. Benjamin Amoani

Supervisor's Signature…… Date……..

Name: Mr. Kwabena Dankwa

ABSTRACT

Schistosoma haematobium infections are well established and endemic in Ghana. The endemicity of the disease also shows a focal distribution, with communities along fresh surface water bodies being the most affected. The impact of these water bodies in communities with respect to the problem of urinary schistosomiasis infection and its effect on immunoepidemiological parameters has not been explored. This study therefore seeks to profile the immunohaematological epidemiology of S. haematobium in the Adansi South District. The study employed a cross-sectional study design and recruited 406 participants from five communities in the district. Urine and stool samples were collected from participants for microscopy, immunological and haematological analysis. A questionnaire was also administered to participants to determine the sociodemographics and risk factors for urinary schistosomiasis. The study showed an overall prevalence of 16.3% of *S. haematobium* infections. Owusukrom had the highest prevalence of 37.0%, while Menang had the lowest prevalence of 1.1%. There was no significant difference in either IgG or IgM levels nor in haematological paramters in either the infected or uninfected controls. Major risk factors associated with infection included contact with freshwater bodies, poor hand and personal hygiene, history of infection, farming activities in waterlogged areas and the use of river/surface water sources for domestic activities. Conclusively, the prevalence of *S. haematobium* infections in the Adansi South District is high and the immunohaematological parameters associated with it are widely varied across individuals.

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DEDICATION

I dedicate this work to my beautiful wife, Grace Afia Owusuwaa, my mother, Madam Doris Akowuah, and my uncles, Mr. Francis Yeboah and Mr. Randolf Akowuah.

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CHAPTER ONE

INTRODUCTION

Schistosomiasis infection is caused by Schistosoma species, namely, *Schistosoma haematobium, Schistosoma mansoni, Schistosoma mekongi, Schistosoma japonicum,* and *Schistosoma intercalatum*. The disease has been a great public health concern in Ghana over the years. It has been endemic in the country and has contributed to ill health and negatively impacted the socioeconomic wellbeing of various families and communities. The fight against the infection has been on but is still far from ending. An understanding of the infection dynamics and immune response across different people will provide data to inform strategies in the fight against schistosomiasis.

This study sought to assess the distribution of immune responses against schistosomiasis in a selected Schistosome endemic area in Ghana. The data from this research will be relevant to public health specialists and stakeholders and the international community to better understand the infection and immune dynamics peculiar to the Ghanaian situation.

Background

Schistosomiasis affects over 250 million individuals globally, with children mostly affected (Hotez & Kamath, 2009. It is one of the main causes of morbidity and mortality in low- and middle-income countries (LMICs), especially in Africa, where poor sanitation, inadequate water and poverty are common (WHO, 2014; Bethony, Brooker, Albonico, Geiger, Loukas, Diemert, et al., 2006). Tropical areas in Africa, the Caribbean, East Asia, South America and the Middle East are hubs for schistosomiasis. According to the WHO (2012), over 700 million persons are at risk of being infected with

schistosomiasis, and more than 207 million individuals are infected globally (Hotez & Ehrenberg, 2010). Interestingly, sub-Saharan Africa records over 90 percent and almost 200,000 deaths yearly of the cases reported worldwide (Hotez & Fenwick, 2009).

Worldwide, *S. haematobium, S. mansoni, S. japonicum, S. mekongi* and *S. intercalatum* are the five commonly distributed species in schistosomiasis endemic regions. Individuals infected with chronic schistosomiasis have reduced work capacity and die in some cases. Mass praziquantel administration is the mainstay of current programs focused on schistosomiasis morbidity control (Fenwick *et al*; 2009). Whereas microscopy has been the basic laboratory diagnostic tool for schistosome detection in stool and urine samples, polymerase chain reaction (PCR) and serology have served as confirmatory diagnostic techniques for the detection of various pathogens, including schistosome(s) parasites, over the years (Pontes *et al*, 2002). A good measure of effective diagnostic methods is its ability to ensure accurate, precise and reliable early detection of infections. Although the detection of schistosome eggs, species identification and infection intensity determination can be performed using microscopy, the gold standard for schistosomiasis diagnosis, there are challenges such as the good recovery of parasite eggs at apt periods, technical errors, time factors and intensive labour needed. All these factors can contribute to downplaying the impact of measures to mitigate schistosomiasis due to imperfect results produced using microscopy (McCarthy *et al*, 2012). These challenges raise concerns about the use of microscopy as the traditional diagnostic tool for schistosome detection. Reports by the CDC (2014) presented a changing paradigm in schistosomiasis infection due to increased snail vector

distribution and migration of travellers, and there has been a shift in the infection from endemic regions to nonendemic regions. Evaluating the immune epidemiology of schistosomiasis is not amiss since it can reveal new epidemiological trends.

Problem Statement

Schistosomiasis continues to be endemic in Ghana, with the most recent nationwide survey reporting a prevalence of 70.9% in 2010 (Susanne, 2015). In Ghana, preventive chemotherapy (PCT) has been an effective schistosomiasis control measure with a primary focus on mass drug administration (MDA) in Neglected Tropical Diseases programmes (Seddoh *et al*, 2013). Communities in the Adansi South District of the Ashanti Region, Ghana, namely, Asarekrom, Owusukrom, Adansi Praso, Wuruyie, Tonkoase 2, Atwereboana, Subriso, Aboi, New Edubiase, and Kojo Mankrong, are within the rainforest belt. Considering that the area has streams and rivers as their source of water and for agricultural purposes, the presence of intermediate host snails is evident around the banks of the rivers and streams. Mass drug administration with praziquantel is done every two years in the district, a measure initiated by the Ghana Health Service to help reduce the disease burden. The last report of the mass drug administration in the district dates back to over five years ago. Interestingly, monitoring and evaluation of these measures depend on infection detection by microscopy. However, according to Pontes *et al*. (2003), microscopy has reduced sensitivity in post-treatment situations in the control of transmission in low endemic areas. The sensitivity of serology-based specific schistosome antigen detection (anti-*Schistosoma* antibodies) is better than that of microscopy in low schistosome endemicity (Coon, 2005).

Many studies that outline the impact of S. *haematobium* have been conducted using animal models (Geiger *et al.*, 2002). However, the immune mechanisms behind this phenomenon are relatively associated with various factors, including cross-reactive antibodies (Behnke, 2008). The impact of antibodies in controlling schistosomiasis is unclear and not well understood. Afrifa, Gyedu, Ofori Gyamerah, Essien-Baidoo & Mensah-Essilfie (2017) reported anaemia in schistosomiasis infections in Yeji. These reports coupled with the documented life cycle activities of the *S. haematobium* parasite present possibilities of unreported anaemias in these communities due to Schistosoma infections. Therefore, understanding the immune and haematological responses to *S. haematobium* infection could be useful in vaccine development and help to control the disease. Additionally, given that the risk factors for the disease are multifactorial and differ from place to place, a poor understanding of the peculiar risk factors in given endemic communities hinders the fight against *S. haematobium* infections.

For almost a decade, no study has been carried out in any of these communities. The constant exposure and contact with these water sources put these communities at great risk of infection. There is therefore the need to undertake investigations to understand the peculiar disease characteristics and immuno-haematological presentations of Schistosoma *haematobium* among inhabitants of these communities in the Adansi South District, Ghana.

General Objective

To determine the immuno-epidemiological parameters associated with schistosomiasis among inhabitants of some selected communities in the Adansi South District, Ghana.

Specific objectives

1. To determine the prevalence of schistosomiasis infection in the selected communities in the Adansi South District.
2. To determine the possible risk factors associated with Schistosoma infection in Adansi South District.
3. To assess the antibody levels (IgG and IgM) against Schistosome crude egg antigen among the study participants
4. To determine the haematological parameters among study participants

Significance of the Study

Parasitic infections such as *S. haematobium* infections are not merely an indication of ill health but also a predictor of the general wellbeing of a population and the socioeconomic status of the community. It tells of the general public health status and the social, economic and political atmosphere in a given population. Information on the prevalence of these infections is used by nations as a record to advise their citizens for or against travel to endemic regions. Ghana has been noted as one such country, with a relatively high endemicity of parasitic infections (Klumpp, 1982). Countries such as the US have some time advised their citizens against travel to Ghana because of *Schistosoma haematobium* infections. There is therefore the need to review our status as far as the infection is concerned and an appraisal of our data, especially with regard to the general immunity of the populace to the infection. This study, which assesses the immunoepidemiology of *S. haemtobium* infections, will provide data to address the need to understand our current status as far as the infection is concerned and the immune profile of the endemic populations concerning the infections.

Large-scale praziquantel administration has been one of the dominant means in the fight against schistosomiasis infection (Fenwick *et al*, 2009). Coupled with repeated exposure and possible infections in these endemic communities, there exists the possibility of a range of immune developments against the infection. The present study, which assayed the various immune responses to infection, exposure, mass treatments, and possible reinfections, will provide information on the effectiveness of the last mass drug administrations among the population and the development of anti-schistosome antibodies among the population. This will further inform the choice of strategies to fight the infection in our country.

Delimitation of the study

This study was designed to cover only some selected communities in the Adansi South District of the Ashanti region of Ghana. The Adansi South District is a well-known Schistosoma endemic community in the Ashanti region, partly due to its marshy areas and farming activities. The specific communities include Bronikrom, Owusukrom, Asarekrom, Tensuani, Dotom and Menang. Other communities in the same district other than these five were not included in the present study.

The immune markers measured in the present study included IgM and IgG. These markers are indicative of the immune response to the onset, recovery and reinfections of schistosomiasis.

Limitations of the study

The study could not assess the possible reinfections in the course of the research. Given that this is an endemic region for the parasite, reinfections could

have occurred at any time during the research. This could greatly influence the findings of the study.

Additionally, the study did not identify the stage of the infection or whether it was a first-time reinfection. Our inability to continually assess the infection status of the participants over time is part of the weakness of the present study. Thus, this research provides a general idea of the situation on the ground and points out the areas of interest for future scientific research.

Organization of work

This is a standard five-chapter thesis. Chapter one introduced the concept of the research. It included the background of the study, the statement of the problem, the purpose of the study, the specific objectives, the research questions, significance of the study, delimitations of the study, limitations of the study, and the organization of the work. Chapter two reviewed the available literature relevant to the study. Chapter three presents the study design, the study area, the study population, sample size, sampling techniques, sample collection and analysis, data analysis, ethical considerations and expected outcome. Chapter four presents the results of the study and discusses the findings of the study with existing knowledge. Chapter five summarizes the findings, draws conclusions, and proposes recommendations based on the findings of the study.

CHAPTER TWO

LITERATUR REVIEW

Introduction

The subject of urinary schistosomiasis has caught the attention of numerous researchers who are constantly exploring new ways of understanding the disease and its causative organism, treatment and prevention strategies and mapping the epidemiology of the disease in different geographical areas. As such, knowledge of urinary schistosomiasis is constantly evolving, with scientists disagreeing on aspects of the current knowledge of the disease or not knowing enough to make definite conclusions. This chapter sought to review the empirical and theoretical knowledge relevant to this subject and thus set the theoretical context in which this study is captured. Subheadings such as the epidemiology of the disease, lifecycle of the causative parasite, pathogenesis, diagnosis, risk factors for infection, prevalence of the disease, and management and treatment of the disease are captured in this section.

Epidemiology and Distribution

Schistosomiasis is a water-borne parasitic disease caused by trematodes of the genus Schistosoma (Mendis, Fukino, Cameron, Laing, Filipe Jr., Leowski, et al, 2007). After malaria and amoebiasis, schistosomiasis is the third leading parasitic disease in the world (Okwori, Sidi, Ngwai, Obiekezie, Makut, Chollom, et al, 2014). In 2007, an estimated 250 million people in 76 countries were known to be infected with the parasite (Mathers, Ezzati & Lopez, 2007). An estimated over 700 million people are at risk of Schistosoma infections worldwide (Youssef, A.I., Uga, S.). In Africa, South America, the Caribbean, the Middle East and Asia, Schistosoma infections is a major contributor of

morbidity and mortality (Magalhães, Biritwum, Gyapong, Brooker, Zhang, Blair, et al, 2011).

Worldwide, 74 countries are Schistosoma endemic (Olveda, Li, Olveda, Lam, PChau, Ham, et al (2013)). Olveda et al. (2013) estimated that 779 million people in these countries are at risk of the disease. Endemicity for schistosomes is closely linked to underdevelopment. This is partly because of the poor healthcare systems that are inadequate in providing basic primary health care (Chitsulo, Engels, Montresor, & Savioli, 2000). The most severely affected countries, according to Mendis, Fukino, Cameron, Laing, Filipe Jr, Leowski et al. (2007), are in Africa: Angola, Central African Republic, Chad, Egypt, Ghana, Madagascar, Malawi, Mozambique, Nigeria, Senegal, Sudan, the United Republic of Tanzania, Zambia; In South America: Brazil; In South-East Asia: Philippines; and In South-West Asia: Yemen Arab Republic. Countries with the lowest prevalence, with infections less than 1,000, include Antigua and Barbuda, India, Indonesia, Jordan, Oman and Saint Lucia (Steinmann, 2008).

Africa remains the worst hit by the parasite. For example, in West Africa, Schistosoma and hookworm infections are said to cause considerable morbidity among children of school-going age (Olveda, Li, Olveda, Lam, PChau, Ham, et al, 2013). In Africa alone, an estimated 300,000 people die annually from Schistosoma infections (Olveda, Li, Olveda, Lam, PChau, Ham, et al, 2013). In the last 20 years, annual deaths from *Schistosoma* infections have increased, making it the third most dreaded neglected tropical disease (Hote, Alvarado, Basáñez, Bolliger, Bourne, Boussinesq, et al, 2014). Africa recorded the highest number of at-risk people of660 million, representing 85% of the worldwide number of at-risk persons. Okwori, Sidi, Ngwai, Obiekezie, Makut,

Chollom et al. (2014) estimated that Africa accounted for 97% of all schistosome infections (mostly *S. haematobium*) globally.

The five species of Schistosoma (*haematobium, mansoni, japonicum, mekongi, intercalatum*) are variedly distributed across different continents. Whereas some are found on more than one geographical continent, others are restricted to particular continents and geographical locations.

Schistosoma mansoni (the causative of intestinal morbidity) is the most prevalent of all Schistosoma species. It is endemic in 55 countries, including the Arab Peninsula, Egypt, Sudan, Libya, Sub-Saharan African countries, Brazil, some Caribbean islands and Venezuela (Barakat, 2013).

Schistosoma haematobium, the second most prevalent Schistosoma species, is endemic in 53 countries in Africa and the Middle East. An estimated 110 million people are thought to be infected with *S. haemtobium* (Barakat, 2013; Olveda, Li, Olveda, Lam, Chau, Ham, et al, 2013).

Schistosoma japonicum originated in Japan but has been eradicated from Japan since 1977 (Chitsulo, Engels, Montresor & Savioli, 2000). At present, it is endemic in China, Indonesia and the Philippines whereapproximately 60 million individuals are at risk of infection, and close to two million are currently infected (Olveda, Li, Olveda, Lam, Chau, Ham, et al, 2013).

Schistosoma mekongi, like *S. japonicum*, derived its name from the place of its first notice. It is endemic along the Mekong River and the lower Mekong basin (Olveda, Li, Olveda, Lam, Chau, Ham, et al, 2013).

Schistosoma intercalatum is endemic in Central Africa, particularly rainforest areas (Barakat, 2013). One of the two main distinct species has been

recognized in the Democratic Republic of Congo and the other in Lower Guinea (mainly Cameroon) (Olveda, Li, Olveda, Lam, Chau, Ham, et al, 2013).

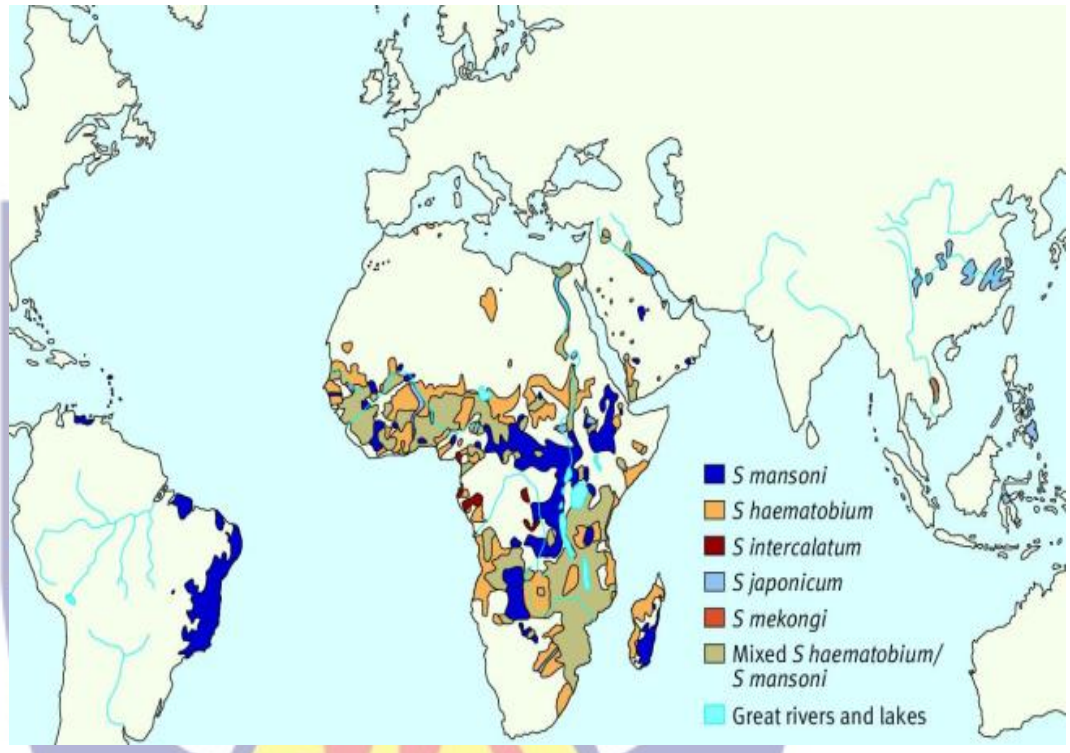


Figure 1: Global distribution of Schistosoma species (Elmorshedy & El Temsahy, 2016)

Ghana is one of the countries in Sub-Saharan Africa that is severely affected by schistosomiasis. Only *S. haematobium* and *S. mansoni* have been reported in Ghana. Cases of schistosomiasis that had been increasing before the construction of the AKosmombo dam still continued to rise steadily even after construction (Mone, Ibikounle, Massougbodji & Mouahid, 2010). In 2008, the Ministry of Health recorded a prevalence of over 90% in some communities along Volta Lake (Ghana Ministry of Health, 2008). Rollinson et al. (2012) estimated a national prevalence of the parasite of 70.9% in 2010. This was slightly lower than the 72.6% estimated in 1986 and 2003 (Rollinson et al, 2012; Utroska, Chen, Dixon, Yoon, Helling-Borda, Hogerzeil, Mott, 1989). Every

region of the country has recorded cases of the disease. *S. haematobium* is endemic in all regions, while *S. mansoni* is found only in the Upper West and Upper East regions (Mone, Ibikounle, Massougbodji & Mouahid, 2010).

Life cycle and transmission of Schistosoma species

Generally, *S. mansoni* and *S. haematobium* are the only species known to infect humans (Carabin, Balolong, Joseph, McGarvey, Johansen, Fernandez et al., 2005; Ross, Bartley, Sleigh, Olds, Li, Williams et al., 2002). The life cycle of schistosomes is divided into two phases: a sexual phase in the definitive host and an asexual phase in the intermediate host.

The intermediate host

The intermediate host for the schistosome parasite is the freshwater snail. Each species of the parasite has a specific snail host that it infects. Whereas *S. haematobium* infects snails of the genus *Bulinus*, *S. mansoni* species infects snails of the genus *Biomphalaria*. Other snail hosts include species of the genera *Oncomelania* and *Neutricula* for *S. japonicum* and *S. mekongi*, respectively (Roquis, Taudt, Geyer, Padalino, Hoffmann, Holroyd et al., 2018). Eggs released by adult worms into freshwater hatch into miracidia upon contact with freshwater. In the water, it remains infective for 6 to 12 hrs. Miracidia infect freshwater snails through the soft tissues of snails. Once inside the snail, the miracidia develop into a mother sporocyst after removing its ciliated plates, which then produces daughter sporocysts by asexual reproduction (Mouahid, Rognon, de Carvalho Augusto, Driguez, Geyer, Karinshak et al., 2018; Gurarie, Lo, Ndeffo-Mbah, Durham & King, 2018). Daughter sporocysts may then produce more daughter sporocysts, also known as sporocytogenous sporocysts, or cercariae, also known as cercariogenous sporocysts (Mouahid, Rognon, de

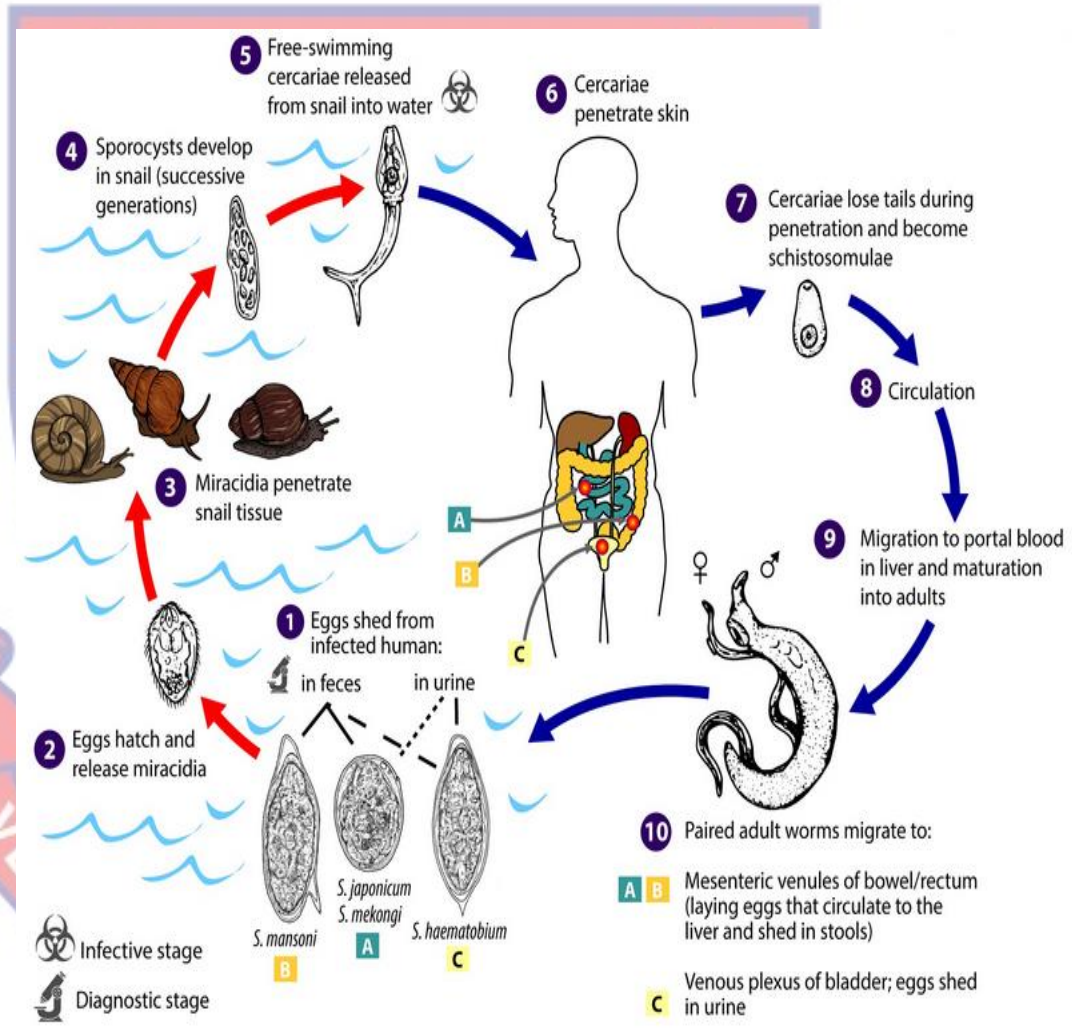
Carvalho Augusto, Driguez, Geyer, Karinshak et al., 2018). The cercaria are fork-tailed and are released into the water upon stimulation by light. *Biomphalaria* snails shed approximately 200 *S. haematobium* cercariae daily, while *Bulinus* snails produce approximately 250 to 600 *S. mansoni* cercariae daily (Braun, Grimes & Templeton, 2018).

The definitive host

Cercariae enter the skin of the mammalian host upon exposure to contaminated water by either mechanical activity or proteolytic enzymes (Ross, Vickers, Olds, Shah & McManus, 2009). On entry, they shed off their forked tail and form a schistosomula (Gurarie, Lo, Ndeffo-Mbah, Durham & King, 2018). Within 48 hrs of skin penetration, the majority of *S. haematobium* schistosomulae find their way to the dermis, and by 72 hrs, the are found in the dermal blood vessels. On leaving the dermis, they are carried in the venules and lymphatic vessels to the heart and lungs. The schistosomula grows into an adult worm as it migrates through various body tissues until it reaches its specific locale. During migration, they are found in large numbers in the lungs as they are held up temporarily in the lung capillaries. In the portal system, adult male and female worms mature sexually and pair up. They then migrate to the pelvic venous plexus for *S. haematobium*. Oviposition occurs at about 9 weeks in *S. haematobium.*

During mating, the adult female is embraced into the male's gynecophoric canal (Ross, Vickers, Olds, Shah & McManus, 2009). The eggs penetrate the vascular walls and enter the intestinal lumen or bladder to be shed in the stool (for intestinal schistosomiasis) or urine (for urinary schistosomiasis). The remainder of the eggs remains in the tissues. The tissue

site of establishment is specific to each schistosome species. *S. haematobium* establishes in the bladder, ureter and sometimes the rectal venules, *S. mansoni* establishes in the small and large intestines, and *S. japonicum* establishes more frequently in the small intestines (Abe, Guan, Guo, Kassegne, Qin, Xu, et al., 2018).



(Centers for Disease Control and Prevention [CDC], 2014)

Figure 2: Life cycle of the Schistosome

Clinical manifestation

Infection by schistosomes gives rise to various pathologies and manifestations. The first manifestation of cercarial skin incursion is dermatitis producing maculopapular eruptions (Kolárˇová, Horák, Skírnisson, Marecˇková

& Doenhoff, 2013.). The status of the disease can be described as either acute, also known as Katayama syndrome, or chronic or advanced schistosomiasis (Gray, Ross, Li & McManus, 2011).

Acute schistosomiasis (Katayama fever)

This occurs primarily from host immune responses to migrating schistosomula, maturing worms, production of eggs and release of egg antigens (Ross, Vickers, Olds, Shah & McManus, 2007). Acute schistosomiasis is largely asymptomatic in persons who live in endemic areas but may present symptoms such as malaise, fever, abdominal pain, headache and eosinophilia in persons who are infected for the first time and are immunologically naïve. (Ross, Olds, Cripps, Farrar & McManus, 2013). Infected persons may recover 2 to 10 weeks post infection. However, the disease may persist in some people with symptoms of abdominal pain, hepatomegaly, diarrhoea, generalized rash and weight loss (Ross, Olds, Cripps, Farrar & McManus, 2013). *S. haematobium is* a less common cause of Katayama fever in endemic areas than other schistosome species (Gryseels, 2012; Ross, Olds, Cripps, Farrar & McManus, 2013).

Chronic schistosomiasis

The chronic phase of the disease is attributed to a granulomatous inflammatory reaction to parasite eggs deposited in specific tissues and organs. The granuloma is made up of neutrophils, mononuclear cells, eosinophils, lymphocytes, macrophages, fibroblasts and multinucleated giant cells. In intestinal schistosomiasis, eggs are mainly deposited in the intestinal walls and liver. The resulting multiple granulomas formed in these tissues and organs can cause polyposis, abscess formation, intestinal mucosal hyperplasia, and

ulcerations. These manifest clinically as perirectal bleeding, abdominal pain, and chronic diarrhoea (Mohamed, al Karawi & Yasawy, 1990).

As the immune response wanes, collagen is deposited in place of granuloma. Uncontrolled collagen deposition can lead to excess collagen, causing fibrosis (Gryseels, 2013; Maguire, 2010). Granulomas and fibrosis are the main causes of mortality in schistosomiasis (Maguire, 2010). In the liver, the formation of granulomas from egg depositions can lead to periportal fibrosis and consequently portal hypertension and hepatosplenomegaly (Gray, Ross, Li & McManus, 2011). In urinary schistosomiasis, the chronic phase is characterized by egg deposition and granuloma formation in the walls of the bladder (Wamachi, Mayadev, Mungai, Magak, Ouma, Magambo et al., 2004). This manifests as dysuria and haematuria. Bladder calcification, urinary tract fibrosis leading to obstructive uropathy and bladder malignancies are possible complications (Hatz, Vennervald, Nkulila, Vounatsou, Kombe, Mayombana et al., 1998). In females, genital schistosomiasis caused by *S. haematobium* has been known to increase the risk of sexually transmitted diseases (Correia da Costa, Vale, Gouveia, Botelho, Sripa, Santos et al., 2014; Kjetland, Hegertun, Baay, Onsrud, Ndhlovu & Taylor, 2014). Genital schistosomiasis may also manifest as contact bleeding, pain and infertility (Kjetland, Leutscher & Ndhlovu, 2012). *S. haematobium* is also a known class 1 carcinogen (Correia da Costa, Vale, Gouveia, Botelho, Sripa, Santos et al., 2014; Kjetland, Hegertun, Baay, Onsrud, Ndhlovu & Taylor, 2014).

Schistosome eggs may also find their way into unconventional sites and cause site-specific complications. For example, eggs deposited in the central nervous system (CNS) give rise to neuroschistosomiasis (Härter, Frickmann,

Zenk, Wichmann, Ammann, Kern et al., 2014). Aside from the specific conditions, schistosome infections may also produce generalized morbidities such as anaemia, malnutrition, growth retardation and impaired development in children (King & Dangerfield-Cha, 2008). Age-specific intensities of the infection have been well noted. In *S. haematobium* infections, maximum intensities of the infection occur in children between 10 and 14 years of age. (El-Khoby, Galal, Fenwick, Barakat, El-Hawey, Nooman et al., 2000; Woolhouse, 1998).

Prevalence of urinary schistosomiasis

Schistosomiasis is one of the most important neglected tropical diseases, especially in Sub-Saharan regions such as Ghana. Worldwide, *S. haematobium* accounts for 200 million schistosome infections (Lengeler, Utzinger & Tanner, 2002). Children have been reported to be the most affected partly because they are more likely to engage in swimming activities in infected water bodies (van der Werf, de Vlas, Brooker, Looman, Nagelkerke & Habbenma, 2003; Cheesebrough, 2005). It is second only to malaria in terms of tropical disease prevalence (Lengeler, Utzinger & Tanner, 2002). As a disease whose spread is influenced by water (Shope, 1992), the recent spate of climate change has the ability to greatly influence its distribution. Consequently, the drying up of surface freshwater sources and the creation of lakes and rivers from melting ice have the potential to shift the distribution of the disease worldwide.

In Sub-Saharan Africa, schistosomiasis is estimated to cause 280,000 deaths every year (King, Dickman & Tisch, 2005). It is a pressing public health concern on the continent. In Ghana, the prevalence of *S. haematobium* infection was estimated to be about 70.9% in 2010 (Rollinson *et al.*, 2012. The focal

nature of the disease distribution means that while prevalence in certain parts of the country, such as communities along Volta Lake, may be high, communities in faraway savanna regions may present lower prevalence. Prevalence reports continue to change over time. In 1963, the prevalence of the disease in some selected communities was between 15 and 20% (WHO, 1987). This prevalence increased to over 60% in the following decade from 1970 to 1980 (WHO, 1987) and to an even higher national prevalence of 70.9% in 2010 (Rollinson *et al.*, 2012; Utroska *et al.*, 1989). The role of individual variation in community prevalence cannot be overemphasized. For example, the Ministry of Health reported that some communities along water bodies could record as high as 90% prevalence of the disease (Ghana Ministry of Health, 2008).

In a review of the incidence of schistosomiasis in the Ashanti region spanning 2000 to 2009, Tay, Amankwa and Gbedema (2011) reported a prevalence of 20.7%. Individual hospitals, however, recorded varying prevalences, such as 40.2% at the Kumasi South Hospital and 4.5% at the Aninwaah Medical Centre, all in the same region. These variations in focal prevalence have been attributed to the varying distribution of risk factors coupled with sociocultural and economic activities peculiar to various communities. These include the sources of water for domestic use, fishing, farming and swimming in surface water bodies and unsanitary conditions in these worst-hit communities (Tay, Amankwa and Gbedema, 2011).

Despite the long-held belief that schistosomiasis is a disease of rural communities, recent evidence shows that it is also prevalent in urban and peri-urban communities. A study in two selected communities in Ghana's capital revealed a 58% prevalence in Mahem, a community along the Weija dam, and

49% in Galilee, a community not close to the same dam (Aboagye & Edoh, 2009). In Nigeria, Okolie and Obaida reported a 17.5% prevalence of the disease in urban Ibadan (Okolie & Obaida, 1999). This highlights the role of a conglomerate of factors that account for the distribution of the disease and not just by virtue of a rural setting.

S. haematobium prevalence has also been shown to have prevalence variations with respect to gender and across seasons. In males, Nsowah-Nuamah, Mensah, Aryeetey, Wagatsuma & Bentil (2001) reported a 55.9% prevalence compared to 3.7% in females. Chimbari and Chirundu (2003) also reported a higher prevalence in Zimbabwean men than in women. These gender distributions are closely linked to the gender roles in African communities, where men are more likely to engage in farming, fishing and swimming than women who may only visit the waterside to draw water. According to Tay, Amankwa and Gbedema (2011), the disease shows seasonal variations in prevalence in the Ashanti region. While they reported a 5.9% to 15.4% prevalence in the drier season, they also reported a much lower prevalence of 6.8% and 9.0% in the rainy season. They also observed a pattern of the spike in infections every three years (in 2000, 2004 and 2008) over the decade-long period of their study. These seasonal variations are likely the result of the parasite's lifecycle and climatic and weather conditions that favour disease transmission.

Risk factors for schistosomiasis

Schistosoma infections are closely associated with contact with contaminated water, such as dams and irrigation sites. Kosinski, Adjei, Bosompem, Crocker, Durant, Osabutey et al. (2012) observed that

socioeconomic factors, nearness to safe and unsafe water sources for domestic use, activities involving contact with watersuch as farming in marshy areas, occupation and age, play crucial roles in disposing individuals to an increased risk of infection. With regard to demography, Agnew-Blais, Carnevale, Gropper, Shilika, Bail, & Ngoma (2010) indicated that age and sex were important variables with respect to the risk of infection. Adolescents aged 10 to 15 had a higher risk because of their likelihood of being involved in water-related activities such as swimming and drawing water from infected water sources. These findings were corroborated by Xue, Gebremichael, Ahmad, Weldu, and Bagtzoglou (2011), who found prevalences of 87.3% among children aged 8 to 11 yrs and 84.1% among children aged 12 to 14 yrs. With regard to sex, adolescent males were found to be more exposed to the risk of infection than females because of their behavioural tendencies to go swimming in infected water bodies (Kosinski *et al*., 2012; M'Bra, Kone, Yapi, Silué, Sy, Vienneau, et al., 2018). In another study by Nsowah-Nuamah, Mensah, Aryeetey, Wagatsuma and Bentil (2001), 55.9% of the infections were males, whereas 3.7% were females.

Proximity to freshwater sources also increases one's risk of being infected (WHO, 2008; Nkegbe, 2010). These waters serve as habitats for the intermediate snail host and stages of the parasite, such as the pupae and larvae. Zarkhary (1997) reported that communities living in and around the Akosombo Dam, built on Volta Lake, saw a rise in prevalence of the infection from 5% in 1964 before the daming to between 80 – 90% prevalence of infection after the daming of the lake in 1971. The growth marches around these water bodies provide breeding grounds to support the life cycle of snail hosts. Other forms of

water bodies include shallow shores and drainage systems containing agricultural run-offs (Zakhary, 1997). Kapito-Tembo, Mwapasa, Meshnick, Samanyika, Banda, Bowie, et al. (2009) and Ugbomoiko, Ofoezie, Okoye, and Heukelbach (2010) observed that there were increased risks in communities with contaminated rivers than communities without rivers at all. Various forms of farming, such as irrigation farming, fish farming and fishing, have been noted to increase the risk of infection (Ugbomoiko, Ofoezie, Okoye, and Heukelbach, (2010); Nkegbe, 2010; WHO, 2010). The levels of exposure to the parasite among these groups of people are high since they spend several hours in these water sources.

Overcrowding and poor standards of living also pose a risk to *Schistosoma* infections (Watts, 2005). Overcrowding is known to be associated with poor sanitation, low standards of living, malnutrition and exposure to various disease risk factors. Poor drainage systems are a common thing in overcrowded settlements. These coupled with the sanitation challenge provide a good ground for the transmission and spread of schistosome parasites across settlements. Movements of people to areas of high prevalence of the infection expose them by increasing their likelihood of getting in contact with the parasite. (Mac-Pherson et al. 2007; Aagaard-Hansen, Nombela & Alvar, 2010).

Diagnosis of Schistosomiasis

Schistosomiasis can be cured based on the ability to accurately diagnose and promptly treat the infection with the right doses of the recommended drugs. It is therefore imperative to employ diagnostic techniques with high sensitivities and specificities to correctly diagnose the disease. In the quest to achieve high sensitivity and specificity in diagnosing infections caused by the parasite,

various techniques have been developed over time, ranging from relatively simple microscopic methods to more complex molecular techniques. These methods used in the diagnosis of schistosomiasis are grouped and discussed below as (i) microscopic diagnosis, (ii) immunological diagnosis, and (iii) molecular diagnosis.

Parasitological diagnosis

The first parasitological diagnostic technique developed involved the direct observation of parasite eggs in the stool or urine of infected persons for intestinal and urinary schistosomiasis, respectively, by microscopy. Several microscopy-based techniques and modified techniques have been developed. However, the Kato-Katz stool smear technique is still the gold standard recommended by the WHO for the quantitative assessment of infection intensity and the diagnosis of intestinal Schistosoma infections (WHO, 2013). This procedure is relatively simpler, less expensive, comparatively less labour intensive and boasts of high specificity. Because of these advantages, it is readily employed in field conditions. Kato-Katz test does not go without its weaknesses. First, the sensitivity of the test is high in persons with high loads of worms and vice versa. As a result, the test may not give accurate results when used in low prevalence areas or among persons with low worm intensity. Additionally, the daily variation in the parasite's egg output (Degarege, Legesse, Medhin, Teklehaymanot & Erko, 2014) may not provide a true reflection of parasite density. Eggs may also clump together and thus be misread, leading to underreporting of parasite density. To address the concerns associated with the sensitivity of the Kato Katz test, the true parasite burden can be estimated as the average of multiple Kato Katz tests over several days

(Engels, Sinzinkayo & Gryseels, 1996). Multiple testing however also comes at extra cost. Other methods include formol-ether sedimentation, salt flotation and centrifugation, the interaction of magnetic microspheres with eggs, and the miracidium hatching test (Engels, Sinzinkayo & Gryseels, 1996). These techniques have several drawbacks that make them either not standardized or out of reach for routine use.

Aside from the detection of parasite eggs, urinary schistosomiasis is also diagnosed with the help of urinalysis. Schistosomiasis is associated with proteinuria and haematuria (Morenikeji, Quazim, Omoregie, Hassan, Nwuba, Anumudu, et al., 2014: Bogoch, Andrews, Dadzie Ephraim & Utzinge, 2012: Ogbonna, Dori, Nweze, Muoneke, Nwankwo & Akputa, 2012). The detection of haematuria may be attributed to the boring activities of the parasite eggs in the urinary bladder and urethra. Proteinuria may result from the leakage of proteins through the renal system as a result of parasite activities.

Immunological diagnosis

Schistosoma infection elicits an immune response over time relative to the time of infection. Immune markers (antischistosomal antibodies) released in response to parasite antigens can be measured in serum, plasma, urine or sputum as indicative of schistosomiasis. Parasite products such as antigens may also be measured in serum, plasma, urine or sputum to diagnose schistosomiasis. With the reduction in parasite densities in many endemic regions because of mass praziquantel administration, parasitological methods are less sensitive. Immunological techniques are more useful in places where parasitological methods give false negatives for persons with light infection (Alarcón de Noya, Ruiz R, Losada, Colmenares, Contreras, Cesari & Noya, 2007). Immunological

methods are also less laborious and more rapid and thus able to be used in point-of-care facilities, endemic regions and field conditions. They are also useful in surveillance and community screenings, especially in places with low infection rates. Various immunological diagnostic methods have been developed and include intradermal tests, antigen detection tests, and antibody detection tests. Antibody tests for schistosomiasis include the circumoval precipitin test (COPT), indirect hemagglutination test (IHT), indirect immunofluorescence assay (IFA) and ELISA.

Intradermal tests were part of the earliest immunological diagnostic tests that were developed to diagnose schistosomiasis. The test involved injecting egg, larval or adult worm antigens into the skin and observing skin reactions (Hunter, Yokogawa, Akusawa, Sano, Araki & Kobayashi, 1982: Keittivuti, D'Agnes, Keittivuti & Viravaidya, 1982.). Although simple and cost-effective, this test has been found to produce false-positive results (Zhu, 2005).

Schistosomal antigen detection from eggs, schistosomula and adult worms has been developed to diagnose schistosomiasis. Antigens such as adult worm antigens (AWA), soluble egg antigens (SEA) and circulating antigens are commonly detected in blood, urine or sputum (Weerakoon et al., 2015). Measurement of circulating cathodic antigen (CCA) and circulating anodic antigen (CAA) has proven to be effective diagnostic markers (Stothard, 2009; Barsoum, Colley & Kamal, 1990; Van Lieshout, Polderman & Deelder, 2000). These tests are based on the principle that worms release genus-specific proteoglycan antigens in the gut epithelium of the host. The measurement of these antigens, therefore, is indicative of infection. Since CCA and CAA are detectable in blood three weeks postinfection, they can be used to detect the

parasite in its earlier stages against parasitological methods that can detect the parasite 4-6 weeks postinfection for intestinal schistosomiasis and 90 days postinfection for urinary schistosomiasis (Barsoum, Colley & Kamal, 1990: Van Dam, Bogitsh, van Zeyl, Rotmans & Deelder, 1996). Rapid diagnostic test kits have been developed to detect CCA in urine for *S. mansoni*. Although these rapid diagnostic kits are easy and more efficient in screening for *S. mansoni* in medium to high endemic regions, they have limitations. For example, kits are limited in detecting *S. haematobium* antigens. Additionally, it has been observed that the sensitivity of the test kit is associated with the endemicity of the parasite. That is, the kit is more sensitive in areas of high endemicity of *S. mansoni*. Therefore, CCA test kits for *S. mansoni* in low endemic regions may give false positive results (Colley, Binder, Campbell, King, Tchuem Tchuenté, Goran, 2013: Coulibaly, Gbesso, Knopp, Guessan, Silué, van Dam & Goran, 2013: Tchuem Tchuenté, Kueté Fouodo, Kamwa Ngassam, Sumo & Dongmo Noumedem, 2012: Van Dam, Odermatt, Acosta, Bergquist, de Dood & Kornelis, 2015). Urinary CCA levels also fluctuate on a day-to-day basis (Stothard, Kabatereine, Tukahebwa, Kazibwe, Rollinson & Mathieson, 2006). An accurate measure of CCA levels will be an average of CCA measurements over several days to reflect the daily changes. Overall, antigen detection techniques have been employed to diagnose acute schistosomiasis. However, these techniques are not readily used in clinical diagnosis (Gray, Ross, Li & McManus, 2011 Van Dam, Xu, Bergquist, de Dood, Utzinger & Qin, 2015: Ochodo, Gopalakrishna, Spek, Reitsma, van Lieshout & Polman, 2015: Van Lieshout, Polderman, Visser, Verwey & Deelder, 1997).

The detection of antibodies produced against antigens of the various stages of the parasite has been used as a diagnostic method over the years. Most antibody detection techniques have been developed using *S. mansoni* antigens. These tests have been applied to diagnose *S. haematobium* and *S. japonicum* infection (Smith, Doenhoff, Aitken, Bailey, Dawson & Gilis, 2012: Van Gool, Vetter, Vervoort, Doenhoff, Wetsteyn & Overbosch, 2002: Hamilton, Klinkert & Doenhoff, 1998). These applications, however, come with reduced sensitivity (Tsang & Wilkins, 1997). Some of these antibody detection methods include the Circumoval precipitin test (COPT), Cercarien Hüllen reaction (CHR), indirect hemagglutination test (IHT), indirect immunofluorescence assay (IFA), and enzyme-linked immunosorbent assays (ELISA).

COPT is based on the principle that lyophilized schistosome eggs, when introduced into a patient's serum, will cause precipitation. COPT is a labour intensive, complex and lengthy procedure that may come with misdiagnosis if not well handled. Nonetheless, its high sensitivity and specificity are arguably among the best (Zhu, 2005, Alarcón de Noya, Ruiz, Losada, Colmenares, Contreras & Cesari et al, 2007, Hillyer, Ruiz Tiben, Knight, Gómez de Rios, 1979.).

Cercarian Hüllen reactions are similar in principle to COPT. Live cercariae are mixed with patient serum. The formation of precipitates and the visible immobilization of larvae are indicative of a positive test. This test, however, is sparingly used now (Ahmed, Hussein & El-Hady, 1993: Smit, 1961)

An indirect hemagglutination test (IHT) is designed to detect the reaction between antibodies in patient serum against schistosomal antigen-

coated red blood cells. IHT remains positive several years after successful treatment of the infection (Yu, de Vlas, Jiang, Gryseels. 2007: Zhou, Yang, Wang, Zhao, Wei, Peng & Jiang, 2007: Sorgho, Bahgat, Poda, Song, Kirsten & Doenhoff et al., 2005). Despite these limitations, IHT is relatively simple and sensitive and has been used in large-scale community screenings (Zhou, Yang, Wang, Zhao, Wei, Peng & Jiang, 2007: Sorgho, Bahgat, Poda, Song, Kirsten & Doenhoff et al., 2005).

IFA detection of IgM and IgG antibodies in acute and chronic schistosomiasis is one of the antibody diagnostic methods (Kanamura, Dias, Da Silva, Glasser, Patucci, Vellosa, Antunes, 1998: Burlandy-Soares, de Souza Dias, Kanamura, de Oliveira & Ciaravolo, 2003: Kanamura, Hoshino-Shimizu, Camargo, da Silva, 1979). It involves the reaction between parasite antigens and anti-parasite antibodies. The test is performed using adult worms, cercariae and eggs (Azab, Safer & Ghaffar, 1984: Kolarova, Sykora & Bah, 1994: Kamiya, Suzuki, Matsuda & Tanaka, 1982). IFA techniques are relatively expensive to set up and run (Burlandy-Soares, de Souza Dias, Kanamura, de Oliveira & Ciaravolo, 2003).

ELISA techniques generally involve the measurement of the reaction between anti-schistosomal antibodies and antigens from the parasite. The most commonly used antigens include AWA, SEA, and larval antigens (100: Sarhan, Aminou, Saad, Ahmed, 2014: Lunde & Ottesen, 1980: McLaren, Draper, Roberts, Minter-Goedbloed, Ligthart, Teesdale et al., 1978). To reduce cross-reactivity with other helminthic infections, purified parasite antigens such as cationic fraction 6 (CEF-6) of eggs (Dunne, Bain, Lillywhite & Doenhoff, 1984: Doenhoff, Butterworth, Hayes, Sturrock, Ouma, Koech et al., 1993) have been

developed for use in ELISA. A major drawback of ELISA, the time-consuming nature of the test, is being addressed by newer and modified ELISA techniques such as Falcon Assay Screening Test (FAST) ELISA and Fraction Antigen (FA) ELISA (Tsang, Hillyer, Noh, Vivas-Gonzalez, Ahn, Pilcher et al., 1997; Abdel-Fattah, Al-Sherbiny, Osman, Charmy, Tsang, 2011).

Molecular methods

Genetic markers unique to schistosomes can be detected in the blood, stool, urine and other body fluids of infected persons. The measurement of these markers is used as a diagnostic tool in investigating schistosomiasis. Schistosome DNA or RNA can be detected using polymerase chain reaction (PCR) techniques. Other modern molecular diagnostic techniques include the detection of egg DNA, circulating cell-free parasite DNA (CFPD), and circulating microRNAs (miRNAs) (Weerakon et al, 2015).

Detection of schistosomal DNA or RNA: Conventional PCR methods have been developed to amplify specific portions and detect parasite DNA in the stool (Gordon, Acosta, Gray, Olveda, Jarilla, & Gobert, 2012: Pontes, Oliveira, Katz, Dias-Neto, Rabello, 2003). For low endemic communities, conventional PCR is more sensitive than routine microscopy (Pontes, Oliveira, Katz, Dias-Neto & Rabello, 2003: Pontes, Dias-Neto & Rabello, 2002: Sandoval, Siles-Lucas, Pérez-Arellano, Carranza, Puente, López-Abán et al., 2006: Oliveira, Santos, Gonçalves, Barreto & Peralta, 2010). Mitochondrial gene segment amplification and detection have also been shown to provide very sensitive results (Lier, Johansen, Hjelmevoll, Vennervald & Simonsen, 2008, Ibironke, Phillips, Garba, Lamine, Shiff, 2011). Over the years, conventional PCR has been improved by the introduction of newer PCR methods, such as

restriction fragment length polymorphism PCR (PCR-RFLP), droplet digital PCR (dd-PCR), multiplex PCR and real-time PCR (qPCR). PCR tests come with huge reagent costs and the requirement of specialized personnel to man the machines. PCR results can corroborate parasitological and serological test results (Sørensen, Bøgh, Johansen & McManus, 1999).

Detection of cell-free parasite DNA in serum and other body fluids: Cell-free parasite DNA (CFPD) is released into serum from tegument shedding worms, dead schistosomula and disintegration of inactivated eggs (Xu, Liu, Guo, Wang, Qiu & Sun, 2013). Unlike schistosome eggs in stool and urine, CFPD is uniformly distributed in plasma. CFPD is also excreted in saliva (Kato-Hayashi, Yasuda, Yuasa, Isaka, Haruki, Ohmae et al., 2013), urine (Kato-Hayashi, Leonardo, Arevalo, Tagum, Apin, Agsolid et al., 2015), cerebrospinal fluid, and other body fluids (Pontes, Dias-Neto & Rabello, 2002). As early as the first week post-infection, PCR-based techniques can be used to detect CFPD in serum (Kato-Hayashi, Kirinoki, Iwamura, Kanazawa, Kitikoon, Matsuda & Chigusa, 2010). CFPD detection in urine and saliva presents non-invasive methods of diagnosis. Nonetheless, like any other PCR-based method, CFPD detection is relatively expensive and requires much expertise to operate (Weerakoon, 2015).

Loop-mediated isothermal amplification: This is a one-step amplification method in which a large pyrophosphate iron by-product reacts with divalent metallic iron ions to form an insoluble salt complex. Manganous iron and calcein were added to visualize the alterations in fluorescence. The naked eye can be used to detect the test endpoints with no need for electrophoresis equipment (Tomita, Mori, Kanda & Notomi 2008). LAMP is a

very sensitive, cost-effective and feasible alternative to PCR techniques. LAMP, however, may produce cross-over contaminations (Tomita, Mori, Kanda & Notomi 2008).

Detection of circulating miRNA: This method is still largely under development. The identification and characterization of miRNAs (a group of noncoding RNAs (Bartel, 2004)) is the principle on which this test is built (De Souza Gomes, Muniyappa, Carvalho, Guerra-Sá & Spillane, 2011: Simões, Lee, Djikeng, Cerqueira, Zerlotini & da SilvaPereira, 2011: Simões, Lee, Djikeng, Cerqueira, Zerlotini, da SilvaPereira et al., 2011). miRNAs are detectable in several body fluids, including plasma and serum. In both high and low parasite endemic communities, detection of circulating miRNA is noted for high sensitivity and specificity (Hoy, Lundie, Ivens, Quintana, Nausch, Forster).

Other molecular diagnostic methods include the detection of cytokine biomarkers, metabolic markers, and other antigens and proteins, as diagnostic methods have been developed and are under investigation.

Immunity to schistosomiasis

The different stages of the life cycle of the parasite evoke different humoral and cellular immune responses to the various antigens peculiar to each stage of the parasite life cycle. In chronic infections, for example, while some of the responses are downregulated, others are upregulated steadily (Colley & Secor, 2014). Various clinical and epidemiological studies have shown that after years of exposure to the parasite, people who live in endemic regions develop some form of immunity against the parasite (Gryseels *et al* 2006). The development of protective immunity against the transformation of schistosomula to adult worms is slow. This is, however, hastened by the death

of the worm (either naturally or by the use of praziquantel) (Colley & Secor, 2014). Dying worms release immunogens that stimulate the release of immune protective responses. These responses over time can mount a strong immune response against invading schistosomula and thus prevent infection (Fitzsimmons, Jones, Pinot de Moira, et al., 2012: Walter, Fulford, McBeath, et a., 2006). Maximum protection of a Schistosoma-infected person is mediated by both humoral and cell-mediated immune responses.

The immune response against soluble egg antigens (SEAs) appears high at the onset of infections and gradually decreases as the infections become chronic. In contrast, immune responses to soluble worm antigenic preparations (SWAP) increase at the onset of infection and maintain steady levels throughout infection (Colley, Cook, Freeman Jr, Bartholomew & Jordan, 1977: Barsoum, Gamil, Al-Khafif, Ramzy, El Alamy & Colley, 1982: Colley, Garcia, Lambertucci, et al., 1986: Grogan, Kremsner, Deelder & Yazdanbakhsh, 1998: Joseph, Jones, Kimani, et al., 2004: Caldas, Campi-Azevedo, Oliveira, Silveira, Oliveira & Gazzinelli, 2008).

During the first 4-5 weeks after exposure to cercariae, the immune response is tailored towards the Th1 response directed against worm antigens. However, when schistosome eggs are produced during infection, the immune response leans towards the Th2 response (Zhang & Mutapi, 2006). The Th2 response delivers circulating IgE, which is activated by IL-4 and IL-13. This causes the degranulation of mast cells, eosinophils and basophils, thereby releasing toxic granular content onto the surface of worms. This creates pores in worms and subsequently kills the worms (Zhang & Mutapi, 2006).

In the event of reinfections, parasite-specific IgE and eosinophils have been known to be positively associated with resistance against reinfections. This does not apply to all immune responses. IgG4 is associated with susceptibility to reinfections. It has been proposed that IgG4 inhibits the action of IgE by serving as a blocking antibody (Hagan, Blumenthal, Dunn, Simpson & Wilkins, 1991: Rihet, Demeure, Bourgois, Prata & Dessein, 1991: Demeure, Rihet, Abel, Ouattara, Bourgois & Dessein, 1993: Satti, Lind, Vennervald, Sulaiman, Daffalla & Ghalib, 1996: Zhaosong, Haiwei, Suchen, et al., 1997: Oliveira, Figueiredo, Cardoso, et al., 2012). The relative difference between children and adults in the secretion of IgG4 and IgE correlates with their susceptibility and resistance to reinfections, respectively. In adults, treatment of schistosomiasis causes an increase in the maintenance of pretreatment levels of IgE and a decrease in IgG4 levels. This correlates with improved chances of resisting reinfection. In children, however, treatment does not significantly affect IgG4/IgE ratios (Colley & Secor, 2014). Infection and treatment of schistosomiasis also affect the cellular (cytokine) immune response. After treatment with praziquantel, IL-4 and IL-5 levels aregenerally increased. These cytokines are associated with stimulation of IgE and eosinophil production (Roberts, Butterworth, Kimani, et al., 1993: Scott, Turner, Mutapi, et al, 2000: Joseph, Jones, Laidlaw, et al, 2004: Joseph, Jones, Walter, et al., 2004: Fitzsimmons, Joseph, Jones, et al., 2004: Grogan, Kremsner, Deelder & Yazdanbakhsh, 1996). Resistance to reinfection has been associated with these responses. Additionally, following treatment with praziquantel, IFN-γ and IL-10 production have been associated with susceptibility to reinfections (Shen, Zhang, Wu, et al., 2003: van den Biggelaar, Borrmann, Kremsner &

Yazdanbakhsh, 2002). This is further explained by the fact that IL-10 is associated with IgG4 production (van de Veen, Stanic, Yaman, et al., 2013). These findings are corroborated by findings in mice that blocking IL-10 receptors during treatment can protect against reinfections (Wilson, Cheever, White, Thompson & Wynn, 2011). Other studies have shown that whereas IgG4, IgG2 and IgM are associated with susceptibility to reinfection, IgE and IgG1 are protective and offer resistance to reinfections (Naus *et al*, 1998; Odongo-Aginya *et al*, 2012). IgG4, IgG2 and IgM act as antibody receptor blockers, thus inhibiting the protective activity of IgE and IgG1.

Prevention and control

Schistosomiasis negatively impacts the environmental, economic and sociopolitical life of countries. Reducing schistosomiasis is therefore not only a public health concern, as it has attracted both national and international attention from the World Health Organization and others who have devoted resources to fight against the disease. Since schistosomiasis is transmitted primarily through freshwater infected with the parasite, the main preventive measure is to avoid contact with such infested water. Some of these preventive measures include vigorous towel drying in the event of exposure to contaminated water; boiling water for at least one minute before drinking or bathing; allowing water to stand for at least 24 hrs before using it; filtering out cercariae from infected water using fine-mesh filters before use and applying insect repellants such as DEET (N, N-Diethyl-meta-toluamide) on the body may also prevent cercariae from penetrating the skin (Montgomery, 2014).

Schistosoma endemic countries have been formulating and implementing policies to eradicate the disease from their countries. Among the

various control measures being implemented across several nations include chemotherapy, vaccine development, genetic manipulations, vector control and public education.

Chemotherapy

The World Health Assembly in 2001 proposed resolution 54.19 to backed the use of chemotherapy through mass drug administration as the main control tool against schistosomiasis. This resolution, however, did not meet its 75%–100% regular chemotherapy coverage for school-aged children (aged 5–14 years) at risk of morbidity target by 2010(1). Of the 51 countries where preventive chemotherapy against schistosomiasis should have been applied by 2010, only 21 million children out of over 108 million children in 28 out of the 51 countries received treatment (WHO, 2012). Mass drug administrations are aimed at reducing the morbidity and mortality related to Schistosoma infections and preventing infections by limiting the reservoirs from which the parasite can be transmitted (Humphries, Nguyen, Boakye, Wilson, Capello, 2012). In essence, by reducing the overall prevalence in a given population, mass drug administration helps to reduce the incidence of new infections of the parasite. A reduction in the number of infected persons will translate into a reduction in the passing out of parasite eggs in stools and subsequently reduced transmission.

Mass drug administration is recommended for all school-aged children and adults who live in high-risk communities where the prevalence of infection is at least 50%, as detected by parasitological methods, or urinary schistosomiasis has a prevalence of 30% or higher based on the history of hematuria. In moderate-risk communities, school-aged children and those in special risk groups such as fishermen should be given a preventive dose every

two years. In low-risk communities, however, only school-aged children should be given preventive chemotherapy (Inobaya et al., 2014). The drug of choice in mass drug administration is praziquantel, a safe and relatively inexpensive drug that has proven to have a significant impact on schistosomiasis prevalence and intensity (Fenwick & Webster, 2006). Issues of non-compliance with treatment, huge cost of mass chemotherapy for large populations, and possibilities of developing drug tolerance and resistance have plagued the use of the drug as a treatment for schistosomiasis (Gray, McManus, Li, Williams, Berqquist & Ross, 2010; Tallo, Carabin, Alday, Balolong, Olveda & McGarvey, 2008; Humphries, Nguyen, Boakye, Wilson & Capello, 2012).

Vector control

Another preventive measure for schistosomiasis is vector control. The snail vector is crucial to the life cycle of the parasite. As a result, methods to eliminate the vector will terminate the life cycle of the parasite, thereby breaking the transmission cycle. Studies in China have shown that 50% and 4% formulations of niclosamide ethanolamide salt wetted powder produced 88% and 93% snail mortality, respectively, in snail populations (Yang et al., 2010). Since no single application of niclosamide produced 100% snail mortality, it indicated that a repeated dose was needed to eliminate all snails in the population. This comes at an extra cost and time requirement, making this method not economically feasible for some nations. Other methods, such as burying snail habitats, flooding snails with water up to several meters in depth, and digging ditches or water drainage tunnels, have been used in some jurisdictions as vector control methods (Sleigh, Li, Jackson & Huang, 1998).

Education

In addition to mass drug administration and snail vector control, schistosomiasis control also requires public education. The World Health Assembly resolution 54.19 also recommended public education, among others, as ways of controlling the infection (WHO, 2013). Access to safe drinking water, sanitation and hygienic practices are important in the fight against Schistosoma infections. Public education holds the key to encouraging these positive attitudes and discouraging negative behaviours such as defecating in water bodies, swimming and fishing in schistosome-infested waters. Public education should be complemented with the provision of basic social amenities and better alternatives for the things that education is advising against. In Ghana, for example, the provision of a swimming pool for school children as an alternative to swimming in the local river significantly reduced the incidence of new infections among the students (Kosinski et al., 2012; WHO, 2011).

Vaccine development

Although at present, there is no vaccine against schistosomes, a vaccine could greatly reduce transmission and illness spectrum (Reta & Erko, 2013; Borrmann et al., 2001). Several vaccine candidates have been proposed, with some still under investigation. A cysteine peptidase-based vaccine was proposed by Tallima et al. (2015) against *S. haematobium.* Studies by You et al. (2018) and You et al. (2018) showed that SjAChE and rSjLD1 could be vaccine or drug candidates against schistosomes. Many more vaccine candidates are still under investigation. However, to date, no vaccine has been approved for use against schistosome infections (Tebeje, Harvie, You, Loukas, McManus, 2016).

Genetic manipulations

The prospects of genetic manipulations could present novel and important measures in the fight against schistosomiasis. Techniques such as the clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) system, adeno-associated virus (AAV) and end-joining homology techniques (EJHTs) provide prospects for future control measures (Nelwan, 2018). In experimental mice, He et al. (2018) showed that rAAV mediated miR-203-3p protection of mice from schistosome infections and assuaged hepatic pathology. The CRISPR/Cas9 system and EJHTs were engineered to edit incorrect genes. These tools can be of significant use in the development of control mechanisms of schistosomiasis.

Chapter Summary

This chapter discussed the empirical literature relevant to the research topic. Epidemiologically, Africa remains the continent with the highest prevalence of infection. Factors such as proximity and activities around freshwater sources such as dams increase one's risk of infection. Once a person is infected, the parasite goes through two major cycles as part of its life cycle: the definitive host in humans and the intermediate host in the snail host. Schistosome infections may either produce an acute infection and/or a chronic infection. In either case, the infection can be treated with praziquantel, a WHO-recommended chemotherapy. Other methods of control and prevention of infection include avoiding contact with infected water, prophylactic chemotherapy, snail vector control, genetic modifications of the parasite, vaccine development and public education.

CHAPTER THREE

MATERIALS AND METHODS

Introduction

The present chapter presents the research instruments, materials, and methods used in the study of the study objectives. It describes the study design, study site, study population and sample size, inclusion and exclusion criteria, the sampling technique and data collection procedures and the laboratory protocol. It also discusses the data management and analysis plan and the ethical considerations that went into the conduct of the research.

Study Design

This community-based cross-sectional study was conducted in the Adansi South district from January to July 2021.

Study Site

The study was conducted in the Adansi South District of the Ashanti region of Ghana. The Adansi South District, created by Legislative Instrument 1752 and Act 462, 1993, is located within latitude 40" north and 6 degrees 22" north and longitude 1 degree west and 1 degree 38" west. It is bordered to the north by the Obuasi Municipality, to the south by the Assin districts and to the east by the Birim district. To the west, the district shares borders with the River Offin and the River Pra to the east.

The district lies within the semi-deciduous forest egological zone. The district is largely an agricultural settlement, with approximately 73% of its inhabitants engaged in commercial and subsistence farming activities (Population and Housing Census, 2010). Common crops produced in the area include cocoa, oil palm and rice. The heavy amounts of rain in this area provide

enough moisture and marshy areas to support the cultivation of foodstuffs such as rice. The natural vegetation, presence of rivers and other waterbodies and animal life (i.e., snails) coupled with farming activities makes this area a haven for the transmission of schistosomiasis. Six (6) communities, namely, Asarekrom, Owusukrom, Menang, Tensuani, Dotom and Bronikrom, within the Adansi South District were selected for this study.

Study Population

The study population included persons aged 5 to 70 yrs living in the study communities. The risk of schistosomiasis is linked to the kind of water the community uses and the daily interactions with freshwater bodies in the community. Fishing in these water bodies exposes inhabitants to snail-infested freshwater. Crop farming, such as rice farming, also exposes farmers to marshy fields that could harbour freshwater snails, the intermediate host of schistosome species. Although many people in the community may be exposed by their use of untreated river water for domestic and agricultural activities, persons at most risk are those of active age, i.e., 5 to 70 yrs who may swim or farm in and/or near snail infested waters. This study population also covers children of school-going age who are likely to engage in swimming activities and adults who are likely engaged in farming and other related activities.

Sample size

The sample size was calculated using the formula:

$n = NZ^2 P(1-P) / d^2 (N-1) + Z^2 P(1-P)$

Where n = Sample size with finite population

N=Population Size (115,378, (PHC, 2010))

Z= Z statistic for a level of confidence [confidence level at 95% (1.96)]

P= Estimated prevalence (50%)

d=Precision [margin of error at 5%(0.05)] (Daniel, 1999).

The minimum sample required for the study was 381.6. A contingency of 5% was calculated of the 381.6 (which gave 19) to cater for drop-offs or researcher withdrawal of participants based on conditions identified in the course of the study. Thus, a total sample size of 400 was used in this study.

Inclusion Criteria

The participants included all individuals aged 5 to 70 years who resided in Adansi South district. Participants with no history of praziquantel use in the past six months were enrolled. Praziquantel, a known anti-schistosomiasis drug, influences immune responses to the parasite. Participants who had not used the drug were included in this study to assess the immune epidemiology of schistosomiasis among the population.

Exclusion criteria

The study excluded inhabitants of selected communities who were 71 years and above or below the age of 5 years. Individuals who did not reside in the study area during the study period were excluded. Additionally, persons who were not sure of their stay within the study area for the period were excluded. This was to reduce the likelihood of loss to follow-ups. Also, participants with other helminthic infections were excluded from the immunological study.

Sampling technique

The study employed purposive and random sampling techniques to recruit and sample participants. Purposive sampling allows the researcher to

intentionally select a participant that will suit his research interest. It reduces the waste of resources in sampling. Random sampling, on the other hand, allowed the researcher to select participants without any bias whatsoever. Purposive sampling was employed to select the communities from the district. Based on data available at the district health directorate, six communities within the district with the highest prevalence of Schistosoma infections in recent years were selected. The study employed a random sampling technique to recruit participants from the selected communities. Of the 406 participants that took part in the study, the sample required from each community was calculated as a function of the ratio of its population relative to the other selected communities.

Data collection instrument and administration

A pre-tested structured questionnaire was used to collect participant information. The questionnaires avoided personal questions. Participants were allowed to answer candidly as much as they could. The questionnaire was administered in the language of the participants' choice. The first part of the questionnaire sought to obtain demographic information. This information included name, sex, gender, educational status and marital status. These data allowed for the interpretation of the research findings with respect to the independent variables. The second part of the questionnaire was used to obtain information about participants' exposure to the various risk factors. These factors included the source of water for domestic activities and exposure to fresh water in rivers and marshy farmlands, among others. The final part of the instrument acquired data about the participants' exposure to praziquantel and/or other forms of treatment of schistosomiasis. Immune responses to

schistosomiasis are closely related to the treatment of the infection. Treatment history also provided an idea of prior infections.

Data collection Procedures

The research team conducted a community entry exercise through the chiefs and opinion leaders. The study was explained clearly in the local language to each participating community. After entry, the research team recruited participants who met the inclusion criteria. The team moved around each community, from house to house to sample participants. In each house, the researcher endeavoured not to recruit more than two people from the same close-knitted house since the characteristics and exposures from such persons may be almost the same. This was to allow for a distribution of the sample across the whole community. The research team explained the details of the study to prospective participants or their guardians in a language of their choice. Only persons who consented to participate in the study were registered for the study.

Upon consent, pre-labelled urine containers with the age, sex, date and participant's unique identification code were given to the participant to produce a clean-catch midstream urine sample. On receiving the samples, they were kept in an ice chest bag at approximately 4 – 6°C and sent to the laboratory within 6 hrs for macroscopic and microscopic examination. Stool samples were also collected from all the participants recruited for the immunoepidemiological study to check for the presence of other helminths by microscopy. All those who tested positive for other helminths were excluded from the immunological study.

Once the infection status of the participants had been established by urine microscopy, five (5) milliliters of venous blood was collected to measure

the association between Schistosoma infection and hematological parameters as well as the immune response. Two (2) milliliters was dispensed into EDTA tubes for hematological analysis, while the remaining blood was dispensed into gel separator tubes to obtain serum. Plasma and serum samples were stored at --80°C and frozen until ready for laboratory analyses.

Laboratory analyses for urine and stool samples

Sedimentation technique

Each urine sample collected was homogenized, and 10 ml was transferred into a new 50 ml tube using a Pasteur pipette. The samples were centrifuged at 1000 rpm for 5 minutes, and the supernatant was carefully decanted, leaving a precipitate at the bottom of the tube. Each sample precipitate was transferred to a slide with a Pasteur pipette and covered with a coverslip. The prepared slides were then examined for schistosome eggs using X10 and X40 objective lenses of an Olympus CX21FS1 (Japan) light microscope. Eggs observed were identified morphologically using the position of their spines to differentiate into species where necessary.

Stool sample preparation using Saline emulsification method

A few drops of saline were added to a small amount of stool in a container, and the solution was mixed. Few drops of the resulting solution were placed on a glass slide and covered with a coverslip. The slide was observed under a microscope using a low-power X10 objective lens. Upon observation of a suspicious helminthic egg, the X40 objective lens was used for clearer identification.

Haematological analysis

Blood samples collected in EDTA tubes were immediately analysed using an Auto hematology analyser mindray BC-2800 (Japan). Details of the

participants, such as age, sex, gender and study code, were entered into the analyser's system. Briefly, the participant's sample was shaken gently on the mixer rollers and fixed in place for the machine to aspirate the sample for analysis. The analyser uses the electrical impedance method to count each blood cell group and the cyanide-free method to measure haemoglobin in the blood. The results of the analyses were expressed in the various units of the individual parameters and a result sheet printed out from the machine.

Antibody Assays

Crude Schistosoma haematobium egg extraction

S. haematobium eggs were suspended in 4°C 1X PBS at a concentration of approximately 500 eggs/ml. The eggs were homogenized on ice using a prechilled homogenizer. The solution was frozen in a cryovial tube in a -80°C freezer, thawed and homogenized 3 times. When approximately 95% (or more) of the eggs were shredded/disrupted, the crude mixture was centrifuged at 4°C at 14000 rpm for 60 minutes. The supernatant was collected and sterilized by passing it through a 0.2 µm filter. They were then aliquoted into 2 ml cryovial tubes and stored at -80 °C until ready to be used for the ELISA analysis.

S. haematobium-specific antibody reactivity in sera

Sera from the participants were screened for *S. haematobium*-specific antibodies by indirect ELISA as described Geiger *et al.*, (2011). Briefly, ELISA plates (Nunc, Maxisorp, Fisher Scientific, USA) were coated with 3.0 µg/ml (100 µl) of S. *haematobium egg* antigen diluted in PBS buffer (137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, KH_2PO_4, pH 7.2-7.4) and left overnight at 4°C. The plates were washed 4 times with washing buffer (phosphate-buffered saline [PBS]/0.1% Tween-20; pH 7.2–7.4) and were then blocked for 1 hour at room

temperature (RT) with 200 ml of blocking buffer (PBS/0.1% Tween-20/5% skim milk). The plates were tapped on a pad and washed 4 times in washing buffer (for each washing step, the plates were filled with washing buffer for 1 minute before they were emptied). Individual test and positive control serum samples were diluted in PBS/0.1% Tween-20/2.5% bovine serum albumin buffer (1:100 for IgG and IgM), and 100 µl were added in duplicate to the respective wells, and plates were incubated for 2 hours at room temperature. A pool of hyperimmune serum samples was titrated two-fold downward with a starting dilution of 1:50, 100 µl was added to each plate as a standard, and PBS buffer blank (serum dilution buffer) was added in duplicate to the wells. The plates were washed (4x), and 100 µl per well of alkaline phosphatase-conjugated detection antibody in PBS/0.1% Tween-20/2.5% bovine serum albumin buffer was added at the following dilutions: 1:3000 for IgG (Life Technologies', Cat #. H10007, USA) and 1:2000 for IgM (Thermo Scientific, Cat #. PA 1-74396). The plates were then washed, treated with Tetramethylbenzidine (TMB-plus One) and incubated as follows: 25 minutes for IgG and 15 minutes for IgM. Then, absorbances were read at 450 nm with a reference wavelength of 620 nm within 10 minutes of stopping the reaction using an automated ELISA reader (SpectraMax 340 PC, Molecular Devices, USA). Gen5 1.09 for Windows (Molecular Devices) was used for data capture. The optical density (OD) values obtained were converted into arbitrary units (AU) using computer software (ADAMSEL, version 1.1 build 40 © 2009 EJ Remarque). The AU values for the ODs were averaged and normalized. The cut-off value for the difference between duplicates was set at 25%. If the difference was higher, the measurement was repeated. Additionally, a positive

control serum from individuals infected with *S. haematobium* from the study population was used to ensure consistency between plates.

Data management

Data were entered into an MS Excel worksheet designed to capture the data of each participant over the experimentation period. Each data entry for each participant (coded as S001 to S406) was cross-checked by a second project assistant to ensure correct entries. Data generated from this study were kept in a password-protected laptop kept by the principal investigator and accessed only by the research team members. The hard copies are kept under lock and key in a safe manner and accessed by only team members. These copies will be kept until 5 years from now to publish research papers from the data collected. All biological samples were disposed of in sealed and named Ziplock bags and deposited in hazardous waste bins provided in the laboratory. These were subsequently handled by waste management organizations in charge of clearing hazardous waste.

Data Processing and Analysis

Data management and analyses were conducted using STATA 14 software. Quantitative variables were determined using normality tests. The median with interquartile range was used to categorize the various characteristics. Independent t-test was used to determine the association between the outcome and independent categorical and continuous variables. The Wilcoxon rank-sum test was employed to compare differences within the various participants since the data were skewed. Categorical data are presented as percentages and frequencies. Comparison of group differences was assessed using Fisher's exact test and/or Pearson's chi-square test where appropriate. In

addition, multivariate logistic regression models were used to determine the association of schistosome infection with other variables using adjusted odds ratios (ORs) with a 95% confidence interval (CI). $P \leq 0.05$ was considered statistically significant.

Ethical Considerations

Ethical clearance for the study was obtained from the University of Cape Coast Institutional Review Board (UCCIRB) (with ID UCCIRB/CHAS/2021/97), Ghana Health Services and authorities of the various communities within the study area. Written informed consent was sought from participants before they were enrolled in the study. Parents/guardians of participants less than 12yrs were contacted to provide consent for their wards to participate in the study. This project was carried out by following the Helsinki Declaration for Research involving human subjects. Careful measures were ensured during sample collection and all research-related activities.

A thorough explanation of the study protocol was made to all prospective participants in a language the prospective participant understood. They were made to understand that at any point in the project, they could opt out without any consequences to them, whatsoever. To protect the identity of participants and encourage participation, it was also ensured that all participants were anonymized and their samples coded instead of using names.

Blood sample collection was carried out by a trained and experienced phlebotomist following the internationally accepted protocol for blood collection via venipuncture. In the event of overly painful blood draw or

swellings, wrapped ice cubes were pressed against the sites of venipuncture to reduce the swelling.

Chapter summary

The present chapter described the various materials and research methodologies that were employed to study the research objectives. The community-based cross-sectional study conducted in the Adansi South district from January to July 2021 sampled 400 participants aged five to fifty residing in the selected communities at the time of sampling. After administering questionnaire to assess sociodemographic and risk of exposure data, urine samples were collected from each participant and tested microscopically for Schistosoma haemtobium eggs. Blood samples were then collected from the 31 S. haematobium positive and 31 S. haematobium negative participants. These were subsequently analyzed to assess the relationship between infection status and haematological parameters. The laboratory results were analyzed and interpreted in the light of sociodemographic data and data on the risk of exposure to S. haematobium.

CHAPTER FOUR

RESULTS AND DISCUSSION

Introduction

Chapter four presents the findings of the research. Research data were analysed using both descriptive and inferential statistics. The results are thus presented in tables. The results are captured under five main headings, while the discussion is captured under four main headings. Each subheading presents and discusses the results in light of empirical evidence, known theories and other relevant research on the subject.

RESULTS

Participants' sociodemographic characteristics

Table 4.1 presents the sociodemographic data of the participants. Of the four hundred and six (406) study participants, the majority (58.9%) were females, whilst 41.1% were males. Owusukrom recorded the highest number of participants, in proportion to its population size, with Tensuani recording forty (40). Our study participants were mainly adult (26.6%) and teen populations (24.9%). Aside from students (62.8%), farmers (28.6%) constituted the major occupation of our participants. An overwhelming 97.4% of our participants were Christians, and 70.0% were not married (single). Additionally, almost all participants had at least basic education (83.3%), with only 38 representing 9.4% of our study participants who had no formal education at all. The majority of the participants lived in the community for approximately 5 to 9 years (25.9%) and over 20 years (24.6%) at the time of sampling.

Table 1: Distribution of Sociodemographic Characteristics of the participants

Variables	Frequency (406)	Percentage (%)
Gender		
Male	167	41.1
Female	239	58.9
Location		
Owusukrom	100	24.6
Dotom	50	12.3
Asarekrom	76	18.7
Tensuani	40	9.9
Menang	90	22.2
Bronikrom	50	12.3
Age		
5-9years	82	20.2
10-14 years	101	24.9
15-19 years	70	17.2
20-24 years	26	6.4
25-29 years	19	4.7
30 years and above	108	26.6
Occupation		
Farming	116	28.6
Artisan	11	2.7
Trading	18	4.4
Student	255	62.8
Other	2	0.5
Unemployed	4	1.0
Ethnicity		
Akan	240	92.7

Ewe	13	5.0
Northerner	6	2.3
Religion		
Christian	228	97.4
Muslim	4	1.7
Traditionalist	1	0.4
None	1	0.4
Marital Status		
Married	105	25.9
Single	285	70.2
Divorced	8	2.0
Widowed	8	2.0
Educational Status		
None	38	9.4
Basic Education	338	83.3
SHS/NVTI	28	6.9
Tertiary	2	0.5
Years been in the community		
1-4 years	49	12.1
5-9 years	105	25.9
10-14 years	98	24.1
15-19 years	54	13.3
Above 20 years	100	24.6

Distribution of Sociodemographic Characteristics of the participants for immunohaematological assays

Table 4.2 presents the sociodemographic characteristics of the participants for the immunohaematological studies. Of the 62 participants

recruited for the haematological and immunological studies, 31 were schistosome infected, and 31 were uninfected. There was no significant difference across gender or age strata. Consequently, the males and females were comparable in terms of statistical power, and therefore, inferential statistics across genders hold valid for equal interpretation across both genders. The same could be said of the age groupings. Additionally, there was no statistically significant difference in the numbers of participants from the various communities. This allowed for a fair interpretation of results across communities, with the assumption that all communities are similar in terms of samples drawn from each community.

Table 2: Distribution of Sociodemographic Characteristics of the participants for immunohaematological assays

Variables	Positive (31)	Negative (31)	Total (62)	P value
Gender				
Male	13 (41.94)	16 (51.61)	29 (46.77)	0.445[a]

Female	18 (58.06)	15 (48.39)	33 (53.23)	
Communities				
Owusukrom	7 (38.89)	11 (61.11)	18 (29.03)	**0.852[a]**
Dotom	4 (100.00)	0 (0.00)	4 (6.45)	
Asarekrom	7 (100.00)	0 (0.00)	7 (11.29)	
Tensuani	3 (50.00)	3 (50.00)	6 (9.68)	
Menang	1 (33.33)	2 (66.67)	3 (4.84)	
Bronikrom	9 (37.50)	15 (62.50)	24 (38.71)	
Age				
Mean ± (SD), SEM	18.26 (13.26)	17.29 (10.48)	17.77 (11.86)	0.751[b]

[a] P-values were generated using Chi-square test for only Owusukrom, Tensuani, Bronikrom. [b] P-values were generated using independent sample t-test

Prevalence of urinary Schistosomiasis in the study area

Table 4.3 presents the results of the prevalence of *S. haematobium* infections in the study communities Out of the 406 participants who took part in the study, sixty-six (66) of them had *Schistosoma* eggs in their urine samples, giving an overall prevalence of 16.3%. Because the populations of the selected communities were different and consequently the sample size for the communities varied, the percentage scores of the community distribution of our recorded prevalence were most indicative of the patterns of infection in the communities. Owusukrom recorded the highest prevalence of 37%, followed by Bronikrom (26%), while Menang had the lowest prevalence of 1.1%.

Table 3: Prevalence and distribution of *S. haematobium* infection among communities

Variables	**Frequency** (N=406)	**Percentage (%)**
Communities		
Owusukrom (N=100)	37	37.0

Dotom (N=50)	4	8.0
Asarekrom (N=76)	7	9.2
Tensuani (N=40)	4	10.0
Menang (N=90)	1	1.1
Bronikrom (N=50)	13	26.0
Overall		
Positive	66	16.3

N=number of participants recruited from the community

Association between Sociodemographic Characteristics and Risk factors against Infection Status

Tables 4.4 and 4.5 present the association between sociodemographic characteristics and risk factors for infection against the likelihood of becoming infected with schistosomiasis. Two sociodemographic variables, that is, location and marital status, were significantly associated with infection with schistosomiasis. With regard to risk factors for schistosomiasis, "Wearing of shoes", "Previous Infection of schistosomiasis", "GHS supplying praziquantel", "Farm located in waterlogged area", washing of hands with soap and water before using the toilet, washing hands with soap and water before eating and washing hands with soap and water after playing were significantly associated with schistosomiasis infection in the study area. All other risk factors and sociodemographic characteristics were not significantly associated with *Shistosoma haematobium* infections.

Table 4: Association between Sociodemographic Characteristics and Schistosomiasis infection

Variables	P value
Sociodemographic Characteristics	
Gender	0.185
Communities	**<0.001**
Age	0.443

Occupation	0.118
Ethnicity	0.415
Marital Status	**0.038**
Educational Status	0.081
Years lived in Community	0.148

P values were generated from the chi-square test of association

Table 5: Association between risk factors and infection status

Risk factors	
Wearing shoes	**0.025**
Heard about schistosomiasis	0.793
Previous Infection of schistosomiasis	**0.013**
If yes, did you go to the hospital	0.708
Did the symptoms to subside by itself	0.778
Duration it took for the symptoms to subside	0.353
Frequency of taking praziquantel	0.598
GHS supplying praziquantel	**0.003**
Coming into contact with waterbodies	0.793
Whether water is treated before use	0.349
Treatment option used	0.058
Wearing protective shoes to farm	0.158
Farm located in waterlogged area	**0.004**
Knowledge of how Schistosomiasis is contracted	0.377
Do you know whether personal hygiene helps prevent schistosomiasis	0.289
Do you wash hands with soap and water after using the toilet	**<0.001**
Do you wash hands with soap and water before eating	**0.001**
Do you wash hands with soap and water after playing	**<0.001**
Source of water for bathing	**0.004**
Source of water for cooking	0.147
Source of water for drinking	0.084

P values were generated from the chi-square test of association. Significant *P* values are boldened.

Logistic regression for the association between the risk factors and infection status

Table 4.6 presents the logistic regression analysis of the association between the risk factors and the presence or absence of the infection in the participants. The crude analysis did not take into consideration the role of possible confounding factors such as age. However, the adjusted analysis corrected for the role of gender, location, age, occupation, ethnicity, marital status, educational status and years lived in community. In the crude analysis, seven out of the assessed risk factors showed significant association with infection status. In the adjusted analysis, six out of the assessed risk factors were significantly associated with infection status. The details of these findings are captured in the table 6 below.

Table 6: Logistic regression of the association between the risk factors and infection status

Risk factors	**Crude Analysis**			**Adjusted Analysis**		
	OR	**95% CI**	**P value**	**OR**	**95% CI**	**P value**
Wearing Shoes						
Never (Ref)		-	-		-	-
Rarely	0.49	0.17, 1.48	0.207	0.47	0.03, 6.47	0.573
Sometimes	0.833	0.30, 2.32	0.727	2.29	0.19, 27.78	0.515
Often	0.84	0.30, 2.33	0.740	1.33	0.10, 16.86	0.827
Always	0.12	0.02, 0.64	**0.013**	0.51	0.03, 8.80	0.646
Heard about Schistosomiasis						
No (Ref)						
Yes	1.07	0.633, 1.82	0.793	1.85	0.62, 5.50	0.268
Previous Infection of Schistosomiasis						

No (Ref)		-	-		-	-
Yes	2.56	1.19, 5.50	**0.016**	4.93	1.38, 17.70	**0.014**
Coming into contact with Waterbodies						
No[a] (Ref)						
Yes[b]	1.14	0.42, 3.07	0.793	1.70	0.20, 14.43	0.625
Wearing protective shoes to farm						
No (Ref)		-	-		-	-
Yes	0.65	0.36, 1.18	0.160	0.24	0.06, 0.94	**0.041**
Is your farm located in waterlogged area						
No (Ref)		-	-		-	-
Yes	0.45	0.26, 0.78	**0.004**	0.47	0.18, 1.19	0.111
Do you know how Schistosomiasis is contracted						
No (Ref)		-	-		-	-
Yes	0.71	0.33, 1.53	0.379	2.30	0.62, 8.56	0.214
Do you know whether personal hygiene helps prevent Schistosomiasis						
No (Ref)		-	-		-	-
Yes	1.47	0.72, 3.02	0.291	2.29	0.43, 12.25	0.332
Do you wash your hands with soap and water after using the toilet						
No (Ref)						
Yes	0.29	0.17, 0.50	**0.000**	0.56	0.22, 0.1.48	0.243

Do you wash your hands with soap and water before eating						
No (Ref)		-	-		-	-
Yes	0.37	0.20, 0.66	**0.001**	0.48	0.18, 1.29	0.146
Do you wash your hands with soap and water after playing						
No (Ref)		-	-		-	-
Yes	0.30	0.15, 0.59	**0.001**	0.27	0.08, 0.88	**0.029**
Source of water for bathing						
Borehole (Ref)		-	-		-	-
Groundwater/River	2.26	1.10, 4.65	**0.027**	13.48	2.73, 66.66	**0.001**
Combination[c]	2.56	1.37, 4.79	**0.003**	2.13	0.53, 8.54	0.288
Source of water for cooking						
Borehole (Ref)						
Groundwater/River	1.82	0.90, 3.66	0.093	14.72	2.85, 76.10	**0.001**
Combination[a]	0.70	0.26, 1.87	0.476	2.46	0.60, 10.04	0.210
Source of water for drinking						
Borehole (Ref)						
Groundwater/River	1.67	0.83, 3.33	0.148	7.38	1.96, 27.79	**0.003**
Combination[a]	0.34	0.08, 1.46	0.147	0.65	0.07, 5.65	0.694

OR- Odds Ratio; Some risk factors were excluded because the models could not be fitted due to multicollinearity; The covariates in the adjusted model were Gender, Location, Age, Occupation, Ethnicity, Marital Status, Educational Status and Years lived in Community; [a]These included responses of Rarely and Never; [b] These included responses of Daily, sometimes and often; [c]Combinations include all water source combinations excluding pipe; [c]Combinations include all water source combinations excluding pipe

Antibody levels among the study participant

Table 4.7 presents the median concentrations of IgG and IgM among the positive group and negative group for the whole study participants. It also includes the variations in immunoglobulins among the positive and control groups in some selected communities. In general, IgM levels were relatively elevated compared with IgG levels in both the case and control groups. Additionally, Tensuani recorded the highest levels of IgG, while Bronikrom recorded the highest levels of IgM. There was no statistically significant difference between the observed means of IgG among the cases and the controls ($p>0.05$), and no observed difference in the means of IgM among the cases and the controls. At the community level, no observed difference in either IgG or IgM among the cases and controls was statistically significant.

Table 7: Mean immunological parameters across infection statuses and selected communities

	IgG [Median, (IQR)]	P value	IgM [Median, (IQR)]	P value
Infection status[a]				
Positive	100.98 (76.89, 137.74)	0.2287	110.64 (101.14, 137.77)	0.5975
Negative	108.79 (81.33, 160.49)		119.58 (103.5, 144.3)	
Communities[b]				
Owusukrom	100.05 (75.11, 152.01)	0.7384	110.31 (102.54, 133.1)	0.3262
Tensuani	119.825 (101.78, 137.74)		110.105 (100.32, 137.77)	
Bronikrom	103.69 (78.3, 154.56)		121.905 (107.11, 171.245)	
Antibody levels within community clusters				
Owusukrom[a]				
Positive	94.53 (75.11, 146.89)	0.5561	110.64 (91.90, 131.14)	0.8919
Negative	101.02 (74.89, 160.49)		110.58 (102.54, 139.41)	
Tensuani[a]				
Positive	127.25 (101.78, 137.74)	0.8273	115.98 (100.32, 137.77)	0.8273
Negative	112.4 (78.82, 162.05)		104.23 (79.15, 159.79)	
Bronikrom[a]				
Positive	108.40 (78.11, 145.45)	0.6547	126.83 (106.03, 150.57)	0.9287
Negative	98.98 (81.33, 171.49)		120.98 (108.19, 198.52)	

[a] P values were generated using Mann–Whitney U test. [b] P values were generated using Kruskal–Wallis Test

Multivariate analyses to assess the effect of infection status and location on antibody levels

Table 4.8 presents the multivariate regression analysis of immunoglobulins against infection status. In the crude analysis, the results showed a negative association of IgG and infection status and a negative association of IgM and infection status. These associations, however, were not statistically significant. The adjusted model (which controlled for location, age and sex) presented slightly weaker inverse associations of IgG and IgM with positive infection status. These associations were also not statistically significant. At the community level, this general picture was replicated with inverse associations between IgG and IgM against positive infection status in the selected communities (Owusukrom and Bronikrom) in both the crude and adjusted analyses. Again, these associations were not statistically significant.

Table 8: Multivariate linear regression of log transformed IgM and IgG levels versus infection status and location

Parameters	IgG			IgM		
			Crude Analysis			
	β	**95% CI**	**P value**	**β**	**95% CI**	**P value**
Infection Status						
Positive[a]	-0.12	-0.30, 0.06	0.185	-0.05	-0.20, 0.10	0.475
Location						
Owusukrom[b]	-0.10	-0.45, 0.25	**0.561**	0.06	-0.21, 0.33	0.663
Bronikrom[b]	-0.06	-0.39, 0.28	**0.734**	0.17	-0.10, 0.43	0.209
			Adjusted Model			
	β	**95% CI**	**P value**	**β**	**95% CI**	**P value**
Infection Status						
Positive[a]	-0.11	-0.29, 0.07	0.221	-0.04	-0.19, 0.11	0.609
Communities						
Owusukrom[b]	-0.11	-0.48, 0.26	**0.552**	0.05	-0.24, 0.33	0.753
Bronikrom[b]	-0.05	-0.41, 0.30	**0.757**	0.16	-0.11, 0.43	0.246

[a] Reference category was negative; covariates used in the adjusted model were location, age and sex. [b] Reference category was Tensuani; covariates used in adjusted model were age and sex

4.1.7 Haematological levels among the study participants

Table 4.9 presents the mean haematological parameters across the positive and negative groups of studies. These are expressed as Mean ± (SD). All parameters of the complete blood count were captured and compared across both groups. It is worth noting that all the measured values for each parameter were within the normal range. There was also no observed significant difference in each of the measured parameters across the two study groups. Consequently, the measured means of the parameters were comparable for both groups.

Table 9: Haematological parameters across *S. haematobium* positive and negative participants

Parameters	Positive (N=31)	Negative (N=31)	P value
White blood cell (*10^3/µL)	6.31±3.28	6.44±3.01	0.9175
Red blood cells (*10^6/ µL)	4.43±0.54	4.39±0.56	0.7982
Haemoglobin (g/dL)	11.80±1.35	11.27±1.13	0.1014
Haematocrit (%)	34.61 ± 3.53	33.68 ±3.15	0.2751
MCV (fL)	78.73±7.54	77.71 ±7.01	0.5838
MCH (Pg)	26.87 ± 3.13	26.02 ± 2.80	0.2634
Platelet (*10^3/µL)	179.98 ±92.45	175.32±86.11	0.8381
PDW (fL)	11.4±4.11	11.52±4.12	0.9070
MPV (fL)	9.77±2.78	9.92±2.84	0.8432
RDW-SD (fL)	38.51±3.89	38.30±4.54	0.8481
RDW-CV (fL)	13.51±1.08	13.51±0.88	0.9898
P-LCR (%)	26.49±10.84	27.6±10.94	0.6896
PCT (%)	0.184±0.092	0.177±0.095	0.7871
Neutrophil (%)	29.60±14.43	32.35±13.42	0.4411
Lymphocyte (%)	52.722 ±11.742	49.44±10.97	0.2602
Monocyte (%)	10.46±6.23	10.71±4.14	0.8539
Eosinophil (%)	5.33±7.10	4.05±2.89	0.3583
Basophil (%)	1.88±2.78	2.38±3.68	0.5488
IG (%)	0.27±0.28	0.34±0.33	0.3968
RET (%)	1.68±0.58	1.67±0.73	0.9871

P values were generated using independent T test

DISCUSSION

Prevalence of urinary schistosomiasis

The distribution of the prevalence of the various schistosome species is wide and subject to various epidemiological factors. In Ghana, *S. haematobium* and *S. mansoni* are the most prevalent. *S. haematobium* infections are usually higher in the middle belt of the country, which has semideciduous rainforests, wetlands, swarms and marshes. This topography is suited for the habitation of the snail intermediate host of the *S. haematobium* parasite. Unlike parts of the Savanah grasslands in northern Ghana where marshes and swarms are scarce, the middle belt where our study was sited is home to several freshwater bodies that constantly keep the lands wet throughout most of the year. This explains the biased prevalence of schistosomiasis in these parts of the country.

However, our observed prevalence of 16.3% is far lower than the estimated national prevalence of 70.9% (Susanne, 2015). Over the years, the estimated national prevalence has seen a decrease from 72.5% in 2003 to 70.9% in 2010 (Rollinson, *et al.* 2013). This largely has been due to the numerous interventions put in place, such as the preventive administration of praziquantel to stem the spat of infections. Schistosomiasis, however, exhibits focal prevalence in a specific epidemiological context. Thus, the national prevalence may not be a true reflection of the focal distribution of the disease. Since the last reported national prevalence in 2010, several interventions over the decade to stem infections have been put in place. In endemic regions, urinary schistosomiasis among adults is estimated to be 20.0% on average (King, 2001). Our reported prevalence in this study is similar to the expected average prevalence among the adult population. This also echoes the fact that our study area is a schistosomiasis endemic region. Our observed prevalence is also

similar to the reported prevalence of 15.5% in adults in three villages northwest of Accra (Koukounari, Webster, Donnelly, Bray, Naples, Bosompem, *et al.*, 2009) and the 20.0% reported prevalence by Nmorsi, Ukwandu, Ogoinja, Blackie, Odike (2007) in Nigeria. However, it is inconsistent with other findings of 95% in Bunuso (Ayeh-Kumi, Obeng-Nkrumah, Baidoo, Teye, Asmah, 2013) and 51.7% in Mozambique (Augusto, Magnussen, Kristensen, Appleton & Vennervald, 2009). These vast variations may be due to differences in study sites, times of the year of the study and other intrinsic factors.

Intrinsic factors such as the economic activity of the communities may not change easily, even over time. The predominant annual farming activities (crop farming and fishing) in these wetlands and swarms are risk factors they are constantly in touch with. These socioeconomic activities are part of the livelihoods of communities. Crops such as rice and yam require a constant water supply, preferably in marshy areas. These marshes also serve as reservoirs for the Bulinus snails that host the parasite cercariae. These factors increase the chance of contact with the parasite and thus increase the risk of infections.

The observed vast difference in the prevalence in Owusukrom (37.0%) and Menang (1.1%) also draws attention to the role of the number of water body- and water body-related activities on the prevalence distribution. Whilst Owusukrom is surrounded by several small streams and water bodies, Menang has more access to clean water sources. This affirms the assertion by Kloos et al. (1997) that relative closeness to freshwater sources translates into higher chances of infection. Studies in Accra reported a higher prevalence in Mahem (58%), which is closer to the Weija dam than Galilee (49%), which is relatively farther from the same dam (Aboagye & Edoh, 2009). Also, a study in the

Ashanti region reported similar findings, where communities surrounding the Atonsu River had a higher prevalence (40.2%) than those farther from the same river, such as Boadi, Ayeduasi and Kokoben. (Tay, Amankwa & Gbedema, 2011).

Risk factors associated with urinary schistosomiasis

We measured over fifteen risk factors in our study ten (10) of which showed higher odds of being associated with *S. haematobium* infections. Our study area is a typical Schistosoma endemic region. The endemicity of this area is closely related to several natural and sociocultural practices that predispose inhabitants to urinary schistosomiasis. Behavioural risk factors are also largely related to the kind of socioeconomic activities. Inhabitants in the study communities are largely farmers engaging in subsistent and sometimes commercial farming. Additionally, while fishing is a part-timejob for some, it is the main economic activity for a few others. It was thus not surprising that "location/community" was a significant risk factor, as recorded in our study. Some communities, for example, Owusukrom, showed a higher prevalence than others, such as Menang. This we attribute largely to the proximity of Owusukrom to the two rivers in the district than Menang is to either of them.

We observed that persons who wore shoes "Always" had 88% reduced odds of being infected with urinary schistosomiasis (OR = 0.12, p = 0.013). Additionally, persons who wore protective shoes to farms had 76% reduced odds of infection. These observations, however, were not statistically significant after adjusting for gender, location, age, occupation, ethnicity, marital status, educational status and years lived in community. Wearing sandals, as basic as it might appear, is not the case among some families and communities. One of

the most common routes of becoming infected with the parasite is via the skin of the feet, as one remains in infected water for some time. Thus, persons whose feet are unprotected stand a higher chance of exposure to the parasite cercariae than those whose feet are covered. This is supported by the present observation that wearing shoes always, which includes the time spent in the waterlogged farms and contaminated water bodies, reduced their odds of getting infected.

Persons who had previous infections had over 150% odds of being infected again. This was true and statistically significant for both the crude and adjusted analyses. This is a significant observation in light of the role of IgE in reinfections (Naus *et al*, 1998; Odongo-Aginya *et al*, 2012). However, this must be understood from two dimensions. The presence of parasite eggs, which defined infection in this study, may not necessarily mean a new infection but the reactivation of a chronic infection. Consequently, this finding may not necessarily be the odds of a new infection but may also include the odds of reactivation of a chronic infection. In either way, it echoes the endemicity of the disease in the region. Persons who were hitherto infected and who had lived in the community for over 5 years were at higher odds of getting the infection again. The prevalence of the same risk factors in the same community that predisposed them to their first infection still persists in the community; thus, it is not surprising that previous infection is more associated with reinfection than someone who had never had the infection before. It has been shown that treatment with praziquantel reduces the risk of reinfections (Mogeni et al., 2020). In the absence of prophylactic treatment or treatment at all, the risk of infection or reinfection increases. Additionally, persons who had previous infections are immune-sensitized and produce IgE. The levels of IgE, for

example, reduce the chances of reinfection (Naus *et al*, 1998; Odongo-Aginya *et al*, 2012). We conclude from our observations that the immune levels of the participants, especially IgE, may be low, resulting in higher odds of reinfection among persons with prior infection.

Persons who washed their hands with soap and water after using the toilet, before eating, and after playing had 71%, 63% and 70% reduced odds of being infected, respectively. These risk factors associated with hand hygiene practices are important risk factors given that ingestion of parasite eggs from contaminated food and water is an important route of infection. Our study suggests that constant and proper cleaning of hands reduces the risk of infection. This is supported by our observed average 70% reduced odds of infection when hand hygiene is properly and routinely performed.

Furthermore, persons whose source of bathing water was groundwater/river water had over 100% odds of getting infected compared to persons who used borehole water to bath. Similarly, persons who used groundwater/river water for cooking had approximately 82% higher odds of infection than persons who used borehole water, while persons who used groundwater/river water for drinking had approximately 67% higher odds of getting infected than persons who used borehole water. Several groundwater sources, such as wells, streams, and slow-moving rivers, are scattered throughout the study area. In some communities, these are the major sources of both domestic and farm water. People swim, fish, wash and collect water from these water bodies for domestic activities. These water sources are also reservoirs that collect running water that washes off domestic waste and human excreta into them. They serve as sources of many pathogens and host freshwater

snails, the intermediate host of the *S. haematobium* parasite. We infer, therefore, that the observed associations between groundwater sources and infection status are a result of the suitability of groundwater sources to host the intermediate host of the parasite and thus a reservoir for parasite transmission. Borehole water, on the other hand, is pumped from deep in the earth from the water table. This water, although contaminated by seeping chemicals, does not contain freshwater snails that host the *S. haematobium* parasite. They are thus relatively safe for domestic use and translate into negative odds of being associated with *S. haematobium* infections.

Our findings are consistent with Satayathum, Muchiri, Ouma, Whalen and King (2006), who also observed among coastal Kenyans that persons with a reduced risk of infection had access to pipe-borne water and acceptable sanitary conditions. The source of water has been closely linked to the risk of infection (Pennance *et al*., 2016; Clennon *et al.* 2004). Similar to our findings of the variations in the prevalence between Menang and Owusukrom, Mogeni et al. (2020) also observed that persons who had access to pipe-borne water were less likely to frequently get in touch with surface freshwater sources, which translates into a reduced risk of infections.

Immunological parameters across *S. haematobium*-positive and S. haematobium-negative participants

The antibody response in schistosomiasis has long been associated with the elicitation of IgM and IgG responses. Not all subtypes of IgG, however, are involved in schistosomiasis. In recurring infections, IgE plays a crucial role. In this study, we measured IgG and IgM levels associated with schistosomiasis. Our study, however, did not measure the subtypes of either immunoglobulin. The median concentrations of both immunoglobulins across the two study

groups, that is, persons who had schistosomiasis (defined by microscopic identification of parasite eggs in urine), also known as the cases/positive group, and schistosomiasis-negative individuals (defined as persons with no microscopic schistosome parasite eggs in urine), also known as the controls/negative group, were similar.

We observed no significant difference in the mean concentrations of IgM in either the cases or the controls. Dying worms release immunogens that stimulate the release of immune protective responses. These responses over time can mount a strong immune response against invading schistosomula and thus prevent infection (Fitzsimmons, Jones, Pinot de Moira, et al., 2012: Walter, Fulford, McBeath, et a., 2006). IgM is produced as the first humoral immune response to invading antigens. Although they tend to have a low antigenic affinity, they can recruit other immune factors, such as T cells, to eliminate the pathogen. They are short-lived, approximately five to six days in circulation. Our observations show that in the absence of active infection (control group), IgM levels persisted. Whereas it is expected that IgM levels in active infections will be high against no infection, our findings show otherwise. This may indicate that the infections were chronic but not recent infections, and thus, IgM may have receded at the time of sampling. We observed a relative increase in IgM in the healthy controls compared to the active infection group, albeit not a statistically significant increase. This apparent deviation from the norm may stem from several factors. First, the persistent presence of schistosome antigens even after recovery from the disease may contribute to the levels of IgM in the control group. Additionally, the absence of schistosome eggs in urine (our definitive marker of infection) does not necessarily mark the absence of parasite

antigens in the person. These antigens have the ability to continually stimulate IgM secretion. The stages of the parasite cycle stimulate the parasite not always be found in urine but tissues and lymph. From these sites, it is still able to elicit an antigenic response. Thus, even though it may not yet appear in urine, it can incite IgM secretion. This is a plausible explanation because the late appearance of the parasite in urine will have marked the decline in IgM levels. This will imply that there may be people who had contracted the parasite who showed an IgM response but had yet to be confirmed positive using urine microscopy.

Our study also observed no significant difference between IgG levels in our cases and control groups. IgG appears late in infection and apparently the last of the known immunoglobulins to respond to infections. It is known as a chronic infection antibody. Although it appears late in infections, it has a longer half-life. Consequently, its presence may not indicate a recent infection. IgG levels increase in schistosomiasis and remain at a steady level over a long period, even after infection. Our findings reiterate this characteristic of IgG. It was relatively the same in both the presently infected persons and the uninfected controls (whom we cannot guarantee did not have the infection in the past). These results present two possible ongoing immune responses in the two groups. First, some in the control group may have had infections in the past and thus still had sustained levels of IgG. This is understandable given that the communities of the study are endemic for schistosomiasis. The second possible explanation is that the presently infected group has had the infection for a considerable number of days to allow for the appearance of IgG or the presence of IgG in the presently infected is as a result of past infections of the parasite.

In schistosomiasis, unlike other infections, past infection does not guarantee immunity against future infections.

Multivariate linear logistic regression analysis of the association between immunoglobulin levels and infection status revealed a weak association between IgG levels and positive infection status. An even weaker association was shown between IgM and positive infection status. All of these associations were not statistically significant. This was true for both the crude and adjusted (age, sex, and/or location) analyses. At the community level, similar relationships were recorded. Thus, there was no significant relationship between IgG and IgM levels and infection status in our study population. It is interesting to note that although there was an overall negative association of either IgG or IgM with infection status, in Bronikrom and Owusukrom, there was a positive association between IgG and IgM. It is also worth noting that when age and sex were controlled for in the adjusted analysis, there was no significant difference in the findings. These findings underscore the fact that age and sex do not contribute to differences in infection patterns. The risk factors for infections are spread almost uniformly across age and sex. These findings from the logistic regression analysis corroborate the findings that there existed no significant difference means of the immunoglobulins in either case. These findings also reflect the already known endemic status of the study communities. The endemicity of the infection translates into the endemicity of antibodies against the parasite. Thus, whether presently infected or not, these immunoglobulins are present in the population.

Haematologcial paramters in urinary schistosomiasis

The life cycle and pathogenesis of *S. haematobium* infection are both related to blood and blood products. Even though it is tagged a urinary disease, parts of its life cycle are spent in blood vessels as the cercariae grow into an adult worm. One defining feature of *S. haematobium* infections is haematuria. The loss of blood from *S. haematobium* infections can be so severe as the parasite eggs are passed out in urine through the urethra. Thus, the effect of the parasite on haematological parameters is an important area of research.

Our observed Full Blood Count (FBC) results appear mostly contrary to the largely reported elevated WBC and reduced RBC and haemoglobin levels. We recorded no difference in the levels of these parameters across both study groups. Afrifa et al., (2017), Mohammed, Eltayeb & Ibrahim (2007), and Dejon-Agobé, Adegnika and Grobusch, (2021) all reported elevated WBC, contrary to our findings of no significant difference in WBC in both cases and control groups. Whereas we anticipated an elevated WBC in response to parasitic infection, as is normal with most infections, we also recognized the role of other infections in the control group, which could also cause elevated WBC levels independent of schistosomiasis. Plasmodium infections in these communities are common. It is thus reasoned that other infections may also independently cause the reported WBC levels in the negative group and thus mask any expected difference in WBC in the cases and controls.

Decreased RBC production coupled with increased RBC loss has been predicted to be responsible for reported cases of decreased haemoglobin in urinary schistosomiasis (Afrifa et al., 2017). Unlike Afrifa et al. (2017) and Dejon-Agobé, Adegnika and Grobusch (2021), who both reported anaemia and decreased haemoglobin, respectively, in infected persons compared with

uninfected persons, our study reported no significant decrease in haemoglobin in the infected persons as against the uninfected participants. Our reported normal haemoglobin levels are similar, however, to Nwabueze, Fagbemi, and Opara's (2008) report of normal haemoglobin among school children in Nigeria. It must, however, be noted that Afrifa *et al*. (2017) and Dejon-Agobé, Adegnika and Grobusch (2021) conducted their respective studies among school-going children only. Our study however was conducted irrespective of age. The haemoglobin levels in the adults may likely have masked a possible low haemoglobin among the infected children. We do not write off the possibility of normal haemoglobin as a result of light infections, which may not cause much destruction of RBCs or hinder the production of the same (Afrifa *et al*., 2017).

We also report in our study slightly elevated platelet counts in the infected group compared with the control group, albeit with an insignificant difference. Thrombocytes are involved in defense mechanisms against adult worms in experimental rats (Joseph, Auriault, Capron, Vorng, Viens, 1983). Dejon-Agobé, Adegnika and Grobusch (2021) also reported a significant increase in thrombocytes in urinary schistosomiasis. Although our observation is not significant, the finding of elevated thrombocytes is an important observation. Our smaller sample size may have masked the full effect of schistosomiasis on thrombocytes.

We also recorded a slight rise in eosinophils among the cases, albeit not significant. Eosinophils have generally been recognized for their role against helminthic infections (Hagan, Wilkins, BlumenthalJ, Hayes & Greenwood, 1985; Issa & Shalaby, 1999). Although the intensity of urinary schistosomiasis

has been linked to leukocytosis and eosinophilia (Mohammed, Eltayeb & Ibrahim, 2007), our study observed relative eosinophilia in the infected participants and no leukocytosis in the same group. Eosinophilia may form part of the range of immune responses against helminths. Other less important immune cells in helminthic infections were either the same in both groups or even lower in the cases than in the controls.

Chapter summary

Chapter four presented the findings of the study and discussed same in the light of relevant literature, the context of the study and the implications of the findings. Our study revealed an overall prevalence of 16.3% (66/406). At the community level, with Owusukrom having the highest prevalence of 37%, followed by Bronikrom, with a prevalence of 26%. Menang recorded the lowest prevalence of 1.1%. We also observed that previous infection of schistosomiasis, not wearing protective shoes to farms, location of farms in waterlogged areas, poor hand hygiene, and using surface freshwater sources for domestic activities were important risk factors for infection with the parasite. These reports which are similar to what is reported in literature in the sub region are important in informing the design of public health policies in the prevention and control of S. haematobium infections in the study area. Finally, neither haematological parameters nor IgM and IgG were influenced by S. haematobium infection status.

CHAPTER FIVE

SUMMARY, CONCLUSION AND RECOMMENDATION

Introduction

This chapter presents the final aspects of this thesis and brings to finality the issues of this research. First, it summarizes the key issues raised in this thesis, the key research paradigms and methodology employed to explore the study objectives and the resulting findings from the research data. The chapter also presents the conclusions drawn from the entire thesis. This conclusion draws from all the data presented in this thesis and the available literature on the subject. Finally, the chapter also presents recommendations for policy, science, research and health and wellbeing. These recommendations draw from the findings and the conclusions of this study.

Schistosomiasis caused by *S. haematobium* is endemic in tropical regions such as Ghana. In Ghana, the disease is endemic in the wetlands, deciduous and semi-deciduous rain forest regions of the middle belt of the country. *S. haematobium* exhibits a parasitic life cycle in humans and freshwater snails. In humans, the disease is characterized by blood in urine and is diagnosed by microscopic, immunological and genetic diagnostic tools. The immune response to acute, chronic or reinfections is complex and not fully understood. In endemic communities, the chances of repeated infections present an even complex dilemma regarding the immune responses at play in the infection. Our study assessed the immunoepidemiological parameters associated with *S. haematobium* infections among people in *S. haematobium* endemic communities in the Adansi South District.

Consequently, we employed a cross-sectional study design to sample and study 406 participants living in five selected communities in the district. We used purposive sampling to select the five communities and simple random sampling to recruit individual participants. We collected urine samples from 406 participants and used microscopy to detect S. haematobium infection. We then obtained blood samples from 31 participants who tested positive for urinary schistosomiasis and from 31 participants who tested negative for urinary schistosomiasis. To assess the immunoepidemiology of the disease in the study area, the serum from the blood was subsequently analysed using ELISA to detect the levels of IgG and IgM specific to the Schistosoma soluble egg antigen. Using an automated full blood count haematological analyser, we also measured the haematological parameters. The data were analysed and interpreted in the context of existing literature and the prevailing socioeconomic and demographic characteristics of the study communities.

Summary of findings

Our study was conducted under four broad specific objectives. For our objective one, which sought to estimate the prevalence of urinary schistosomiasis among the study population, our study revealed an overall prevalence of 16.3% (66/406). At the community level, Owusukrom had the highest prevalence of 37%, followed by Bronikrom, with a prevalence of 26%. Menang recorded the lowest prevalence of 1.1%.

The second objective of the study sought to examine the risk factors associated with *S. haematobium* infection. We observed that previous infection of schistosomiasis, not wearing protective shoes to farms, location of farms in waterlogged areas, poor hand hygiene, and using surface freshwater sources for

domestic activities were important risk factors for infection with the parasite. Persons who were exposed to any of these risk factors had over 60% odds of being infected compared to those without the risk factors.

The third objective sought to examine the effect of *S. haematobium* infection on haematological parameters measured as full blood count. Unlike other studies that recorded anaemia and leukocytosis in schistosomiasis, our study did not record any anaemia or leukocytosis in either schisotosomisis-positive participants or their negative counterparts. There was no statistically significant difference in the measured parameters infected and uninfected participants.

Objective four also assessed the effect of infection with schistosomes on IgG and IgM levels. IgM and IgG levels were independent of Schistosoma infections status as levels of IgM and IgG were statistically similar in both infected and uninfected persons.

Conclusion

In this study, there was an overall *Schistosoma haematobium* prevalence of 16.3% in five selected communities in the Adansi South district of the Ashanti region of Ghana. The highest disease prevalence of 37.0% was recorded in Owusukrom, while the lowest prevalence of 1.1% was recorded in Menang. We also reported that persons who did not always wear closed foot wear to farms, those who had previous infection of the disease, those who used fresh surface water for domestic activities, those who did not practice personal hygiene, and persons who farmed in waterlogged areas had increased odds of being infected with the disease. Additionally, IgG and IgM levels were not significantly different among the positive and negative Schistosoma-infected

individuals, which is not surprising since the research was carried out in an endemic community, and the participants may have had previous exposures and mounted immunity to the infection.

Recommendation

From the findings of our research, we propose the following:

1. Governments, nongovernmental organizations (NGOs), and stakeholders in health in the district and beyond should increase public education in the district about the disease, its risk factors and prevention. The common risk factors reported in this study may be partly due to ignorance on the part of the communities on how their actions and sociocultural practices predispose them to the infection. Such public education will bring enlightenment and raise awareness of the disease and how to prevent themselves from being infected.
2. Stakeholders in the community and beyond should take pragmatic steps in providing potable drinking water for the reach of the communities. Sources of water for drinking, cooking and washing were important risk factors reported in this study. These infested water bodies serve as sources of water for domestic and household activities. This is not just a risk factor for schistosomiasis but also other waterborne diseases. The provision of potable drinking water to communities will stem the spread of the disease by eliminating these risk factors
3. Preventive chemotherapy in the community has stalled since the early 2000s. It has been shown that persons who take the recommended dose of praziquantel have a better chance of not developing the disease in the event of an infection. The restoration of this vital public health

preventive intervention will go a long way to reduce the infections in these communities. We also suggest mass drug administration of praziquantel in the study communities to treat many infected persons and serve as prophylactic treatment.

Suggestion for future research

The present research, although sound in scientific principle, was deficient in some areas. Future research may attempt to repeat similar research while taking into consideration stratifying the participants according to the intensity of the infection. This will allow for the comparison of immune and haematological responses across different intensities of the infection. Additionally, a larger sample size for both the positive participants and negative control participants will bolster the statistical power of the research and allow for generalization of the findings to the larger population in the district.

We also suggest that future research should aim to measure the subtypes of IgG and IgE against both crude egg and adult worm antigens. This is because the immune response that will be mounted against these antigens in the human host may be different. In addition, the sub-IgG types may respond differently to these antigens. Again, the antibody response to helminth infection is normally associated with stronger IgG4 and IgE responses. The reported total IgG levels in our study may not provide a clear picture of how each subtype responds to the infection and how that response to infection may either serve good or harmful purposes in the patient.

Finally, we suggest that this research be replicated in other communities in the district, particularly to observe the variations in the prevalence in those communities closer to water bodies than others. From this study, we observed

that Owusukrom had a higher prevalence than Menang, which we hypothesize may be due to the proximity of Owusukrom to water bodies than Menang. This hypothesis, however, remains to be proven in the case of the Adansi South district of the Ashanti region.

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APPENDIX A – QUESTIONNAIRE

QUESTIONNAIRE

University of Cape Coast

College of Health and Allied Science

School of Medical Science

Department of Microbiology

GPS address……

Community name……

Questionnaire

Dear respondent, this survey is designed to investigate immuno-epidemiology of *schistosoma* infection in some selected communities within Adansi south district. Please I can assure you that this data is purely for academic purposes and under no circumstance will it be used for any purpose. Your anonymity and confidentiality are therefore assured.

Please respond to the items in this survey questionnaire by either ticking (√) or writing in the space provided. Please you can tick multiple choices where necessary

1. Location of respondent ……
2. Age of respondent
    a. 5 – 9
    b. 10 - 14
    c. 15 - 19
    d. 20 - 24
    e. 25 - 29
    f. 30 above

3. Gender
    a. Male
    b. Female

4. Occupation
    a. Farming
    b. Small scale mining
    c. Petty trading

   d. Pupil/student
   e. Other specify…….

5. What ethnic group do you belong to
   a. Akan
   b. Ewe
   c. Northerner
   d. Others
6. What religion do you belong to
   a. Christianity
   b. Islam
   c. Traditional religion
   d. None

7. Marital status
   a. Married
   b. Divorce
   c. Widow
   d. Single

8. Educational status
   a. Basic education
   b. SHS/NVTI
   c. Tertiary
   d. None

9. How many years have you been in this community?
   a. 1 – 4years
   b. 5 – 9years
   c. 10 – 14years
   d. 15 – 19years
   e. Above 20years

10. Participant owns shoe
   a. Yes
   b. No

11. Wears shoes
   a. Always
   b. Often
   c. Sometimes
   d. Rarely
   e. Never

12. Have you ever heard of schistosomiasis infection/bilharzia before?
   a. Yes
   b. No

13. If yes did you go to the hospital?
    a. Yes
    b. No

14. Did you use herbal preparations?
    a. Yes
    b. No

15. Did signs and symptoms of schistosoma infection/bilharzia stop by itself?
    a. Yes
    b. No

16. If no, how many weeks or months did it take for the signs and symptoms to disappear?
    a. 1month
    b. 2months
    c. Others specify……

17. How often do you take praziquantel?
    a. Every 6 month
    b. Yearly
    c. Above 5 years

18. Has GHS supply praziquantel to the community before?
    a. Yes
    b. No

19. Have you been baptized in a water body before?
    a. Yes
    b. No

20. How many years now
    a. 1 – 6months
    b. 7 – 11months
    c. 1 – 5years
    d. 6 and above

21. Do you come in Contact with water bodies?
    a. Yes
    b. No

22. Source of drinking water
    a. Pipe water
    b. Borehole

c. Well
d. Rainwater tank
e. River
f. Others specify……

23. Source of water for bathing
a. Pipe water
b. Borehole
c. Ground water from well
d. Rainwater tank
e. River
f. Others specify……

24. Source of water for cooking
a. Pipe water
b. Borehole water
c. Ground water from well
d. Rainwater tank
e. River
f. Others specify……

25. Water source location
a. In own house
b. < 50meters from house
c. 50meters or more

26. Do you do anything to water before used?
a. Yes
b. No

27. If yes
a. Boil
b. Add alum
c. Strain through cloth
d. Others specify……

28. Do you wear protective shoe when going to the farm?
a. Yes
b. No

29. Is your farm located in a waterlogged area?
a. Yes
b. No

30. Do you know how one can get schistosomiasis infection/bilharzia?
a. Yes

b. No

31. Do you think personal hygiene can be used to prevent schistosomal infection?
    a. Yes
    b. No

32. Do you wash your hands with soap and water after visiting the toilet?
    a. Yes
    b. No

33. Do you wash your hands with soap and water before eating?
    a. Yes
    b. No

34. Do you wash your hands with soap and water after playing?
    a. Yes
    b. No

APPENDIX B: ETHICAL CLEARANCE

UNIVERSITY OF CAPE COAST
INSTITUTIONAL REVIEW BOARD SECRETARIAT
TEL: 0558093143 / 0508878309
E-MAIL: irb@ucc.edu.gh
OUR REF: UCC/IRB/A/2016/1172
YOUR REF:
OMB NO: 0990-0279
IORG #: IORG0009096

26TH NOVEMBER 2021

Mr. Enoch Ameshia
Department of Microbiology and Immunology
University of Cape Coast

Dear Mr. Ameshia,

ETHICAL CLEARANCE – ID (UCCIRB/CHAS/2021/97)

The University of Cape Coast Institutional Review Board (UCCIRB) has granted Provisional Approval for the implementation of your research titled **Immune-Epidemiology of *Schistosoma* Infection in some Selected Communities within Adansi South District, Ghana.** This approval is valid from 26th November 2021 to 25th November, 2022. You may apply for a renewal subject to submission of all the required documents that will be prescribed by the UCCIRB.

Please note that any modification to the project must be submitted to the UCCIRB for review and approval before its implementation. You are required to submit periodic review of the protocol to the Board and a final full review to the UCCIRB on completion of the research. The UCCIRB may observe or cause to be observed procedures and records of the research during and after implementation.

You are also required to report all serious adverse events related to this study to the UCCIRB within seven days verbally and fourteen days in writing.

Always quote the protocol identification number in all future correspondence with us in relation to this protocol.

Yours faithfully,

Samuel Asiedu Owusu, PhD
UCCIRB Administrator

ADMINISTRATOR
INSTITUTIONAL REVIEW BOARD
UNIVERSITY OF CAPE COAST