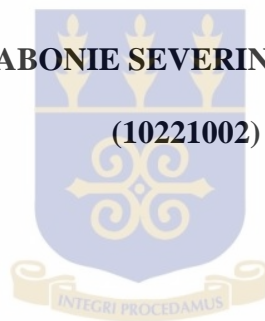


**IMPACT OF CONCURRENT *SCHISTOSOMA MANSONI* AND
S. HAEMATOBIIUM INFECTIONS ON SCHISTOSOMIASIS
DEMOGRAPHICS AND PATHOLOGY IN AN ENDEMIC COMMUNITY IN
GHANA**

BY

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(10221002)



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OF MPhil ZOOLOGY DEGREE**

JULY, 2013

DECLARATION

I hereby declare that except for references to other people's work, which I have duly acknowledged, this exercise is a result of my own original research, and this project, either in whole, or in part, has not been presented for another degree in this University or elsewhere.

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DEDICATION

This work is dedicated to the Almighty God, my parents and siblings. I also dedicate it to all scientists committed to the fight against schistosomiasis.



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Sincere thanks to the Almighty God, for helping me complete this work.

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ABSTRACT

Schistosomiasis remains endemic in Ghana, with *Schistosoma mansoni* and *S. haematobium* being the two dominant species of the schistosome parasites. In some parts of Africa, previous works indicated the occurrence of pairings between these two species, resulting in the production of non-viable eggs (parthenogenesis). In Ghana, although both species occur in sympatry, it is yet to be shown whether this can lead to hetero-specific pairing within the definitive host leading to consequent effects on disease outcome. In this study, the effects of concurrent *S. mansoni* and *S. haematobium* infections on the outcome of schistosomiasis is determined. Pupils from Peace International School, aged between 0 – 26 years were recruited for the study. Urine and stool samples were collected and screened for schistosome eggs, and snail vectors sampled for cercarial shedding. Laboratory bred snail vectors were infected with miracidia and induced to shed cercariae. Experimental mice were subsequently singly and concurrently infected with cercariae to determine the pathological effect. Questionnaires were also administered to assess the knowledge base of the people on the disease and determine water contact activities that predispose pupils to infections. The overall prevalence of urogenital and intestinal schistosomiasis observed were 66.8% (135/202) and 90.1% (163/181) respectively. It was revealed that both single and concurrent infections occurred among pupils, with the latter being more prevalent (50 and 131 respectively). Of the 131 individuals with concurrent infections, 50 presented with both *S. mansoni* in urine and *S. haematobium* in stool (type A), with the remaining 81 presenting with both parasites in urine and/or stool (type B). Whereas 65 individuals had heavy infection intensities in their urine, only 2 had heavy infections recorded in their stool. *Biomphalaria pfeifferi* and *Bulinus truncatus* snails were the vectors found hosting human infective cercariae, as well as two other

morphologically distinct cercariae unknown in the community. The presence of unknown cercariae types suggest possible bovine or avian schistosomiasis and the likelihood for zoonotic transmission of the disease in the community. Apparently, the high prevalence recorded for *S. mansoni* could be indicative of rapid spreading and colonizing of new areas that were earlier reported to be *S. haematobium* endemic. Also the high loads of *S. mansoni* eggs observed in urine samples relative to *S. haematobium* eggs in stool may suggest greater inter-specific mating ability by *S. haematobium* males than *S. mansoni* males. Concurrent infections in mice resulted in more granuloma formation compared to single infections. Studies of concurrent schistosome infections should be expanded to other endemic parts of the country while assessing its pathological impact in humans to facilitate disease control and eventual elimination.

CHAPTER ONE

INTRODUCTION

1.1 Background

Schistosomiasis is a major global health concern as it ranks second to malaria (Waknine-Grinberg et al., 2010). Over 200 million persons are infected worldwide, with 85% of the cases occurring in Africa (World Health Organization, 2013). The disease is caused by the blood flukes of the genus *Schistosoma*, with 3 main species infecting man (*S. mansoni*, *S. japonicum*, and *S. haematobium*). Other species such as *S. intercalatum* and *S. mekongi* are also known to cause disease in man (Schmitt, 2006; Steinmann et al., 2006; Ayanda, 2009).

Schistosomes are dioecious in their definitive hosts, and studies have shown that they exhibit polygamous characteristics (Tchuem Tchuente et al., 1995), with no physiological barriers to prevent changing of partners as well as inter-specific pairings (Southgate et al., 1995; Tchuem Tchuente et al., 1995; Webster et al., 1999; Cosgrove & Southgate, 2003a & 2003b).

Schistosomiasis remains endemic in Ghana (Yirenya-Tawiah et al., 2011), and two of the three main human species, *S. mansoni* and *S. haematobium* are known to be dominant in various parts of the country and cause disease (Bosompem et al., 2004). While *S. haematobium* causes urogenital schistosomiasis with its associated symptoms of haematuria and dysuria, abdominal pains and bloody stool associated with intestinal

schistosomiasis is caused by the *S. mansoni* species (Danso-Appiah et al., 2010).

The migration of infected individuals, with either or both parasite species, to new and endemic areas with freshwater bodies harbouring host snails compatible with parasite species, will continue to ensure the spread of both species in the same habitat in some parts of the country (Ampofo & Zuta, 1995). These conditions with time may facilitate simultaneous infection with likelihood for hetero-specific mating competition among species, since both parasite species have similar diurnal cercarial shedding pattern, and therefore can infect a definitive host at the same time (Mouahid et al., 1991). So far in Ghana, it has not been established whether this can lead to hetero-specific pairing in the definitive host. In the most likely event that this situation occurs, at least two types of interaction could be expected: 1) production of viable eggs that will lead to hybridization and 2) hetero-specific pairing with production of parthenogenetic eggs.

In recent times, reports indicate that there is geographical overlap of the two species in some schistosomiasis endemic communities (Feldmeier et al., 1998). Phylogenetically, the two species are distantly related that they utilize different snail vector species- *Bulinus* species for *S. haematobium* and *Biomphalaria* species for *S. mansoni*; yet both have been found in the same habitat. The utilization of the same fresh water body by different host snails harbouring different parasite species that infect similar definitive hosts may have consequent influence on the epidemiology of schistosomiasis in endemic areas in the country in the near future. Also, the sympatric nature of these species and their vectors calls for the need to assess the level of competition for survival among the

snails in the water body, as well as potential for mixing among sexes of the parasites in human hosts, so as to determine levels of infection, virulence and/or pathology.

It is most likely that the competitive interaction of the two parasite species will result in the production of viable eggs, which will lead to the establishment of a hybrid species. The presence of a hybrid species will therefore lead to the progressive exclusion of one or both species from the habitat. However at present, no sufficient data is available to support such occurrences. This possible development necessitates assessment of the changing patterns of prevalence and intensity of infections in the human population in the environments with existence of both the two parasite species as well as their respective vector snails.

In some parts of Africa, some works over a decade ago did indicate that pairings involving two different species such as *S. haematobium* and *S. mansoni* do occur and results in parthenogenesis, therefore the production of non-viable eggs (Southgate et al., 1982; Southgate & Rollinson, 1987; Tchuem Tchuente et al., 1994; Jourdane et al., 1995; Khalil & Mansour, 1995; Southgate et al., 1995).

A research work in the laboratory has shown mating competition between *S. mansoni* and *S. haematobium* with the formation of heterospecific and homospecific pairs (Webster et al., 1999). Also, Ernould (1996) reported that *S. mansoni* eggs were passed in the urine of some people in areas where the two parasite species are sympatric.

Nevertheless, though areas with both species have been identified in Ghana, there is rarely any information on hetero-specific pairing and hence passage of *S. mansoni* eggs in urine or *S. haematobium* eggs in stool. This calls for investigation, as hetero-specific pairing of parasites species could have implications for transmission of the disease or progressive exclusion of one or both parasite/vector species as well as virulence/pathology within definite host due to infection and mating competition that may result in hybridization or parthenogenesis.

The control of schistosome vector snails and hence schistosomiasis in endemic communities is of great importance to the sustenance of freshwater bodies in these communities. On one hand, water contact activities, mainly fishing and farming, pre-dispose humans to the disease. On the other hand, human activities such as passing out urine and stool into these water bodies pre-dispose the aquatic vector snails to infection.

Thus, human water contact activities could influence schistosomiasis transmission dynamics, which in turn, could affect sustainable use and management of water resources in endemic communities as well as impair the health of persons in these areas, ultimately perpetuating the cycle of poverty. It is therefore imperative to ascertain water use activities that influence schistosomiasis transmission parameters in these communities by assessing the infection status of both the intermediate and definitive hosts of the disease, while determining factors that could potentially exacerbate severity of the disease condition. This will inform inhabitants on good water usage practices and in the long term eliminate the disease from endemic communities.

1.2 Justification

Schistosomiasis continues to be a disease of public health importance in Ghana despite an increase in the number of control strategies. Several studies have looked at the prevalence and intensity of either *S. haematobium* or *S. mansoni* in endemic communities of Ghana, with very little information on hetero-specific infections compared to reports from some African countries where vectors of both species are found in the same water body (Tchuem Tchuente et al., 1995, Webster et al., 1999).

The presence of these two parasite species in the same habitat with their respective vectors creates the opportunity for hetero-specific infection with the potential for production of viable eggs and subsequently hybridization both at the community and individual level.

The present study seeks to identify mating interactions between *S. mansoni* and *S. haematobium* and determine the effects of mixing between these two species on the distribution and pathology of disease.

1.3 Objectives

1.3.1 Aim:

The aim of this study was to determine the effects of concurrent *S. mansoni* and *S. haematobium* infections on the outcome of schistosomiasis.

1.3.2 Specific objectives:

1. To determine prevalence and intensity of single and concurrent infections of the two schistosome species among people in the study community.
2. To establish the presence of snail host species in the study community
3. To determine water contact activities that pre-dispose persons to infection.

CHAPTER TWO

LITERATURE REVIEW

2.1 The disease schistosomiasis

Schistosomiasis, commonly known as bilharziasis, is a parasitic disease caused by blood flukes of the phylum Platyhelminthes, super family schistosomatoidae and belong to the genus *Schistosoma* (Gryseels et al., 2006; WHO 2013). A wide range of schistosome species under the genus *Schistosoma* parasitize both vertebrates and invertebrates. Five schistosome species of vertebrates have been identified as *S. intercalatum*, *S. mekongi* (Roca et al., 2002), *S. mansoni*, *S. haematobium*, and *S. japonicum* (Schmitt, 2006; Steinmann et al., 2006; Ayanda, 2009), with the latter three causing major pathology in man. Apart from *S. haematobium* which dwells in the venous plexus of the bladder and causes urogenital schistosomiasis, all the other four human schistosome parasites dwell in mesenteric vesicles, where they cause intestinal schistosomiasis (WHO, 2013).

Schistosomes are atypical flukes; the only dioecious species among all blood flukes (Loker & Brant, 2006; Webster et al., 2013), with worms showing significant dimorphism (Brant & Loker, 2005). These parasites complete their life cycle using two hosts; an intermediate host and a definitive host, unlike the three-host system used by majority of the flukes (Loker & Brant, 2006). They invade their definitive hosts by penetrating their skin directly, before migrating through blood vessels to their final dwelling sites.

Male worms carry female worms in a specialized feature located on their ventral side, the gynaecophoric groove, where mating occurs. Once paired, female worms produce eggs throughout their lifetime (Gryseels et al., 2006), which they pass into the environment through either the urine (i.e. *S. haematobium*) or stool (i.e. *S. mansoni*). *Schistosoma haematobium* female worms produce about 100 eggs daily (Basch, 1991) while those of *S. mansoni* produce approximately 300 eggs (Loker, 1983; Bogitsh, 2005). Adult worms have a lifespan of between three and five years on the average (Gryseels et al., 2006).

The disease and pathological conditions presented by schistosome parasites are classified as either urogenital or intestinal schistosomiasis and are mainly due to the presence of eggs in tissues (Brant & Loker, 2005; Barnabas et al., 2012).

Urogenital schistosomiasis is primarily characterized by the presence of blood in the urine, a condition known as haematuria (WHO, 2013), which may be accompanied by dysuria. In women, it may cause lesions in the cervix and vagina leading to vaginal bleeding, a risk factor for HIV transmission (Kjetland et al., 2006; Yirenya-Tawiah et al., 2011). In men, urogenital schistosomiasis can cause prostate cancer and damage to the seminal vesicles. Generally, chronic or advanced stages of the disease presents with bladder and ureteral fibrosis, hydronephrosis as well as cancer of the bladder (Parkin, 2008). Intestinal schistosomiasis is normally characterized by abdominal pains, diarrhoea and bloody stool. The liver and spleen enlarge, a condition known as hepatosplenomegaly in chronic stages of the disease (Parkin, 2008).

Generally, schistosomiasis causes anaemia, stunted growth, reduced learning and working ability as well as severe organ damage (King & Dangerfield-Cha, 2008).

The disease infects persons of all ages who come into contact with freshwater infested with cercariae (Jordan & Webbe, 1993; Ross et al., 2002). Children are at a higher risk of infection compared to adults (Utzinger et al., 2009; Barnabas et al., 2012). Activities such as farming, fishing (WHO, 2002), playing and bathing along freshwater bodies increase human contact with the site of infection and play a role in disease transmission (Useh & Ejezie, 1999; WHO Expert Committee, 2002; Wagatsuma et al., 2003). High occurrence of snail vectors and intense human activities along freshwater bodies in endemic areas indicate active transmission of the disease (Yirenya-Tawiah et al., 2011).

Nduka et al. (1995) and Musa et al. (2010) have reported children aged between 12 and 15 as showing the highest infection rates of urogenital schistosomiasis. Recent works by Deribe et al. (2011) on urogenital schistosomiasis and Barnabas et al. (2012) on both urogenital and intestinal schistosomiasis also reported children aged between 10 and 14 years as showing the highest infection rates. All these works confirmed boys have higher infection rates compared to girls. According to Jordan and Webbe (1993), schistosomiasis decreases with age, thus adults in endemic areas generally record very low infection rates. This condition may be associated with increased immunity against the disease, a possible result of continuous exposure to infection.

2.1.1 Life cycle of schistosome parasites

Schistosoma spp. complete their life cycle utilizing two host species, intermediate hosts which are freshwater snails and definitive hosts which are humans and other animals. Following schistosome infections in the human hosts, eggs are expelled into the environment through urine in the case of *S. haematobium* infections and through stool during *S. mansoni* and *S. japonicum* infections (figure 1). Upon contact with water, the eggs hatch, releasing miracidia which swim and penetrate suitable vector snails in the water body. Snails of the genus *Bulinus* host miracidia from *S. haematobium* eggs while *Biomphalaria* snails harbour miracidia from *S. mansoni* eggs. Miracidia of *S. japonicum* eggs infect snails of the genus *Oncomelania* (Jordan et al., 1993; Waine & McManus, 1997).

In the snail hosts, the miracidia develop into mother and daughter sporocysts and further develop into cercariae, the free-swimming stages. At 4 to 6 weeks post-infection, the snails shed cercariae into the water body in the presence of light and temperature range of 10 and 30°C. The free swimming cercariae then penetrate human skin which comes into contact with the cercariae infested water. Upon penetration, cercariae lose their forked tails and transform into schistosomulae, which are transported in blood vessels to the liver, where worms mature into adults and pair. Paired *S. haematobium* and *S. mansoni* worms migrate from the liver and finally dwell in the venous plexus of the urinary tract and/or the mesenteric venules of the intestines where they lay and deposit eggs (Jordan et al., 1993; Waine & McManus, 1997).

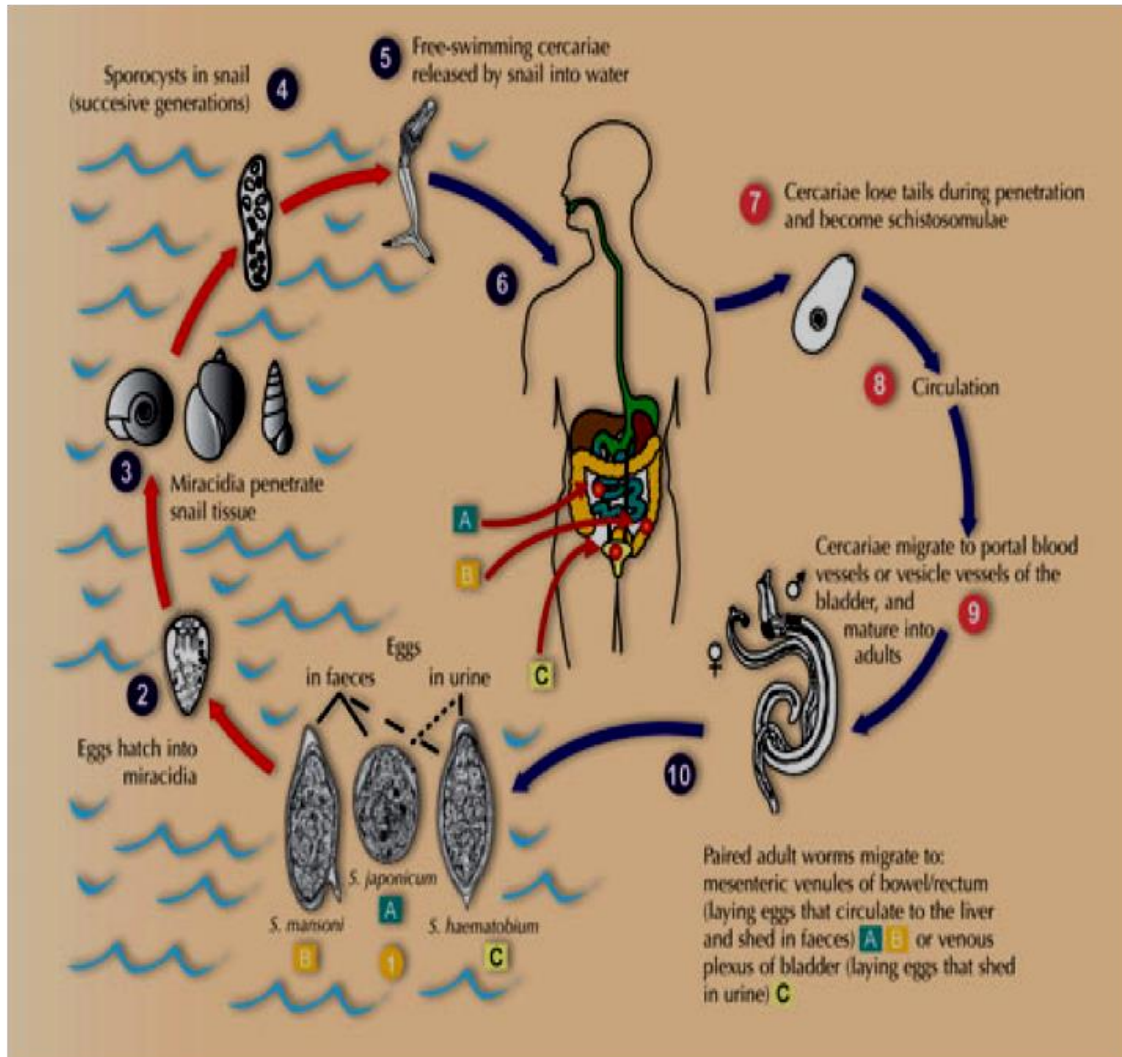


Figure 1: Life cycle of schistosome parasites

Source: <http://www.scienceinschool.org>

2.2 Epidemiology of schistosomiasis

2.2.1 Global epidemiology

It is estimated that about 780 million people worldwide stand the risk of schistosomiasis disease (Hotez et al., 2006; Steinmann et al., 2006), of which more than 207 million people currently have the disease in 78 countries worldwide (Barnabas et al., 2012; WHO, 2013). This indicates the continuous spread of the disease worldwide; Schmitt (2006), Steinmann et al. (2006), Fenwick (2006 and 2009) and WHO (2010) earlier reported an estimated number of 200 million people with the disease in 74 countries. Of the over 200 million infected persons, 90% live in 46 African countries (Barnabas et al., 2012; WHO, 2013).

Schistosoma haematobium which causes urogenital schistosomiasis is dominant in Africa and the Middle East (Schmitt, 2006) and has been documented in 53 countries (figure 2). According to Risikat and Ayoade (2012), about 90 million people living in these countries are positive for the disease. Intestinal schistosomiasis caused mainly by *S. mansoni* and *S. japonicum* is distributed in 54 countries (WHO, 1993b; Southgate, 1997; Urbani et al., 1997; Chitsulo et al., 2000). While *S. mansoni* is widely distributed across the globe, *S. japonicum* is confined to Asian countries.

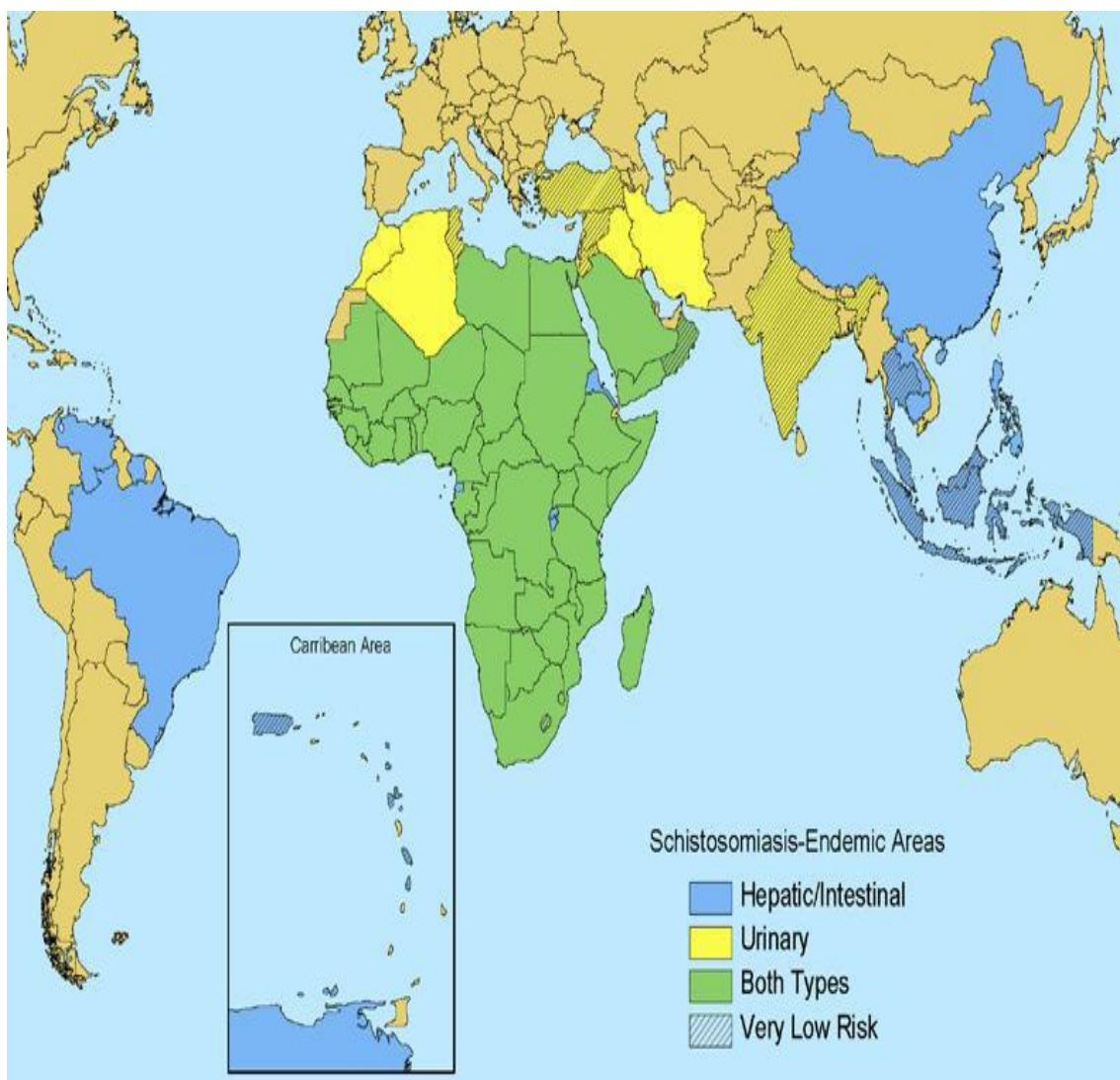


Figure 2: Global distribution of schistosomiasis

Source: United States of America (USA) Centers for Disease Control and Prevention

2.2.2 Epidemiology of schistosomiasis in Ghana

Schistosomiasis is most prevalent in many developing countries in Africa, including Ghana. Urogenital schistosomiasis, the most common form of the disease in Ghana, was first reported in 1895 while intestinal schistosomiasis was first reported in 1920 (CEGET-CNRS /OMS-WHO, 1987). Only two of the main parasites, *S. haematobium* and *S. mansoni* cause disease in Ghana (Bosompem et al., 2004). Epidemiological data on the two forms of the disease were first documented between 1952 and 1955. Before 1955 it was revealed that 20% of Ghana's total population suffered from urogenital schistosomiasis at some point in their lives (McCullough, 1954).

The creation of the Volta lake in 1964 and 1981 at Akosombo and Kpong respectively (Zakhary, 1997; Aryeetey et al., 2000), the Weija lake in 1979 (Ampofo & Zuta, 1995) and other water reservoirs has led to the outbreak of the disease in the country, as the sudden reduction in the flow rate of these reservoirs, provided suitable breeding sites for the snail intermediate hosts to thrive (Nkegbe, 2010). Settlements along these lakes have since been described as endemic areas, with high infection rates recorded among the local populace (Ampofo & Zuta, 1995; Zakhary, 1997).

Extensive epidemiological surveys and field researches have been carried out on the disease in Ghana, spanning over five decades. Works of McCullough, (1959); Obeng, (1997); Aryeetey et al. (2000); Bosompem et al. (2004); Aboagye and Edoh, (2009); Nkegbe, (2010); Yirenya Tawiah et al. (2011) and many others along several freshwater bodies in the country, notably the Volta and Weija Lakes, have contributed to the

knowledge on the disease. These studies have reported on the prevalence of both intestinal and urogenital schistosomiasis, snail vectors responsible for hosting parasites, as well as human activities that influence the distribution of the disease. Efficacy of drugs for treatment and measures for control of the disease have also been explored and documented.

Report from the World Schistosomiasis Risk Chart (2012) indicates that, the disease caused by both parasites, is present in the country as a whole. However, the distribution is variable, with communities recording single infections of either *S. haematobium* or *S. mansoni*, or both. Lack of proper sanitation and the availability of conducive habitats that favour the survival of the vector snails contribute to the endemicity of the disease in many parts of the country.

Generally, there are no data on the overall prevalence of schistosomiasis in Ghana. Prevalences recorded are based on the estimates of studies carried out in various parts of the country. Prevalence of *S. haematobium* infection has been found to range between 52% and 60% (Aryeetey et al., 2000; Nsowah-Nuamah et al., 2001; Aboagye & Edoh, 2009). On the other hand, prevalence of *S. mansoni* infection ranges between 10 and 49% (WHO, 2010), but this is likely to increase as this species is colonizing new areas which were initially noted for *S. haematobium* infections only (figures 3 and 4).

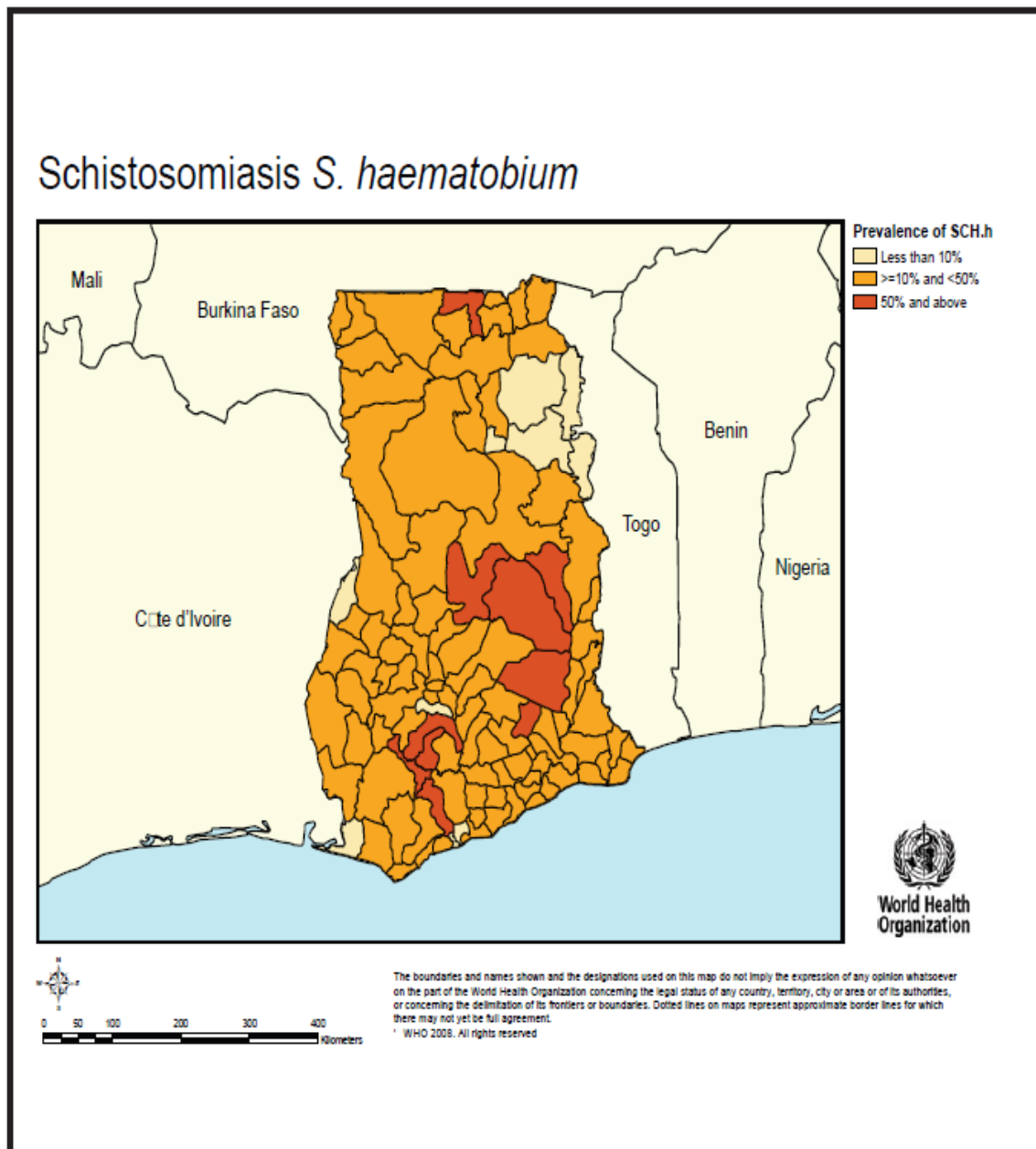


Figure 3: Distribution of *S. haematobium* in Ghana

Source: WHO, 2010

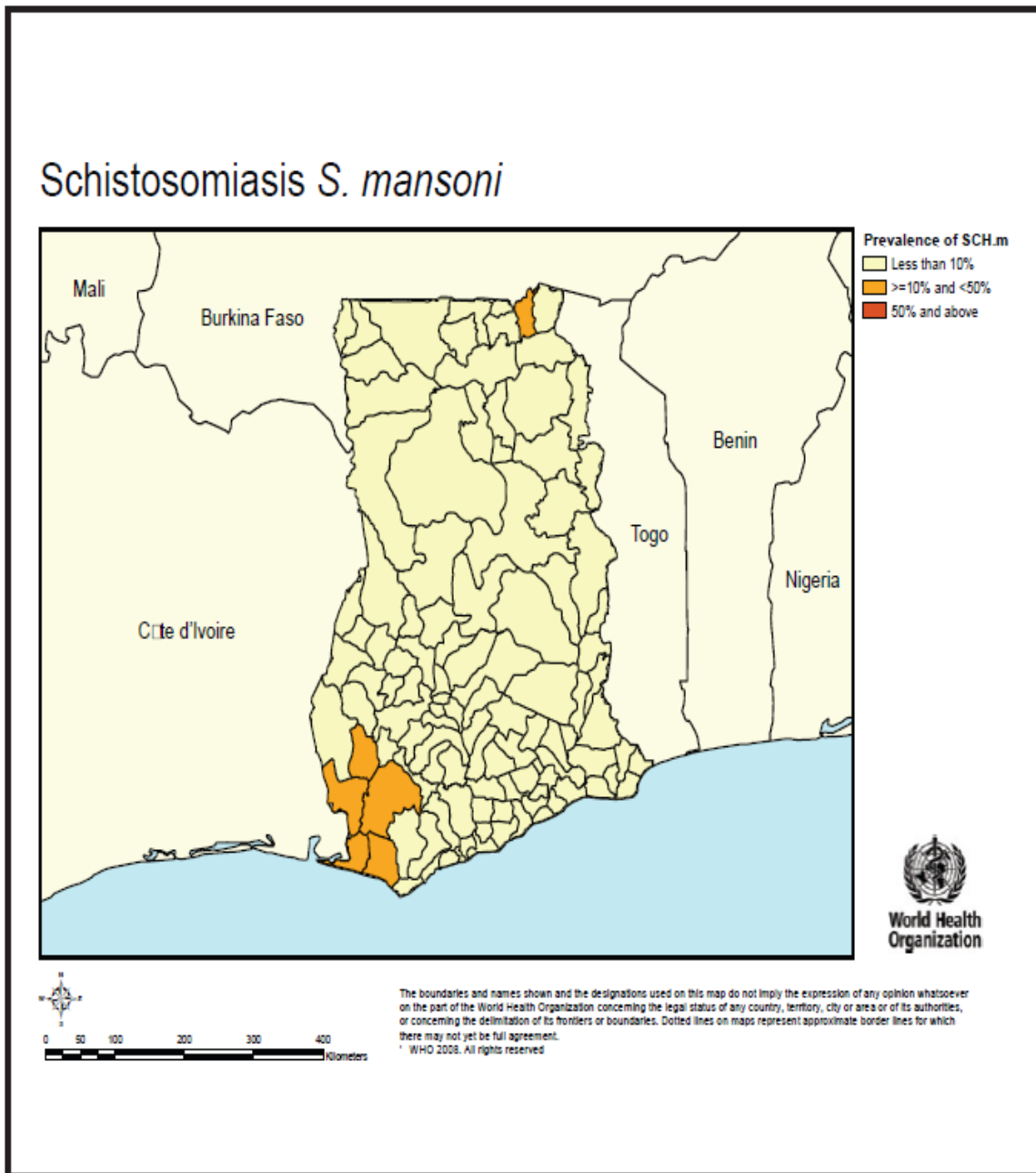


Figure 4: Distribution of *S. mansoni* in Ghana

Source: WHO, 2010

2.3 Morphology of schistosome parasites and eggs

2.3.1 Adult worms

Schistosoma parasites are cylindrical in shape. Adult male worms of *S. mansoni* and *S. haematobium* measure ~1cm by 1 mm in size. They possess a complex tegument, a blind digestive tract as well as oral and ventral suckers which they use for attachment to their definitive host. Coarse tubercles cover the entire tegument of *S. mansoni* male worms as opposed to the finer tubercles possessed by *S. haematobium* male worms. Tubercles are absent in *S. japonicum* male worms. Generally, male worms are shorter, stouter and possess a ventrally placed gynaecophoric canal (figure 5) in which they carry females (figure 6) for mating (Stewart, 2010).

Conversely, female adult schistosome worms are generally longer and thinner than male worms (Danso-Appiah, 2009) (figure 6). *S. mansoni* females measure ~1.1cm by 0.2mm and *S. haematobium* females measure ~1cm by 1mm. Female worms also possess suckers; an oral sucker near the oesophageal gland and a ventral sucker near the genital pore, though these are not as pronounced as those found in male worms (Stewart, 2010).

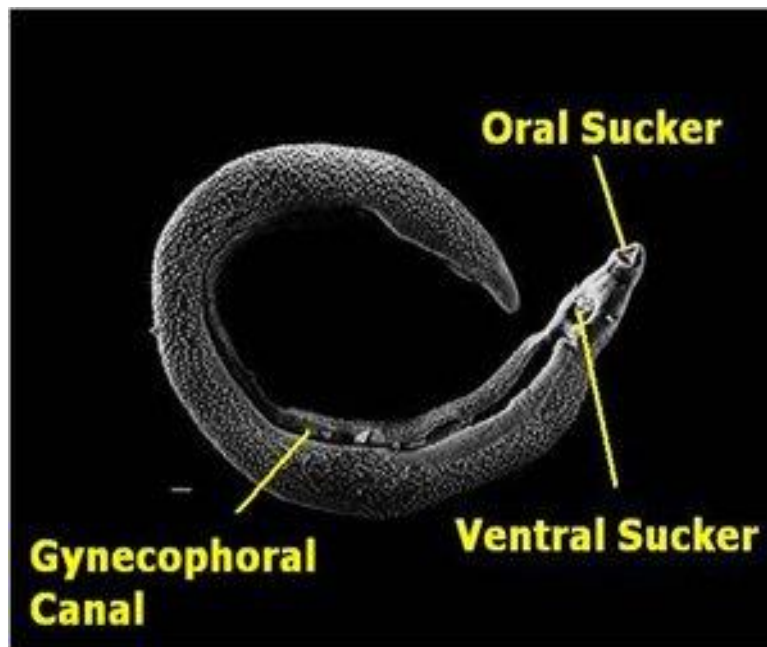


Figure 5: Morphology of male schistosome worm

Source: <http://www.mines45.multiply.com>

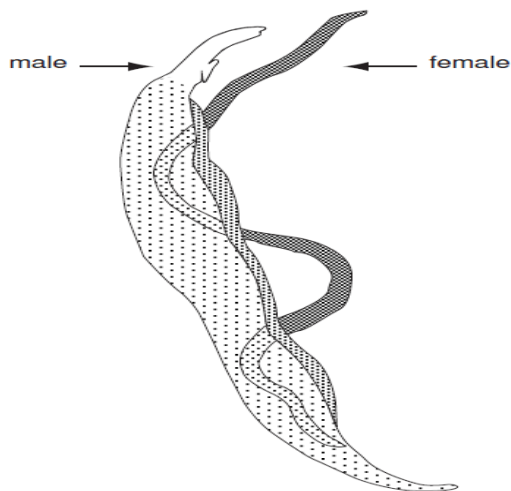


Figure 6: Male and female schistosome worms in copulation

Source: Lewis (1998)

2.3.2 The miracidium

Miracidium is a non-feeding, sexually differentiated form of the parasite that hatches out of the egg upon contact with water. This stage of the parasite is infective to the vector snails. It is a free-swimming stage of the parasite that measures 150-180 μm in length and 70-80 μm in width (Stewart, 2010). As shown in figure 7, the body of a miracidium is covered with cilia for rapid movement in water. It possesses a terebratorium, located at its apex, which aids attachment to the snail host for effective penetration. The parasite also has a nervous system comprising of photoreceptors and chemoreceptors that help in locating and penetrating its snail host (Walker, 2011).



Figure 7: Morphology of a miracidium

Source: <http://www.nhm.ac.uk>

2.3.3 The cercaria

Cercaria, the human infective stage of the schistosome parasite, develops from daughter sporocysts in the vector snail hosts. Akin to miracidium, it is a non-feeding and free-swimming stage of the parasite which is sexually differentiated. It measures $\sim 325\mu\text{m}$ in

length and is characterized by a head region and a bifurcated tail region (figure 8) that propels it in water (Lewis, 1998). Cercaria is wholly covered in a single continuous layer by a carbohydrate-rich tegument which is made up of three layers of plasma membrane consisting of spines and sensory papillae. These help in the location of its host, man (Stewart, 2010). It also possesses an acetabulum that forms its ventral sucker, a pre-acetabular gland which contains enzymes such as proteases essential for efficient penetration of its host skin, a post-acetabular gland that secretes proteases and mucus for adherence to its host surface and an excretory system made of flame cells which help in osmoregulation (Walker, 2011).

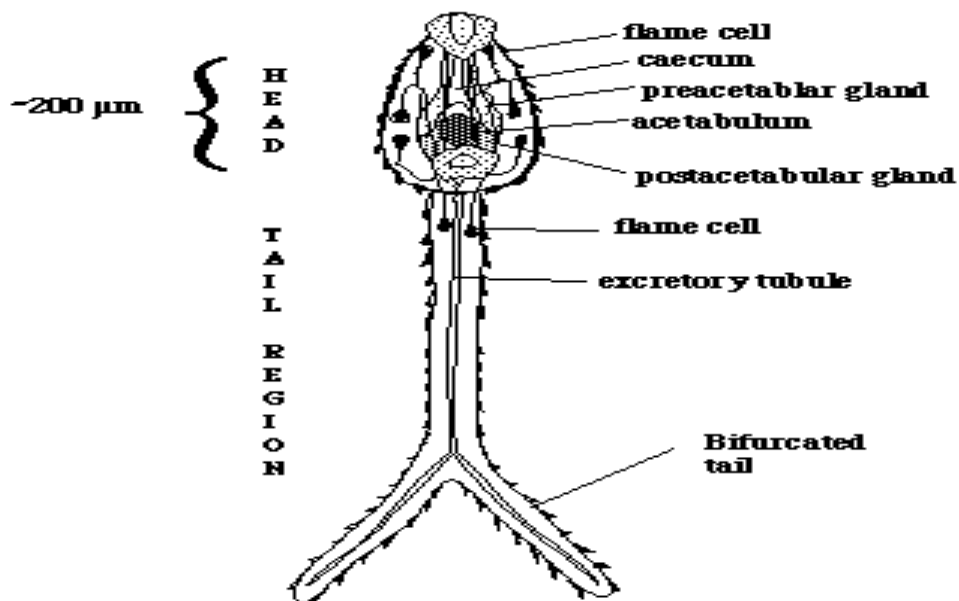
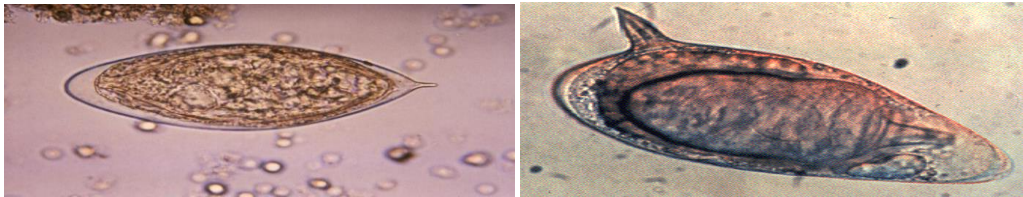


Figure 8: Morphology of a cercaria

Source: <http://www.path.cam.ac.uk/>

2.3.4 Human schistosome eggs

Presence of schistosome eggs in urine and/or stool are used for diagnoses of the disease in man (Shane et al., 2011). Based on the presence and position of spines, eggs are classified as either *S. haematobium*, *S. mansoni* or *S. japonicum*. *Schistosoma haematobium* eggs are large, non-operculate, and have a transparent shell, with terminal spines located at their posterior end (figure 9a). They measure between 112- 170 μ m in length (Kjetland et al., 2012). *Schistosoma mansoni* eggs are also large, possessing lateral spines close to their posterior ends (figure 9b). They measure between 114- 180 μ m in length (Silva et al., 2002). *Schistosoma japonicum* eggs are smaller, measuring between 68- 100 μ m in length (Centers for Disease Control and Prevention). These eggs also possess spines, although reduced (figure 9c). Most of the pathology associated with schistosomiasis are caused by the presence of eggs in tissues (Wynn et al., 2004; Danso-Appiah, 2009).



a) *S. haematobium* egg
<http://www.path.cam.ac.uk>

b) *S. mansoni* egg <http://www.commonswikimedia.org>



c) *S. japonicum* egg
<http://www.dpd.cdc.gov>

Figure 9: Morphology of the three main schistosome eggs of public health importance

2.4 Vectors of schistosomiasis

Vectors of schistosomiasis are freshwater snails belonging to various genera. *Oncomelania* spp. are the intermediate hosts of *S. japonicum*, while *Biomphalaria* and *Bulinus* spp. host *S. mansoni* and *S. haematobium* respectively (Madsen et al., 2008; Ayanda, 2009). They thrive and breed in stagnant freshwater bodies or slow sections of swift flowing water (Jones, 1993; Obeng, 1997; Yirenya-Tawiah et al., 2011), with aquatic vegetation at the fringes providing food, microhabitats, protection from strong water currents and cache for their eggs (Zakhary, 1997; Ofori, 1999). Clearing of aquatic vegetation may therefore help in controlling of vector populations at transmission sites (Yirenya-Tawiah et al., 2011). Vectors require favourable physiochemical conditions, including suitable pH and temperature to thrive well in the water bodies.

Biomphalaria pfeifferi (figure 10) is responsible for the transmission of intestinal schistosomiasis in Ghana, while two species of the genus *Bulinus*: *B. truncatus* (figure 11) and *B. globosus*, have been identified as transmitting urogenital schistosomiasis, with the latter confined to small streams in Ghana (Chu et al., 1978).



Figure 10: *Biomphalaria pfeifferi* snails feeding on aquatic vegetation



Figure 11: *Bulinus truncatus* snails feeding on aquatic vegetation

2.5 Mating interactions

Mating interactions among schistosome parasites in concurrent infections have been studied. These interactions have resulted in the formation of homospecific and/or heterospecific pairs. Works by Southgate et al. (1995), Webster et al. (1999), Webster and Southgate (2003) have reported mating interactions between *S. haematobium*, and *S. mattheei*, *S. mansoni* or *S. intercalatum* respectively. These studies have shown that *S. haematobium* males have a greater heterospecific mate preference compared to males of the other species. This occurrence may be attributed to the presence of *S. mansoni* eggs in urine of some individuals as stated in a study by Cunin et al. (2003). The study also revealed, schistosome eggs eliminated through unusual routes may be due to high infections with that particular schistosome species; causing a spill-over of eggs. Similar to works done by Webster et al. (1999) in Senegal, there are areas in Ghana where *S. haematobium* and *S. mansoni* occur in sympatry, though the two parasites utilize vectors of different species.

2.6 Clinical signs and symptoms of schistosomiasis

Clinical signs and symptoms of schistosomiasis may vary with the species of parasite, intensity and stage of infection. The disease can be manifested in three main forms; cercarial dermatitis (swimmers itch), acute schistosomiasis (katayama fever) and chronic schistosomiasis (Pearce & MacDonald, 2002). Cercarial dermatitis occurs mostly in 'naive' individuals few minutes after cercariae penetrate the skin. This is usually

characterized by the formation of urticarial rash at the site of penetration which is able to persist for several days in the form of papules (Gryseels et al., 2006).

2.6.1 Acute phase

Acute schistosomiasis or katayama fever is generally an asymptomatic phase of the disease. It is a hypersensitive immune response against migrating schistosomulae (Gryseels et al., 2006) and progresses to the stage when female worms begin laying eggs (Stewart, 2010). Symptoms generally associated with this phase include fever, fatigue, myalgia (muscle pain), malaise, non-productive cough, eosinophilia infiltrates on thorax radiography (Gryseels et al., 2006; Caldas et al., 2008) and in severe cases bloody diarrhoea.

2.6.2 Chronic phase

Individuals with chronic schistosomiasis, show symptoms months or years after initial exposure to cercariae. All chronic disease conditions of schistosomiasis are caused by the eggs trapped in tissues. Immune response by the hosts system against these eggs involve the release of cytokines, antibodies, and immune cells, including epithelial cells, macrophages and T cells, to mount an appropriate effector response (Wynn et al., 2004). This results in cellular inflammation of tissues leading to granuloma formation and subsequently fibrosis mostly in the liver and spleen (Stewart, 2010). This condition also leads to organ damage in the abdomen and hence ascites (accumulation of fluid in the abdomen) (Manzella et al., 2008).

2.7 Pathology associated with *Schistosoma* spp. infections

2.7.1 Pathology of *Schistosoma mansoni* infection

Individuals presenting with *S. mansoni* infections manifest the disease in two main forms: intestinal and hepatosplenic (Danso-Appiah, 2009). Eggs trapped in the intestines are engulfed by immune cells which cause inflammation resulting in granuloma formation. Some eggs are carried in the bloodstream to the liver where they get trapped in the liver tissues. Here again, immune cellular inflammatory response to the eggs leads to granuloma formation in the liver (figure 12). Over a long period of time, the granulomatous liver thickens and becomes fibrotic with an associated enlargement of the spleen resulting in a condition known as ‘hepatosplenomegaly’ (figure 13) (Cheever, 1978; Cheever et al., 1978).

As the liver continues to be inflamed due to immune reactions, liver cirrhosis sets in inhibiting blood flow and subsequently leading to portal hypertension. Once blood flow is obstructed, fluid accumulates in the vessels resulting in ascites and subsequent enlargement of the hepatic arteries forming varicose veins which eventually rupture causing anaemia as so much blood is lost. An infected person presenting with these symptoms may suffer from repeated variceal bleeding and haemorrhagic shock and may eventually die (Danso-Appiah, 2009).

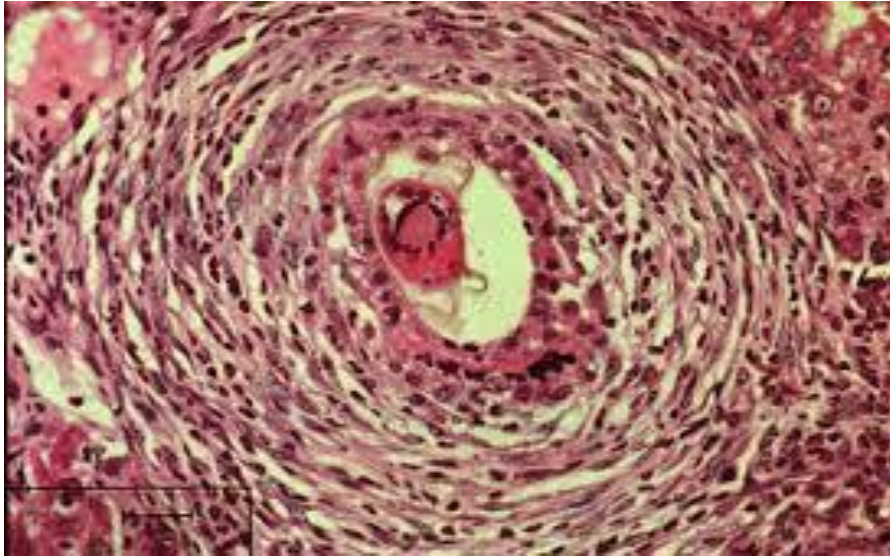


Figure 12: Schistosome egg granuloma in human liver

Source: [http:// www.path.cam.ac.uk/](http://www.path.cam.ac.uk/)



Figure 13: Hepatosplenomegaly resulting from *Schistosoma* infection

Source: [http:// www.path.cam.ac.uk](http://www.path.cam.ac.uk)

2.7.2 Pathology of *Schistosoma haematobium* infection

Individuals with *S. haematobium* infections normally develop ulcerations in the walls of the bladder causing haematuria (bloody urine), dysuria (painful urination) and pollakisuria (frequent urination) as well as cystitis (inflammation of the bladder) over a long period of time (Saathoff et al., 2004). Eggs in the ureter may cause obstruction in the lower ends leading to conditions such as hydroureter and hydronephrosis (Badmos et al., 2009). In advanced stages of the disease, cancerous bladder develops and blood loss from the genitourinary tract results in anaemia in infected individuals. Infections with *S. haematobium* also cause genital disease in both men and women resulting in infertility (Yirenya-Tawiah et al., 2011) as eggs pass through and cause lesions with their spines in the spermatic ducts of men and the cervix as well as vagina of women. These lesions present in the reproductive organs of infected persons predispose them to acquiring and transmitting HIV during sexual intercourse (Kjetland et al., 2006; Yirenya-Tawiah et al., 2011). This ensures the spread of the virus and subsequently AIDS in an already schistosomiasis diseased population.

2.8 Granuloma formation in schistosomiasis infections

The formation of granulomas in schistosomiasis infections is due to a delayed hypersensitivity reaction by the host immune response mechanism. It is a protective mechanism against severe schistosomiasis disease (Zuim et al., 2012) which results in the destruction of schistosome eggs (Warren & Domingo, 1970). A number of immune cells in granulomatous lesions play a role in the sequestration of toxins/antigens, released by

the trapped eggs. These include eosinophilic granulocytes, lymphocytes, fibroblasts and macrophages (Andrade & Azevedo, 1987; Lenzi et al., 1998; Baptista & Andrade, 2005). In liver tissues, infiltration of granulocytes and lymphocytes is a common feature of the egg induced granulomatous lesions. These lesions have been reported by Perrin and Philips (1989) to decrease in size as the disease progresses from an acute phase to a chronic phase. The length and size of granuloma lesions differ from one schistosome parasite to the other and are proportional to the persistence of eggs in tissue and the ability of host immune cells to destroy them (Lichtenberg, 1962, 1968).

S. mansoni infections have been extensively studied using mice as animal models to ascertain the immune responses associated with the disease. Apart from mice showing a high compatibility with the parasite, their availability and low cost have added to their increase use in laboratory experiments (Farah et al., 2000) although the strain of mice influences disease progression to a large extent (Warren & Peters, 1967; Cheever et al., 2002). *S. haematobium* infections on the other hand, have been less studied in animal models due to their compatibility with limited animal models such as hamsters (Webster et al., 1999). According to McLaren and Smithers (1987), *S. haematobium* parasites rarely mature into adult worm stages when introduced into animal models.

2.9 Diagnosis and treatment of schistosomiasis

Schistosomiasis can be diagnosed in four main ways including direct, indirect, immunological and molecular methods (Kahama et al., 1998).

2.9.1 Direct and indirect methods of schistosomiasis diagnosis

Diagnosis of schistosomiasis is based primarily on the examination of urine and stool samples for eggs of *Schistosoma* parasites using microscopy, a direct method (Gryseels et al., 2006). *Schistosoma* eggs and other intestinal helminthes in stool can be ascertained by microscopy using Kato-Katz technique, direct faecal smears and faecal concentration procedures. On the other hand, filtration techniques and sedimentation techniques are used to identify *Schistosoma* eggs in urine using microscopy. Although microscopy is inexpensive, straightforward (Feldmeier et al., 1998), and considered a standard method for diagnosing *Schistosoma* eggs (Gryseels et al., 2006) and other helminthes, it may not be useful particularly during the early stages of infection and when infection loads are low. Results obtained from microscopy, however, may not be a true representation of the egg/worm burden in an individual (Gryseels et al., 1996). Only urogenital schistosomiasis can be diagnosed using an indirect method, reagent strips. However, this method can also detect ulcerations caused by other infections other than urinary schistosomiasis, thus may not be effective for diagnosis.

2.9.2 Immunological and molecular diagnostic methods

Immunologic diagnostics of schistosomiasis include serologic tests and immunoassays such as ELISA. These methods can measure levels of specific antibodies such as IgG and IgE. Antibodies are directed against soluble egg antigens or adult worm antigens in the serum of an individual. Since antibodies can be detected even after cure, this diagnostic tool is not able to differentiate an active infection from a passive one (Rabello et al., 1997). Circulating soluble egg antigens in the urine and blood can be tested using ELISA. This technique is highly sensitive and thus reliable as it gives a clear indication of egg load and pathology status of an individual. (Kahama et al., 1998; Stothard et al., 2009). Polymerase chain reaction, a molecular technique, is highly sensitive and can be specifically used in diagnosing schistosomiasis. It is however very expensive and thus its use is not widespread compared to microscopy.

Molecular tests show positive results between one to two months after initial exposure to the parasite, and positive results may also indicate past exposure. Tissue biopsy is useful in identifying eggs that are lodged in tissues. In addition, ultrasound, chest X-rays, CT scans and MRI may be used to assess the damage of organs as inflicted by the presence of the parasites or their eggs.

2.9.3 Treatment

Currently, praziquantel is the drug of choice for the treatment of schistosomiasis (Shekhar, 1991; Fallon & Doenhoff, 1994; Badmos et al., 2009). The drug is only effective in eliminating the adult worms, but not the eggs which cause most of the pathology associated with the disease. Other drugs such as oxamniquine and metrifonate are also useful in treating schistosomiasis (Shekhar, 1991; Fallon & Doenhoff, 1994). Currently, there are no vaccines for schistosomiasis and there is also the need to develop new drugs, particularly in areas where parasites have developed resistant to praziquantel.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area and study population

The study was carried out in Tomefa, a coastal savannah vegetational zone, located along the Weija Lake, 17 km west of Accra in the Greater Accra region, with GPS coordinates N 05.57379° and W 000.37714°. The nearest health facility is about 10km from the community whose inhabitants are predominantly Ewes and Ga-Adangbes numbering approximately 1,500 (Danso-Appiah et al., 2010). The major occupation of men in the community is fishing with the women mostly trading in fish among other wares. Tomefa has one school (Kindergarten to Junior high school) with a population of about 200 pupils who were recruited as subjects for the study. The pupils were aged between 0 and 26years.

3.2 Sample collection

Informed consent was sought from community leaders, teachers, parents and adult pupils. Questionnaires were administered to the pupils to get information on their knowledge, practices and attitudes of schistosomiasis. Parasitological data were also collected to estimate the prevalence and intensity of the disease.

3.2.1 Urine sample collection and analysis

The study participants were given 50ml labelled screw cap tubes to provide 15ml or more of urine. They were instructed on the appropriate sample collection procedures to follow to avoid mix-ups and contamination. Urine samples were then kept at 4°C in a cool box and transported to the Laboratory of the Parasitology Department of Noguchi Memorial Institute for Medical Research (NMIMR). Urine samples were later analyzed using the sedimentation and filtration methods and examined by microscopy to detect schistosome parasite eggs.

3.2.1.1 Sedimentation technique

Each urine sample was homogenized and 10ml transferred into a new 50ml tube using a Pasteur pipette. The samples were then centrifuged at x1000 rpm for 5 minutes and the supernatant carefully decanted leaving a precipitate at the bottom of each tube. Each sample precipitate was transferred onto a slide with a Pasteur pipette and covered with a cover slip. The prepared slides were then examined for schistosome eggs using the x10 and x4 objectives of the Olympus CX21FS1 light microscope. Eggs observed were identified morphologically using the position of their spines to differentiate them into species. They were then counted and the numbers of each species recorded.

3.2.1.2 Filtration technique

Urine samples were homogenized and for each sample, 10ml was drawn using a syringe. Each drawn sample was then filtered through a 25mm diameter Millipore filter

membrane of pore size 12 μ m secured in a swinnex support chamber to trap schistosome eggs. The membrane was then carefully removed from the chamber, using a pair of forceps, and placed on a microscope slide with the side on which eggs were trapped being in contact with the slide surface. The slides were then examined for schistosome eggs using the x10 and x4 objectives of the Olympus CX21FS1 light microscope. Eggs observed were identified morphologically using the position of their spines to differentiate them into species. They were then counted and the numbers of each species recorded.

3.2.2 Stool sample collection and analysis

Participating pupils were each given a labelled plastic container to provide fresh stool sample. They were taken through appropriate procedures for sample collection to ensure the expected amount was received and contamination avoided. Collected stool samples were transported in a cool box to the laboratory (Parasitology Department, NMIMR) and stored at 4°C. Stool samples were later analyzed using the Kato-Katz and faecal concentration methods and examined microscopically to detect schistosome parasite eggs and eggs of other soil-transmitted helminths.

3.2.2.1 Kato-Katz technique

Hydrophilic cellophane strips measuring 25 x 35mm² in size were soaked in 50% glycerol-malachite green solution for about 24 hours (hrs) before use. For each collected specimen, a gram of stool was transferred onto a pergamon paper using an applicator stick in a hood. A nylon mesh was then pressed on top of the sample and a flat-sided

wooden applicator stick used to scrape across the upper surface of the mesh to sieve the stool sample. The sieved sample was used to fill the hole of a 6mm diameter template placed on a clean microscope slide. This was then levelled with the applicator stick and the template removed carefully to leave a cylindrical sieved stool sample on the slide. The sample on the slide was covered with the treated cellophane strip, inverted, and the sample firmly pressed against the cellophane strip on a smooth surface to evenly spread out. The prepared slide was then placed in a slide holder for about 30minutes (mins) to clear, after which it was examined for eggs using the x10 and x4 objectives of the Olympus CX21FS1 light microscope. Observed eggs were identified, counted and recorded.

3.2.2.2 Faecal concentration technique

One gram of stool was added to 10ml of 10% formalin solution in a centrifuge tube and stirred using an applicator stick to form a suspension. The suspension was then strained through two layers of gauze into another centrifuge tube. More 10% formalin solution was added to the strained suspension to bring the volume to 10ml. Three milliliters (3mm) of ethyl acetate was then added to the suspension in the tube and vigorously shaken for 10 seconds, after which it was centrifuged for 3 mins at 500g. Three layers of filtrate are formed in the tube; a layer of formalin at the bottom, a middle plug of faecal debris and a layer of the ethyl acetate on top. Using an applicator stick, the plug of faecal debris was carefully 'freed' from the walls of the tube, after which the filtrate was decanted in a single movement. The remaining sediment was examined under an

Olympus CX21FS1 light microscope at x10 and x4 objectives. Observed eggs were identified, counted and recorded.

3.2.3 Snail sample collection and exposure to determine infectivity

Schistosome vector snails were collected from the Weija Lake bordering the community with a scoop net into containers with water from the lake and transported to the laboratory (NMIMR). On arrival at the laboratory, the snails were separated, according to their species, into labelled aquaria containing distilled water and allowed 24hrs to adjust to laboratory conditions. Thereafter, snails were singly placed in each well of a 24-well plate containing distilled water. The plates were then placed under an artificial source of light for 2hrs to ensure maximum cercarial shedding by snails.

After exposure, snails were observed under x10 and x4 objectives of a CK30-F200 inverted microscope. Snails that were found to have shed cercariae were noted in addition to type of cercariae shed. Snails that shed no cercariae were returned to their respective aquaria containing fresh distilled water and placed in a dark environment. Cercariae shedding snails were also transferred from the well plates to new aquaria labelled according to the snail species and type of cercariae shed. These snails were also put in a dark environment to minimize rate of cercariae shedding. Exposure was repeated for 4 weeks for non-shedding snails to allow enough time for snails with patent infection at field sampling time to begin cercariae shedding. Snails were observed for cercariae shedding in intervals of two days to isolate those that had begun shedding.

3.3 Hatching of miracidia and infection to laboratory snails

3.3.1 Miracidium hatched from eggs in urine

Remaining urine samples containing only *S. haematobium* eggs were pooled in a test tube and spun at x1000 rpm for 5 mins. The supernatant was decanted leaving pelleted eggs, which were re-suspended in normal saline and again spun at x1000rpm for 5 mins. Once again the supernatant was decanted leaving pelleted eggs. The eggs were re-suspended in distilled water and transferred gently unto the bottom of a conical flask containing distilled water. The conical flask was then covered with an aluminium foil up to the neck region leaving a portion for light penetration. More distilled water was then added to fill the conical flask up to the uncovered portion and left to stand on the laboratory bench at room temperature for at least 30 mins for egg hatching into miracidium.

3.3.2 Miracidium hatched from eggs in stool

Stool from positive samples with only *S. mansoni* eggs were pooled and emulsified using normal saline in a beaker. This was then filtered using three different sieves of mesh sizes 150 μ m, 75 μ m and 45 μ m. Most of the debris in the stool was trapped by the sieves of mesh sizes 150 μ m and 75 μ m, with sieve of 45 μ m mesh size trapping mainly eggs. The contents of the sieve of mesh size 45 μ m was then washed with saline into a 50ml tube and spun at x1000rpm for 5 mins. Following, the supernatant was decanted leaving sedimented eggs which were re-suspended in normal saline and spun at x1000rpm for 5 mins. The supernatant was again decanted and the eggs re-suspended in distilled water which was then gently transferred unto the bottom of a conical flask containing distilled water. An aluminium foil was used to cover the conical flask up to the neck region

leaving a portion for light penetration. More distilled water was later added to fill the conical flask up to the uncovered portion and left to stand on the laboratory bench at room temperature for at least 30 mins for egg hatching into miracidium.

3.3.3 Miracidia infection to laboratory snails

Five to six miracidia of *S. mansoni* and *S. haematobium* in solution were separately pipetted into 20 wells of two 24-well plates. Each plate contained miracidia of only one schistosome parasite species. Twenty (20) snails each of laboratory bred *B. pfeifferi* and *B. globosus* were singly placed in these wells containing their respective schistosome parasites. The snails were left in the miracidia solution for at least 3hrs to ensure maximum infection after which they were transferred to their respective aquaria. Snails used were three months old F2 generations obtained from uninfected wildtype species sampled from the study community.

3.4 Cercariae shedding by infected snails

At four weeks post-parasite infection to *B. pfeifferi* and *B. globosus* snails respectively, the snails were transferred into a dark environment for 48hrs. The snails were then placed singly in wells of 24-well plates containing 1ml of distilled water. For at least 2hrs, the snails were exposed to an artificial source of light to induce cercarial shedding.

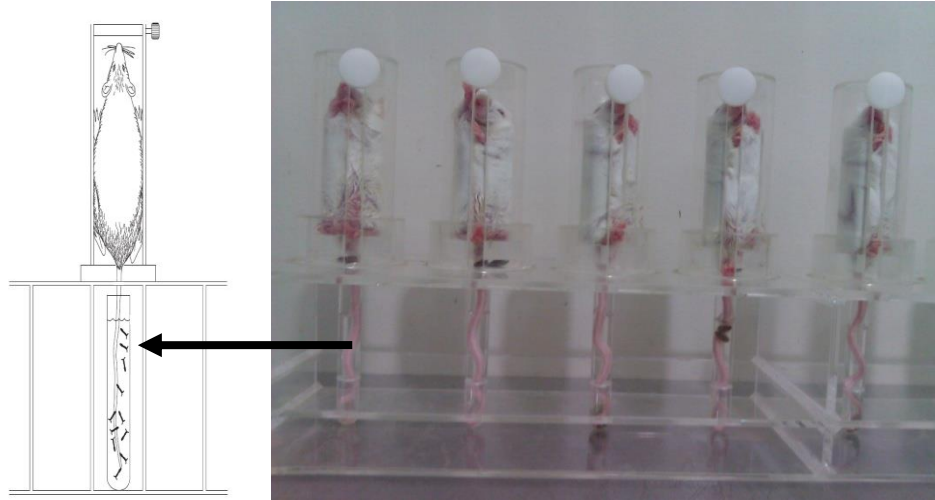
Exposed snails were then observed with the x4 and x10 objectives of the CK30-F200 inverted microscope to check for those that shed cercariae. Snails found not to have shed were returned to their respective aquaria.

Cercariae counts were made for snails shedding cercariae after which they were placed in new aquaria according to their species. All snails were again placed in a dark environment for 48hrs and later observed for cercarial shedding.

Cercariae were pooled into a test tube with the aid of a Pasteur pipette. About 20 μ l of cercariae solution was pipetted onto a slide and the cercariae counted. The counts were repeated three times to estimate the average number of cercariae in 20 μ l of cercariae solution.

3.5 Cercariae infection to laboratory mice

Cercariae solution was pipetted into restrainer test tubes and distilled water added to about three-quarters the volume of each test tube. The tubes, each containing between 150-200 human infective cercariae, were then placed in a restrainer rack and mice infected through their tails (figure 14). In all, 9 female ICR mice, (3 mice/ group) were each infected for an hour to ensure maximum infection. Group A mice were infected with *S. mansoni* cercariae only, whereas mice in group B were infected with only *S. haematobium* cercariae. The last group of mice, group C, were infected simultaneously with cercariae of *S. mansoni* and *S. haematobium*.

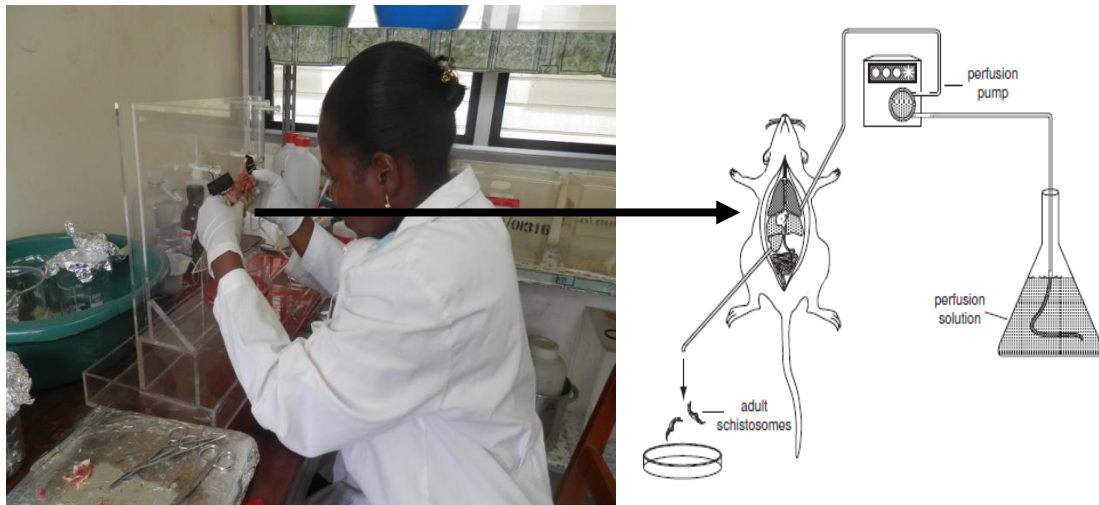


Source: Lewis (1998)

Figure 14: An illustrative diagram and picture of cercariae infection to mice through their tails.

3.6 Perfusion of mice

Infected mice were euthanized after 8 weeks and 12 weeks for *S. mansoni* and *S. haematobium* infections respectively. Mice infected simultaneously with *S. mansoni* and *S. haematobium* cercariae were also euthanized at 12 weeks post- parasite infection. Euthanized mice were dorsally placed onto a dissecting board and dissected to reveal the hepatic portal vein and mesenteric venous systems. Their livers were then cleaned by perfusing with citrate-saline buffer (prepared from 0.85% of sodium citrate and 0.9% of sodium chloride) and schistosome worms collected in the process (figure 15). Granulomatous livers and intestines of perfused mice were harvested, processed histologically and examined microscopically.



Source: Lewis (1998)

Figure 15: A picture and an illustrative diagram of the perfusion of a mouse.

3.7 Schistosome eggs from perfused mice

Cut portions of granulomatous liver and intestines of perfused mice were harvested and macerated in saline solution in an electric blender. The macerated tissue in solution was then sieved using three different sieves of mesh sizes 150 μ m, 75 μ m and 45 μ m. Most of the tissue was trapped by the sieve of mesh size 150 μ m, with sieves of 75 μ m and 45 μ m mesh sizes trapping mainly eggs. The contents of the sieves of mesh sizes 75 μ m and 45 μ m was then washed with saline into a 50ml tube and spun at x1000rpm for 5 mins. Following, the supernatant was decanted leaving sedimented eggs. The sedimented eggs were collected and hatched into miracidia using the process for hatching eggs from urine and stool. Miracidia obtained were then used to infect laboratory snails, following which infection to laboratory mice were made with cercariae obtained from the infected snails to maintain the life cycle in the laboratory.

3.8 Sampling and processing of mice liver and mesenteric vessels for histology

Granulomatous liver and mesenteric tissues harvested from infected mice were fixed in 20ml of 10% buffered formalin. Fixed tissues were then taken through routine histological processes of dehydration, embedding, sectioning, de-waxing, rehydration, staining, dehydration and mounting.

In dehydrating, tissues were passed through graded alcohol series of 50%, 70%, 95% and 100%. However before being embedded in paraffin wax, tissues were put in xylene, a solution that is easily miscible with wax to allow for proper embedding of tissue. Dehydrated tissues were then infiltrated with wax at 58°C after which they were put in labelled cassettes, filled with molten wax and allowed to cool to obtain a block of tissue embedded in wax.

Tissue blocks were kept at -5°C in a refrigerator to further harden before sectioning with a Leica 2235 rotary microtome at a thickness of 5.0 µm. Sectioned tissue blocks were placed in a water bath of temperature 48°C to spread out and then picked with labelled microscope slides and allowed to dry in an oven.

Each sectioned tissue on a slide was de-waxed by dipping in xylene and then rehydrated by passing through graded alcohol of 100%, 95%, 70% and 50%.

Rehydrated sectioned tissues were then stained with haematoxylin and eosin (H&E) and dehydrated again in serial solutions of alcohol of 50%, 70%, 95%, 100% followed by

dipping in xylene. Stained tissue sections on slides were then prepared for microscopic examination by mounting with dibutylphthalate in xylene, DPX.

3.9 Data analysis

Parasite distribution according to sex and age of study participants were entered onto Microsoft Excel Spreadsheets and the prevalence and intensities of infection determined. All data were assessed for normality, following which non-normal data were normalized through transformations. ANOVA and unpaired t-tests were used to test for statistical significance. ANOVA was carried out to determine variations in mean intensities of parasite eggs among the various age groups of participants. A two sample unpaired t-test was also used to determine statistical significance between snail vectors shedding cercariae. All analyses were carried out at a set alpha (α) level of 0.05. Genstat Statistical Software (version 9.2) was used in all analyses except for questionnaire data. Data obtained from questionnaires administered were analyzed using Statistical Package for Social Science (SPSS) version 21.

3.9.1 Prevalence of infection

The prevalence of both urogenital and intestinal schistosomiasis is measured in percentage (%). Infection prevalence among age groups and sex was determined as the ratio of positive individuals to the total number of study participants and mathematically represented as:

$$\text{Prevalence} = \frac{\text{Number of subjects testing positive}}{\text{Number of subjects investigated}} \times 100\%$$

3.9.2 Intensity of infection

The intensity of urogenital schistosomiasis was measured as the number of eggs in 10ml of urine (ep10ml). Positive urine samples were classified as light intensity (1–49ep10ml) or heavy intensity (≥ 50 ep10ml).

In the case of intestinal schistosomiasis, intensity was measured as total egg count in one gram of stool and classified as either light (1–99epg), moderate (100–399epg) or heavy (>400 epg) infections.

The mean egg loads were also calculated and expressed as:

$$\text{Mean egg} = \frac{\text{Sum of each individual epg}}{\text{Number of subjects being investigated}}$$

3.10 Definition of terms

Single infections were defined as individuals with only one type of schistosome parasite egg present in either urine or stool. Concurrent infections were defined as individuals with both schistosome parasite eggs irrespective of route of elimination.

CHAPTER FOUR

RESULTS

4.1 Demography and sample analysis of study participants

As shown in table 1, a total of 202 pupils consisting of 102 males and 100 females from the Peace International School, Tomefa, who were aged between 0 and 26 years (yrs) were involved in the study. All the study participants gave urine samples whereas only 181 comprising of 91 males and 90 females provided stool samples (table 1). Following parasitological examination for the presence of schistosome eggs in the 202 stool samples, 163 tested positive for schistosome parasite eggs; this consisted 146 *S. mansoni* single infection and 17 concurrent infections of both *S. mansoni* and *S. haematobium* (*S. mansoni*+*S. haematobium*) (table 2). As shown in table 3, of the 202 children who presented urine samples, 135 were positive for schistosome eggs, comprising of 59 *S. haematobium* single infections, 3 *S. mansoni* single infections and 73 concurrent infections (*S. mansoni*+*S. haematobium*).

Table 1: Demographic data of study participants.

Age group (yrs)	Urine samples (+ve individuals)		Stool samples (+ve individuals)	
	Male	Female	Male	Female
0 – 5	5 (3)	12 (2)	5 (5)	10 (7)
6 – 8	18 (12)	17 (9)	18 (17)	15 (14)
9 – 11	9 (7)	18 (10)	9 (9)	17 (16)
12 – 15	51 (38)	45 (35)	48 (43)	40 (36)
>15	19 (12)	8 (7)	11 (10)	8 (6)
Total	102 (72)	100 (63)	91 (84)	90 (79)
Overall total	202 (135)		181 (163)	

+ve = positive

Table 2: Infection status of intestinal schistosomiasis by age range of study participants.

Age group (yrs)	Number examined	Infection status of positive individuals	
		Sm single infections	Sm+Sh infections
0 – 5	15	9	3
6 – 8	33	30	1
9 – 11	26	23	2
12 – 15	88	69	10
>15	19	15	1
Total	181	146	17

Sm = *Schistosoma mansoni* Sh = *Schistosoma haematobium*

Table 3: Infection status of urogenital schistosomiasis by age range of study participants.

Age group (yrs)	Number examined	Infection status of positive individuals	
		Sh single infections (Sm only)	Sm+Sh infections
0 – 5	17	1 (1)	3
6 – 8	35	9 (2)	10
9 – 11	27	4 (0)	13
12 – 15	96	35 (0)	38
>15	27	10 (0)	9
Total	202	59 (3)	73

Sm = *Schistosoma mansoni* Sh = *Schistosoma haematobium*

(Sm only) = individuals with only Sm eggs in urine

4.2 Prevalence of schistosomiasis

4.2.1 Age distribution of intestinal schistosomiasis

Prevalence of single *S. mansoni* infections, ranged between 59% and 91%, with the highest prevalence occurring in the (6–8) yrs age group. On the other hand, prevalence of both parasite species (*S. mansoni*+*S. haematobium*) at the same time in stool samples ranged between 2-21%, with the highest prevalence being recorded in the (0–5) yrs age group (figure 16). Overall prevalence of intestinal schistosomiasis among the pupils was 90.1% (163/181), and all age groups presented both concurrent and single infections in stool (table 2 and figure 16).

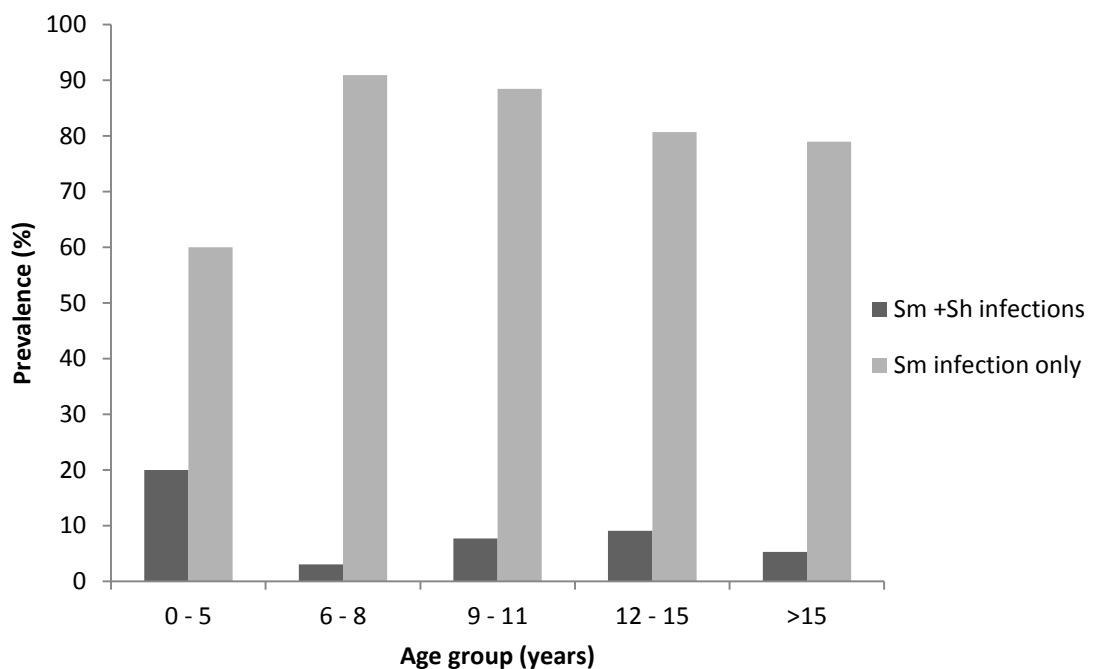


Figure 16: Prevalence of intestinal schistosomiasis of single and concurrent infections by age range.

4.2.2 Age distribution of urogenital schistosomiasis

Prevalence of *S. haematobium* single infections, ranged between 5-45%, with the highest prevalence occurring in the >15yrs age group. In the case of infections with both parasite species, the highest prevalence was recorded in the (9–11) yrs age group, with a range of between 23-49% across all age groups studied (figure 17). Generally, prevalence of urogenital schistosomiasis determined among the pupils was 66.8% (135/202), with all age groups presenting both *S. mansoni*+*S. haematobium* concurrent infections as well as *S. haematobium* single infections as shown in table 3 and figure 17.

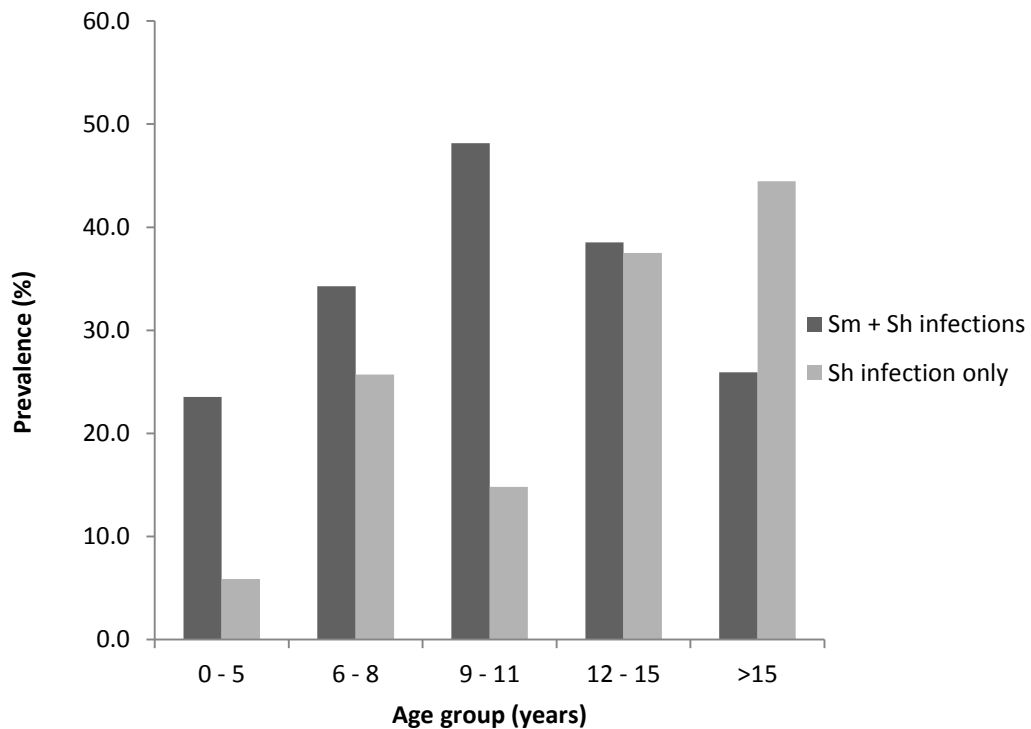


Figure 17: Prevalence of urogenital schistosomiasis of single and concurrent infections by age range in study participants.

4.2.3 Sex distribution of intestinal schistosomiasis

The prevalence of intestinal schistosomiasis based on the sex of study participants showed males had higher prevalence of infection (92.3%) compared to females (87.8%) among all the age groups (figure 18 and table 1). Whereas female infection prevalence was highest in the ages ranging from 6–15 yrs; male prevalence was generally high for all the age groups studied.

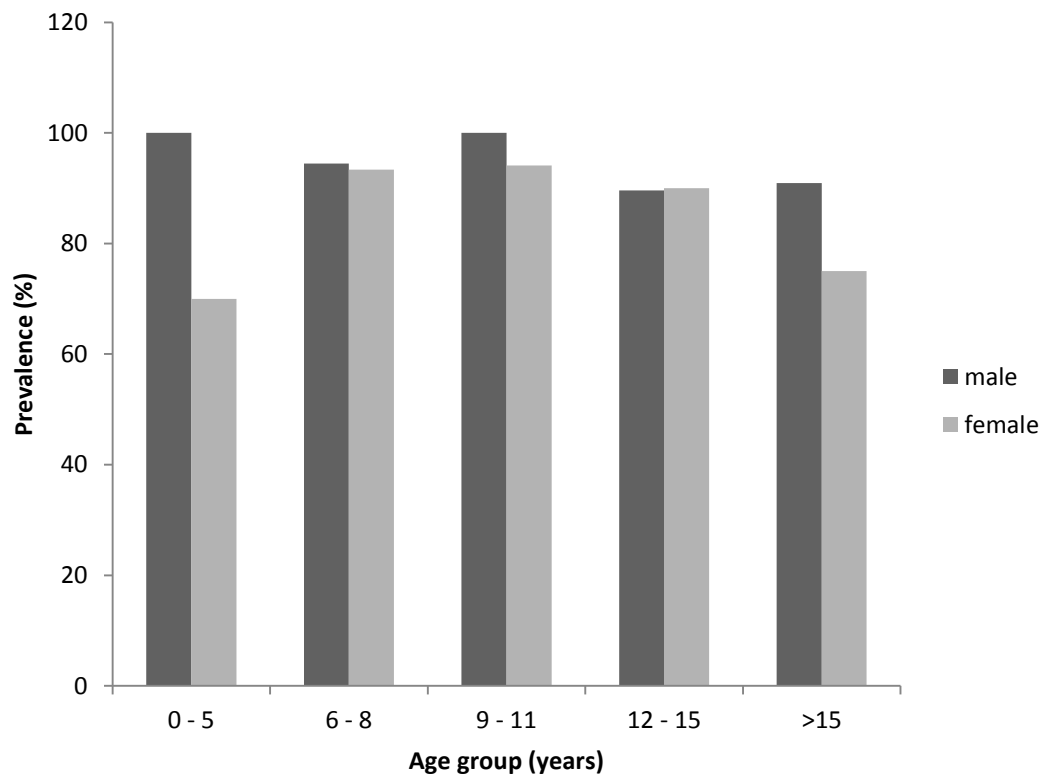


Figure 18: Prevalence of intestinal schistosomiasis by sex across age groups in study participants.

4.2.4 Sex distribution of urogenital schistosomiasis

Prevalence of urogenital schistosomiasis increased steadily from the lowest age group for both sexes, with peaks at the (9–11) yrs age group for males and >15yrs age group for females as shown in figure 19.

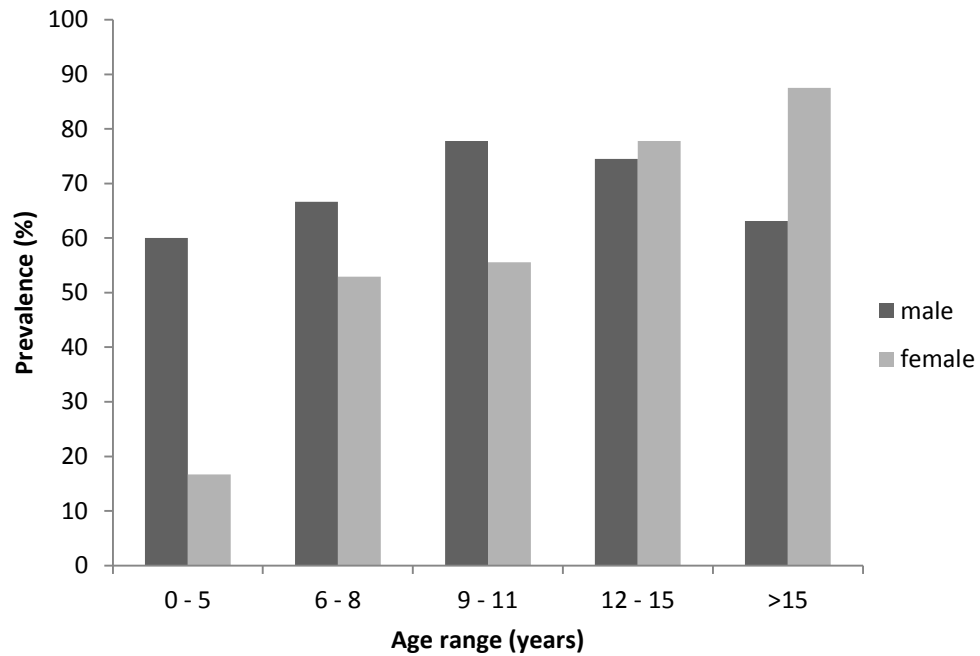


Figure 19: Prevalence of urogenital schistosomiasis by sex across age groups in study participants.

4.3 Single and concurrent *Schistosoma* spp. infections in study participants

As shown in table 4, majority of the study participants (131) showed concurrent infections relative to single infections (50). Of the 131 individuals with concurrent infections, 50 had *S. mansoni* eggs in stool and *S. haematobium* eggs in urine (type A concurrent infections), with the remaining showing both *S. mansoni* and *S. haematobium* eggs in either urine or stool, or both (type B concurrent infections).

Table 5 further shows the distribution of parasites in individuals classified as type B concurrent infections. Among the 81 individuals, 63 had both *S. mansoni* and *S. haematobium* eggs in their urine only, with 6 individuals presenting both parasites in stool only. The remaining 12 individuals had both *S. mansoni* and *S. haematobium* eggs present in their urine as well as in the stool.

Table 4: Single and concurrent *Schistosoma* spp. infections in study participants.

Types of infections		Number of individuals infected	Total
Single infections	Sm eggs only	43	50
	Sh eggs only	7	
Concurrent infections	Type A	50	131
	Type B	81	
Total		181	181

Sm = *S. mansoni* Sh = *S. haematobium*

Single infections = individuals with only one type of parasite egg in stool/urine.

Concurrent infections (type A) = individuals with both *S. mansoni* eggs in stool and *S. haematobium* eggs in urine.

Concurrent infections (type B) = individuals with both *S. mansoni* and *S. haematobium* eggs in urine and/or stool.

Table 5: Categorization of type B concurrent infections in study participants.

Category of type B concurrent infection	Number of individuals
Sm and Sh in urine	63
Sm and Sh in stool	6
Sh and Sm in urine and stool	12
Total	81

Sm = *S. mansoni* Sh = *S. haematobium*

4.4 Intensity of schistosomiasis infection

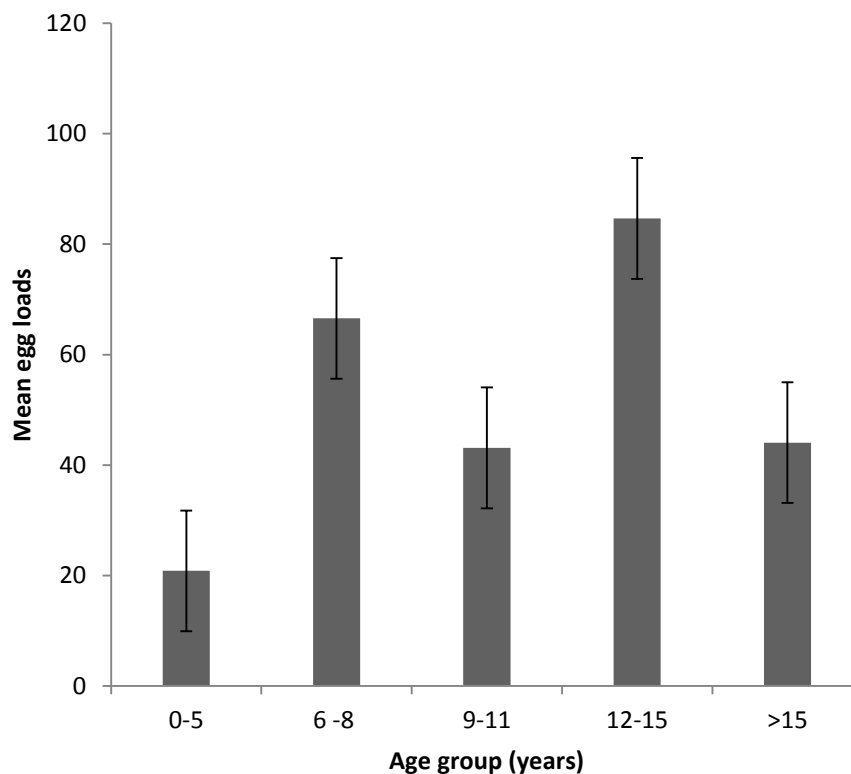
4.4.1 Intestinal schistosomiasis intensities and mean egg loads

As presented in table 6, among the 163 egg positive individuals, 131 individuals presented light infections, including all 12 individuals in the (0–5) yrs age group. The highest number of individuals with the infection intensity threshold described as moderate intensity of infection (30 positive individuals) belonged to the 6–8 and 12–15 age groups. Again, only two individuals, each from the same age groups (6–8 and 12–15) yrs, had heavy intensity of infection. Although the calculated difference in the mean egg loads among all the age groups was not statistically significant ($p=0.19$), individuals in the (12–15) yrs age group had the highest mean egg load, with individuals in the (0–5) yrs age group presenting the lowest as shown in figure 20.

Table 6: Intensity of intestinal schistosomiasis by age range of study participants.

Age range (yrs)	Infection Intensity Thresholds		
	Light (1 - 99 epg)	Moderate (100 – 399 epg)	Heavy (\geq 400 epg)
0 – 5	12	0	0
6 – 8	25	5	1
9 – 11	23	2	0
12 – 15	57	21	1
>15	14	2	0
Total	131	30	2

epg = eggs per gram

**Figure 20:** Mean egg loads of intestinal schistosomiasis in different age groups of study subjects.

4.4.2 Urogenital schistosomiasis intensities and mean egg loads

From table 7, study participants from all age groups had either light or heavy infections. Seventy (70) positive individuals presented light infections as against 65 with heavy infections. Of the heavy infections recorded, majority of those individuals were in the (6–8 and 12-15) yrs age group. For the calculated mean egg loads, individuals in the (6–8) yrs age group had the highest while the least was recorded in the >15 yrs age group (figure 21). The difference in the mean egg loads among all the age groups was however not statistically significant ($p=0.59$).

Table 7: Intensity of urogenital schistosomiasis among the different age ranges of study participants.

Age range (yrs)	Infection intensity thresholds	
	Light (1 - 49 ep10ml)	Heavy (≥ 50 ep10ml)
0 – 5	3	2
6 – 8	10	11
9 – 11	10	7
12 – 15	36	37
>15	11	8
Total	70	65

ep10ml = eggs per 10ml

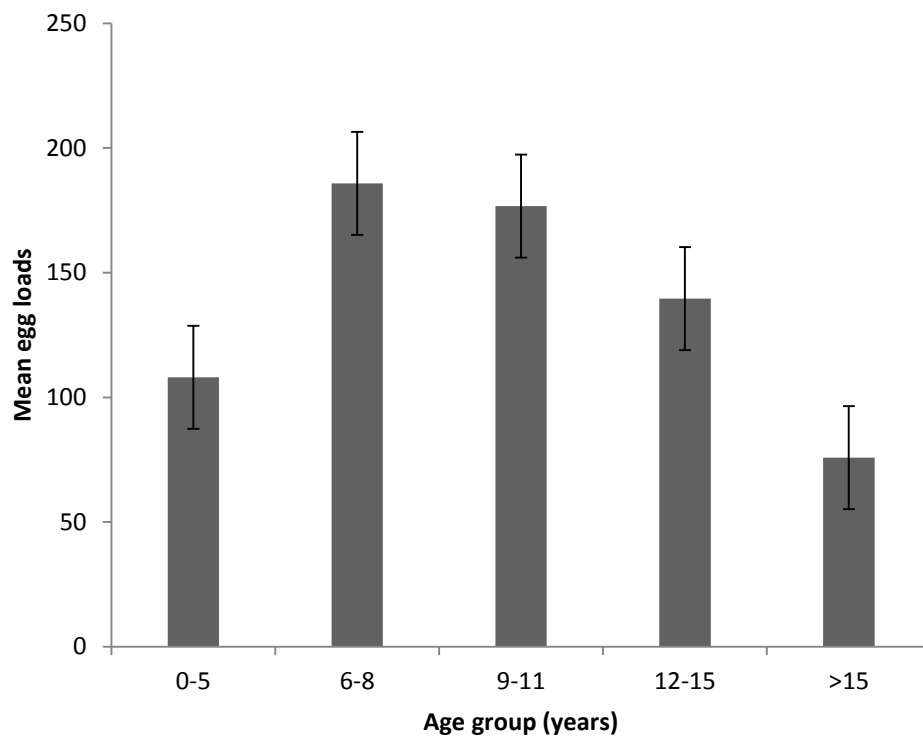


Figure 21: Mean egg loads of urogenital schistosomiasis.

4.5 Water contact activities of study participants and sewerage facilities available in study community

Table 8 shows the various activities undertaken by male and female study participants at the side of the lake bordering the community. Fishing, fetching of water, washing and playing were the four main activities study participants engaged in along the lake. Generally, more males (102) engaged in fishing in the lake compared to females (7), with nearly an equal number of both sexes (95 males and 96 females) fetching water from the lake for domestic purposes, including cooking and bathing. All respondents of the questionnaire, 102 males and 100 females (table 1) stated the lake to be the main source of water supply to the community.

Table 9 presents the various sewerage facilities utilised by the study population. It was evident in the study that there were inadequate toilet facilities in the community. Study participants used either of two main sewerage facilities- pit-latrines or nearby bushes mainly at the banks of the lake.

Table 8: Water Contact Activities of Study Participants

Water contact activities	Males	Females	Total
Fishing	102	7	109
Fetching water	95	96	191
Washing	75	85	160
Playing	46	12	58

Table 9: Sewerage facilities used by Study Participants

Sewerage facility	No. of individuals
Flush toilet	0
Pit-latrine	133
Bush area	127

4.6 Field snail vectors identification and collection

Species of snail vectors sampled from the part of the lake used by the study community mainly for fishing and domestic activities were identified as *B. pfeifferi* and *B. truncatus*. Snail vectors were found mostly attached to aquatic vegetation, including *Nymphaea lotus* (figure 22) and *Ceratophyllum demersum* (figure 23). Other aquatic vegetation found at the banks of the lake where snail vectors were sampled from included: *Ipomea aquatica*, *Digitari insularis* and *Floscopa africana*.

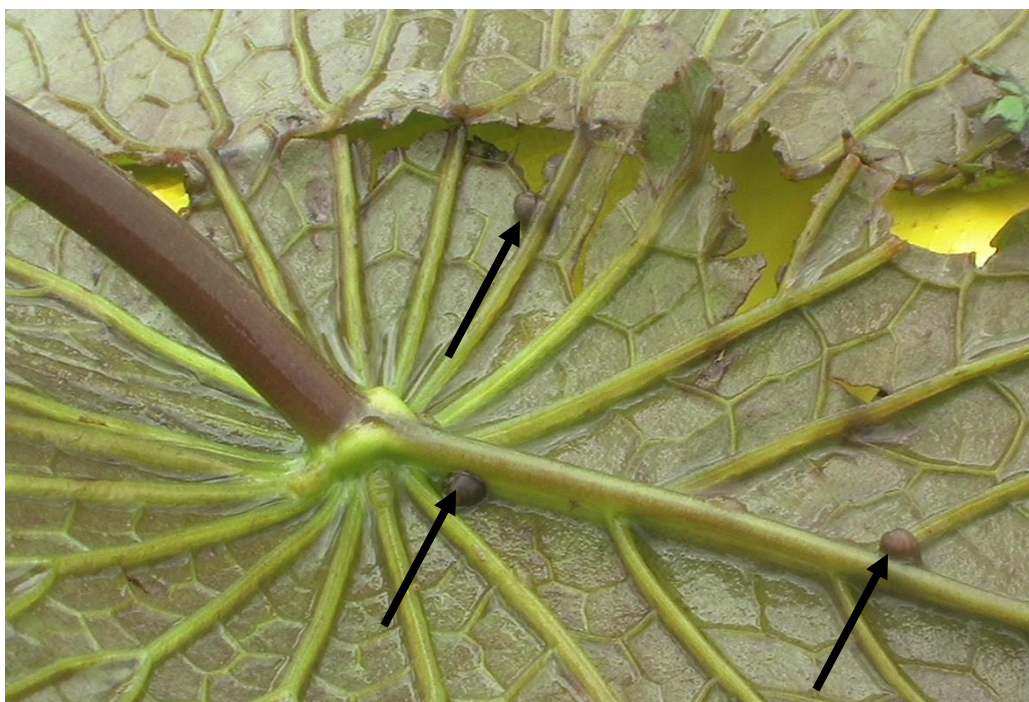


Figure 22: *Bulinus truncatus* snails (indicated by arrows) attached to *Nymphaea lotus*.

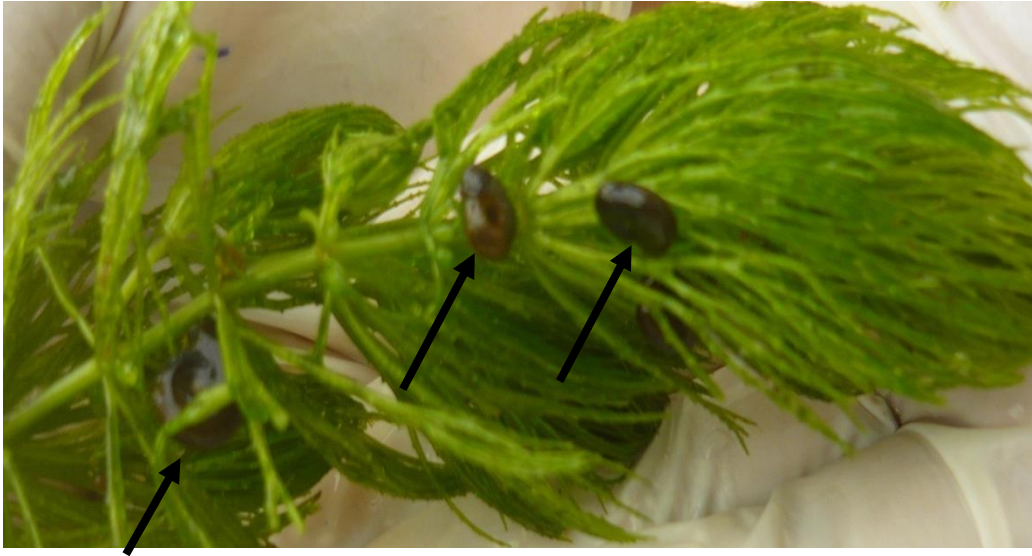
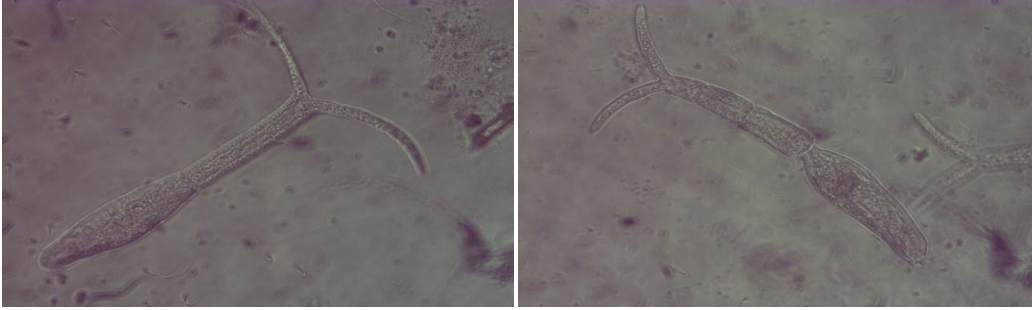
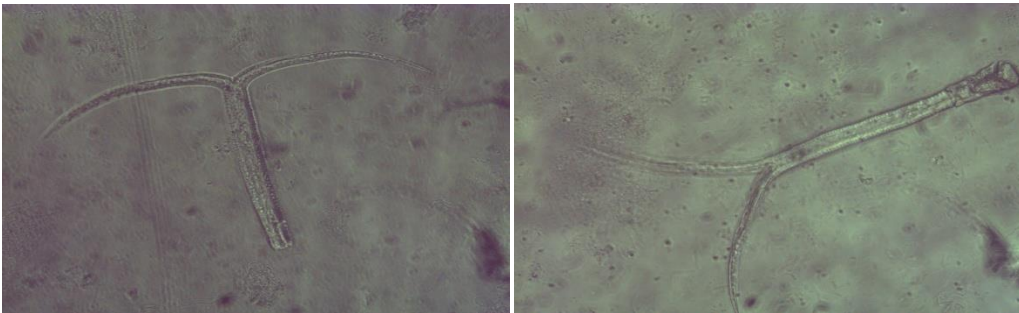


Figure 23: *Biomphalaria pfeifferi* snails (indicated by arrows) attached to *Ceratophyllum demersum*.

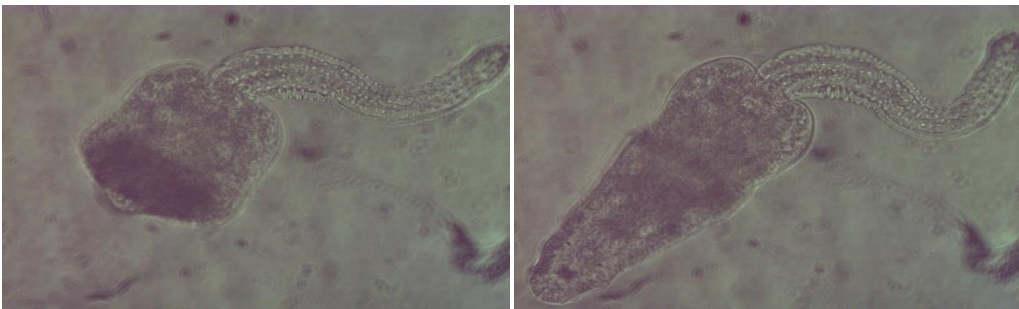
In table 10, a total of 1,676 snails consisting 780 *B. pfeifferi* and 896 *B. truncatus* were sampled from the lake. Although the numbers of *B. truncatus* snails were higher than that of *B. pfeifferi* snails, the difference was not statistically significant ($p = 0.81$). A hundred and fifty-six (156) *B. pfeifferi* out of the 780 collected and 85 out of the 896 *B. truncatus* snails were observed to be shedding at least one of three morphologically different cercariae parasites (figure 24a,b&c), of which 14 (8.9%) and 7 (8.2%) of the infected *B. pfeifferi* and *B. truncatus* snails respectively, shed cercariae infective to human only. The calculated means of snails of either species found to shed only human cercariae were equal ($p = 0.24$). Interestingly, one *B. truncatus* and one *B. pfeifferi* were each found to shed both human and one other cercariae not known to be infective to man (figure 24a&b).



a) Human infective cercariae shed by both *B. truncatus* and *B. pfeifferi* snails



b) Unknown cercariae type 1 shed by both *B. truncatus* and *B. pfeifferi* snails



c) Unknown cercariae type 2 shed by both *B. truncatus* and *B. pfeifferi* snails

Figure 24: Three morphologically distinct cercariae types shed by both *B. truncatus* and *B. pfeifferi* snails

Table 10: Infection status of snails collected from the field.

Snail vector species	Number examined	Number of snails shedding cercariae			
		Human only	Other	Human+other	Total
<i>Bulinus truncatus</i>	896	7	77	1	85
<i>Biomphalaria pfeifferi</i>	780	14	141	1	156
Total	1676	21	218	2	241

4.7 Infections to laboratory-bred snail vectors

As shown in table 11, of a total of 57 second filial generation (F2) snails consisting of 32 *B. pfeifferi* and 25 *B. truncatus* that were infected with human cercariae, eight (25%) of the *B. pfeifferi* snails shed cercariae whereas none of the *B. truncatus* snails shed cercariae after 4 weeks.

Table 11: Infection status of laboratory-bred snails at four weeks post-infection.

Snail vector species	Snail numbers		
	Infected	Alive at exposure	Shedding at exposure
<i>Biomphalaria pfeifferi</i>	32	31	8
<i>Bulinus truncatus</i>	25	25	0
Total	57	56	8

4.8 Infection status of laboratory mice infected with human *Schistosoma* cercariae

Table 12 shows the infection status of 3 groups of mice. Group A mice (*S. mansoni* infected) gave a total of 127 schistosome worms, comprising 45 single and 41 paired worms. Fifteen worms consisting 11 single and 2 paired ones were obtained from group B mice (*S. haematobium* infected). Of a total 235 worms collected from group C mice (*S. mansoni*+*S. haematobium* infected), 90 were paired and 55 were single.

Table 12: Schistosome worms recovered from laboratory mice infected with human infective schistosome cercariae

Infection group	Mouse number	Single worms collected	Paired worms collected	Total
Group A	1	5	8	21
	2	20	13	46
	3	20	20	60
	Total	45	41	127
Group B	1	2	0	2
	2	9	2	13
	3	0	0	0
	Total	11	2	15
Group C	1	13	3	19
	2	22	32	86
	3	20	55	130
	Total	55	90	235

4.9 Processing and staining of infected mice tissues (liver and intestines)

Upon dissection of infected mice, the intestines were found enlarged and the livers hardened with clear granulomatous lesions. Further processing of the tissues histologically, revealed granuloma formations around schistosome eggs trapped in the tissues.

As shown in figures 25a&b, 26a&b, 27a&b, 28a&b, 29a&b and 30a&b granuloma lesions formed around eggs varied for the three mice groups.

In comparison with tissues of mice infected with only *S. mansoni* (group A) and *S. haematobium* (group B) cercariae, tissues of mice infected with cercariae of both species (group C) showed gross pathology revealing an up-regulation of inflammatory cells (mainly eosinophils, basophils, mast cells and neutrophils) in the entire tissue. The granulomatous lesions around mixed (*S. mansoni* and *S. haematobium*) parasite eggs were also larger in size due to the high intensity of infiltration of inflammatory cells.

Whereas granuloma formation was large in livers and intestines of group A mice which were presented with *S. mansoni* eggs, quite smaller granulomas were found in those of group B mice which presented no schistosome eggs but only a worm in the liver.

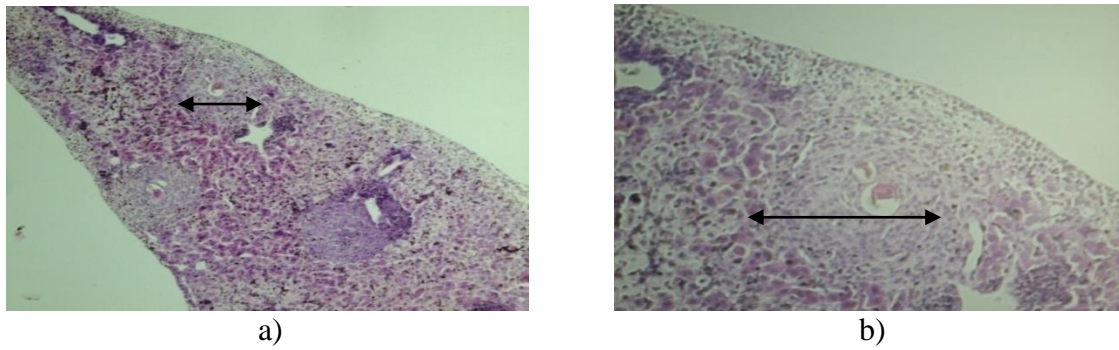


Figure 25: Liver of mice infected with cercariae of only *S. mansoni* parasites.

Panels a) and b) show respectively, x40 and x100 magnifications of sectioned mice liver stained with haematoxylin and eosin. Arrows indicate size of granuloma formations surrounding *S. mansoni* eggs in mice liver.

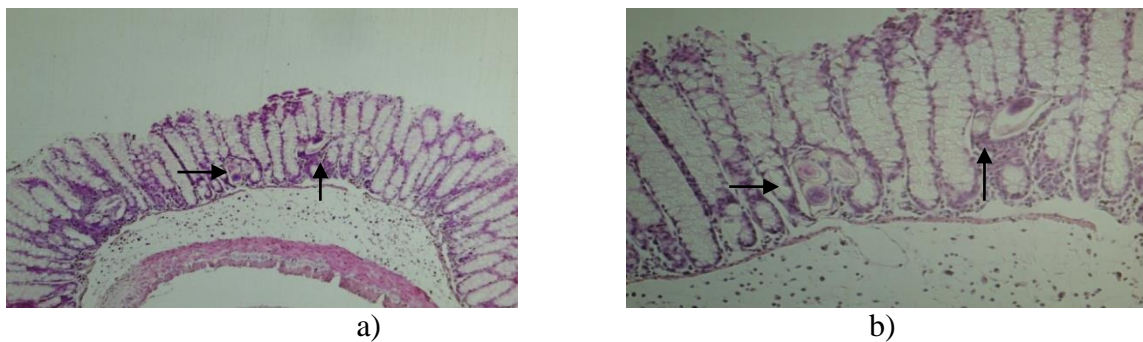


Figure 26: Intestines of mice infected with cercariae of only *S. mansoni* parasites. Panels a) and b) show x40 and x100 magnifications respectively of sectioned mice intestines stained with haematoxylin and eosin. Arrows indicate granuloma formations surrounding *S. mansoni* eggs.

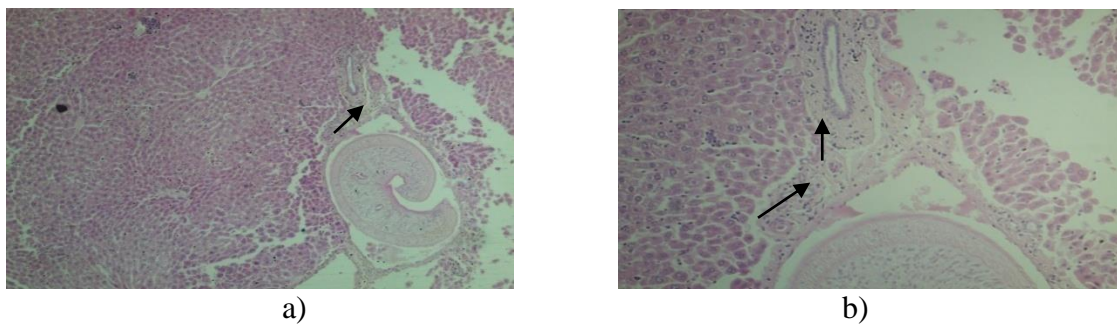


Figure 27: Liver of mice infected with cercariae of only *S. haematobium* parasites.

As shown in panels a) and b) of x40 and x100 magnifications respectively, sectioned liver tissues were stained with haematoxylin and eosin. Arrows indicate granulocytes surrounding a worm.

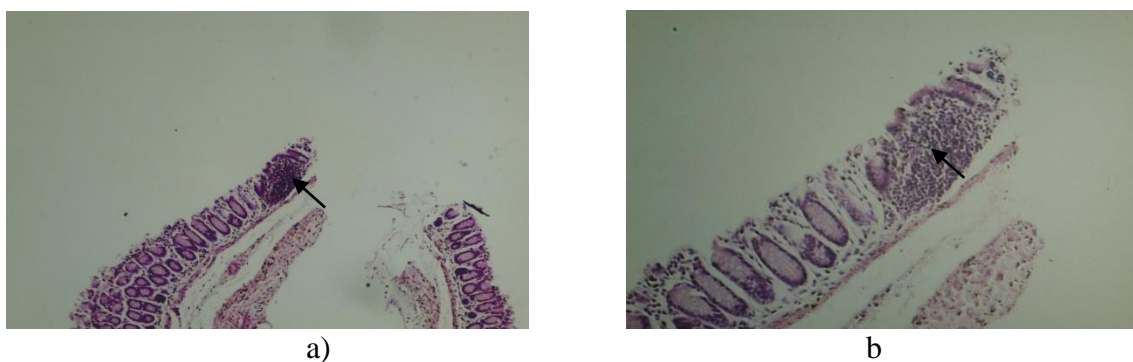


Figure 28: Intestines of mice infected with cercariae of only *S. haematobium* parasites.

Panels a) and b) show x40 and x100 magnifications respectively of sectioned mice intestines stained with haematoxylin and eosin. Tissues were obtained from mice infected with cercariae of only *S. haematobium* parasites. Arrows indicate aggregations of granulocytes.

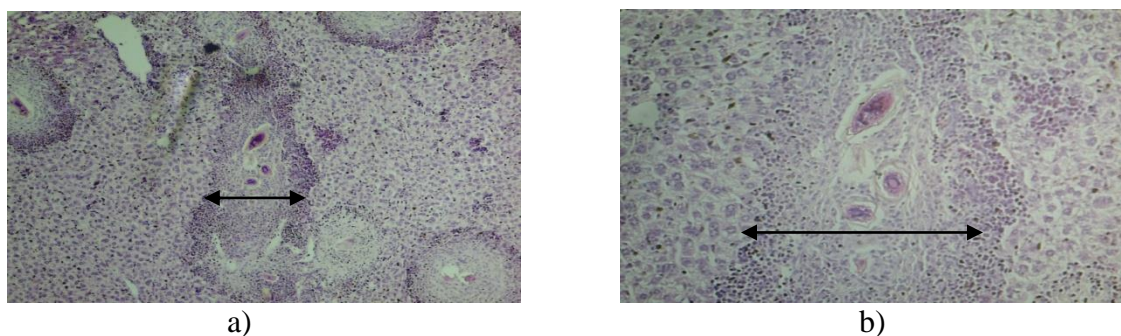


Figure 29: Liver of mice infected with cercariae of both *S. mansoni* and *S. haematobium* parasites.

Panels a) and b) show sectioned liver viewed under x4 and x10 objective lens of a light microscope. Sectioned liver tissues were stained with haematoxylin and eosin. Arrows show intense infiltration of tissue with granulocytes as well as trapped schistosome eggs.

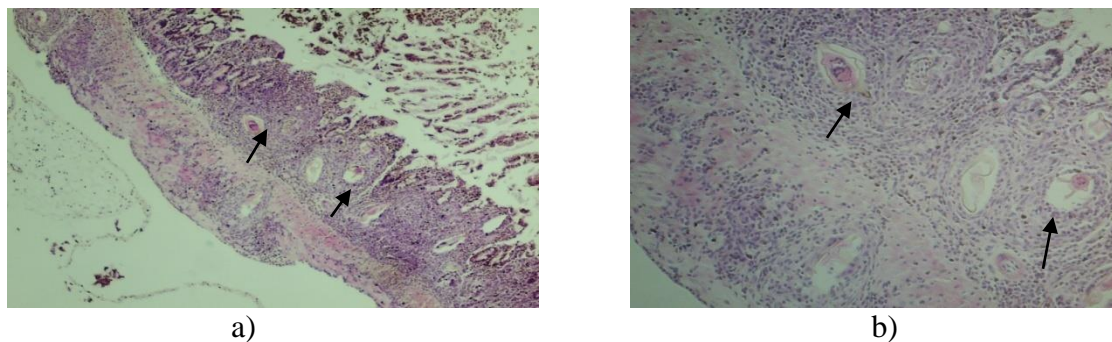


Figure 30: Intestines of mice infected with cercariae of both *S. mansoni* and *S. haematobium* parasites.

Panels a) and b) show x40 and x100 magnifications respectively of sectioned mice intestines stained with haematoxylin and eosin. Arrows show intense infiltration of tissue with granulocytes as well as trapped schistosome eggs.

CHAPTER FIVE

DISCUSSION

5.1 Discussion

Schistosoma mansoni and *S. haematobium* remain the dominant parasite species causing schistosomiasis in Ghana. Vectors of these parasites were found to occur in sympatry along the banks of the Weija lake bordering Tomefa, the study community. Prior to this study, no data existed on the sympatric nature of these vectors and their possible effect on disease outcome. This study thus sought to ascertain the occurrence of concurrent infections of schistosome parasites and the outcome of the disease using both field and laboratory investigations.

In Ghana, urogenital schistosomiasis is reported to be dominant over intestinal schistosomiasis (CEGET-CNRS /OMS-WHO, 1987), with higher infection prevalence in endemic communities of between 52-60% (Aryeetey et al., 2000; Nsawah-Nuamah et al., 2001; Aboagye & Edoh, 2009). The prevalence calculated for urogenital schistosomiasis in this study was slightly higher than results from previous works. On the other hand, the study recorded very high prevalence of intestinal schistosomiasis contrary to the low prevalence of between 10% and 49% reported for previous works in various parts of the country (WHO, 2010). This suggests both infections are spreading, however, *S. mansoni* may be spreading rapidly and colonizing new areas.

Transmission of schistosomiasis in Ghana is reported to be facilitated by *Biomphalaria* and *Bulinus* spp. of aquatic snails (Yirenya-Tawiah et al., 2011). In the current study, *B. pfeifferi* and *B. truncatus* snail vectors were found in sympatry in the community, to be responsible for the maintenance of the disease in the suitable aquatic habitat. The absence of *B. globosus* in the study community confirms work by Chu et al. (1978) who reported this species as being confined to small water bodies, considering the Weija Lake as a large water body.

The study also revealed some of the snail vectors harbouring other types of cercariae in addition to human cercariae, indicative of compatibility of snail vectors to different cercariae species. Again, presence of different cercariae types suggests that there could be schistosome infections to other animals like cattle and birds (McManus & Loukas, 2008 and Webster et al., 2013) apart from man and therefore possible zoonotic transmission of the disease in the community. Snail vectors were found predominantly attached to aquatic vegetation, namely *N. lotus*, *C. demersum*, *I. aquatica*, *D. insularis* and *F. Africana*, with *B. truncatus* snails mostly attached to *N. lotus* while *B. pfeifferi* snails were mostly attached to *C. demersum* in the water body. Generally, snails depend on these aquatic vegetations for food and micro-habitation (Zakhary, 1997 & Ofori, 1999).

Water contact activities along the lake included fishing, swimming and fetching of water for domestic purposes. The questionnaire investigations revealed that males generally spent longer periods in the lake while fishing and playing compared to females who mostly frequent the lake to fetch water for domestic purposes. This is reflected in the

higher prevalence of both intestinal and urogenital schistosomiasis among males relative to females (Deribe et al., 2011; Barnabas et al., 2012). Overall, human water contact activities in addition to cercariae shedding by snails suggest active transmission of the disease in the study community (Yirenya-Tawiah et al., 2011).

Fishing, playing and fetching of water for domestic purposes formed the main water contact activities that predisposed individuals to infection (Useh & Ejezie, 1999; Aryeetey et al., 2000; WHO, 2002; and Wagatsuma et al., 2003). Lack of adequate sewerage facilities in the community also contributes to high infection rates. Defecating and urinating into the lake facilitate snail infections, which in turn lead to the lake teeming with cercariae, thereby perpetuating the life cycle of the parasite. Perhaps, the provision of potable water and proper sewerage facilities will reduce water contact and ultimately, minimize the rate of infection in the community. Also, removal of aquatic vegetation along the banks of the lake could reduce populations of aquatic snails, thus truncating the life cycle of the parasite.

The study revealed children of all age groups understudied had either single infections of intestinal and urogenital schistosomiasis or concurrent infections. Although children between the ages 0–5 yrs are mostly excluded from schistosomiasis research and treatment regimes, the study revealed that this group also presented both single and concurrent infections; thus, establishing infant schistosomiasis in the study community and necessitating further studies along this line (Bosompem et al., 2004).

In some individuals with concurrent infections, schistosome eggs may be eliminated through unusual routes i.e. *S. haematobium* eggs in stool or *S. mansoni* eggs in urine, which may be accounted for in two ways. Firstly, the presence of *S. mansoni* eggs in urine or *S. haematobium* eggs in stool may be as a result of a spill-over, i.e. the higher the infection intensities of *S. haematobium* in urine and/or *S. mansoni* in stool, the greater the probability that schistosome eggs will be eliminated through these unusual routes (Cunin et al., 2003). On the other hand, concurrent infections of definitive hosts with both parasite species creates the opportunity for heterospecific pairing/mating interactions, with consequent passage of eggs through atypical channels as reported by Webster et al. (1999). Results from this study showed more cases of *S. mansoni* eggs in urine than *S. haematobium* eggs in stool with more heavy infections recorded in urine than in stool. In the event that these occurrences stemmed from spilling-over of eggs, one would have expected more cases of *S. haematobium* eggs in stool relative to *S. mansoni* in urine, but this was not the case. Since no relation was found between infection intensities and passage of eggs through unusual routes, there is little chance that these occurrences resulted from spilling-over. Consequently, mating interactions between the two schistosome species were explored for evidence to elucidate these occurrences. Available literature suggest that *S. haematobium* males have a greater propensity for inter-specific pairing than males of *S. mansoni* (Southgate et al., 1995; Webster et al., 1999; Webster & Southgate 2003) and other *Schistosoma* spp. This greater competitive ability of *S. haematobium* males may have accounted for the higher frequency of elimination of *S. mansoni* eggs in urine relative to *S. haematobium* eggs in stool. Heavy infections in urine may also be a risk factor for HIV acquisition and transmission in the community as

passage of eggs cause lesions in reproductive organs (Kjetland et al., 2006; Yirenya-Tawiah et al., 2011).

The study also revealed more cases of concurrent infections than single infections, highlighting the need to assess the impact of concurrent infections on the outcome of disease. Schistosome infections to mice were very successful with cercariae of *S. mansoni* alone and a combination of both *S. mansoni* and *S. haematobium*. Infections were however not that successful with cercariae of *S. haematobium*. This supports earlier works which used hamsters (Webster et al., 1999) instead of mice for *S. haematobium* infections.

Andrade and Azevedo (1987), Lenzi et al. (1998) and Baptista and Andrade, (2005) showed that eosinophilic granulocytes, lymphocytes, fibroblasts and macrophages formed granulomatous lesions around eggs to protect organs from toxins/antigens released by trapped eggs. Sizes of granulomatous lesions however differed among schistosome parasite species (Lichtenberg, 1962, 1968). Liver and intestines harvested from mice infected with either of the schistosome parasites in the study, showed intense granulocytes. Histological preparations of liver and intestines of mice infected with both parasites, revealed intense granuloma lesions with eggs of both *S. mansoni* and *S. haematobium* trapped in tissue compared to those of mice infected with single parasites. Granulomatous lesions observed in tissues of mice infected with concurrent schistosome parasites appeared to be larger than those found in tissues of mice infected with single parasites. This may be attributed to the additive effect of the two schistosome parasite eggs in the tissues.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Concurrent infections of *S. mansoni* and *S. haematobium* formed more granulomas than single infections of either parasite species in mice, suggestive of an associated greater pathology. Snail vectors identified were *B. pfeifferi* and *B. truncatus*, which is a first report of its kind of the two species occurring in sympatry in Ghana. The snail vectors shed both human and unknown cercariae types, pointing to compatibility of snail vectors to different cercariae species and possible zoonotic transmission of the disease in the community. Prevalence of intestinal schistosomiasis was higher (90.1%) than that of urogenital schistosomiasis (66.8%), suggesting that *S. mansoni* may be spreading rapidly and colonizing new areas. Eggs of *S. mansoni* in urine were more common than eggs of *S. haematobium* in stool, possibly a result of *S. haematobium* males being more competitive than *S. mansoni* males. Fishing, playing and fetching of water were the main activities that pre-disposed study participants to cercariae shed by snails. Defecating and urinating into the lake in turn facilitated infection of snails with parasites.

6.2 Recommendations

Infants should be included in schistosomiasis research and control programmes since results from this study showed them (children <5 yrs old) to be harbouring infections just as older children. *S. mansoni* may be spreading rapidly and colonizing new areas, given the high prevalence recorded, contrary to earlier reports. It is of interest to monitor this development over a period. Apparently, concurrent infections were associated with more pathology relative to single infections in mice. Studies of concurrent schistosome infections should be expanded to other endemic parts of the country while assessing its pathological impact in humans to facilitate disease control and eventual elimination.

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APPENDICES

RESEARCH CONSENT FORM

Abonie Severin Dzifa
Department of Animal Biology and Conservation Science
University of Ghana

Title: Concurrent infections of *Schistosomamansoni* and *S. haematobium* in an endemic community in Ghana: Impact on disease distribution and pathology.

I am a Master of Philosophy student of the above named institution carrying out a research on the effect of concurrent infections of two *Schistosoma* parasites on humans and the parasites in an endemic community. This research involves processes such as the collection of urine and stool samples which will cause no harm to subjects who voluntarily participate. It is known that both species of *S. mansoni* and *S. haematobium* occur in sympatry in the same water body. Thus, there is a likelihood of mixing of the two species which can possibly result in hybridization. The outcome of this study will make known mating interactions between *S. mansoni* and *S. haematobium* and also outline the effects of mixing between these two species on the distribution and pathology of the disease.

The information obtained from this research will not be made available to anyone who is not directly involved in the study. The answered questionnaires will also be kept away from the public by locking them in a safe place. Participation is voluntary and subjects have the right to withdraw their consent or discontinue their involvement at any time. In case of any doubts or questions about this research, please contact Doc. William Anyan (NMIMR, WACIPAC).

Declaration by Study Participant

I confirm that I have thoroughly read and understood the information sheet provided and thus, (I/My ward) agree to take part in the above research voluntarily.

.....

(Signature/name of subject) (Date)

.....

(Signature of researcher) (Date)

Questionnaire
Department of Animal Biology and Conservation Science

University of Ghana

Title: Concurrent infections of *Schistosoma mansoni* and *S. haematobium* in an endemic community in Ghana: Impact on disease distribution and pathology.

Please respond appropriately

Please tick (✓) in the boxes where appropriate

1. Personal details:

i) ID # ii) Age..... iii) Class.....

iv) Sex: Male Female

2. Do you go to the lake? Yes No

If **YES** answer the following/ If **NO** continue from question 3

i) Once a week? Yes No

ii) Twice a week? Yes No

iii) Three times a week? Yes No

iv) Every day of the week? Yes No

3. What activities do you engage in at the lake?

- i) Fishing Yes No
- ii) Washing Yes No
- iii) Playing Yes No
- iv) Cattle rearing Yes No
- v) Fetching water Yes No

4. What do you use the lake water for:

- i) Drinking? Yes No
- ii) Cooking? Yes No
- iii) Washing? Yes No
- iv) Bathing? Yes No

5. What other sources of water supply is available for use:

- i) Pipe borne? Yes No
- ii) Well? Yes No

iii) Borehole? Yes No

iv) River water/ Stream? Yes No

6. Which of the following diseases do you think are associated with this/these water source(s):

a) Pipe borne

i) Diarrhoea ii) Guinea worm disease

iii) Schistosomias iv) Cholera v) Skin rashes

b) Well

j) Diarrhoea ii) Guinea worm disease

iii) Schistosomias iv) Cholera v) Skin rashes

c) Borehole

k) Diarrhoea ii) Guinea worm disease

iii) Schistosomias iv) Cholera v) Skin rashes

7. What type of toilet facilities do you use?

- i) Flush toilet ii) Pit latrine and KVIP
iii) Bush iv) Lakeside v) Others

8. Have you ever seen blood in your urine? Yes No

9. Have you ever seen blood in your stool? Yes No

10. Do you know of schistosomiasis? Yes No

11. What do you think causes schistosomiasis?

- i) Witchcraft ii) Curse iii) Drinking lake water
iv) Adulthood v) Bathing lake water vi) Playing in the lake

12. Which of these do you think contributes to the transmission of schistosomiasis?

- i) Fish ii) Water snails iii) Worms

13. What are the signs and symptoms of schistosomiasis?

i) Blood in urine ii) Fatigue iii) Painful urination

v) Blood in stool vi) Abdominal pains iv) Fever

vii) Joint pains viii) Diarrhoe ix) Headache

14. Do you think schistosomiasis can affect other mammals? Yes No

15. Which other mammals do you know are infected with schistosomiasis?

i) Cattle ii) Cat iii) Dog

iv) Goat v) Sheep

16. What are some of the ways of controlling schistosomiasis?

i) Filtering water ii) Boiling water

iii) Avoiding urination in lake iv) Removal of weeds from lake

v) Avoiding defecation in lake and bushes

17. Where do you go when you detect you have schistosomiasis?

i) Herbalist

ii) Spiritualist

iii) Pharmacy

iv) Hospital

18. What do you use to treat schistosomiasis?

i) Herbs

ii) Pain killers

iii) Other drugs

19. Have you received any treatment on schistosomiasis before?

Yes

No

20. If yes, how long since you received the last treatment?

i) 6 months

ii) 12 months