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UNIVERSITY OF GHANA

COLLEGE OF HEALTH SCIENCES

GENETIC VARIATIONS IN *SCHISTOSOMA HAEMATOBIUM*, RESISTANCE AND REINFECTION IN THE GREATER ACCRA REGION

BY

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DECLARATION

I hereby declare that except for references to other people's work, which I have duly acknowledged this work is a result of my own research under the able supervision of Dr. Patience Borkor Tetteh-Quarcoo and Rev. Prof. Patrick Ferdinand Ayeh-Kumi, both of the Department of Medical Microbiology, College of Health Sciences, University of Ghana. This work neither in whole nor in part had been submitted for another degree elsewhere.

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DATE: ……

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DEDICATION

I dedicate this thesis to the Lord Almighty for how far He has brought me, my mother: Mrs. Agnes Antwiwaa Owadee and my best friend, Obed Akwasi Aning.

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ACKNOWLEDGEMENT

I express my sincere appreciation to God Almighty for allowing all these efforts coupled with hard work to be a reality. I would like to single out the mastermind behind this wonderful research work, my supervisor, Dr Patience Borkor Tetteh-Quarcoo for all her guidance, corrections, constructive criticisms and financial support in making this research successful. I am also grateful to my second supervisor, Prof. Patrick Ferdinand Ayeh-Kumi for taking time to supervise this research. I acknowledge the staff, parents, guardians and pupils of Nadel Academy School and Christ Mission International School, all of the Zenu community and also staff, parents, guardians and pupils of Assured Future International School, in the Weija community for allowing me to take samples from them. My gratitude goes to Prof. Kwabena M. Bosompem, the Deputy Director and the Head of the Parasitology unit of the Noguchi Memorial Institute for Medical Research for granting access for some part of the laboratory work to be done in such a wonderful facility and also Isabella and Jeffery all of Noguchi Memorial Institute for Medical Research who helped with the fluorescent microscopy. I share my happiness with my colleagues and staff of the Department of Medical Microbiology, University of Ghana, for their immense support in one way or the other in completing this work. Of particular mention are Robert Armah, Robert Aryee, Benjamin Konney, Emmanuel Afutu, Kantanka Addo-Osafo, Irene Amoakoh Owusu and John Kofi Nakoja for helping in the laboratory work and proof reading. My sincere gratitude goes to Prof. Yaw Afrane and Dr Simon. K. Attah, all of the Parasitology Unit, Department of Medical Microbiology, University of Ghana, for their words of encouragement and support. Finally, to all staff of Narh-Bita college, Medical Laboratory Department.

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ABSTRACT

Background: Schistosomiasis is among the major parasitic diseases following malaria in the category of neglected tropical diseases (NTD) especially in Africa sub-Saharan region. About 93% of Schistosomiasis is believed to occur in Africa. The genus *Schistosoma* is the parasitic trematode worm responsible for this disease. *Schistosoma haematobium* is responsible for causing Urogenital Schistosomiasis. The WHO recommended drug for schistosomiasis is Praziquantel (a single dose of 40mg/kg). Resistance of Schistosomiasis using Praziquantel (PZQ) has been reported in Egypt and Senegal. However, in endemic areas, reinfection after treatment is common. Genetic variants in Schistosomes have been observed in countries like Mali and Sudan. Presently in Ghana little is known about trends of genetic variants, as well as the possibility of resistance development and reinfection.

Aim: The aim of this study is to determine genetic variation in *Schistosoma haematobium* (ITS2, NAD1, COX1), assessment of resistance and reinfection.

Methodology: Urine samples of school children were obtained with consents from parents and guardians in the two communities (Zenu and Weija) in the Greater Accra region. Microscopy with egg count as well as viability test (modified hatchability technique, vital stains, and fluorescent microscopy) was performed on the spun urine sediments after macroscopic examination. The study procedure included a follow up collection of urine samples after treatment to assess resistance and reinfection. Genetic variation was assessed from sequences of the PCR amplified products (ITS2, NAD1) by comparing with sequences from other countries.

Results: The percentage reinfection was 10.4% and there was no resistance observed in this study. The mean percentage viability of all the methods used were 70% and 30% in live and dead eggs respectively at baseline. At post-treatment, the percentage viability of live and dead eggs was 13.3% and 86.6% respectively. For the ITS 2 sequences, there was genetic variation from the Kenyan strain and for the NAD 1 sequences it varied from Madagascar, Mauritius and Tanzania.

Conclusion: Reinfection was recorded among the study participants however there was no resistance observed. There was effect of treatment on the viability of *Schistosoma haematobium* eggs using the modified hatchability technique which was complemented by the vital stains and the fluorescent microscopy. Genetic variants were identified in relation to other countries.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Schistosomiasis is the second major parasitic disease next to malaria especially in Sub-Saharan African. (Gryseels *et al*., 2006). The genus *Schistosoma* is the parasitic trematode worm responsible for this disease and about six species are known to parasitize humans. These include; *S. haematobium, S. mansoni, S. japonicum. S. mekongi, S. malayensis* and *S. intercalatum* (Gryseels, 2012).

The estimated number of people infected with schistosomiasis in the world is 207 million with 93% occurrence in Africa (WHO, 2010). Ghana is known to be part of the highly prevalent countries with this disease (WHO, 2012). Hepatic and intestinal diseases and complications is associated with *Schistosoma* species such as; *Schistosoma mansoni, Schistosoma japonicum,* and *Schistosoma mekongi* (Elbaz *et al.,* 2013). Urogenital Schistosomiasis is caused by *Schistosoma haematobium*. The presence of macro-haematuria is an indicator of an active infection and acts as a sign of urinary bladder damage. *S. haematobium* is the only known species responsible for urinary complications. Disease can either be asymptomatic or patients may present anemia, haematuria and dysuria (Sousa-Figueiredo *et al*., 2012). There is the formation of granuloma lesions as well as calcification and fibrosis of ureters and bladders. Squamous cell carcinoma may also occur in the bladder (Sousa-Figueiredo *et al*., 2012).

In endemic communities, urogenital schistosomiasis affects all age groups (Sousa-Figueiredo *et al*., 2012). Occupation, sex, age, water contact activities, socioeconomic status, unsafe water sources and distance to water source are some of the risk factors associated with this disease (Kosinski *et al.,* 2012). The highest prevalence, intensity and

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morbidity occur in school-aged children, adolescents and young adults. Indiscriminate excretory habits such as urination into water bodies paves way for children who gets into the water to be infected as well as serve as reservoirs of the parasite (Woolhouse, 1998). Conversely, a high occupational exposure associated with farming or fishing may result in the maintenance of high intensities of infection in adult individuals (Kabeterine *et al*., 1999). Reinfection has been implicated mostly to endemic sites as a result of poor health education and lack of potable water to inhabitants of such areas (WHO, 2012).

Chemotherapy has been one of the effective ways of controlling schistosomiasis (WHO, 2012). The aim of the chemotherapy is to cure the patient by eliminating the adult worms, and this leads to cessation of egg deposition. In the endemic areas, the assessment of chemotherapy can be done in patients during post treatment using urine and stool specimen for analysis. Ultrasonography and endoscopy can also be used (Baik *et al*., 2006).

Praziquantel is a broad-spectrum anthelminthic drug active against all the species of *Schistosoma* including *Schistosoma haematobium*. According to WHO, it is the recommended drug of choice for the treatment of schistosoma species (WHO, 2010). Although the mechanism of action of Praziquantel (PZQ) is not well understood it is said to act on the adult stages of the schistosomes. Praziquantel has also been known to have some effect on the viability of *Schistosoma haematobium* eggs (Richards *et al* ., 1989; Matsuda *et al* ., 1983; Elfaki *et al* ., 2015). Reported cases of treatment failures could be as a result of developing resistance to the antischistosome drug (WHO, 2010). The recommended dose for the treatment of both intestinal and urogenital schistosomiasis is 40 mg/kg in a single dose.

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In order to effectively eradicate any surviving schistosomes, a second dose is recommended. The reduction of clinical cure and treatment failure of schistosomiasis using Praziquantel has been reported in Egypt and Senegal (Stelma *et al* ., 1995; Gryseels, 1994; Ismail *et al* ., 1996; 1999). This may be due to resistance development in schistosomes to Praziquantel. There could also be re-infection briefly after successful treatment which may seem as treatment failure. Treatment failure has often been used to explain the ineffectiveness of a drug to any species of schistosomes. Treatment failure has been attributed to the development of Praziquantel resistance (King *et al* ., 2000).

Resistance can be explained as an acquired reduction in drug sensitivity to adult worms of *Schistosoma haematobium* and the persistence of viable eggs after the sixth week following therapy with Praziquantel (Prichard *et al.* 1980; Coles and Kinoti, 1997). Reinfection can also be explained as the evidence of total egg clearance by the sixth week and the re-appearance of viable eggs by the sixth month after the treatment with Praziquantel (N'Goran *et al* ., 2001). Reinfection with current drug of choice Praziquantel, has been reported among school children which was largely predicted by the degree of water contact (Mutapi *et al* ., 1999).

Using diverse molecular tools, there have been substantial studies pointing to the presence of genetic variants in schistosomes across the world especially in Africa using diverse molecular tools (Bowles *et al* ., 1993, Agola *et al* ., 2009, Webster *et al* ., 2012, Shalaby *et al* ., 2010, Sady *et al* ., 2015, Yin *et al* ., 2015). Considerable genetic variations have been noted in natural population of schistosomes, of which a diverse number of genotypes is exhibited by naturally infected host of single status. (Ezeh *et al* ., 2015, Rollinson *et al* ., 1986, Durand *et al* ., 1997 and Brouwer *et al* ., 2001). This has been possible as a result of available molecular tools such as Restriction Fragment Length Polymorphism (RFLP), microsatellite markers and Random Amplified Polymorphic

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DNA (RAPD) (Minchella *et al* ., 1994). Southern blotting analysis using the SM 750 gene as a probe has been used to differentiate 14 strains of *S. mansoni* (Minchella *et al* ., 1994).

These molecular tools help scientists and researchers determine whether changes in gene frequencies and variations affect treatment effectiveness, and help them to gain knowledge on the impacts treatment has on the population structure and gene pool of the *Schistosoma* genus. It also helps them to monitor whether new parasites are introduced as a result of movement of people from non-treatment areas into local areas (Rollinson, 2009). Variations in the genome of the parasites population plays a vital role in their ability to have negative impact on the human population by making standard treatment regimen ineffective in disease conditions (Brouwer *et al.,* 2003). The sequencing of Internal transcribed spacer 1(ITS1) and Internal transcribed spacer 2 (ITS2) have been reported by several groups on various species of *Schistosoma* and used the derived information on the analysis of phylogenicity (Rollinson *et al.*, 1997). The schistosome mitochondrial genome is known for its characteristic high level polymorphism, a reason that supports its use as an ideal marker for studies on phylogenicity (Després *et al*., 1992). The ITS 2 in the rDNA repeat and the cytochrome oxidase I in the mitochondrial genome (COX I) were used in the genetic diversity analysis of *S. japonicum* which served as good markers for genetic variation analysis in other schistosome species as well (Bowles *et al*., 1993).

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1.2 Problem statement

Infection with *Schistosoma haematobium* which is responsible for urogenital schistosomiasis, is widespread in Ghana and causes morbidity to different categories of people including school children in endemic areas. Infected individuals are normally reinfected in endemic areas (De Clercq *et al*., 1997; Webster *et al*.,2013).

The current state of reinfection in Ghana is unknown. Praziquantel which is the drug of choice, has widely been used for treatment over the years paving way for selective pressure on the drug. Praziquantel resistance in Ghana is currently unknown. There have been reports of substantial genetic variation in Schistosomes, by researchers in Cameroon, Tanzania, Zambia, Niger, Nigeria, as well as East Africa countries (Ezeh *et al*., 2015, Betson *et al* ., 2013, Webster *et al* ., 2012) but there is no idea what the situation is in Ghana. Reports are made about the persistence of *S. haematobium* infection even after repeated treatment (Alonso *et al* ., 2006; Silva *et al* ., 2005). Also reports have been made on the effect of Praziquantel treatment on the viability of eggs of *S. haematobium* in Sudan (Richards *et al* ., 1989; Matsuda *et al* ., 1983, Elfaki *et al* ., 2015) but there is no idea what the situation is in Ghana.

1.3 Justification

In Ghana, there seem to be no idea of the presence of genetic variations in Schistosomes, neither is it apparent that, there might be possible existence of developing resistance, to the antischistosome drugs. Meanwhile there is evidence of substantial genetic variation in *Schistosomes*, reported by researchers in Cameroon, Tanzania, Zambia, Niger, Nigeria, as well as East Africa countries (Ezeh *et al* ., 2015, Betson M *et al* ., 2013, Webster *et al* ., 2012). This study will provide some

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information of the state of genetic variants as well as show whether there are circulating resistance strains and also the effect of viability on the eggs in the Greater Accra region. The detection and identification of resistant related variants of *Schistosoma haematobium* in Ghana will also provide information which will determine the actions that would be taken, when a new drug is manufactured for the treatment of schistosomiasis. Such actions might vary from what will be considered at other geographical locations (Cameroon, Sudan, Nigeria, Egypt and other East African Countries), since different variants of *Schistosoma haematobium* might be observed as seen in different parts of the world, especially Africa (Bowles *et al*.,1993, Agola *et al*.,2009, Webster *et al*.,2012, Shalaby *et al*.,2010, Sady *et al*.,2015, Yin *et al*.,2015). Data in regards to genetic variants will provide pertinent information which will help in the control and elimination of the disease from Ghana and Africa.

1.4 Aim

- The main aim of this study is to determine genetic variations in *S. haematobium* (ITS2, NAD1, COX1), assessment of resistance and reinfection among *Schistosoma haematobium* infected individuals in the Greater Accra Region.

1.5 Objectives

The objectives of the study are;

- To determine possible resistance, reinfection and the effect of Praziquantel treatment on the viability of eggs amongst *S. haematobium* infected individuals.
- To determine variation in genes (ITS 2, NAD 1, COX 1) of *S. haematobium* in the Greater Accra Region in comparison with other countries.

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CHAPTER TWO

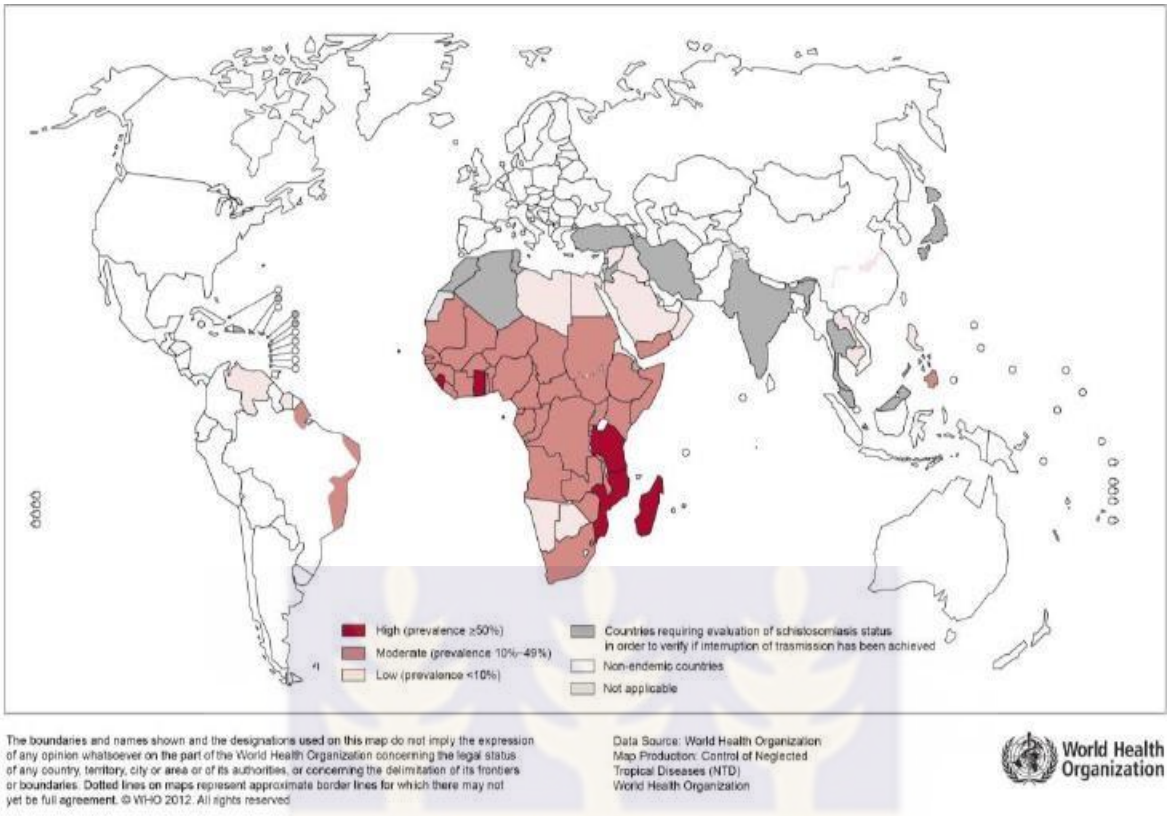
2.0 LITERATURE REVIEW

2.1 Schistosomiasis

Schistosomiasis is an endemic and one of the most prevalent parasitic disease caused by species of schistosomes (Gryseels *et al*., 2006). The evolving nature of this infection to disease was first noticed at the River Nile (Nozais,1987; Nozais ,2003). A German physician known as Theodor Maximilian Bilharz was the first to identify the egg of schistosome, the disease was named after him (bilharzia). Eggs of schistosomes were first identified in Egyptian mummies in 1910 (Cox, 2002). There are several species of schistosomes but only six have been identified to parasitize man which includes; *Schistosoma haematobium, Schistosoma mansoni, Schistosoma japonicum, Schistosoma intercalatum, Schistosoma malayensis* and *Schistosoma mekongi* (Gryseels, 2012). The ecology of the snail hosts plays a vital role in the identification of different species of schistosome in various geographical distributions. Typical sources of schistosomiasis infection include; artificial waters such as dams and irrigation canals as well as natural streams, lakes and ponds (Steinmann *et al*., 2006).

Schistosomiasis is a waterborne disease known for debilating cause in adults and children. In children, schistosomiasis is known to cause anaemia, stunted growth and impaired cognition (Friedman *et al* ., 2005; Hall *et al* ., 2008; Stephenson *et al* ., 2000). There are two (2) main forms of schistosomiasis; intestinal schistosomiasis known to be caused by *Schistosoma mansoni, Schistosoma intercalatum, Schistosoma japonicum* and *Schistosoma malayensis*. Urogenital schistosomiasis is known to be caused by *Schistosoma haematobium*.

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Source: http://gamapserver.who.int/mapLibrary/Files/Maps/Schistosomiasis_2012.png

Figure 2.0: Distribution of global schistosomiasis, 2012. Credit WHO Map Library

2.2 Urogenital schistosomiasis

Schistosoma haematobium is the etiologic agent of urogenital schistosomiasis, throughout African, Middle east and the Southern Europe (Hotez *et al* ., 2008). The geographical distribution of this disease and its transmission zone is closely related to the fresh water intermediate host snails such as the *Bulinus* and *Physopsis* species (Stothard *et al* ., 2009; Robert & Janovy, 2000). This parasite is known to cause diseases and infection in asymptomatic individuals. The lifestyle and behaviour of children swimming in large water bodies infected are among at risk individuals (Jordan *et al*., 1993). Urogenital schistosomiasis is known to cause dysuria, haematuria, nutritional deficiencies, risk of bladder cancers and growth retardation in children of school going age (Mostafa *et al* ., 1999).

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Haematuria is known to be the main marker for diagnosis of urogenital schistosomiasis, microhaematuria has frequently been identified with urogenital schistosomiasis compared to macrohaematuria which is a visible sign during the severe stage of this disease. Patients with heavy infection of urogenital schistosomiasis had a higher percentage (95%) of microhaematuria compared to macrohaematuria which was detected in just 45% of the total sample (Ugbomoiko *et al* ., 2009). *Schistosoma haematobium* is known to have a complicated life cycle differentiating it from most of the other digenean life cycles. The separate sexes of *Schistosoma haematobium* makes it distinct from other trematodes. The association between the male and female adult worms remains a key factor for fertilization. The male adult worms have to locate the female worm by moving within the host's body as well as long contact time together which makes copulation difficult. The venous plexus of the bladder is the site for the deposition of eggs by the female worms. Averagely it takes 9.5 weeks from infestation by the cercariae to the formation of eggs. (Ghandour, 1976 ; Matsunaga, *et al* ., 1987; Agnew, *et al* ., 1988 ; Roberts and Janovy, 2000).

2.2.1 Life Cycle Overview of *Schistosoma haematobium*

The life cycle involves two phases; the sexual phase which involves man as the definitive host and the asexual phase which involves the snail (Bulinus species) as the intermediate host. The stages of the life cycle involve the eggs (ova), the miracidia, the cercariae, the juvenile worms (schistosomulae) and the adult worms (male and female). Man gets infected when the cercariae from a water body penetrates the skin upon sufficient contact time with the surface of the skin. A single miracidium can develop into as many as 100,000 cercariae. The cercariae then develop to juvenile worms upon maturation. The juvenile worms migrate undisturbed

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from the derma of the lungs where it matures to adult forms sexually. The adult forms can stay up to 30 years (Hornstein *et al*., 1990).

After 6 to 12 weeks, the adult worms (male and female) normally pair in the peri-bladder veins (venous plexus) where they lay characteristic oval eggs with a prominent terminal spine. *Schistosoma haematobium* is generally known to lay between 20 to 200 eggs per day (CDC, 2012). The miracidium, which is a free-living ciliated stage in the eggs are known to be sensitive to light and water and normally individuals infected with *Schistosoma haematobium* serve as reservoir and infect water bodies through urination into these water bodies. The miracidia will have to find a specific intermediate host between 8 to 12 hours where they further develop into sporocysts (mature and daughter).

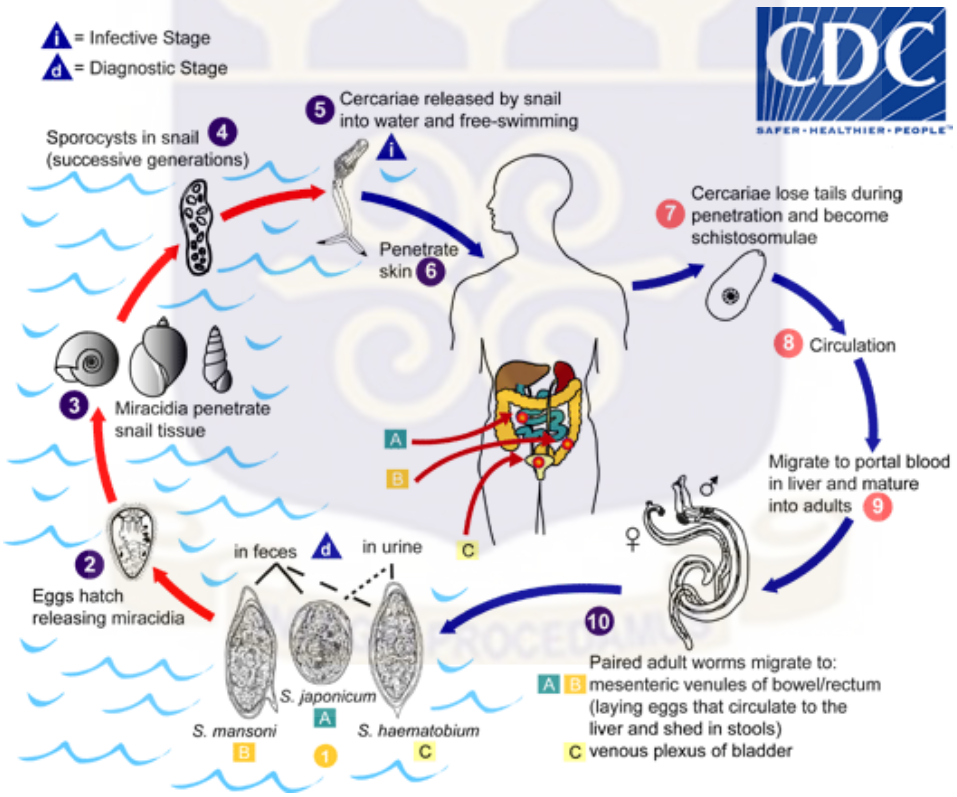


Figure 2.1: Life cycle of schistosome species. Credit CDC, 2012

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2.2.2 Prevalence and Intensity of Urogenital Schistosomiasis

Reports suggests that 436 million people at risk of infection of which 112 million people are infected with urogenital schistosomiasis in the sub-Saharan Africa (WHO, 2012). In Africa, Nigeria and Ghana have recorded variable prevalences of urogenital schistosomiasis in different categories of people even though these areas have been noted to be endemic. Hyperendemicity of urogenital schistosomiasis has been reported among school children in the Guma Local Government Area, Nigeria (Amuta; Houmsou, 2014). In Nigeria, a study conducted on the prevalence of *Schistosoma intercalatum* and *Schistosoma haematobium* in a rural community of Ogun State recorded a prevalence of 62% compared to 38% respectively which suggests higher prevalence compared to the work under study (Otuneme *et al* ., 2014).

Other prevalence studies conducted in Nigeria, one of the highly endemic countries has also recorded high prevalences among different categories of people (Engels *et al* ., 2002, Ishaleku *et al* ., 2012, Akeh *et al*., 2010, Nwosu *et al* ., 2005, Akinwale *et al* ., 2010).

Ghana is known to be an endemic site of schistosomiasis and widely distributed in many areas around major water bodies (Wright, 1973). A prevalence rate of 33.2% was reported along an irrigation scheme in the Kassena-Nankana district of the northern region (Anto *et al*., 2013). There are other research works that have reported various prevalences along the Zenu dam and the lower Volta basin with rates 30.7% and 2 to 21% respectively (Nkegbe, 2010; Tetteh-Quarcoo *et al* ., 2013). A prevalence rate of 30 % was reported using reagent strip among the areas surveyed for infant schistosomiasis of which the Efutu Senya District of the central region of Ghana was part. This was compared to an 11.2% which was reported based on microscopy (Bosompem *et al* ., 2004). In Weija, a higher prevalence of urinary schistosomiasis of 76.0% was recorded among school children (Anim-Baidoo *et al* ., 2017).

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2.3. Morphological variations of *Schistosoma haematobium*

Schistosoma haematobium has been found to interbreed with its closely related sister species resulting in a process where one organism invades the genome of another, this process is known as hybridization (Hélène Moné *et al*.,2012). During concurrent natural human infection, hybridization occurs between schistosome species (Southgate, 1978). Such occurrence has been detected between *S. haematobium*, *S. mattheei* and *S. intercalatum*. *Schistosoma haematobium* is known to be the only pathogenic schistosome species to cause urogenital schistosomiasis in humans. Its ova (egg) is described as having a characteristic oval shape with the presence of a terminal spine however, species like the *Schistosoma intercalatum* and *Schistosoma mattheei* have similar egg morphology which has raised questions to the identification of *Schistosoma haematobium* in urine of humans (Wright & Ross, 1980). The geographical distribution of *S. haematobium, S. intercalatum* and *S. mattheei* have been known to overlap since they are all found mostly in Africa (Mahmoud, 2001). *S. haematobium* and *S. mattheei* also share a common intermediate host. Countries like Zimbabwe and South Africa are known to have overlap of contact sites with open river systems which allow easy snail movements (intermediate host) enhancing diversity of *S. haematobium* as a result of exposure (Dabo *et al* ., 1977). Evidence of hybridisation of *S. haematobium* among animal and human schistosomes in the South-East of Africa suggests the exchange of native genotypes and imported genotypes results in new parasitic strains (Shiff *et al* ., 2000). The movement of people and animals for settlement during civil wars has contributed to the effectiveness of diversity being established between *Schistosoma haematobium* species. Reservoirs of *S. haematobium* have contributed to the free transmission of imported genotypes of these species to a new environment which enhances diversity among these species which have a major effect on treatment.

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Research conducted across Cameroon on the compatibility of *S. intercalatum* and *S. haematobium*, and their hybrids with the intermediate host Bulinus truncatus found out that, hybrids produced from *S. haematobium* and *S. intercalatum* are more viable and have more enhanced characteristics during the life cycle phase such as increase infectivity of cercariae and miracidia (Webster, 2012). Another research conducted on the hybridisation of *S. haematobium* and *S. mattheei* indicated that hybrids of such kind are more aggressive and resistant to drugs such as oxamniquine (Pitchford & Lewis, 1978). In Benin a conducted research on the natural interactions existing between *Schistosoma guineensis* and *Schistosoma haematobium* concluded that the presence of three egg morphotypes (*S. haematobium*, *S. guineensis*, and intermediate) were detected by ITS2 rDNA HRM analysis. The results of the morphological analysis were confirmed by the three genotypes: *S. haematobium*, *S. guineensis*, and a hybrid (Moné *et al* ., 2012).

2.4 Treatment of Schistosomes

Chemotherapy has been one of the most reliable ways of combating schistosomiasis (WHO, 1993). Before the introduction of drugs for schistosomes, the quality of life of millions of people in endemic areas was reduced due to health problems caused by schistosomiasis. During the second world war, western soldiers who were posted to China, Phillipines and other Asian countries made schistosomiasis gain an international attention as a result of a lot of soldiers been diagnosed with the infection upon return to their native countries (Sandbach, 1976). People with their abodes in endemic areas got constantly infected due to continuous exposure to water which served as habitats for the infective forms of this parasite (Mahmoud, 2001). Non-facilitation of compliance, ineffectiveness of the drug and expensive nature of the drug were among the reasons for failure of some drugs. Metrifonate and Oxaminquine were the first drugs to achieve

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success in the destruction of worms (*S . haematobium* and *S. manson*). Drugs of diverse kind were not able to achieve successes before Metrifonate and Oxaminquine were introduced. However, after a couple of years these drugs were studied in both experimental animals and human populations and were discarded due to the high toxicity and poor patient tolerance (Miele, 2014). There are three (3) recognised delivery systems by which treatment is reached out or recognized; mass treatment, targeted chemotherapy and selective population treatment. Mass treatment is explained as treatment of all inhabitants living in endemic areas avoiding the cost of individual diagnosis. In the case of targeted chemotherapy there are differences in observations of individual worm loads in endemic populations and prescribes that treatment should be reserved for those most in need. Selected population treatment deals with treatment of various sectors of the population for example those with a pattern of particularly high water contact (Mahmoud, 2001).

When Praziquantel was introduced (Davis & Wenger., 1979) it immediately became a potent drug for treatment especially in the endemic areas (Gönnert and Andrews 1977; Seubert *et al* ., 1977). Currently Praziquantel remains the number one drug for the treatment of schistosomiasis (WHO, 2006). Praziquantel usage started in the late 1970s which was produced by Merck, in Germany and after two decades proved to provide the obvious advantage of reduction in morbidity while incapable of influencing transmission significantly. Reinfection is the problem that has remained unsolved among endemic areas that lack safe clean water even after mass treatment with Praziquantel.

Praziquantel is a broad spectrum anthelminthic drug effective against the adult forms of the schistosome worms. The tablets of praziquantel (PZQ) contain 600 mg of the active ingredient however in Egypt, epiquantel the syrup formulation of Praziquantel is suitable

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for small children who cannot take the tablets (Doenhoff *et al* ., 2009). The tablet is bitter tasting white crystalline, insoluble in water and has a good absorption rate (75 %-100%).

The mechanism of action of praziquantel is not well understood nevertheless it provides the major effects on the adult forms of the parasite which includes; the spastic paralysis of the parasite musculature, possibly arising as a consequence of an influx of Ca^{2+} into the worm as well as vacuolation and degeneration of the worm (Jeziorski *et al* ., 2006). Praziquantel has side effects such as abdominal pain, headache, anorexia, fever, myalgia, epigastric pain, vomiting, diarrhoea with or without blood, dizziness, sleeplessness, nausea and rarely skin rash with edema in participants with heavy burden of disease (Cioli *et al* ., 1984; Jaoko *et al* ., 1996; Berhe *et al* ., 1999). Praziquantel has proven to have high curative power against schistosomes, when used orally in a single dose or as a series of double doses depending on the endemicity of the area (Coura *et al* ., 2010).

The number of doses given to the infected individuals is based on the body weight or height of such individuals. It has been used for both short- term prevalence control and for medium to long-term morbidity control. Its extensive usage has called for concerns about the development of tolerant *Schistosoma* species resistant to treatment (Fallon & Doenhoff, 1994).

A decade after Praziquantel was introduced, the use of artemisinin derivatives such as artemeter, artemisone, artelinic, artesunic acids among others, against *Schistosoma* species was evaluated for the first time by Chinese groups, these proved effective against the juvenile worms and not the adult worms however, treatment remained ineffective as a result of risk of interference with malaria treatment in other areas. In China Arthemeter and Artesunate has been used as "prophylactics" against *S. japonicum* infection (Doenhoff *et al* ., 2009). Arthemeter derivatives were investigated further by testing it

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against different species of schistosomes that parasitize humans. In 1984, it was demonstrated that the effectiveness of the derivatives against the juvenile worms, a period in which the treatment with Praziquantel failed, (Lu *et al* ., 2010; Yue *et al* ., 1984) but this was not effective against the adult forms of the parasite which demonstrated the limitation of this derivative. In China a study conducted, proposed that a combined use of Praziquantel and Arthemether would be useful in endemic areas, where reinfection frequently occurs (Liu *et al* ., 2011). However, Hua *et al*. (2010) reported the Arthemether sensitivity of *S. japonicum* decreasing for over the past 10 years in China.

According to WHO, the high-risk groups of which treatment should be mainly focused on include individuals such as fishermen, irrigation workers, women involved in domestic tasks, as well as adolescents and school-age children. (WHO, 2002).

In Ethiopia, a study on the morbidity markers after seven weeks of Praziquantel treatment demonstrated the reduction of proteinuria and haematuria from 94.07% to 48.7% and 100% to 40.8% respectively (Mekonnen *et al* ., 2013). A similar study was organised among children with Zimbabwe origin to determine the single Praziquantel treatment effect on *Schistosoma haematobium*-related morbidity markers, microhaematuria significantly reduced 12 months after treatment. Proteinuria and albuminuria reduced in post-treatment compared to baseline (Wami *et al* ., 2016).

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2.4.1 Treatment Successes

The ideal clinical outcome of treatment with Praziquantel is to provide treatment successes in infected individuals. The World Health Organization records Praziquantel to be the recommended drug for the treatment of schistosomes due to the fact that it has proven to provide a high percentage of efficacy against the target of schistosomes (adult worms) (WHO, 2006).

Ojurongbe *et al*. (2014) conducted a research on the efficacy of Praziquantel in the treatment of *Schistosoma haematobium* among school children in the rural communities of Abeokuta, Nigeria which concluded the reliability of Praziquantel in the treatment of *S. haematobium* infected individuals. The egg reduction rate of infected individuals ranged from 57.1% during the first week (1^{ST}) to 100% in the twelfth week (12^{TH}). In Senegal, a similar research was conducted on the efficacy of Praziquantel on urogenital schistosomiasis among school children and similarly the outcome was that Praziquantel showed expected efficacy of reducing the intensity and prevalence of infection (Senghor *et al* ., 2015).

An examination of the long-term efficacy of Praziquantel was carried out on *S. haematobium* in the Msambweni area of Coast province, Kenya during a school-based treatment programme. The observation from this study was that results indicated substantial year-to-year variation in drug efficacy, ranging from cure rate of 65% to a cure rate of 96% from 1986 to 1990 (King *et al* ., 2000). A study conducted in Kenya among school children infected with *S. mansoni* also indicated 92.6% cure rate when Praziquantel was administered. This study proved that Praziquantel has a good reduction rate in parasite burden (Kihara *et al* ., 2007). In Cameroon a study was conducted to determine the Praziquantel efficacy against *S. haematobium* (Tchuente *et al* ., 2004).

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Their results proved that a single treatment with Praziquantel is actively efficacious due to the fact that the sixth (6^{TH}) and ninth (9^{TH}) weeks post-treatment cure rates was from 83 to 88.6% and the egg reduction rate was 98%. Another evidence of the efficacious ability of Praziquantel against *S. haematobium* was carried out in Zimbabwe with an egg reduction rate and cure rate of 98.2% and 88.3% respectively (Midzi *et al* ., 2008). This particular work can be compared to a similar research conducted among pre-school children in the same province, the overall prevalence in this province was 51.8% which was reduced to 7% after treatment with Praziquantel (Mduluza *et al* ., 2001). The potency of Praziquantel was further demonstrated in regards to the cure rate and egg reduction rates given higher percentages among *S. haematobium* and *S. mansoni* infected school children in Cote d'Ivoire (Coulibaly *et al* ., 2012). In Uganda, the efficacy of Praziquantel was achieved among treatment naïve individuals infected with *S. mansoni* after subsequent follow-ups (ERR-92.1% to 99.1%) (Sousa Figueiredo *et al* ., 2012). In another study, after Praziquantel treatment among school children, the mean egg count per 10 ml of urine reduced from baseline (84.8 eggs/10ml) to (0.3 eggs/10ml) in post-treatment survey (Stete *et al* ., 2012).

Two studies in Ethiopia refuted the facts of possible Praziquantel resistance in their research conducted in *S. mansoni* infected individuals after achieving cure rates between 83.2% and 94% (Degu *et al* ., 2002, Berhe *et al* ., 1999). These could explain the possible reason for Praziquantel to be the recommended drug for schistosomes. A single dose of 40mg/kg has proven to provide effective treatment against *Schistosoma intercalatum* even though older drugs such as antimonials and niridazole were selectively less in effective treatment compared to Praziquantel (Chen, & Mott, 1989; Almeda *et al* ., 1994). The various data from different countries suggests to the fact that Praziquantel has over the years been associated with a significant decrease in the prevalence, incidence and

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intensity of infections caused by schistosomes. The Global performance of Praziquantel is considered satisfactory but some exceptions occur in hyper-endemic areas with schistosomiasis (Mutapi *et al* ., 2011; Metwally *et al* ., 1995). An overall effect of Praziquantel treatment success has to do with interruption of transmission in hyper-endemic areas by reducing eggs passed by reservoirs of this disease.

2.4.2 Treatment Failures.

Treatment failure can be explained as unresponsiveness of an ideal therapeutic dosage of a drug in-vivo or in-vitro to a parasite. This term has been used in relation to a couple of drugs for the treatment of parasite-infected individuals, however in Praziquantel, terms like suboptimal treatment, therapeutic failure and low level potency have often been used to explain the meaning of this term. These terms can be explained differently depending on the decreasing effect of the Praziquantel against the schistosome species in question.

2.4.2.1 Suboptimal Treatment.

Suboptimal treatment can be explained as treatment of *Schistosoma* infected *individuals* with Praziquantel at a recommended dose which will not produce the best results of complete destruction of adult worms (Giboda *et al* ., 1992). Genetic variation can be one of the possible reasons for the failure of standard treatment regimens. The selective pressure exerted by Praziquantel (PZQ) has led to decreased genetic variability in *S. mansoni (*Coeli *et al* ., 2013). A study conducted in the Niger, a West African country showed that after 3 years of Praziquantel (PZQ) treatment (40mg/kg) the intensity and prevalence of *S. haematobium* was significantly lower than the baseline (Senghor *et al* ., 2015), however in cases of endemicity reinfection is highly possible which could be a contributing factor in increasing the selective pressure of the drug to be susceptible to the schistosomes resulting in suboptimal treatment.

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According to Toure *et al*. (2008), the prevalence of *S. haematobium* infection in school-age children was still at a significantly lower level than at baseline and, infection remained at a low level two years after treatment (40mg/kg). After 1 year of treatment with Praziquantel (40mg/kg), there was significant reduction of infection caused by *S. haematobium* in Central part of Sudan.

Another study carried out in Ghana, the intensity of *S. haematobium* infection was reduced by 80–99%, 12 months after Praziquantel treatment and remained very low in two of three study areas after 24 months of treatment (Nsowah-Nuamah *et al* ., 2004). Resistance can be as a result of selective pressure due to the fact that this drug has been in existence since 1970. Research has demonstrated that the single dose has a cure rate of about 88% and the double dose has a cure rate of 100%. According to National control programmes more than a single dose can be used for the control of Schistosomiasis depending on the endemicity of the area. The question is although a single dose of Praziquantel is able to lower eggs below baseline, is the dosage regimen enough to completely destroy the adult worms producing the eggs?

According to Doenhoff *et al*. (2009), there are growing number of cases of treatment failure using Praziquantel, acquired by travellers and military men in endemic areas.

Failure of standard treatment with Praziquantel was observed in two Spanish returned travellers from Mali and Senegal with genitourinary schistosomiasis caused by *S. haematobium* even after repeated doses (Alonso *et al* ., 2006). Morphologically viable eggs of *S. haematobium* were found in patients treated with Praziquantel even between six and twenty-four months' post-treatment (Silva *et al* ., 2005). Praziquantel again failed to complete its purpose of complete destruction of adult worms.

A recent survey conducted in the Ndumo area of uMkhanyakude district, KwaZulu-Natal, South Africa among school children indicated higher cure rates but lower egg reduction rates. This suggested the ineffective efficacy of the Praziquantel drug (Kabuyaya *et al* ., 2017).

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2.4.2.2 Resistance and Genes

Antimicrobial resistance has been a major concern for the past 70 years. Over some time now, many microorganisms have adapted to the drugs designed to destroy them, making the products less effective. Microbes such as viruses, bacteria, fungi and parasites have all been involved in antimicrobial resistance (WHO, 2014). Macrocyclic lactones, imidazothiazoles, benzimidazoles and Praziquantel have been used to treat diseases caused by helminths with Praziquantel as the recommended drug for the treatment of Schistosomes since 1970 (WHO, 2010). The dependence of treatment on one main drug, Praziquantel, remains a major concern. There have been reports of resistance development among the Schistosomes species to Praziquantel but little is known in the genes associated with resistance. Presently there is no direct evidence for the development of Praziquantel resistance to *Schistosoma haematobium*, however there are reports of failure of standard repeated doses caused by S. haematobium (Herwaldt *et al* ., 1995; Alonso *et al* ., 2006). Disease-endemic areas such as Egypt and Senegal have reported treatment failures to Praziquantel suggesting the development of drug resistance (Olliaro *et al* ., 2011). Resistance can be experimentally induced in laboratory where the parasite is exposed to sub-lethal concentrations to produce worms less sensitive to drugs (Ismail *et al* ., 1999). In the Nile Delta region of Egypt, there was a report of *Schistosoma mansoni* resistant to Praziquantel among villagers in that region (Ismail *et al* ., 1996). After treatment with 3 successive doses between 6 and 8 weeks, 41 patients were not responding to Praziquantel treatment. Praziquantel cured 25 out of 28 patients infected with *S. mansoni*, and in the Philippines 60 of 75 people infected with *S. japonicum*.

Praziquantel resistance has been recorded in Egypt, Senegal and even Brazil (Mwangi *et al* ., 2014). The unstable nature of isolates of this parasite to Praziquantel response is a reason to be worried about. Currently resistance markers of *S. haematobium* is not known worldwide.

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2.5 Reinfection

Reinfection can be explained as the evidence of total egg clearance by the sixth week and the reappearance of viable eggs even at the sixth month after Praziquantel treatment (N'Goran *et al* ., 2001). Reinfection is common in the endemic areas however research has shown that children are most likely to be affected by this condition. Children living in endemic areas are most likely to be affected throughout their lives due to continuous exposure to infected water (IARC, 1994; Mostafa *et al* ., 1999).

Reinfection must be highly linked to seasonal and ecological factors (N'Goran *et al* ., 2001). Contact with water after treatment with Praziquantel remains the main reason for reinfection however the period for water contact plays a crucial role in the determination of reinfection (IARC, 1994; Mostafa *et al* ., 1999). Senghor *et al*. (2015), researched on monitoring of reinfection in Senegalese school children and found out that the percentage reinfection was 12.6% which was higher in males than females. He concluded that transmission of urinary Schistosomiasis is seasonal and has a significant effect on the occurrence of reinfection.

Studies have also shown how proximity to water source in terms of settlements is a vital too in risk of infection. Knowledge of haematuria status upon study entry was also a significant predictor of infection or reinfection risk (Satayathum *et al*., 2006).

N'Goran *et al*. (2001) confirmed that *S. haematobium* reinfection patterns is largely dependent on the epidemiology setting which is of importance to ensuring treatment strategies that are well adapted to these diverse settings. A study was conducted on pupils to determine the reinfection patterns within a year and 68% of pupils were reinfected after two doses of Praziquantel indicating the area to be a highly endemic site (Oniya and Odaibo, 2006).

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In Sudan, a study was conducted on school children to determine the prevalence of *Schistosoma haematobium* after a single Praziquantel treatment and the rate reduced from 51.4% to 8.6% which indicated low percentage reinfection (Ahmed *et al* ., 2012). Etard *et al*. (1995), showed that as exposure and pretreatment increases, the reinfection risk decreases with age. A recent study recorded a reinfection rate of 8.03% and 8.00% at 20 weeks and 28 weeks post-treatment respectively (Kabuyaya *et al* ., 2017).

2.6 Genetic Polymorphism in Schistosomes

The biological interaction between Schistosomes and their host is influenced by their respective genetic makeup (Simpson *et al* ., 1995). For a parasite to adapt to its changing environment, and pressures from control interventions, genetic variation plays a key role (Rollinson *et al*., 1997). Genetic diversity has been known to have a vast influence on parasite related-characteristics such as infectivity, virulence, transmissibility (Morand *et al* ., 1996). Investigations on the genetic variability of *Schistosoma haematobium* is understudied compared to *Schistosoma mansoni* due to the absence of specific markers. The evidence of genetic variations among Schistosomes is a contributing factor to the development of drug-resistance species thus the emergence of unsusceptible genotypes. Genetic variation analysis has been done across many countries (Ezeh *et al* ., 2015; Betson *et al* ., 2013, Webster *et al*., 2012) with the notion of finding the molecular epidemiology of *Schistosoma* species. The discovery of various molecular markers has helped to gain insights into the genetic diversity of various schistosome species (Simpson *et al* ., 1995). These tools have been helpful to detect and quantify genetic differences among schistosomes.

In recent years, the developments in tools for molecular analysis, and advances in sequencing of DNA have allowed greater recording and exploration of the genetic diversity of schistosome species and their hosts (Webster *et al* ., 2012).

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The quantities of DNA genome from individual parasites play a significant role in Schistosome population studies. Genetic variations have not only been dominant among *S. mansoni* and *S. haematobium* but in *S. japonicum* as well (Chen *et al* ., 2015; Rudge *et al* ., 2009; Zhou *et al* ., 2013).

Thiele *et al*. (2008), studied earlier on, what they believe was the largest study of population genetics of schistosomes to assess the degree of genetic subdivision of *S. mansoni* within a human host population. They used seven (7) microsatellite loci markers in two villages in Brazil. In the village of Virgem das Graças, out of 585 worms that were genotyped, 5 to 27 alleles were observed per locus, with a mean of 14.1 alleles per locus. In another village, Melquíades, a total of 14 to 24 alleles was observed per locus, with a mean of 18.1 alleles per locus. The outcome from their studies could only compare with that of Curtis. A study conducted on the genetic diversity of *S. haematobium* eggs in Sudan found that the S. *haematobium* population isolated in Sudan was made up of genotype belonging to the Pan African *S. haematobium* as well as *S. haematobium* belonging to a Kenyan strain which was *attributed to* possible inflow due to the proximity of geographical locations (Quan *et al* ., 2015).

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2.6.1 Mitochondrial Markers

Mitochondria is known as an organelle with its own genome responsible for energy production (Hafeti, 1985). The genome of the mitochondrial consists of the mitochondrial DNA (mDNA). The mitochondrial gene of the six species (*S. haematobium*, *S. mansoni*, *S. japonicum* , *S. malayensis*, *S. intercalatum* and *S. mekongi*) of Schistosomes that parasitize man have been sequenced (Nahum *et al* ., 2012). Mitochondrial genes have been used as molecular markers for species identification and strain, which is key to a variety of studies such as phylogenetics, biogeography, molecular ecology and population genetics (Zarowiecki *et al* ., 2007). These molecular markers have been identified as the preferred elements for analysis of population subdivisions for epidemiological studies (Curtis *et al* ., 2001). The genome sizes of these schistosomes range from 13,503 to 16,901 bp (Le *et al* ., 2002). Mitochondrial markers that have been involved in research for various purposes which include NADH-dehydrogenase subunit 1(NAD 1) and Cytochrome oxidase subunit 1 (COX 1) genes. Studies show that the mitochondrial genome of Asian schistosomes, *S. mekongi* and *S. japonicum* demonstrates the same gene order as other Cestoda and Digenea (Lawton *et al*., 2011). In China, research conducted using mitochondrial markers such as NADH-dehydrogenase subunit 1 (Nad1) and NADH-dehydrogenase subunit 4 (Nad4) were used to demonstrate high genetic diversity within populations infected with *S. japonicum* which proved successful in distinguishing variants in mainland China and neighbouring countries around China (Yin *et al* ., 2015). Another research on the Genetic diversity within *Schistosoma haematobium* using DNA barcoding to reveal two distinct groups namely, the African mainland and the Indian Ocean Islands and neighbouring Africa coastal regions, actually found low sequence variation within the mitochondrial cytochrome oxidase subunit 1 (Cox 1) and the NADH-dehydrogenase subunit 1 (Nad1) (Webster *et al* ., 2012).

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In Sudan, mitochondrial genes NAD 1 and Cox 1 were used as markers of genetic diversity which was confirmed in *Schistosoma haematobium* eggs (Quan *et al* ., 2015).

2.6.2 Internal Transcribed Spacer (ITS)

The ribosomal RNA gene cluster of the Internal transcribed spacers regions are one of the sort after nuclear markers in eukaryotic cells used to specifically demonstrate phylogenetic relationships between species, families and genus (Warwick *et al* ., 2010).

The Internal transcribed spacer consists of two spacers, ITS1 and ITS2 distinguished by the 5.8S rRNA gene. This region is known to be conserved within a genus or species (Jousson *et al*.,1998). Studies have shown how ITS have not been analysed as a whole, rather it is broken into two (2) main components, ITS1 and ITS2.

The sequences as well as the length of the ITS regions of rRNA repeats varies due to its fast evolving nature. This has made the ITS region an interesting marker to be used for phylogenetic studies (Baldwin, 1993; Suh *et al* ., 1993; Hsiao *et al* ., 1994; Dubouzet and Shinoda, 1999).

A research conducted in Brazil, on 26 isolates of ectomycorrhizal fungi involving the polymorphism in the Internal transcribed spacer (ITS) of the ribosomal DNA which concluded that this marker is an important tool for taxonomic study of eukaryotes (Gomes *et al* ., 2002). Recently, the nuclear ribosomal internal transcribed spacer (ITS) region was used as a universal DNA barcode marker for fungi and it proved successful and helped in phylogenicity (Schoch *et al* ., 2012), however in the case of a research on the secondary structure analyses of the nuclear ribosomal RNA Internal Transcribed spacers and assessment of its phylogenetic utility across Brassicaceae it was suggested that there were problematic issues with using these regions as the sole markers for phylogenetic studies (Edger *et al* ., 2014). This research

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also recommended that additional markers should be employed in future studies to estimate phylogenetic relationships (Edger *et al* ., 2014).

In a study carried out in Ghana, involving circulating-antigen strip test and real-time PCR in comparison with microscopy where Schistosoma-specific primers were used for real-time PCR on the basis of the internal transcribed spacer 2 (ITS 2) sequences for *S. haematobium* (Gen Bank accession DQ-677661), 89% of cases were found to be PCR-positive (Obeng *et al*., 2008). The Internal transcribed spacer has been used as a marker in most research works especially in Schistosomes. The detection and analysis of interspecific hybrids as well as species identification in *Schistosoma* have been proven useful using the Restriction Fragment Length Polymorphisms (RFLP) within the spacer regions of the ribosomal RNA gene complex (Walker, 1986; Rollinson *et al*., 1990). A study conducted particularly in Sudan used the nucleotide sequences of the ITS2 gene in *S. haematobium* and compared to that discovered in Kenya earlier and it demonstrated 99% similarity (Quan *et al* ., 2015).

2.7 Detection and Viability of *Schistosoma haematobium* Eggs

The gold standard for the detection of schistosomes is the microscopic examination of urine or stool for the presence of schistosome eggs (Kardoush *et al* ., 2016). In Khartoum State-Sudan, a recent study was conducted on the effect of Praziquantel treatment on the number of viable and dead eggs of *Schistosoma haematobium* and it suggested that there were more dead eggs than viable (live) eggs after treatment compared to pre-treatment samples which had more viable (live) eggs than dead eggs. This was distinguished using methylene blue and trypan blue stains (Elfaki *et al* ., 2015).

In Brazil another study distinguished morphological eggs of *S. mansoni* using vital stains such as trypan blue and neutral red and a fluorescent probe known as Hoechst 33258.

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The Hoechst probe 33258 was considered a vital tool for differentiation of viable and non-viable eggs (Sarvel *et al* ., 2006).

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CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study site

The study was conducted among participants in the Zenu and Weija communities. These two communities have lakes and rivers which provide beneficial purposes to the inhabitants as well as reservoir for urogenital schistosomiasis.

3.1.1 Zenu Community

Zenu is a small community situated specifically in the south of Ashiaman municipality (a town found around the outskirts of Tema) which is a suburb of Accra, the capital city of Ghana. It has its geo-graphical coordinates as 5°42'0" North, 0°20'0" West (Tetteh-Quarcoo *et al* ., 2013). Subsistent farming and fishing remains the main economic activities as compared to most rural areas within the region with a few people to be civil servants. The presence of a lake in the community might be the reason for most of the settlers in this community (Figure 3.0 A). The lake serves as a beneficial purpose to inhabitants of the community such as drinking, washing, bathing and other domestic purposes. It became imperative to screen children for the infection since they are mostly vulnerable and affected. The Zenu community is a naïve area in terms of schistosomiasis studies and drug administration by the Neglected Tropical Disease control program and that justifies the reason for choosing this site.

3.1.2 Weija Community

Weija is a community that lies at the South-West of Accra and is interconnected to the Accra Metropolitan Area to the South-East, Ga Central to South-East, Akwapim South to the North-East, Ga West to the East, West Akim to the North, Awutu-Senya to the West, Awutu-Senya

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East to the South-East, Gomoa to the SouthWest and the Gulf of Guinea to the South (Figure 3.0 C).

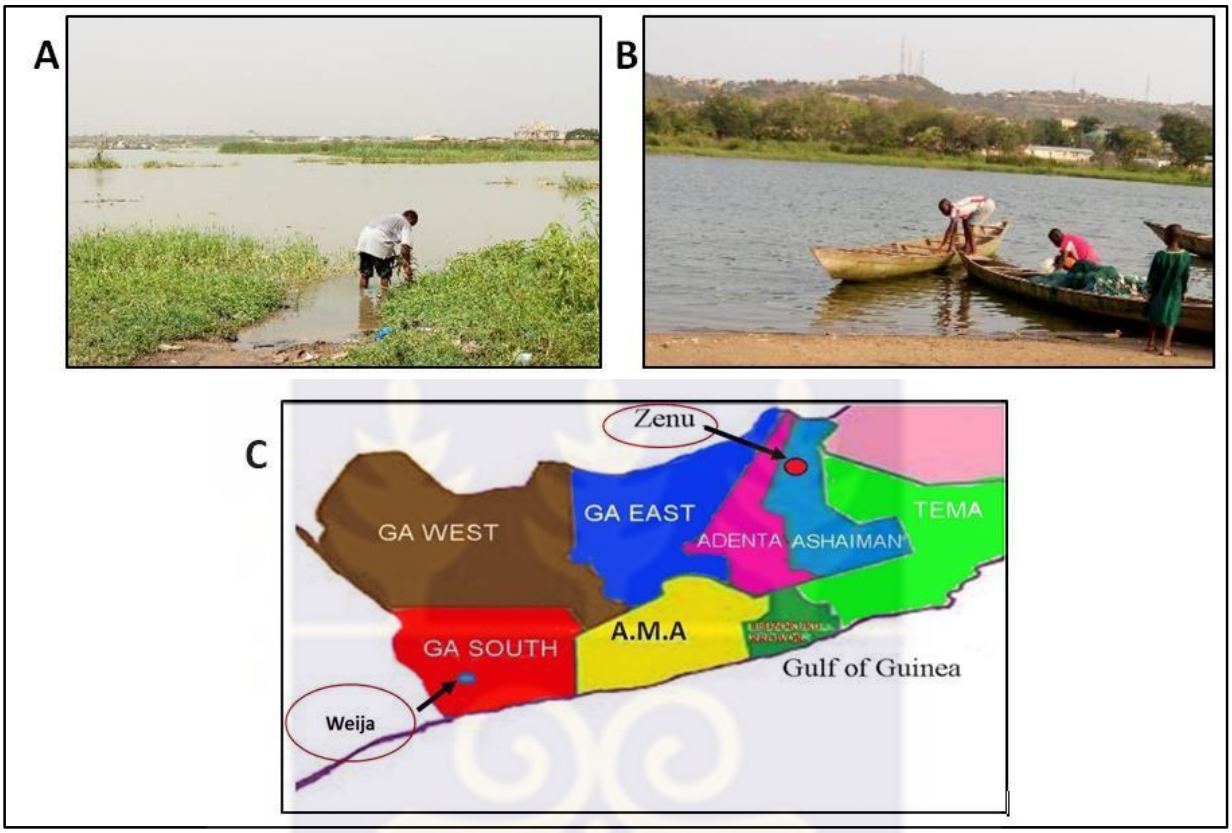


Figure 3.0: Study Locations:(A) Zenu Lake site at the outskirts of the Zenu Community (B) A lake located at the Weija community (C) Geographical location of the Zenu and Weija communities in the Greater Accra region (Adapted and modified from en.wikipedia.org/wiki/Ashaiman Municipal District).

It is the administrative capital of the Ga South since 2012 according to legislative instrument 2134 in July when the Ga south was carved out from the Ga West district. It has about 95 settlements which occupies a total land area of about 341.838 square kilometers. It lies in the dry equatorial climatic zone, with annual average temperature ranging from 25.1^{0}C in August and 28.4^{0}C in February and March (Dickson and Benneh, 2001). There are two main rivers namely, the Ponpon and Densu River, which drain the Municipality. Densu is one of the main sources of water supply to more than half of the

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population of the Accra Metropolis. There are several small lakes created by these rivers in the community (Figure 3.0 B).

The choice of Weija in this study was due to a number of studies (Ampofo & Zuta, 1995, Aboagye & Edoh, 2009, Anim-Baidoo *et al* ., 2017) conducted and consistence drug administration in the area by Neglected Tropical Disease control programme of Ghana. Prevalence from Weija (Mahem) was 49 % (Aboagye & Edoh, 2009).

3.2 Study design

The design was a longitudinal study, involving a baseline sample and subsequent follow-ups. Urine samples were collected from consenting school children, as a baseline to identify those infected with *S. haematobium*. Praziquantel was then administered after food by professionals from the Kasoa Polyclinic and the Zenu Community Hospital all of the Neglected Tropical diseases as part of routine hospital treatment, after which, follow-up (longitudinally) samples, at one (1) week interval for the first four weeks and two weeks interval for subsequent weeks up to twelfth (12) week were collected to assess resistance. For the assessment of reinfection, resampling was done at the sixth month. The data (on egg counts and viability) from each batch of samples (baseline and longitudinal follow-ups) was compared to deduce the effect of the drug. The study was conducted from August, 2016 to June, 2017.

3.3 Inclusion and Exclusion Criteria

Primary and Junior High School children who have not taken medication on schistosomiasis were included in this study whiles those who took medication for schistosomiasis three weeks prior to and during the data collection were excluded.

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3.4 Sample size

The sample size was three hundred and sixty (360). The sample size determination was based on previous prevalence studies conducted in a known endemic area in the Greater Accra Region. The detailed calculation can be found below;

From recent study prevalence rate of *Schistosoma haematobium* in a community in the Greater Accra Region was recorded as 30.7% (Tetteh-Quarcoo *et al*., 2013). Thus, this was used as the estimated prevalence rate.

Minimum sample size was calculated using the formula,

$$n = \frac{Z^2.\ P\ (1 - P)}{m_2}$$

Where n = minimum sample size

Z = standard value at a certain confidence level = 1.96 (95% Confidence Interval CI) P = estimated prevalence = 30.7% = 0.37, *m*= margin of error = 0.05

$$n = \frac{1.96^2 \times 0.37\ (1 - 0.37)}{(0.05)^2} = 358 \sim 360$$

Meanwhile, 420 urine samples were collected for this study.

3.5 Study Procedure

The overview of the study procedure included sample collection, microscopic examination for egg count and viability testing, gene extraction, PCR amplification, and sequencing.

For the assessment of reinfection, resampling was done for four hundred and twenty study participant's baseline samples at the sixth month to identify study participants infected with *S. haematobium* for the first time and study participants with persistent *S. haematobium* ova in the urine for the second time but showed evidence of total egg clearance by the sixth week.

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Reinfection can be described as evidence of total egg clearance by the sixth week and the reappearance of viable eggs (ova) by the sixth month. Egg counts is expected to achieve zero count at the sixth week, and resurface at the sixth month. Live viable eggs are seen at the sixth month as compared to the previous follow-ups.

For the assessment of resistance, school children diagnosed of *S. haematobium* in their urine from a baseline sampling of three hundred and sixty were given Praziquantel treatment after which follow up was done weekly for the first four weeks and two weeks interval from the fourth week to the eighth week and finally at the twelfth week.

Therefore, **Resistance** can be described as evidence of persistent viable eggs (ova) by the sixth week after Praziquantel treatment which will still not clear up to the twelfth week. Egg count is expected to be present even after the sixth week, and still not cleared after the twelfth week. Live viable eggs are expected to be more than dead eggs after Praziquantel treatment.

Epidemiologic and demographic information was gathered by the administration of questionnaire, which covered areas like: age, sex, duration of residence in the community, exposure to the water source, distance of house to the water body, duration of clinical symptoms and history of treatment.

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3.6 Laboratory investigation

The Laboratory procedures employed in the current study included; Urine Sampling and Processing, Microscopic and Macroscopic analysis, Viability testing as well as Molecular analysis (Figure 3.1). All laboratory safety precautions were followed accordingly.

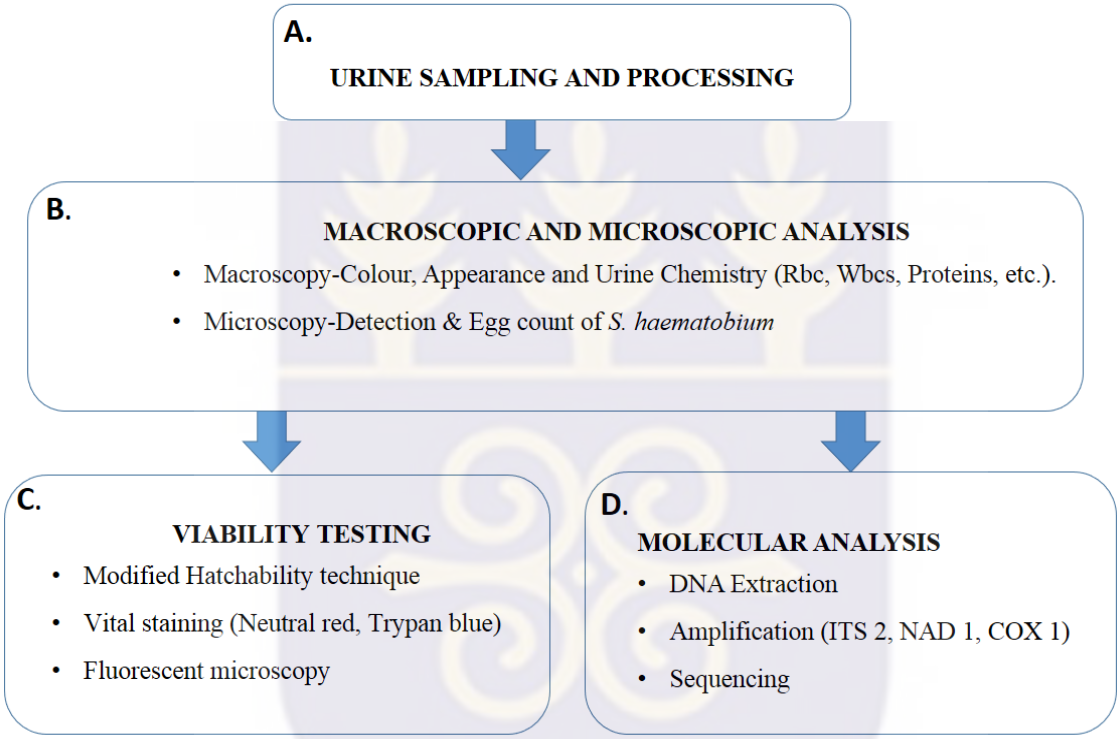


Figure 3.1: Overview of laboratory procedures. A: Urine Sampling & Processing. B: Macroscopic and microscopic analysis (included; macroscopy-colour, appearance and urine chemistry. Microscopy-detection & Egg count of *S. haematobium*). C: Viability testing (included: Modified hatchability technique, Vital staining, Fluorescent microscopy). D: Molecular analysis (included: DNA extraction, Amplification, Sequencing). A, B and C were needed for Objective 1 of the current study whiles A, B and D were needed for objective 2.

3.6.1 Urine Sampling and Processing

Clean, dry, leak-proof, wide mouthed containers were given to each study pupil to provide urine with adequate instructions. Urine sample collection was done between 10:00 am and 12:00 pm for maximum yield (Weber *et al.*, 1967). The urine samples were then transported on ice immediately to the Parasitology Laboratory of the Medical Microbiology Department, University of Ghana, for laboratory investigation.

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3.6.2 Preparation, detection and Egg Count of *S. haematobium*

Before urine samples were examined microscopically, macroscopy was done to determine the colour and the appearance of the urine. This was followed by the use of the urine reagent test strips (URIT 10V, URIT Medical Electronic Co., Ltd, China) to determine infection morbidity parameters such as haematuria, proteinuria, leucocytes (Figure 3.1) as well as other parameters (pH, specific gravity, glucose, ketones, bilirubin, urobilinogen). The collected urine samples were then examined microscopically by pouring 10 ml of the urine into a centrifuge tube. They were then spun at 3000 rpm for 3 to 5 minutes and the supernatant was discarded until the 0.5 ml mark. Fifty microlitres (50 µl) of the sediment was transferred onto clean glass slides. This was done to lookout for the presence of *S. haematobium* ova which is described by its characteristic oval shape and a terminal spine. The egg count, which was determined by the number of eggs present / 10 ml of urine, was useful in assessing the effect of the drug on the subjects (Samie *et al* ., 2010).

In successful treatment, there was gradual reduction in egg count in the follow-up samples, until undetectable levels were achieved, as a result of the destruction of the female adult worm. In the case of resistance however, egg counts did not show any pattern of reduction indicating the lack of destruction of female adult worms. The presence of these eggs is an indication of the inability of the Praziquantel to destroy the adult worms.

In the case of reinfection there would be gradual reduction of egg counts until about the 6th week when there is evidence of total egg clearance but there the re-appearance of persistent viable eggs (ova) at the 6^{th} month.

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3.6.3 Determination of Egg Viability of *S. haematobium*

The viability of the eggs was determined by the modified hatchability test, vital staining, and fluorescent staining (Figure 3.1). Whenever the infection is active, viable eggs are produced, while old infections or treated patients result in nonviable eggs and absence of miracidia in follow up samples (Cook, 2009).

Low levels of viable eggs would be detected, in follow up samples, when treatment is successful. Therefore, in the case of resistance, high levels of viable eggs would be consistently seen microscopically from follow-up samples especially after the 6th week. While the viability of eggs will reduce gradually over the weeks in the case of reinfection, but viable eggs would re-appear at the 6th month. This interpretation is based on the suggestions that, Praziquantel could have effect on the viability of eggs in some Schistosomes (*S. haematobium*, *S. mansoni* and *S. japonicum*) (Richards *et al* ., 1989; Matsuda *et al* ., 1983; Elfaki *et al* ., 2015).

3.6.3.1 Hatchability test

Several methods have been adopted for the identification of eggs demonstrating viability. Miracidial hatching was originally described by Fulleborn in 1921 which was used for chemotherapeutic studies because of its effectiveness in post-treatment evaluation in clinical trials. The procedure adopted for this research was simply done by placing 50 µl of the urine sediment on a clean grease free slide, 20 µl of distilled water was added and covered with a cover slip. It was then observed with x40 objective lens but this time it was exposed to maximum light from the illuminator. The presence of flame cell activity was the initial factor considered for viability, followed by movement of the embryo in the egg. After constant exposure of light and the presence of water, miracidia hatch out of the viable eggs indicating eggs of such activity were viable. On each slide, the number of viable eggs were counted

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against the number of non-viable eggs and the percentage viability was determined. This was calculated as the number of eggs in each slide to be viable or non-viable divided by the total egg count of that slide multiplied by 100.

3.6.3.2 Vital Staining (Trypan blue and Neutral red)

The vital stains 0.4% Trypan blue and 1% Neutral red were used to distinguish dead eggs from live eggs (Sarvel *et al* ., 2006) and this was done by pipetting 50 µl of the 0.4% Trypan blue as well as 1% neutral red which were added to slides containing cell suspension after which a cover glass was applied and incubated in a Petri dish containing moist cotton for 5 minutes. After the 5 minutes it was then observed under the optical microscope using the x10 and x 40 objective to check for stain retention in the eggs for the classification of the eggs as either dead or alive.

In the case of the 0.4% trypan blue stain, live eggs (viable) did not show much stain retention however eggs that showed much stain retention (blue colouration) were classified as dead eggs (ova). For the 1% Neutral red, live eggs retain the red stain whereas the dead eggs do not retain the stain (Sarvel *et al*., 2006). On each slide, the number of live eggs (viable) were counted against the number of dead eggs and the percentage viability was determined. This was calculated as the number of eggs in each slide to be live or dead divided by the total egg count of that slide multiplied by 100.

3.6.3.3 Fluorescent staining

The fluorescent label Hoechst 33258 (bisbenzamide) (2.4 hydroxyphenil 5.4 methyl,1 piperazine 2.5 biH-benzymidazol) is a hydrophilic and fluorescent probe that is permeable to the nuclei and binds to the minor groove of double stranded DNA of cells. It is a cell stain that is ideally used for the visualization of viable cells (viability).

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Hoechst dyes have often been used as a substitute for other dyes such as DAPI due to its less toxicity and enhanced viability of cells. Fluorescent Hoechst 33258 has been considered a useful tool for the differentiation between dead and live eggs (Sarvel *et al.*, 2006). The fluorescent probe Hoechst 33258 was diluted in 0.85% saline and 1 mg/ml of the stock solution was obtained. For every 1 ml of egg suspension, 10 µl of the probe was added, this was prepared on grease free clean slides and incubated at room temperature for twenty (20) minutes. Prepared slides were examined after incubation with the fluorescent microscope with a reading filter of 460nm (Oliveira *et al.*, 2006). Viable eggs fluoresce blue and non-viable eggs do not fluoresce. On each slide, the number of viable eggs were counted against the number of non-viable eggs and the percentage viability was determined. This was calculated as the number of eggs in each slide to be viable or non-viable divided by the total egg count of that slide multiplied by 100.

3.6.4 DNA extraction

Genomic DNA was extracted from *Schistosoma haematobium* eggs using quick-g DNA miniprep kit (ZYMO, RESEARH) protocol after allowing the -80^{0}C stored urine samples to thaw.

3.6.5 Amplification of the ITS2 region by PCR.

PCR amplification of the ITS2 fragment (ITS2-PCR) from *S. haematobium* eggs was performed in a 25-µl volume using primers ITS2F (5´-GAA TTA ATG TGA ACT GCA TAC TGC TT-3´) and ITS2R (5´-TTC CTC CGC TTA TTG ATA TGC TT-3´). The 25 -µl volume was made up of 12.5 µl of master mix (10× Ex Taq buffer, dNTP mixture, TaKaRa Ex Taq DNA Polymerase), 2.5 µl each of ITS2F (5´-GAA TTA ATG TGA ACT GCA TAC TGC TT-3´) and ITS2R (5´TTC CTC CGC TTA TTG ATA TGC TT-3´), 6.0 µl of genomic DNA

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and 1.5 µl of nuclease free water using a PCR Thermal Cycler. All PCR assays were performed using 1 cycle of 94˚C for 30 seconds and 40 cycles of 98˚C for 10 seconds, 60˚C for 30 seconds, and 72˚C for 30 seconds, followed by 1 cycle at 72˚C for 7 minutes and a final hold at 4˚C.

Agarose gel electrophoresis (1.5%) with ethidium bromide (5µl) staining was used to visualize the ITS2-PCR products under an ultra violet light illuminator. The expected band size of 468bp, was measured using 100bp and 50-bp DNA ladders (New England Biolabs Inc.).

3.6.6 Sequencing and analysis of the ITS2 and NAD 1 region

The purified ITS2 and NAD 1 PCR products were sequenced directly using the primers described previously. The complete ITS2 and NAD 1 sequences for *S. haematobium* were compared to those isolated from other countries using the Bioedit software, version 7.0. The consensus sequences for each sample was then aligned, and any variant (polymorphic) positions between individuals, was visualised in comparison with the original sequence chromatograms. The confirmation of the identity was done using the Basic Local Alignment Search Tool (NCBIBlast).

3.6.7 Amplification of NADH dehydrogenase subunit (NAD1) gene to determine genetic variation in *S. haematobium*

Amplification of NAD 1 gene of *Schistosoma haematobium* was used to determine genetic variation in *S. haematobium* in the urine deposits. Amplification was performed in a 25-µl volume, which consists of 6.0 µl of genomic DNA from each sample, 12.5 µl of master mix (10× Ex Taq buffer, dNTP mixture, TaKaRa Ex Taq DNA Polymerase), 2.5µl each of *S. hnad1F* 5´GGC TGA TGT TCG TGA TCA AA-3´ and *S. hnad1R* 5´-CGA AGT CGA GAA

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AAT GAA CCA-3′ and 1.5 µl using a PCR Thermal Cycler. All PCR assays was performed using 1 cycle of 94˚C for 30 seconds and 50 cycles of 98˚C for 10 seconds, 58˚C for 30 seconds, and 72˚C for 60 seconds, followed by 1 cycle at 72˚C for 10 minutes and a final hold at 4˚C. Agarose gel electrophoresis (1.5%) with ethidium bromide (5 µl) staining was used to visualize the NAD 1 PCR products. The expected band size of 431-bp, was measured using 100bp and 50-bp DNA ladders (New England Biolabs Inc.).

3.6.8 Amplification of Mitochondrial Cytochrome Oxidase 1 Subunit 1

Amplification of COX 1 gene of *Schistosoma haematobium* was used to determine genetic variation in *S. haematobium* in the urine deposits. A 25-µl reaction volume was used for amplification, 2.5 µl each of *S. h cox1 F 5'* -AAA AGC TGT GGG TCT CGT GT- 3' and *S. h cox1 R* 5'- AAT GAA GAA GCG GAG AAA GC- 3' was used,12.5 µl of master mix (10× Ex Taq buffer, dNTP mixture, TaKaRa Ex Taq DNA Polymerase) of each sample,1.5 µl of nuclease free water and 6.0 µl genomic DNA of each sample added up to the 25 µl reaction mixture for PCR using a thermal cycler. All PCR assays was performed using 1 cycle of 94˚C for 30 seconds and 40 cycles of 98˚C for 10 seconds, 58˚C for 30 seconds, and 72˚C for 60 seconds, followed by 1 cycle at 72˚C for 10 minutes and a final hold at 4˚C. Agarose gel electrophoresis (1.5%) with ethidium bromide (5 µl) staining was used to visualize the COX 1 PCR products. The expected band size of 110-bp, was measured using 100bp, and 50bp DNA ladders (New England Biolabs Inc.).

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3.7 Statistical analysis

The data was analysed by using GraphPad Prism version 501. The questionnaires were analysed by simple proportions and the chi-square test was used to determine associations.

The egg counts between baseline and follow-ups were used to calculate the arithmetic means of the baseline and post-treatment which was eventually used to calculate the egg reduction rate (ERR). The egg reduction rates of the baseline and follow-up samples was calculated by subtracting one from the products of the arithmetic mean egg counts per 10 mL of urine before and after treatment and multiplying it by 100 (Ojurongbe *et al*.,2014). This was used as the main evaluating tool for resistance assessment.

Chi-square test was used as comparative analysis of the methods used to determine viability. P values < 0.05 was considered statistically significant.

For genetic variations, amplified Nad 1, Cox 1 and ITS 2 between the individual samples was evaluated by simple proportions. Sequence results from the forward and reverse strands (Nad 1, Cox 1 and ITS 2) was manually edited to remove any ambiguity. The ITS2 and NAD1-PCR products were sequenced using the primers described previously. The complete ITS2 and NAD1 sequences for *S. haematobium* was compared to those isolated from other countries using the Bioedit 7.0.

The consensus sequences for each sample was then aligned, and any variant (polymorphic) positions between individuals, was visualised in comparison with the original sequence chromatograms. The confirmation of the identity was done using the Basic Local Alignment Search Tool (NCBIBlast).

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3.8 Scientific Research and Ethical Clearance

Approval of this study was done by the Ethics and Protocol Committee of the College of Health Sciences, University of Ghana (Appendix IX). Urine samples were collected from study participants with consent from parents and guardians as well as teachers. Assent was also sought from school children.

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CHAPTER FOUR

4.0 RESULTS

4.1 Overview of Reinfection Assessment

This section of the results is focused on a summary of how reinfection was assessed taking into consideration the baseline and follow-ups period after Praziquantel treatment. At the sixth month when resampling was done, twenty-one (21) were infected out of the 360 sampled, of which eight (10.4%) of the study participants were reinfected and thirteen were newly infected. The seventy-seven (77) of the study participants identified to be positive for *S. haematobium* at baseline gradually reduced in the follow-ups up to the third week (week 1-66 positives, week 2-26 positives, week 3-19 positives) after which there was no detectable *S. haematobium* ova from the fourth week to the twelfth week (Figure 4.1). The percentage reinfection was 10.4% (Figure 4.1).

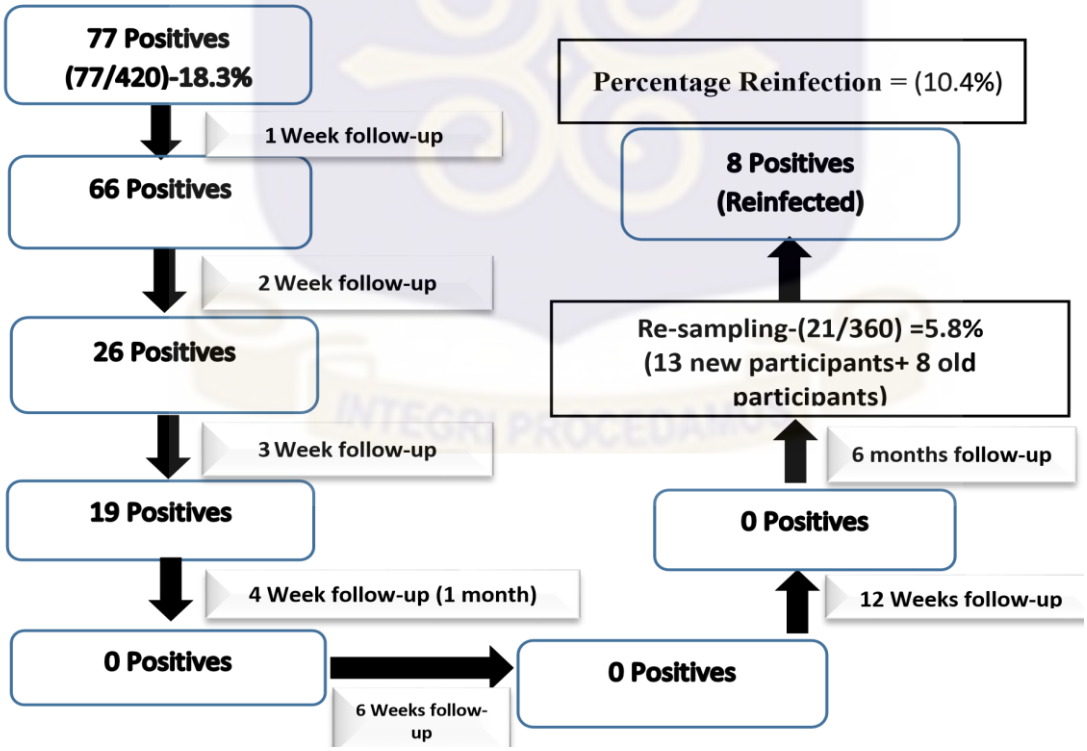


Figure 4.1: Assessment of Reinfection. A Flow chart demonstrating the gradual reduction of *S. haematobium* eggs for a period of 12 weeks. Resampling done at the 6th month with 8 positives (10.4%) found indicate reinfection.

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4.1.1 *S. haematobium* ova in reinfected study participants

The ova of the eight reinfected individuals varied morphologically by their shape and terminal spine (Figure 4.2). The ova of picture F which appeared uniquely different from the others and looks like *S. intercalatum* was from a study participant with an Asian descent from Zenu.

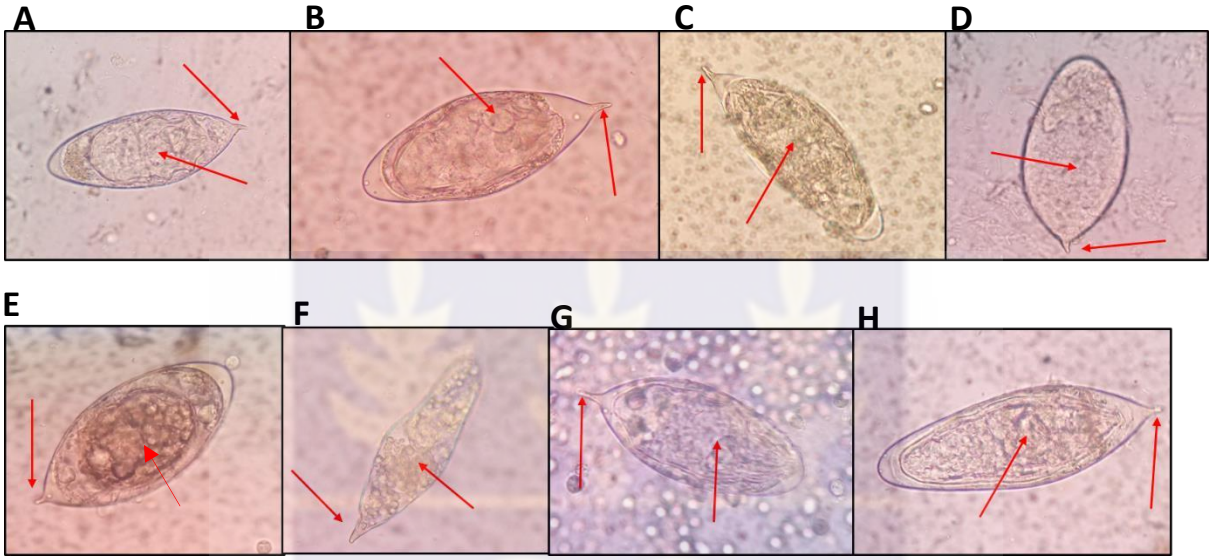


Fig 4.2: *Schistosoma haematobium* eggs (ova) during wet mount microscopy in reinfected study participants. Arrows indicate the shape and the position of the terminal spine as well as the miracidium. Microscopy done using x40 objective lens. A: characteristic oval shape with short curved terminal spine. B: A characteristic oval shape with a terminal spine slightly curved with intermediate size. C: A characteristic oval shape with a pointed terminal spine. D: a characteristic round shape with a short curved terminal spine. E: A characteristic oval shape with short terminal spine. F: A characteristic elongated shape with a terminal spine G: A characteristic oval shape with a terminal spine. H: A characteristic oval shape with a pointed terminal spine.

4.1.2 Re-visit to the water source (dam-site) /Study sites and Reinfection

Questionnaire gathered from the study revealed that, out of the seventy-seven infected, fifty responded (50) 'Yes' to re-visit the water source (dam-site) of which 62.5% (5/8) were reinfected. Twenty-seven (27) of the infected participants responded 'No' to re-visit the water source (dam-site) of which 37.5% (3/8) were reinfected. For participants who responded 'Yes' to re-visit the water source (dam-site), the water contact activities included; swimming, fetching water and selling. The percentage reinfection in Zenu and Weija were 10% (6/60) and 11.8% (2/17) respectively.

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There is no association between re-visit to the dam and reinfection (Chi-square value calculated χ^2= 0.0233 is less than the tabulated value χ^2 =3.84).

4.2 Overview of Resistance assessment

For the assessment of resistance, out of the three hundred and sixty (360) study participants that were sampled at baseline, twenty-one (21) were identified with *S. haematobium* ova in their urine (Figure 4.3). However, there was gradual reduction of *S. haematobium* ova in the follow-ups after Praziquantel treatment for the first 4 weeks (week 1-9 positives, week 2-4 positives, week 3-3 positives, week 4- 0 positives). Subsequent follow ups from the 6th week to the 12 th week demonstrated no evidence of *S. haematobium* ova in any of the study participants indicating egg clearance (Figure 4.3).

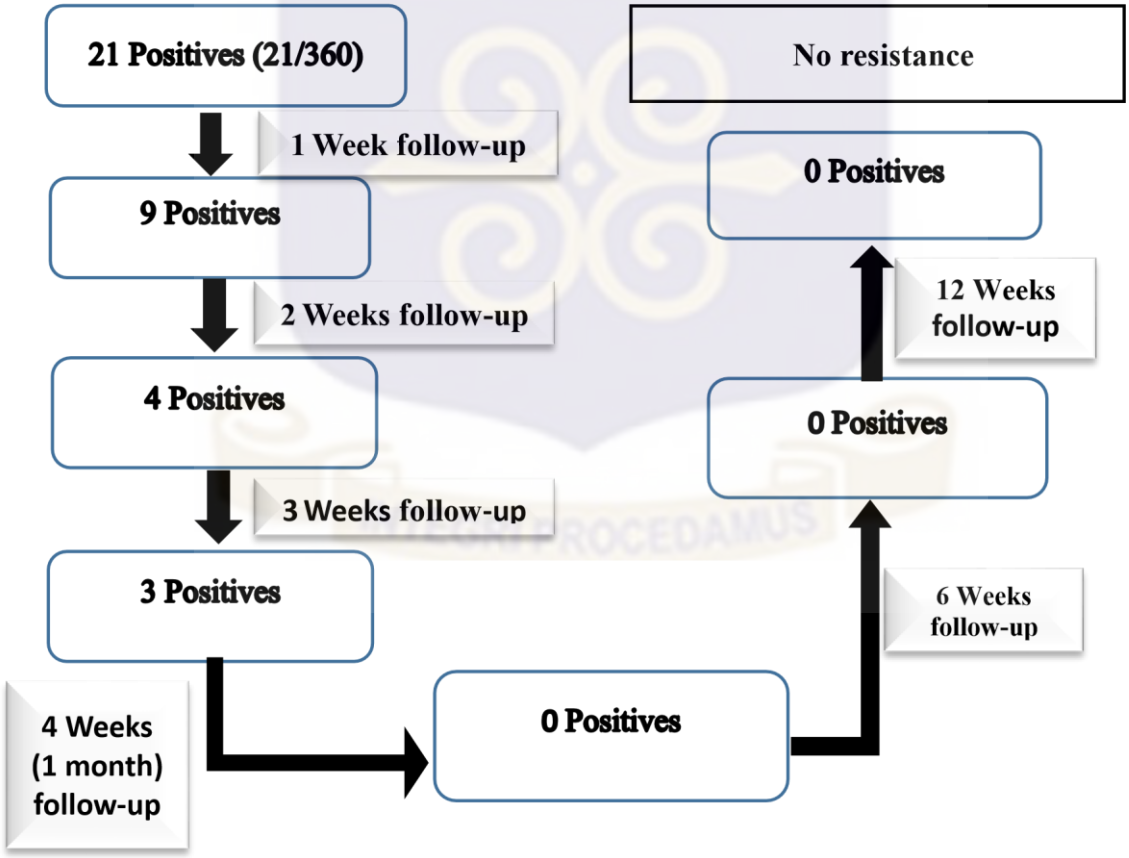


Figure 4.3: Overview of Resistance Assessment. A Flow chart indicating a summary of *S. haematobium* egg reduction in study participants from the 1st week to the 12 th week. Week 4 to 12 indicates no positives in these follow up period.

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4.2.1 Egg Reduction Rates of Post-Treatment Weekly

Of the 21 (twenty-one) participants treated with Praziquantel, resistance was assessed by the Egg Reduction Rates (E.R.R) in the follow-ups. By the sixth (6^{th}) week, no ova were seen in the urine samples of all 21 (twenty-one) participants up to the 12^{th} week. The Egg Reduction Rate of the first week could not be calculated as a result of the remarkable high egg count. The Egg Reduction rates at week 2, week 3 and week 4 were 81.24%, 94.37% and 90% respectively (Figure 4.4 B). Egg reduction rate for both the sixth and eighth week was 100% (Figure 4.4 B).

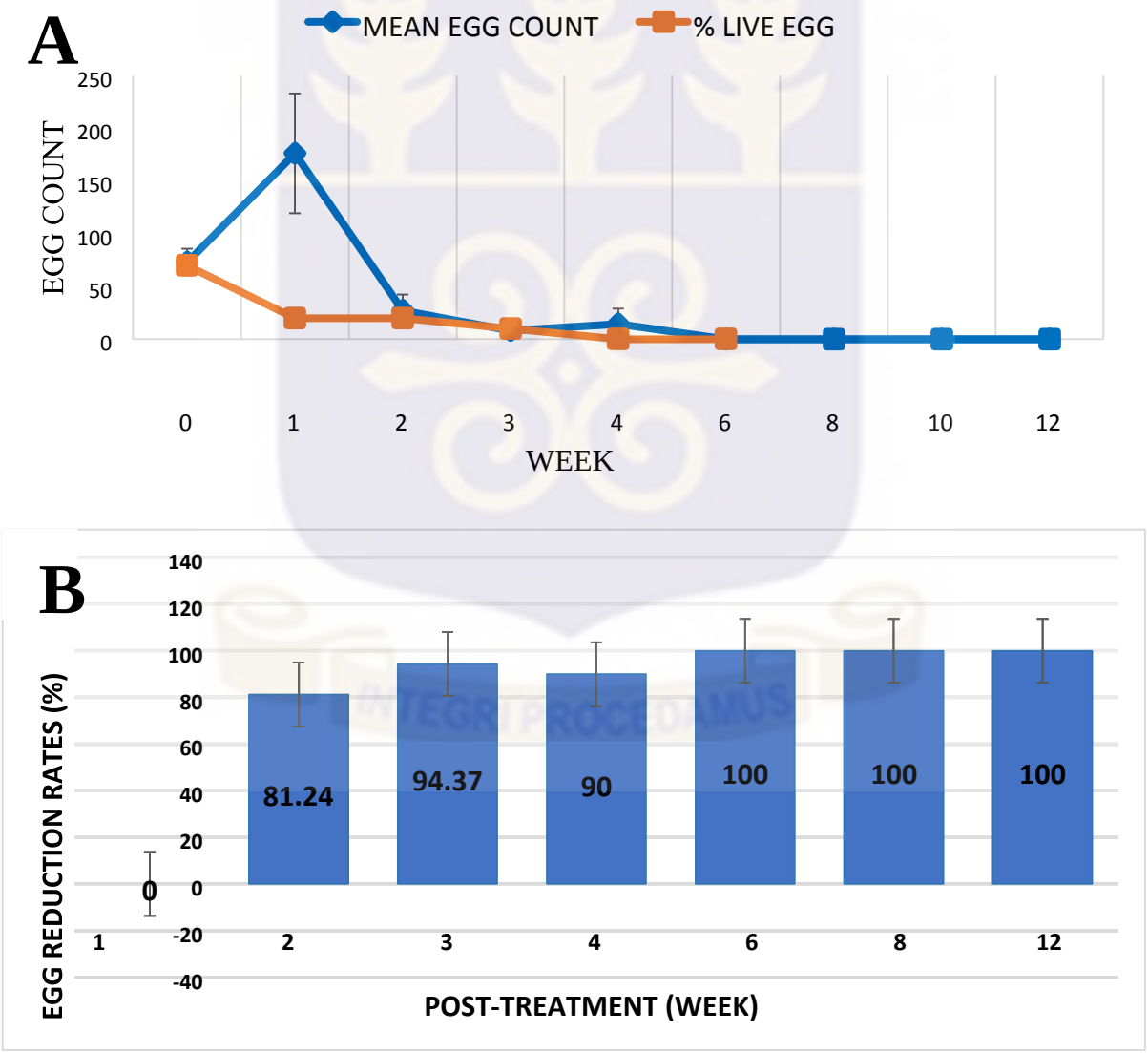


Figure 4.4: Mean egg count and Egg Reduction Rates. A: A Line graph showing mean egg count and percentage live viability trend during post-treatment. B: A bar graph showing Egg Reduction Rates at post-treatment for resistance assessment**.**

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4.2.2 Egg count and percentage live viability at Post-treatment

The mean egg count recorded at baseline to be 76.9 was lower than the mean egg count of the first week of follow-up (post-treatment) which was recorded to be 79.05. From the second week of follow-up to the 4th week, there was a drastic reduction of mean egg count (week 2-14.29, week 3-4.29, week 4-7.62 and week 6-0.0) (Figure 4.4 A). From the 6th week to the 12th week, mean the egg count was zero (Figure 4.4 A). The percentage live viability at baseline (70%) was higher than in the follow-ups (week 1-20%, week 2-20%, week 3-10%, week 4-0%). From week 6 to week 12, the percentage live viability was 0.0%.

4.2.3 Assessment of *S. haematobium* infection morbidity parameters

The infection morbidity parameters assessed include haematuria (red blood cells in urine), proteinuria (protein in urine) and pyuria (white blood cells in urine). Haematuria and proteinuria were detected at baseline among study participants although pyuria was not detected. For haematuria, the percentage of the participants infected at baseline was higher (76.2%) compared to the follow-ups (57.1% at week 1 and 0% in subsequent weeks). Proteinuria was lower at baseline (19.19%) compared to the first week which was higher (71.4%). There were undetectable levels in the subsequent follow-ups (0.0%). In the case of pyuria which was not detected at baseline, there were higher stable detectable levels during the first to the third week post-treatment (week 1-95.2%, week 2-95.2%, week 3-95.2%) and low level in the fourth week (4.8%) (Figure 4.5). There were no detectable levels from the 6th week to the 12th week (Figure 4.5).

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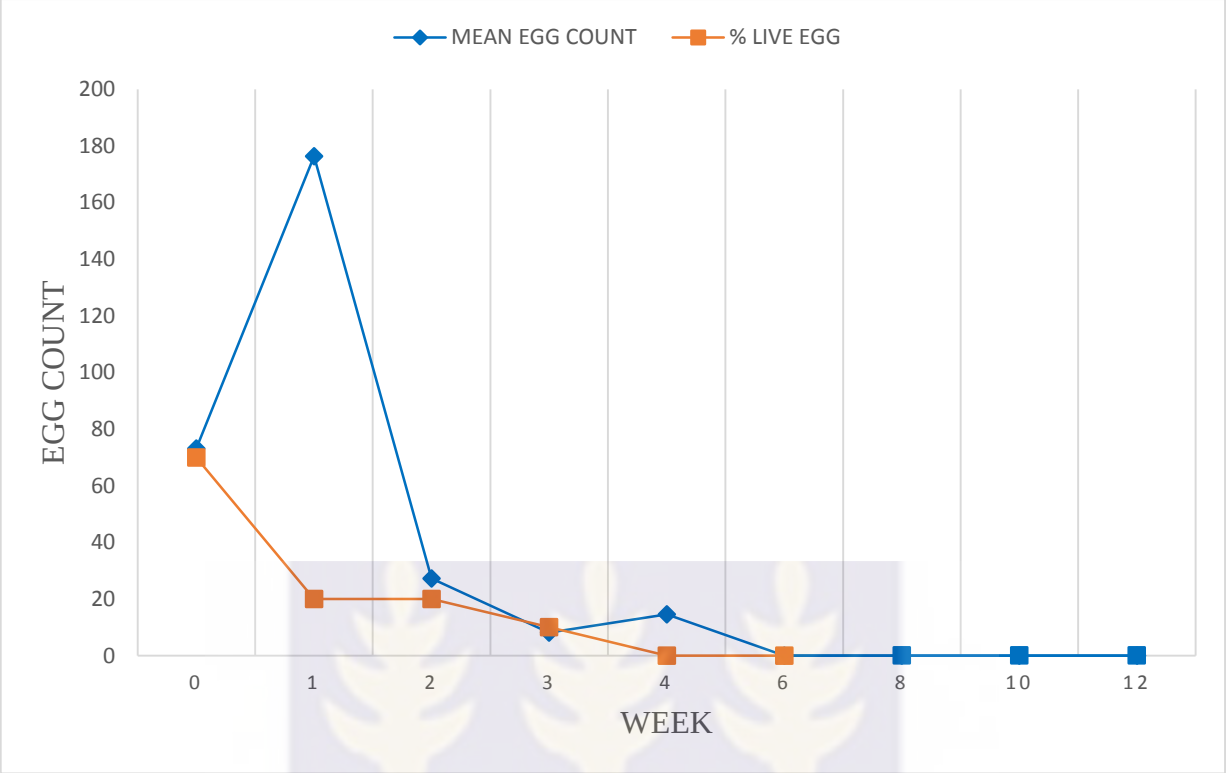


Figure 4.5: A line graph showing *S. haematobium* infection morbidity parameters assessment in follow-ups.

4.3 Viability at baseline and post-treatment

The proportion of live eggs to dead eggs at baseline was higher compared to that at post-treatment considering all the methods (modified hatchability technique, the vital staining and the fluorescent microscopy) used to determine viability (Table 4.1). All the methods used to determine viability of *S. haematobium* ova in the study complemented each other. The mean percentage viability of live eggs and dead eggs at baseline was 70% and 30% respectively (Table 4.1). However, 13.25% live eggs and 86.5% dead eggs was recorded at post-treatment. All the methods used to determine viability were statistically the same (Chi-square value calculated χ^2=1.1822 is less than the tabulated value χ^2 =7.81).

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Table 4.1: VIABILITY AT BASELINE AND POST-TREATMENT

METHOD USED	BASELINE (PRE-TREATMENT)		POST-TREATMENT	
	LIVE EGGS	DEAD EGGS	LIVE EGGS	DEAD EGGS
TRYPAN BLUE	60%	40%	15%	85%
NEUTRAL RED	70%	30%	13%	87%
FLUORESCENT MICROSCOPY	80%	20%	12.5%	87.5%
MODIFIED HATCHABILITY TECHNIQUE	70%	30%	12.5%	87.5%
MEAN AVERAGE	**70%**	**30%**	**13.25%**	**86.5%**

4.3.1 Viability by Modified Hatchability technique

Eggs showing the presence of flame cells and movement of the embryo normally hatched out of the egg shell (Figure 4.6 A, B, C), an indication of a morphologically viable egg (live eggs). Dead eggs did not show any characteristics such as the presence of flame cells as well as movement of the embryo. Matured eggs considered to be viable hatched out of the egg shell. Eggs of such activity have the miracidia in a different field after swimming for a while (Figure 4.6 D, E).

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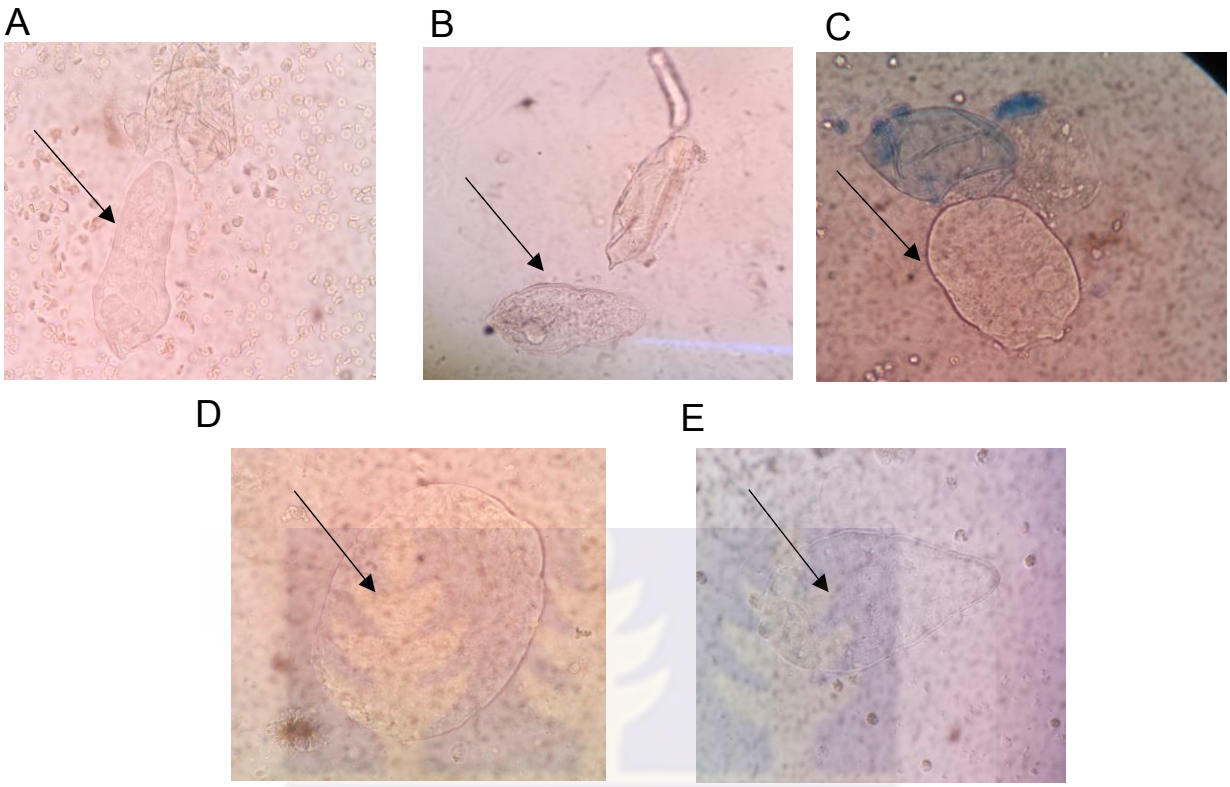


Figure 4.6: A microscopic view of miracidium using the Modified Hatchability technique: Miracidium hatched out of an *S. haematobium* ova with an egg case beside it. D & E: A miracidium observed using x40 objective lens during the modified hatchability technique

4.3.2 Viability by vital staining

The Trypan blue stain was effective in staining the eggs considered to be dead (Fig 4.7.B) whiles eggs considered to be morphologically alive did not pick up the stain (Fig 4.7.A). Incubation time played a critical role in distinguishing this differences between the eggs. The Neutral red stain was able to stain eggs considered to be viable (live) indicating the nuclei of the eggs to stain red (Fig 4.7.C). The nuclei of the eggs considered to be dead could not retain the stain with the surrounding cells showing red colouration (Fig 4.7 D).

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4.3.3 Viability by Fluorescent microscopy

The fluorescent probe (Hoechst 33258) used to stain the eggs was able to differentiate live eggs (viable) from dead eggs (non-viable). The live eggs were able to show fluorescence in a blue colour (Fig 4.8.A) whiles the dead eggs did not show fluorescence indicating to be non-viable (Figure 4.8. B).

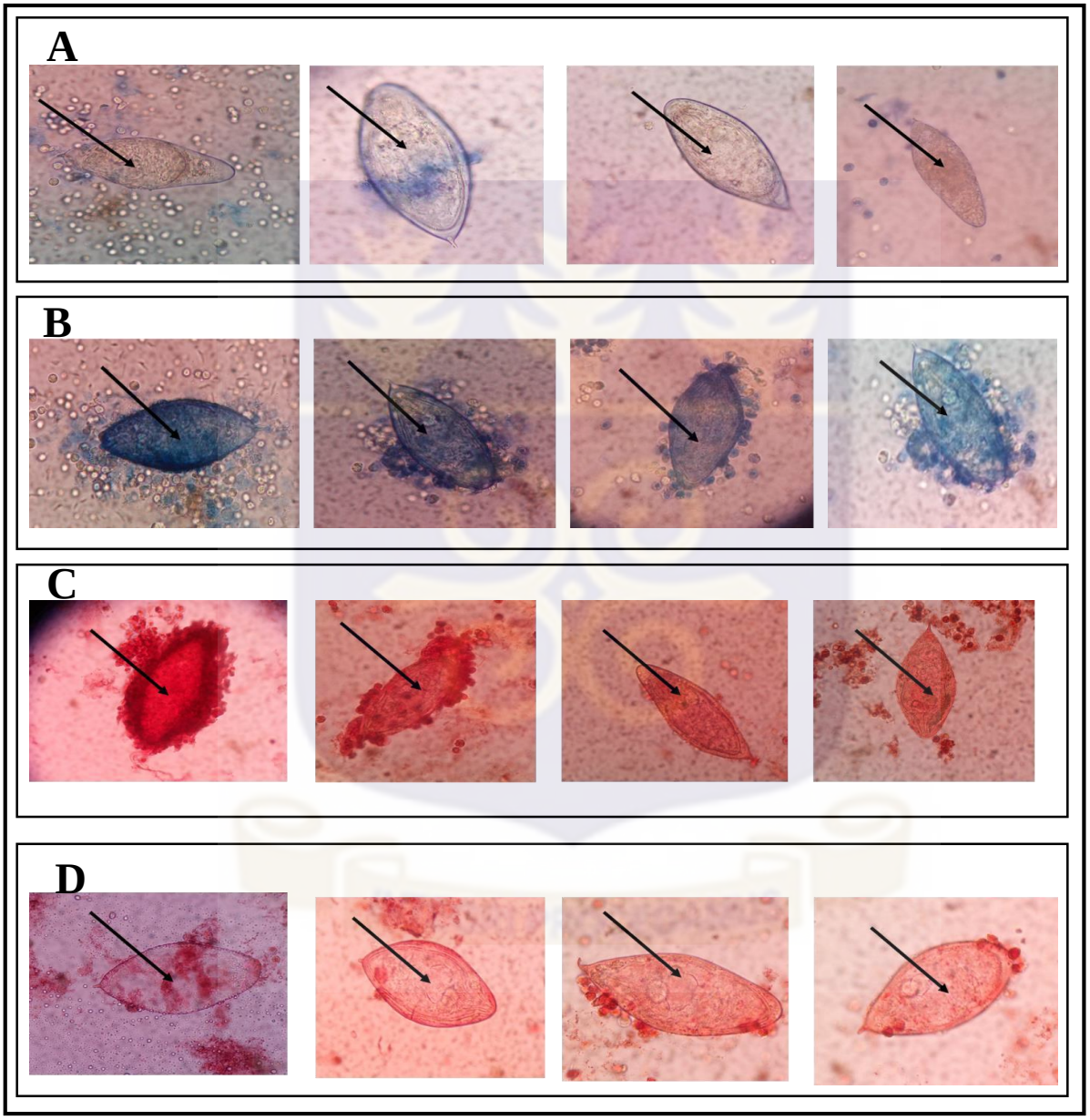


Figure 4.7: *Schistosoma haematobium* eggs (ova) stained by vital stains (0.4% trypan blue and 1% neutral red at x40 Objective lens. Arrows indicative of area of assessment of the stain. (A): A lane demonstrating four eggs with non-retention of 0.4% trypan blue indicative of live eggs of *S. haematobium* (viable). **(B):** A lane demonstrating four eggs with the retention of 0.4% trypan blue stain indicative of dead eggs (non-viable). **(C):** A lane demonstrating four eggs with the retention of 1% neutral red indicative of live eggs of *S. haematobium* (viable). **(D):** A lane demonstrating four eggs with non-retention of 1% neutral red stain indicative of dead eggs (non-viable).

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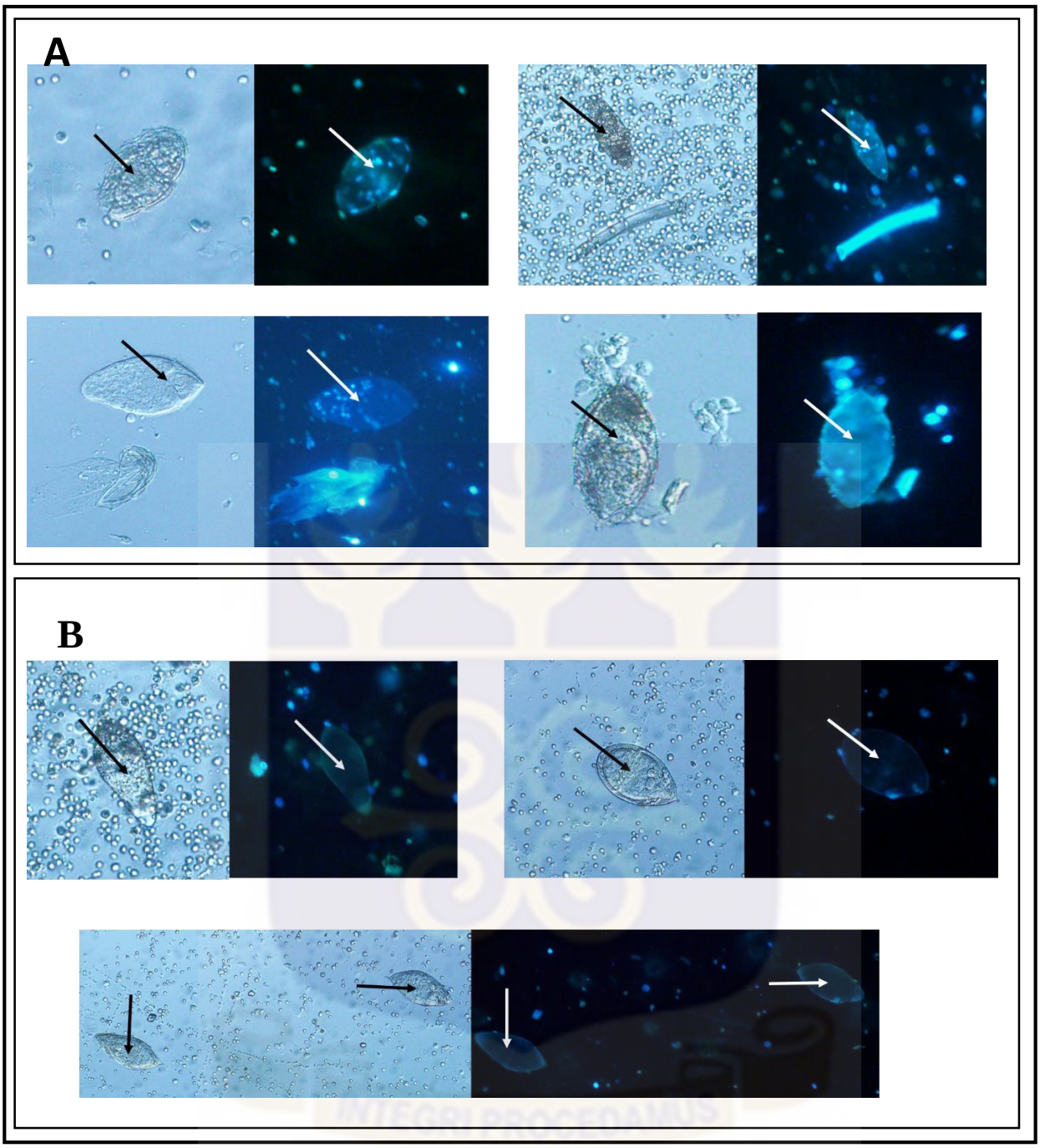


Figure 4.8: *Schistosoma haematobium* ova observed under x20 objective lens by first, light microscope and fluorescent microscopy using the cell stain (Hoechst 33258) A: A lane demonstrating four eggs in light microscopy followed by fluorescent microscopy using Hoechst 33258 and showing fluorescence (blue) indicative of viable eggs. **B**: A lane demonstrating five eggs in light microscopy followed by fluorescent microscopy using Hoechst 33258 and demonstrating no fluorescence indicative of non-viable eggs.

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4.4 Genetic Variation

4.4.1 Summary of Genetic Variation

This section focuses on results obtained from evaluating the following genes; ITS 2, NAD 1 and COX 1. Genomic DNA obtained from urine samples that were confirmed by microscopy were amplified by PCR. Fifteen (71.4%) of the samples were amplified in ITS 2, 9 (42.9%) in NAD 1 and 3 (14.3%) in Cox 1. All samples were run by species specific primers in ITS2, NAD 1 and COX 1.

4.4.2 Amplification of Internal Transcribed Spacer 2

Out of 21 positive urine samples, 15 (71.4%) showed the band size of 468 bp in the ITS 2 gene (Fig 4.9.1). This made it possible to look out for the detection of genetic variation in ITS 2 of *Schistosoma haematobium*. Amplification of ITS 2 gene is shown in the gel (Fig 4.9.1 A).

4.4.3 Amplification of Nicotinamide Adenine Dinucleotide Dehydrogenase subunit 1 gene (NAD 1)

Nine (42.9%) samples showed 431 bp band size of the NAD 1 gene. Amplification of this gene made it possible to find out whether genetic variation was present in the NAD 1 gene. A gel showing the amplification of the NAD 1 gene is (Fig 4.9.1 B).

4.4.4 Amplification of Mitochondrial Cytochrome Oxidase 1 subunit 1

Three (14.3%) samples showed a 110 bp band size of the COX 1 gene (Fig 4.9.1 C). Amplification in these samples showed the presence of the gene and the likelihood of possible genetic variation.

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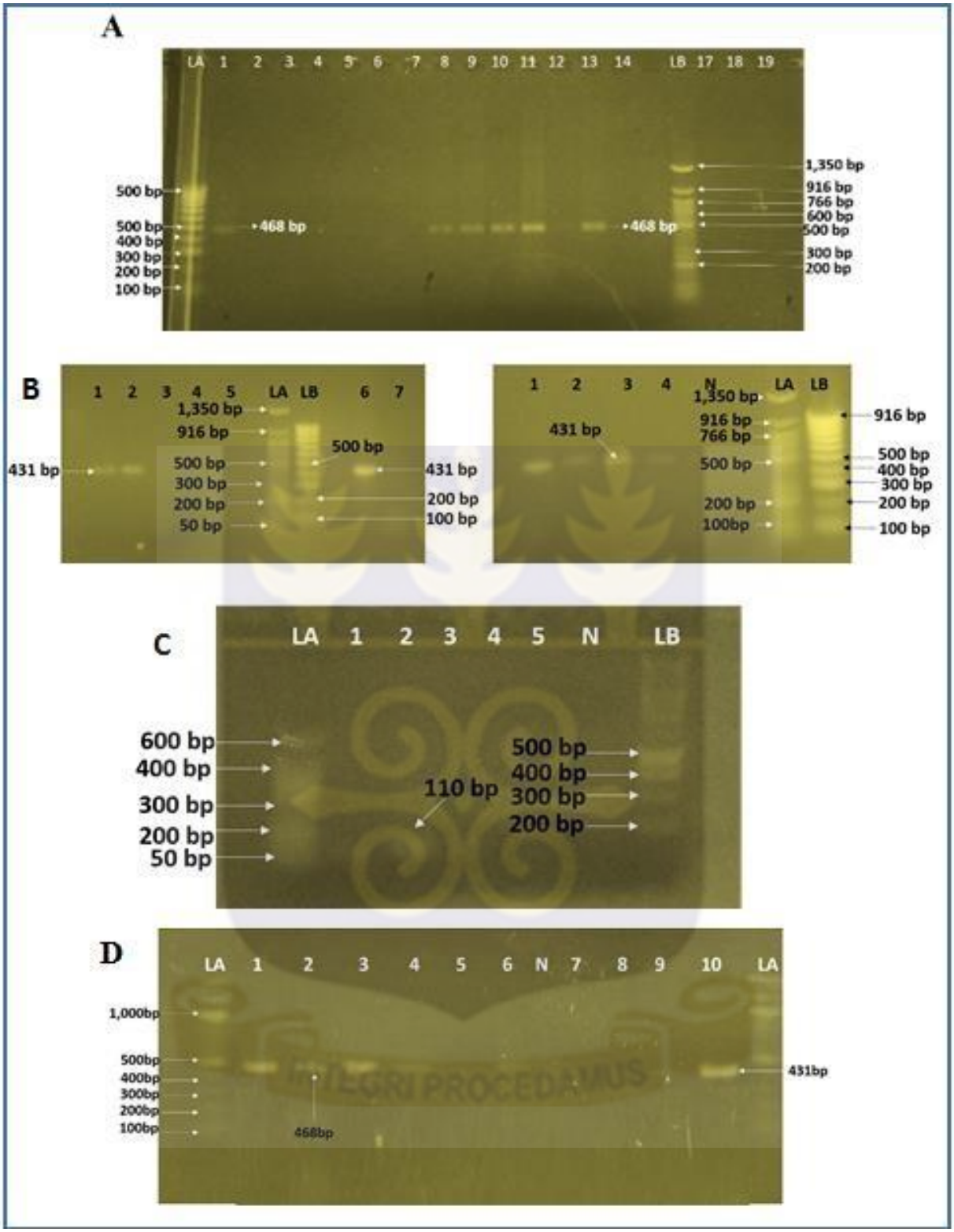


Figure 4.9.1: Gels of amplified products. A: Agarose gel of amplified 468 bp ITS 2 region of *S. haematobium* DNA extracted from urine samples A**: ITS 2, LA- 100bp ladder, LB- 50bp ladder. B:** Agarose gels of amplified 431bp NAD 1 gene of *S. haematobium* DNA extracted from urine samples**: B: NAD 1, LA- 50bp ladder, LB- 100bp ladder. N- Negative sample. C:** Agarose gel of amplified 110 bp COX 1 gene of *S. haematobium* DNA extracted from urine samples**: C: COX 1, LA- 50bp ladder, LB-100bp ladder. N- Negative control. D:** Agarose gel of amplified 468 bp in ITS 2 and 431 bp in NAD 1 gene of *S. haematobium* **DNA extracted from urine samples: D**: ITS 2 & NAD 1, LA- 100 bp ladder, **N**-Negative sample.

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4.4.5 Amplicons of ITS 2 and NAD 1 that qualified for Sequencing

Out of the 15 samples that amplified for ITS 2, 8 (53.3%) showed further amplification with 3µl of the PCR product on the gel. Nine samples amplified for NAD 1 and out of that, 4 (44.4%) were further amplified with 3µl of the PCR product on the gel (Fig 4.9 D). These criteria made these samples qualify for sequencing.

4.5 Sequencing and Analysis of Internal Transcribed Spacer 2 (ITS 2) Gene

The eight (8) samples that were sequenced were first compared among themselves to verify if any variation (change in position) exist between them but analysis done by Bioedit 7.0 proved 100% identities. When the 468 bp ITS 2- PCR fragments were sequenced and compared to isolates from other African countries (Kenya-AF146038, Guinea-Bissau-JQ397404, Senegal-FJ588861, Cameroon-JQ397406, Tanzania-GU257398, Egypt-JQ397407, Madagascar- JQ397414, and Malawi -JQ39741), they were similar, having the same exact sequences (Fig 4.9.2). The eight (8) samples that were sequenced from the amplified products (Ghana) were similar to sequences from other African countries known as the Pan-African *S. haematobium* genotype showing similar identities between them. However, the sequences were different from that of Kenya with a 'C' at position 843 instead of a 'T' (Fig 4.9.2).

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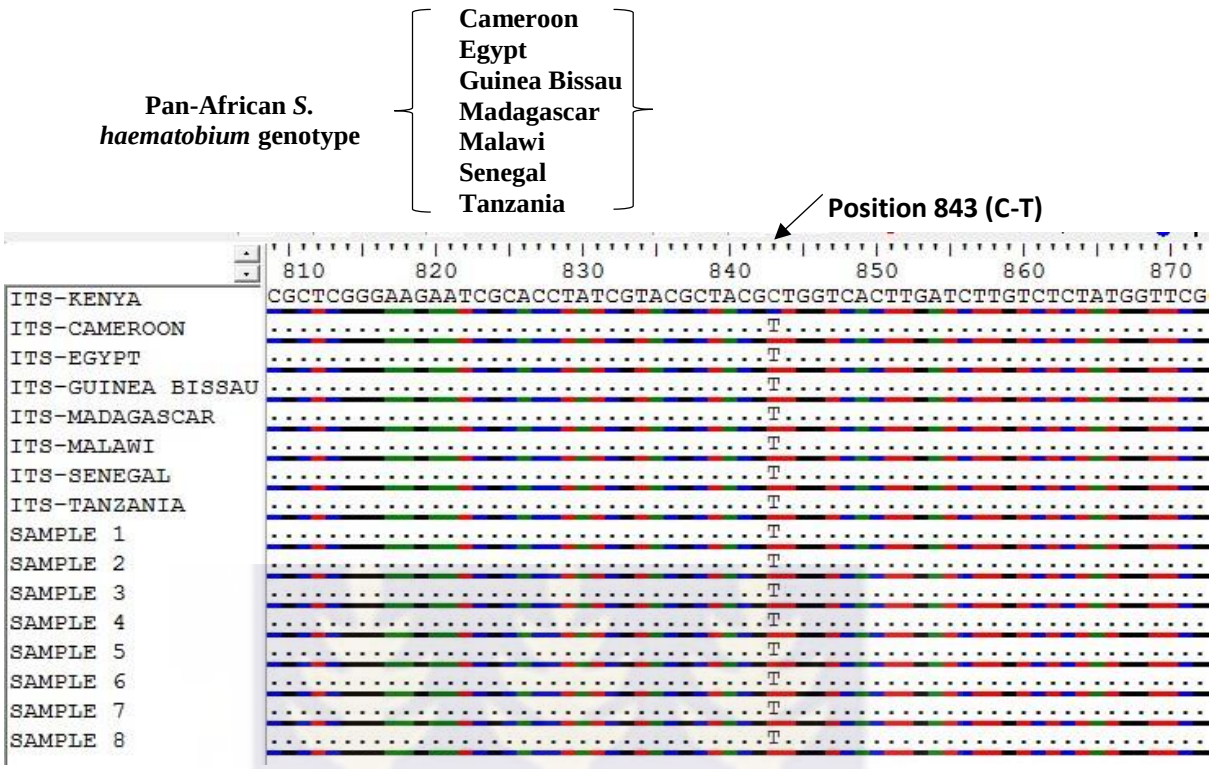


Figure 4.9.2: Comparative ITS 2 Sequences. The ITS2 nucleotide sequences of *S. haematobium* from 8 positive samples obtained from PCR products compared with a GenBank sequence isolated from Kenya (accession no. AF146038) and other African countries (Cameroon, Tanzania, Egypt, Madagascar, Malawi, Senegal, Guinea-Bissau). Base changes in interspecific positions (position 843 C-T). Base Homologies are indicated with a dot (.).

4.6 Sequencing and Analysis of Nicotinamide Adenine Dinucleotide Dehydrogenase subunit 1 Gene

The four samples sequenced showed individual similarities between them. Sequences were also similar to other countries such as Senegal- JQ595387, Mali- JQ595389, Nigeria- JQ595393, Gambia- JQ595390, Liberia- JQ595391, Guinea-Bissau- JQ595392, Cameroon- JQ595394, Sudan- JQ595396, South Africa- JQ595401, Egypt- JQ595395 and Zanzibar- GU257375. However, they were different from Mauritius- JQ595403 and Madagascar- JQ595404 (position 391, 396, 410, 432, 474, 486, 487, 579, 600 and 619) and Tanzania- JQ595402 (position 577).

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Fig 4.9.3: Comparative NAD 1 Sequences. The NAD 1 nucleotide sequences of *S. haematobium* from 4 positive samples obtained from PCR products compared with a GenBank sequence isolated from other African countries (Cameroon, Gambia, Guinea-Bissau, Liberia, Nigeria, South-Africa, Sudan, Tanzania, Senegal, Madagascar and Mauritius). Base Homologies are indicated with a dot (.).

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CHAPTER FIVE

5.0 DISCUSSION

5.1 Reinfection Assessment

Findings of this study which indicated a percentage reinfection of 10.4% suggests that school children living in endemic areas could still be exposed to *S. haematobium* infection even after a single dose of Praziquantel treatment. A low percentage reinfection of 10.4% observed in the current study agrees with similar work done by Senghor *et al*. (2015) and Kabuyaya *et al*. (2017) who also had low reinfection rates of 12.6% and 8.1% respectively. On the contrary, a high percentage reinfection of 58% has been reported by Oniya and Odaibo (2006). Low percentage reinfection of *S. haematobium* has been attributed to effectiveness of treatment in especially, school children (King *et al* ., 2006; Ahmed *et al* ., 2012) and the season in which sampling was done (Kabuyaya *et al* ., 2017; Midzi *et al* ., 2008). In the study by Kabuyaya *et al*. (2017), the low percentage reinfection observed was associated to persistent drought throughout their study period. Also, Midzi *et al*. (2008) showed that a considerable number of the transmission hotspots (water sources) dried up during their study period, thus limiting the exposure of children to water contact, and might have contributed to low percentage reinfection. Similarly, the major part of the current study was conducted in a period (September - March), where rains were not recorded in the coastal region of the country (including Accra) and most of the water sources were almost dried up. Therefore, reasons from Kabuyaya *et al*. (2017) and Midzi *et al*. (2008) might explain the similar observations in the current study with regards to reinfection rate. On the other hand, many factors including ecological, seasonal and being in an area with high intensity infection have been incriminated in the occurrence of high re-infection rates observed in other studies (Oniya and Odaibo, 2006; N'Goran *et al*., 2001; Kabuyaya *et al*., 2017). According to Kabuyaya and colleagues, people might get re-infected when they revert to their previous daily activities involving contact with

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water infested with snail intermediate hosts after a successful treatment with Praziquantel (Kabuyaya *et al* ., 2017).

In this study, the assessment of reinfection at the 6th month after there have been evidence of total egg clearance by the 6th week agrees with a similar study in which reinfection patterns were determined among *S. haematobium* infected individuals proving that the 6th month was the best period for monitoring reinfection (Webster *et al* ., 2013). Pre-patent period (infection to excretion of eggs by host) of *S. haematobium* is known to be between eight to ten weeks (Sathoff *et al* ., 2004), which explains why there was evidence of egg clearance by the 12th week although some study participants were reinfected at the 6th month. Continuous exposure to water as well as water contact activities which was reported in this study could be probable contributing factors to the occurrence of reinfection (Mostafa *et al* ., 1999).

According to this study, the ova in a reinfected study participant with an Asian descent had features which made it difficult to distinguish the ova of *S. haematobium* from *S. intercalatum*. This is similar to a study by Wright and Ross. (1980) in which species like *S. intercalatum* have similar egg morphology with *S. haematobium* in human urine samples attributing the reason to hybridisation between the two species.

5.2 Resistance Assessment

The zero resistance recorded in this study after treatment with Praziquantel in the follow up samples suggests the effectiveness of the drug in the treatment of urinary schistosomiasis. This finding agrees with work done by Ojurongbe *et al*. (2014) in Nigeria who also could not detect the presence of viable eggs in the follow up samples after Praziquantel treatment of their study participants. A study conducted elsewhere, however, revealed the presence of persistent viable eggs after Praziquantel treatment in the follow up samples of the study participants, suggesting a possible reduced efficacy of the drug (Doenhoff *et al* ., 1999;

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Alonso *et al* ., 2006; Silva *et al* ., 2005; Kabuyaya *et al* ., 2017). Parameters such as Egg Reduction Rate (Tchuente *et al* ., 2004, Ojurongbe *et al* ., 2014, Midzi *et al* ., 2008, Kabuyaya *et al* ., 2017) and viability (Elfaki *et al* .,2015) used as tools in follow-ups to assess resistance in this study gave consistent results. The Egg Reduction Rates recorded in this study from the 3rd week to the 12 th week (94.37% to 100%) agrees with a study conducted at Abeokuta with an Egg Reduction Rate of 57% to 100% till the 12th week (Ojurongbe *et al* ., 2014). This demonstrates the excellent performance of Praziquantel. According to Tuente *et al*. (2004), the excretion of dead eggs in follow ups could falsely reduce the Egg Reduction Rates. In contrast to this study, low Egg Reduction Rates of 64% and 80% in males and females were achieved in South Africa attributing the reason not to resistance but rather low drug absorption and high level of catabolism (Kabuyaya *et al* ., 2017).

In this study, the percentage viability of the live eggs being lower in the first, second and third weeks (20%, 20% and 10% respectively) follow-ups compared to that of the baseline (70%) after treatment indicates the impact of Praziquantel treatment on the viability of *S. haematobium* eggs. This observation is similar to a study by Elfaki *et al*. (2015) who reported that, the mean number of viable eggs in treated patients is lower than the mean number of viable eggs in untreated patients. The possible reason accounting for the reduction of *S. haematobium* live eggs in treated patients could be as a result of destruction of the adult worms by the drug (Praziquantel).

According to this study, the mean egg count at baseline was higher than that in the subsequent follow ups. This agrees with a study conducted in Cote d'Ivoire which reported a higher mean egg count of 84.8 eggs /10 ml at baseline, relative to a mean egg count in the range 40.5 to 0.3 eggs/10ml post treatment (Stete *et al* ., 2012). The monitoring of the egg counts in connection with the assessment of viability in follow-ups gives a good indication in evaluating the effect of Praziquantel on the adult worms. Thus, a reduction of live eggs.

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5.3 *Schistosoma haematobium* infection morbidity parameters

In this study, the infection morbidity parameters such as haematuria, proteinuria and pyuria (leucocytes) were associated with *S. haematobium* infection and this agrees with earlier workers who also reported the association of urogenital schistosomiasis with haematuria, proteinuria and pyuria (Gryseels *et al* ., 2006, Stete *et al* ., 2012, Mekonnen *et al* ., 2013 and Wami *et al* ., 2016).

Proteinuria and haematuria were detected at baseline and post-treatment, whereas pyuria (leucocytes) was detected only at post-treatment in study participants infected with *S. haematobium*. The detection of proteinuria and haematuria (19.19% and 76.2% respectively) at the baseline is not surprising because of the damaging effect of the terminal spine of the ova on the bladder walls leading to the release of the red blood cells. into the urine Therefore the percentage decrease in haematuria in follow up samples could be attributed to the activity of Praziquantel on the parasites leading to gradual healing of the damaging effect of the terminal spine. This agrees with an earlier work done by Wami *et al*. (2016) in which proteinuria and haematuria reduced after Praziquantel treatment. Another study has also indicated high prevalence of haematuria and proteinuria (88% and 73% respectively) at baseline, compared to the 16% and 4% respectively recorded in the follow ups (Stete *et al* ., 2012). Mekonnen *et al.* (2013) also recorded higher prevalence in baseline compared to post-treatment for haematuria and proteinuria (100% to 40% and 94.1% to 48.7% respectively). The detection of leucocytes in urine (95.2% reducing to 4.8%) can be attributed it to inflammations and lesions in the urinary tract during the response of the host to the effect of the drug on the parasite, as explained in a study conducted Kenya (Kahama *et al* ., 1999). This observation is also similar to a study conducted in Cote D'Ivoire which reported a prevalence of 71% in leucocytes and reduction to 2% five weeks' post-treatment (Stete *et al* ., 2012). The high number of leucocytes in urine in the first week after treatment which was recorded to

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have zero prevalence of proteinuria, haematuria and pyuria 4 weeks post-treatment can be attributed to the effectiveness of praziquantel treatment even on these morbidity parameters.

5.4 Viability

The higher mean percentage viability at baseline relative to post treatment suggests the efficiency of the drug on the viability of *S. haematobium* ova. This finding is similar to work done by (Elfaki *et al* ., 2015) in Sudan on evaluating the effect of Praziquantel treatment on the eggs of *S. haematobium.* Their study revealed more viable eggs in baseline samples as compared to posttreatment samples and more non-viable eggs in post-treatment samples in comparison to baseline samples (Elfaki *et al* ., 2015).

The methods used for viability determination which included the modified hatchability technique, the vital stains and the fluorescent microscopy complemented each other. This agrees with Sarvel *et al*. (2005), who suggested that the fluorescent dye (Hoechst 33258) was a good tool for differentiating live eggs from dead eggs. The vital stains, 0.4% and 1% neutral red were able to distinguish live eggs from dead ones. Dead eggs were best stained by the 0.4% trypan blue stain (blue colouration) whereas live eggs were best stained by the 1% neutral red stain (red colouration). The modified hatchability technique was the best tool to identify the live activity of the eggs as a result of the visualisation of the presence of flame cells and movement in the embryo. The fluorescent dye (Hoechst 33258) fluorescence blue in viable cells because of its ability to be a cell stain used for visualization of viable cells.

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5.5 Genetic variation of the ITS 2 gene

The amplification of the Internal transcribed spacer 2 (ITS 2) in 15 samples confirms the presence of *S. haematobium*, highlighting its importance as an interesting marker for species identification and phylogenetic studies (Baldwin, 1993; Suh *et al* ., 1993; Hsiao *et al* ., 1994; Dubouzet and Shinoda, 1999). The sample of the Asian descent child was among the samples that amplified for the ITS 2 and this suggests that the ova was *S. haematobium* although it looked like *S. intercalatum* morphologically. However, the remaining of the samples that could not be amplified cannot be attributed to misdiagnosis of *S. haematobium* but speculations that eggs might be from adult worms hybrid in nature (Wright and Ross, 1980). ITS 2 have been found to be an important marker for 100% amplification compared to other methods such as microscopy and circulating catholic antigen (CCA) as reported by Obeng *et al*. (2008). With the exception of the Kenya type (AF146038), the similarities between the sequences of the amplified products to that of the other African countries [Senegal (FJ588861), Guinea Bissau (JQ397404), Cameroon (JQ397406), Tanzania (GU257398), Egypt (JQ397407), Madagascar (JQ397414) and Malawi (JQ397410)] suggests the sequences from Ghana is the type common to majority of the African countries. This supports the findings of the study conducted from Sudan where 12 out of 13 sequences had the same sequence similarities across other African countries with the exception of one which was similar to a Kenyan type (Quan *et al* ., 2015). In this study, the change in an intraspecific base at position 843 of a 'T' across other countries as compared to a 'C' which was identified in that from Kenya agrees with another study which grouped the *S. haematobium* that has base 'T' at position 843 as the Pan-African genotype (Quan *et al* ., 2015). A variation in the ITS 2 can therefore be associated with the difference in the base position 843 (C-T) as displayed by the Kenyan type in comparison with the Pan- African genotype (Ezeh *et al* ., 2015).

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5.6 Genetic variation of the NAD 1 gene

The Nicotinamide adenine dinucleotide dehydrogenase subunit 1 (NAD 1), is a mitochondrial marker that have been used for species and strain identification (Zarowiecki *et al* ., 2007). Species identified by this molecular marker have been known to be involved in high energy activity as a result of its mitochondrial DNA (Hafeti, 1985). In this study, amplification of 9 samples of the NAD 1 gene suggests that some samples had *S. haematobium* eggs but couldn't be amplified using this mitochondrial gene and the probable reason could be due to lack of the gene, non-expression in detectable quantities or the possession of a variant type which could not be amplified with the primers used. The sample of the Asian descent school child from Zenu, which did not amplify for this gene, could be probably because it possesses a variant type of NAD 1, which might be involved in low energy production (Hafeti, 1985).

Besides, the sequences of Mauritius (JQ595403) and Madagascar (JQ595404), the similarities in the sequences of the samples with other African countries [(Senegal- JQ595387, Mali- JQ595389, Nigeria- JQ595393, Gambia- JQ595390, Liberia- JQ595391, Guinea-Bissau- JQ595392, Cameroon- JQ595394, Sudan- JQ595396, South Africa- JQ595401, Egypt- JQ595395 and Zanzibar-GU257375)] suggests the sequences from Ghana is a type common to that in the other African countries grouped as the African mainland. Besides a 'G' substituted with an 'A' at position 577 in the case of the Tanzania sequence, all the African countries including that from Ghana (out of this study) had similar sequences which supports the findings of Webster *et al*. (2012) in which all these countries were grouped under the African mainland (haplotype 1).

Based on the findings of the current study, Ghana therefore joins the African mainland group of the NAD 1 gene. The demonstration of massive gene variation in the sequences of Mauritius and Madagascar at diiferent positions [391 ('T 'for a 'A'), 396 ('C' for a 'T'), 410 ('C' for a 'T'), 432 ('G' for an 'A'), 474 ('A' for a 'G'), 486 ('G' for 'A'), 487 ('C' for a 'T'),

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579 (‘G’ for ‘A’), 600 (‘C’ for a ‘T’) and 619 (‘G’ for ‘A’)] agrees with the findings of Webster *et al*.(2012) in which Mauritius and Madagascar were grouped separately into the Indian ocean island and coastal African countries with a unique haplotype.

A study conducted by Razafindrazaka et al. (2010) on complete mitochondrial DNA sequences using the Polynesian motif as a marker to trace the late Holocene expansion of Austronesian speaking populations explain the reason why Mauritius and Mauritania had similar sequences. This was due to the settlement of first French with slaves during the 18th century in the island of Mauritius. It is known that majority of the slaves were imported from Madagascar.

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CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

The percentage reinfection of 10.4% was recorded among the 8 study participants. There was no resistance recorded among the study participants demonstrating the effectiveness of Praziquantel for the treatment of *Schistosoma haematobium*. *S. haematobium* infection morbidity parameters such as haematuria, proteinuria and pyuria were associated with *S. haematobium* infections.

There was effect of Praziquantel treatment on viability of *S. haematobium* eggs using the modified hatchability technique which were complemented by the vital stains (0.4% Trypan blue, 1% neutral red) and the fluorescent stain (Hoechst 33258).

For ITS 2, genetic variants were detected in relation to that of Kenya but sequences of the samples were similar to sequences from the other Africa countries (Pan-African *S. haematobium* genotype). For NAD 1, genetic variants were detected with other countries such as Madagascar and Mauritius (Indian Ocean Island, Group 2) but similar to countries belonging to the African mainland (Group 1) except Tanzania.

6.2 RECOMMENDATIONS

This study will go a long way in adding immense contributions and knowledge to existing works and new findings. The following are recommendations being made:

1. More research could be done in more endemic regions to determine genetic variation, reinfection and resistance possibilities.
2. Health education on water contact activities and transmission of the parasite should be carried out in the study areas.

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APPENDICES

Appendix I

Primers suspension

To obtain a concentration of one hundred micro-molars (100 µM), appropriate volumes of nuclease free water were added to specific lyophilized primers as indicated below.

Primers	amount of substance (n mol)	Volume of nuclease free water added (µL)
COX 1 F	55.15	551.5
COX 1 R	46.08	460.8
S. h NAD 1 F	52.62	526.25
S. h NAD 1 R	45.72	457.23
ITS 2 F	25.24	252.35
ITS 2 R	36.5	365.02

The primers were reconstituted to 10M as a working solution by adding 90 µl of nuclease free water to 10 µl of the 100M stock solution.

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Appendix II

DNA Extraction

To lyse a sample, add 400 µl of Genomic Lysis Buffer to 100 µl of urine sample. The samples were vortex for 4 to 6 seconds for 5 to 10 minutes. It was then transferred to a Zymo-Spin Column in a collection tube. Centrifugation was done for one minute. The supernatant in the collection tube was discarded. The Zymo-Spin Column was transferred to collection tube. 200 µl of DNA Pre-Wash buffer was added to the spin column. Centrifugation was done for 1 minute. 500 µl of g-DNA Wash Buffer was added to the spin column. Centrifugation was done for one minute.

The spin column was transferred to a clean micro centrifuge tube. 50 µl of DNA Elution Buffer was added to the spin column. It was then incubated for 5 minutes at room temperature and then spun at top speed for 30 seconds to elute the DNA.

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Appendix III

Preparation of 1.5% Agarose Gel

This was prepared by weighing 1.5g of agarose powder and adding it 100 ml of 1X TAE. It was then mixed and heated to boil to dissolve completely. 5 µl of Ethidium bromide was added to the mixture after it has cooled. It was mixed uniformly. The gel was then poured into a gel tray with a comb fixed across it vertically. The comb was gently removed after the gel has cooled at room temperature and solidified.

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Appendix IV

Preparation of TAE buffer

A stock solution of X10 was prepared by weighing 48.4g of the Tris powder into a conical flask.

11.4 ml of glacial acetic acid was added and allowed to dissolve. 3.7g of EDTA was added and

500ml of deionized water was finally added, mixed very well and stored in a bottle. To

prepare X1 from the X20 stock prepared, thus 1 litre was prepared by measuring 100 ml

of the X20 TAE stock solution and adding 900 ml of distilled water to it.

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Appendix V

SEQUENCING RESULTS

1. ITS 2-64

Schistosoma haematobium isolate HB336 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

CCTGTGGCCACGCCTGTCCGAGGGTCGGCTTTTCATCTATCACGGCGCACATTGAGTCGTGGA
TTGG
GCGAGTGCCTGCCGGCGTGTATACCCGCATATCAACGCGGGTTGCTGGTCGAAGGCTCCGTC
CTAAT
AGTCCGGCCACAGCCTAGTCCGGTCTAGATGACTTGATCGAGATGCTGCGGTGGGTTGTGCT
CGAGT
CGTGGCTTAATGACATTATACGCGCTCGGGAAGAATCGCACCTATCGTACGCTACGTTGGTC
ACTTG
ATCTTGTCTCTATGGTTCGGTCTACGGTTTGTACCGATGGTGTGTGTAATACGCACGAATTGT
ATAAT TGACCCTGACCTCGGATCAGACGTGATTACCCGCTGAACTTAAGCATATCATA

Query 1
CCTGTGGCCACGCCTGTCCGAGGGTCGGCTTTTCATCTATCACGGCGCACATTGAGTCGT
60
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 69
CCTGTGGCCACGCCTGTCCGAGGGTCGGCTTTTCATCTATCACGGCGCACATTGAGTCGT
128

Query 61
GGATTGGGCGAGTGCCTGCCGGCGTGTATACCCGCATATCAACGCGGGTTGCTGGTCGAA
120
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 129
GGATTGGGCGAGTGCCTGCCGGCGTGTATACCCGCATATCAACGCGGGTTGCTGGTCGAA
188

Query 121
GGCTCCGTCCTAATAGTCCGGCCACAGCCTAGTCCGGTCTAGATGACTTGATCGAGATGC
180
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 189
GGCTCCGTCCTAATAGTCCGGCCACAGCCTAGTCCGGTCTAGATGACTTGATCGAGATGC
248

Query 181
TGCGGTGGGTTGTGCTCGAGTCGTGGCTTAATGACATTATACGCGCTCGGGAAGAATCGC
240
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 249
TGCGGTGGGTTGTGCTCGAGTCGTGGCTTAATGACATTATACGCGCTCGGGAAGAATCGC
308

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Query 241
ACCTATCGTACGCTACGTTGGTCACTTGATCTTGTCTCTATGGTTCGGTCTACGGTTTGT
300
||||||||||||||| ||||||||||||||||||||||||||||||||||||
Sbjct 309
ACCTATCGTACGCTACGCTGGTCACTTGATCTTGTCTCTATGGTTCGGTCTACGGTTTGT
368

Query 301
ACCGATGGTGTGTGTAATACGCACGAATTGTATAATTGACCCTGACCTCGGATCAGACGT
360
||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 369
ACCGATGGTGTGTGTAATACGCACGAATTGTATAATTGACCCTGACCTCGGATCAGACGT
428

Query 361 GATTACCCGCTGAACTTAAGCATATCA 387
||||||||||||||||||||||||
Sbjct 429 GATTACCCGCTGAACTTAAGCATATCA 455

2. ITS 2-175

Schistosoma haematobium isolate HB336 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence Sequence ID: AF146037.1Length: 468

GAGTCGTGGATTGGGCGAGTGCCTGCCGGCGTGTATACCCGCATATCAACGCGGGTTGCTGG
TCGAA
GGCTCCGTCCTAATAGTCCGGCCACAGCCTAGTCCGGTCTAGATGACTTGATCGAGATGCTGC
GGTG
GGTTGTGCTCGAGTCGTGGCTTAATGACATTATACGCGCTCGGGAAGAATCGCACCTATCGT
ACGCT
ACGTTGGTCACTTGATCTTGTCTCTATGGTTCGGTCTACGGTTTGTACCGATGGTGTGTGTAAT
ACGC
ACGAATTGTATAATTGACCCTGACCTCGGATCAGACGTGATTACCCGCTGAACTTAAGCATA
TCAAT AAGCGGAGGAAA

Query 1
GAGTCGTGGATTGGGCGAGTGCCTGCCGGCGTGTATACCCGCATATCAACGCGGGTTGCT
60
||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 122
GAGTCGTGGATTGGGCGAGTGCCTGCCGGCGTGTATACCCGCATATCAACGCGGGTTGCT
181

Query 61
GGTCGAAGGCTCCGTCCTAATAGTCCGGCCACAGCCTAGTCCGGTCTAGATGACTTGATC
120
||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 182
GGTCGAAGGCTCCGTCCTAATAGTCCGGCCACAGCCTAGTCCGGTCTAGATGACTTGATC
241

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Query 121
GAGATGCTGCGGTGGGTTGTGCTCGAGTCGTGGCTTAATGACATTATACGCGCTCGGGAA
180

Sbjct 242
GAGATGCTGCGGTGGGTTGTGCTCGAGTCGTGGCTTAATGACATTATACGCGCTCGGGAA
301

Query 181
GAATCGCACCTATCGTACGCTACGTTGGTCACTTGATCTTGTCTCTATGGTTCGGTCTAC
240

Sbjct 302
GAATCGCACCTATCGTACGCTACGCTGGTCACTTGATCTTGTCTCTATGGTTCGGTCTAC
361

Query 241
GGTTTGTACCGATGGTGTGTGTAATACGCACGAATTGTATAATTGACCCTGACCTCGGAT
300

Sbjct 362
GGTTTGTACCGATGGTGTGTGTAATACGCACGAATTGTATAATTGACCCTGACCTCGGAT
421

Query 301 CAGACGTGATTACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAA 347

Sbjct 422 CAGACGTGATTACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAA 468

3. ITS 2-212

Schistosoma haematobium isolate HB336 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence Sequence ID: AF146037.1Length: 468

GCCTGTCCGAGGGTCGGCTTTTCATCTATCACGGCGCACATTGAGTCGTGGATTGGGCGAGTG
CCTG
CCGGCGTGTATACCCGCATATCAACGCGGGTTGCTGGTCGAAGGCTCCGTCCTAATAGTCCG
GCCAC
AGCCTAGTCCGGTCTAGATGACTTGATCGAGATGCTGCGGTGGGTTGTGCTCGAGTCGTGGCT
TAAT
GACATTATACGCGCTCGGGAAGAATCGCACCTATCGTACGCTACGTTGGTCACTTGATCTTGT
CTCTA
TGGTTCGGTCTACGGTTTGTACCGATGGTGTGTGTAATACGCACGAATTGTATAATTGACCCT
GACCT CGGATCAGACGTGATTACCCGCTGAACTTAAGCATATCATA

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Query 1
GCCTGTCCGAGGGTCGGCTTTTCATCTATCACGGCGCACATTGAGTCGTGGATTGGGCGA
60

Sbjct 80
GCCTGTCCGAGGGTCGGCTTTTCATCTATCACGGCGCACATTGAGTCGTGGATTGGGCGA
139

Query 61
GTGCCTGCCGGCGTGTATACCCGCATATCAACGCGGGTTGCTGGTCGAAGGCTCCGTCCT
120

Sbjct 140
GTGCCTGCCGGCGTGTATACCCGCATATCAACGCGGGTTGCTGGTCGAAGGCTCCGTCCT
199

Query 121
AATAGTCCGGCCACAGCCTAGTCCGGTCTAGATGACTTGATCGAGATGCTGCGGTGGGTT
180

Sbjct 200
AATAGTCCGGCCACAGCCTAGTCCGGTCTAGATGACTTGATCGAGATGCTGCGGTGGGTT
259

Query 181
GTGCTCGAGTCGTGGCTTAATGACATTATACGCGCTCGGGAAGAATCGCACCTATCGTAC
240

Sbjct 260
GTGCTCGAGTCGTGGCTTAATGACATTATACGCGCTCGGGAAGAATCGCACCTATCGTAC
319

Query 241
GCTACGTTGGTCACTTGATCTTGTCTCTATGGTTCGGTCTACGGTTTGTACCGATGGTGT
300

Sbjct 320
GCTACGCTGGTCACTTGATCTTGTCTCTATGGTTCGGTCTACGGTTTGTACCGATGGTGT
379

Query 301
GTGTAATACGCACGAATTGTATAATTGACCCTGACCTCGGATCAGACGTGATTACCCGCT
360

Sbjct 380
GTGTAATACGCACGAATTGTATAATTGACCCTGACCTCGGATCAGACGTGATTACCCGCT
439

Query 361 GAACTTAAGCATATCA 376

Sbjct 440 GAACTTAAGCATATCA 455

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4. ITS 2-V293
Schistosoma haematobium isolate HB336 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer
2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Sequence ID: AF146037.1Length: 468Number of Matches: 1

AGTCGTGGATTGGGCGAGTGCCTGCCGGCGTGTATACCCGCATATCAACGCGGGTTGCTGGT
CGAAG
GCTCCGTCCTAATAGTCCGGCCACAGCCTAGTCCGGTCTAGATGACTTGATCGAGATGCTGCG
GTGG
GTTGTGCTCGAGTCGTGGCTTAATGACATTATACGCGCTCGGGAAGAATCGCACCTATCGTAC
GCTA
CGTTGGTCACTTGATCTTGTCTCTATGGTTCGGTCTACGGTTTGTACCGATGGTGTGTGTAATA
CGCA
CGAATTGTATAATTGACCCTGACCTCGGATCAGACGTGATTACCCGCTGAACTTAAGCATATC
AATA AGCGGAGGAAA
Query 1
AGTCGTGGATTGGGCGAGTGCCTGCCGGCGTGTATACCCGCATATCAACGCGGGTTGCTG
60

Sbjct 123
AGTCGTGGATTGGGCGAGTGCCTGCCGGCGTGTATACCCGCATATCAACGCGGGTTGCTG
182

Query 61
GTCGAAGGCTCCGTCCTAATAGTCCGGCCACAGCCTAGTCCGGTCTAGATGACTTGATCG
120

Sbjct 183
GTCGAAGGCTCCGTCCTAATAGTCCGGCCACAGCCTAGTCCGGTCTAGATGACTTGATCG
242

Query 121
AGATGCTGCGGTGGGTTGTGCTCGAGTCGTGGCTTAATGACATTATACGCGCTCGGGAAG
180

Sbjct 243
AGATGCTGCGGTGGGTTGTGCTCGAGTCGTGGCTTAATGACATTATACGCGCTCGGGAAG
302

Query 181
AATCGCACCTATCGTACGCTACGTTGGTCACTTGATCTTGTCTCTATGGTTCGGTCTACG
240

Sbjct 303
AATCGCACCTATCGTACGCTACGCTGGTCACTTGATCTTGTCTCTATGGTTCGGTCTACG
362

Query 241
GTTTGTACCGATGGTGTGTGTAATACGCACGAATTGTATAATTGACCCTGACCTCGGATC
300

Sbjct 363
GTTTGTACCGATGGTGTGTGTAATACGCACGAATTGTATAATTGACCCTGACCTCGGATC

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422

Query 301 AGACGTGATTACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAA 346

Sbjct 423 AGACGTGATTACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAA 468

5. ITS 2-366

Schistosoma haematobium isolate HB336 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence Sequence ID: AF146037.1Length: 468

GCCTGTCCGAGGGTCGGCTTTTCATCTATCACGGCGCACATTGAGTCGTGGATTGGGCGAGTG
CCTG
CCGGCGTGTATACCCGCATATCAACGCGGGTTGCTGGTCGAAGGCTCCGTCCTAATAGTCCG
GCCAC
AGCCTAGTCCGGTCTAGATGACTTGATCGAGATGCTGCGGTGGGTTGTGCTCGAGTCGTGGCT
TAAT
GACATTATACGCGCTCGGGAAGAATCGCACCTATCGTACGCTACGTTGGTCACTTGATCTTGT
CTCTA
TGGTTCGGTCTACGGTTTGTACCGATGGTGTGTGTAATACGCACGAATTGTATAATTGACCCT
GACCT CGGATCAGACGTGATTACCCGCTGAACTTAAGCATATCATA

Query 1
GCCTGTCCGAGGGTCGGCTTTTCATCTATCACGGCGCACATTGAGTCGTGGATTGGGCGA
60

Sbjct 80
GCCTGTCCGAGGGTCGGCTTTTCATCTATCACGGCGCACATTGAGTCGTGGATTGGGCGA
139

Query 61
GTGCCTGCCGGCGTGTATACCCGCATATCAACGCGGGTTGCTGGTCGAAGGCTCCGTCCT
120

Sbjct 140
GTGCCTGCCGGCGTGTATACCCGCATATCAACGCGGGTTGCTGGTCGAAGGCTCCGTCCT
199

Query 121
AATAGTCCGGCCACAGCCTAGTCCGGTCTAGATGACTTGATCGAGATGCTGCGGTGGGTT
180

Sbjct 200
AATAGTCCGGCCACAGCCTAGTCCGGTCTAGATGACTTGATCGAGATGCTGCGGTGGGTT
259

Query 181
GTGCTCGAGTCGTGGCTTAATGACATTATACGCGCTCGGGAAGAATCGCACCTATCGTAC
240

Sbjct 260
GTGCTCGAGTCGTGGCTTAATGACATTATACGCGCTCGGGAAGAATCGCACCTATCGTAC

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319

Query 241
GCTACGTTGGTCACTTGATCTTGTCTCTATGGTTCGGTCTACGGTTTGTACCGATGGTGT
300

Sbjct 320
GCTACGCTGGTCACTTGATCTTGTCTCTATGGTTCGGTCTACGGTTTGTACCGATGGTGT
379

Query 301
GTGTAATACGCACGAATTGTATAATTGACCCTGACCTCGGATCAGACGTGATTACCCGCT
360

Sbjct 380
GTGTAATACGCACGAATTGTATAATTGACCCTGACCTCGGATCAGACGTGATTACCCGCT
439

Query 361 GAACTTAAGCATATCA 376

Sbjct 440 GAACTTAAGCATATCA 455

6. ITS 2-188

Schistosoma haematobium isolate HB336 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

CCTGTGGCCACGCCTGTCCGAGGGTCGGCTTTTCATCTATCACGGCGCACATTGAGTCGTGGA
TTGG
GCGAGTGCCTGCCGGCGTGTATACCCGCATATCAACGCGGGTTGCTGGTCGAAGGCTCCGTC
CTAAT
AGTCCGGCCACAGCCTAGTCCGGTCTAGATGACTTGATCGAGATGCTGCGGTGGGTTGTGCT
CGAGT
CGTGGCTTAATGACATTATACGCGCTCGGGAAGAATCGCACCTATCGTACGCTACGTTGGTC
ACTTG
ATCTTGTCTCTATGGTTCGGTCTACGGTTTGTACCGATGGTGTGTGTAATACGCACGAATTGT
ATAAT TGACCCTGACCTCGGATCAGACGTGATTACCCGCTGAACTTAAGCATATCATA

Query 1
CCTGTGGCCACGCCTGTCCGAGGGTCGGCTTTTCATCTATCACGGCGCACATTGAGTCGT
60

Sbjct 69
CCTGTGGCCACGCCTGTCCGAGGGTCGGCTTTTCATCTATCACGGCGCACATTGAGTCGT
128

Query 61
GGATTGGGCGAGTGCCTGCCGGCGTGTATACCCGCATATCAACGCGGGTTGCTGGTCGAA
120

Sbjct 129
GGATTGGGCGAGTGCCTGCCGGCGTGTATACCCGCATATCAACGCGGGTTGCTGGTCGAA

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188

Query 121
GGCTCCGTCCTAATAGTCCGGCCACAGCCTAGTCCGGTCTAGATGACTTGATCGAGATGC
180

Sbjct 189
GGCTCCGTCCTAATAGTCCGGCCACAGCCTAGTCCGGTCTAGATGACTTGATCGAGATGC
248
Query 181
TGCGGTGGGTTGTGCTCGAGTCGTGGCTTAATGACATTATACGCGCTCGGGAAGAATCGC
240

Sbjct 249
TGCGGTGGGTTGTGCTCGAGTCGTGGCTTAATGACATTATACGCGCTCGGGAAGAATCGC
308

Query 241
ACCTATCGTACGCTACGTTGGTCACTTGATCTTGTCTCTATGGTTCGGTCTACGGTTTGT
300

Sbjct 309
ACCTATCGTACGCTACGCTGGTCACTTGATCTTGTCTCTATGGTTCGGTCTACGGTTTGT
368

Query 301
ACCGATGGTGTGTGTAATACGCACGAATTGTATAATTGACCCTGACCTCGGATCAGACGT
360

Sbjct 369
ACCGATGGTGTGTGTAATACGCACGAATTGTATAATTGACCCTGACCTCGGATCAGACGT
428

Query 361 GATTACCCGCTGAACTTAAGCATATCA 387

Sbjct 429 GATTACCCGCTGAACTTAAGCATATCA 455.

7. ITS 2-295

Schistosoma haematobium isolate HB336 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence Sequence ID: AF146037.1Length: 468

GCCTGTCCGAGGGTCGGCTTTTCATCTATCACGGCGCACATTGAGTCGTGGATTGGGCGAGTG
CCTG
CCGGCGTGTATACCCGCATATCAACGCGGGTTGCTGGTCGAAGGCTCCGTCCTAATAGTCCG
GCCAC
AGCCTAGTCCGGTCTAGATGACTTGATCGAGATGCTGCGGTGGGTTGTGCTCGAGTCGTGGCT
TAAT
GACATTATACGCGCTCGGGAAGAATCGCACCTATCGTACGCTACGTTGGTCACTTGATCTTGT
CTCTA
TGGTTCGGTCTACGGTTTGTACCGATGGTGTGTGTAATACGCACGAATTGTATAATTGACCCT
GACCT CGGATCAGACGTGATTACCCGCTGAACTTAAGCATATCATA

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Query 1
GCCTGTCCGAGGGTCGGCTTTTCATCTATCACGGCGCACATTGAGTCGTGGATTGGGCGA
60

Sbjct 80
GCCTGTCCGAGGGTCGGCTTTTCATCTATCACGGCGCACATTGAGTCGTGGATTGGGCGA
139

Query 61
GTGCCTGCCGGCGTGTATACCCGCATATCAACGCGGGTTGCTGGTCGAAGGCTCCGTCCT
120

Sbjct 140
GTGCCTGCCGGCGTGTATACCCGCATATCAACGCGGGTTGCTGGTCGAAGGCTCCGTCCT
199

Query 121
AATAGTCCGGCCACAGCCTAGTCCGGTCTAGATGACTTGATCGAGATGCTGCGGTGGGTT
180

Sbjct 200
AATAGTCCGGCCACAGCCTAGTCCGGTCTAGATGACTTGATCGAGATGCTGCGGTGGGTT
259

Query 181
GTGCTCGAGTCGTGGCTTAATGACATTATACGCGCTCGGGAAGAATCGCACCTATCGTAC
240

Sbjct 260
GTGCTCGAGTCGTGGCTTAATGACATTATACGCGCTCGGGAAGAATCGCACCTATCGTAC
319

Query 241
GCTACGTTGGTCACTTGATCTTGTCTCTATGGTTCGGTCTACGGTTTGTACCGATGGTGT
300

Sbjct 320
GCTACGCTGGTCACTTGATCTTGTCTCTATGGTTCGGTCTACGGTTTGTACCGATGGTGT
379

Query 301
GTGTAATACGCACGAATTGTATAATTGACCCTGACCTCGGATCAGACGTGATTACCCGCT
360

Sbjct 380
GTGTAATACGCACGAATTGTATAATTGACCCTGACCTCGGATCAGACGTGATTACCCGCT
439

Query 361 GAACTTAAGCATATCA 376

Sbjct 440 GAACTTAAGCATATCA 455

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8. ITS 2-153
Schistosoma haematobium isolate HB336 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence Sequence ID: AF146037.1
Length: 468

GAGTCGTGGATTGGGCGAGTGCCTGCCGGCGTGTATACCCGCATATCAACGCGGGTTGCTGG
TCGAA
GGCTCCGTCCTAATAGTCCGGCCACAGCCTAGTCCGGTCTAGATGACTTGATCGAGATGCTGC
GGTG
GGTTGTGCTCGAGTCGTGGCTTAATGACATTATACGCGCTCGGGAAGAATCGCACCTATCGT
ACGCT
ACGTTGGTCACTTGATCTTGTCTCTATGGTTCGGTCTACGGTTTGTACCGATGGTGTGTGTAAT
ACGC
ACGAATTGTATAATTGACCCTGACCTCGGATCAGACGTGATTACCCGCTGAACTTAAGCATA
TCAAT
AAGCGGAGGAAA

Query 1
GAGTCGTGGATTGGGCGAGTGCCTGCCGGCGTGTATACCCGCATATCAACGCGGGTTGCT
60

Sbjct 122
GAGTCGTGGATTGGGCGAGTGCCTGCCGGCGTGTATACCCGCATATCAACGCGGGTTGCT
181

Query 61
GGTCGAAGGCTCCGTCCTAATAGTCCGGCCACAGCCTAGTCCGGTCTAGATGACTTGATC
120

Sbjct 182
GGTCGAAGGCTCCGTCCTAATAGTCCGGCCACAGCCTAGTCCGGTCTAGATGACTTGATC
241

Query 121
GAGATGCTGCGGTGGGTTGTGCTCGAGTCGTGGCTTAATGACATTATACGCGCTCGGGAA
180

Sbjct 242
GAGATGCTGCGGTGGGTTGTGCTCGAGTCGTGGCTTAATGACATTATACGCGCTCGGGAA
301

Query 181
GAATCGCACCTATCGTACGCTACGTTGGTCACTTGATCTTGTCTCTATGGTTCGGTCTAC
240

Sbjct 302
GAATCGCACCTATCGTACGCTACGCTGGTCACTTGATCTTGTCTCTATGGTTCGGTCTAC
361

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Query 241
GGTTTGTACCGATGGTGTGTGTAATACGCACGAATTGTATAATTGACCCTGACCTCGGAT
300

Sbjct 362
GGTTTGTACCGATGGTGTGTGTAATACGCACGAATTGTATAATTGACCCTGACCTCGGAT
421

Query 301 CAGACGTGATTACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAA 347

Sbjct 422 CAGACGTGATTACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAA 468

1.NAD-C63

Schistosoma haematobium isolate SE1 NADH dehydrogenase subunit 1 (nad1) gene, partial cds; mitochondrial Sequence ID: JQ595387.1Length: 756

GAGTAATGTTATTCATAGCACCTTTAGCTTTTATTGCATGGTTAATAGTGTTATTAAGAGAGA
GTAAT
CGTACACCATGTGACTATTCCGAATCTGAAAGTGAGCTAGTAAGAGGAATTAGAGTGGAATA
TAGG
AGAGTATTATTTTTAGTTATATTTGCTTGCGAGTATTTAATAATGTTTATATTTAGCTGAGTGA
GATTT
ATAGTATTTTGAAGCATAAATGAAATATTGATAGTAATAAACTTGATGTTATTTGTAGTAATG
CGTG GTTCATTTTCTCGACTTCGA

Query 1
GAGTAATGTTATTCATAGCACCTTTAGCTTTTATTGCATGGTTAATAGTGTTATTAAGAG
60

Sbjct 383
GAGTAATGTTATTCATAGCACCTTTAGCTTTTATTGCATGGTTAATAGTGTTATTAAGAG
442

Query 61
AGAGTAATCGTACACCATGTGACTATTCCGAATCTGAAAGTGAGCTAGTAAGAGGAATTA
120

Sbjct 443
AGAGTAATCGTACACCATGTGACTATTCCGAATCTGAAAGTGAGCTAGTAAGAGGAATTA
502

Query 121
GAGTGGAATATAGGAGAGTATTATTTTTAGTTATATTTGCTTGCGAGTATTTAATAATGT
180

Sbjct 503
GAGTGGAATATAGGAGAGTATTATTTTTAGTTATATTTGCTTGCGAGTATTTAATAATGT
562

Query 181
TTATATTTAGCTGAGTGAGATTTATAGTATTTTGAAGCATAAATGAAATATTGATAGTAA
240

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Sbjct 563
TTATATTTAGCTGAGTGAGATTTATAGTATTTTGAAGCATAAATGAAATATTGATAGTAA
622

Query 241 TAAACTTGATGTTATTTGTAGTAATGCGTGGTTCATTTTCTCGACTTCGA 290
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 623 TAAACTTGATGTTATTTGTAGTAATGCGTGGTTCATTTTCTCGACTTCGA 672

2. NAD–Z156

Schistosoma haematobium isolate SE1 NADH dehydrogenase subunit 1 (nad1) gene, partial cds; mitochondrial
Sequence ID: JQ595387.1Length: 756

GAGTAATGTTATTCATAGCACCTTTAGCTTTTATTGCATGGTTAATAGTGTTAT-
TAAGAGAGAGTAATCGTACACCATGTGACTATTCCGAATCTGAAAGTGAGCTAGTAAGAGGA
ATTA
GAGTGGAATATAGGAGAGTATTATTTTTAGTTATATTTGCTTGCGAGTATTTAATAATGTTTA
TATTT
AGCTGAGTGAGATTTATAGTATTTTGAAGCATAAATGAAATATTGATAGTAATAAACTTGAT
GTTAT
TTGTAGTAATGCGTGGTTCATTTTCTCGACTTCG
Query 1
GAGTAATGTTATTCATAGCACCTTTAGCTTTTATTGCATGGTTAATAGTGTTATTAAGAG
60
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 383
GAGTAATGTTATTCATAGCACCTTTAGCTTTTATTGCATGGTTAATAGTGTTATTAAGAG
442

Query 61
AGAGTAATCGTACACCATGTGACTATTCCGAATCTGAAAGTGAGCTAGTAAGAGGAATTA
120
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 443
AGAGTAATCGTACACCATGTGACTATTCCGAATCTGAAAGTGAGCTAGTAAGAGGAATTA
502

Query 121
GAGTGGAATATAGGAGAGTATTATTTTTAGTTATATTTGCTTGCGAGTATTTAATAATGT
180
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 503
GAGTGGAATATAGGAGAGTATTATTTTTAGTTATATTTGCTTGCGAGTATTTAATAATGT
562

Query 181
TTATATTTAGCTGAGTGAGATTTATAGTATTTTGAAGCATAAATGAAATATTGATAGTAA
240
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 563
TTATATTTAGCTGAGTGAGATTTATAGTATTTTGAAGCATAAATGAAATATTGATAGTAA
622

Query 241 TAAACTTGATGTTATTTGTAGTAATGCGTGGTTCATTTTCTCGACTTCG 289

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4. Schistosoma haematobium isolate SE1 NADH dehydrogenase subunit 1 (nad1) gene, partial cds; mitochondrial Sequence ID: JQ595387.1Length: 756

GAGTAATGTTATTCATAGCACCTTTAGCTTTTATTGCATGGTTAATAGTGTTATTAAGAGAGA
GTAAT
CGTACACCATGTGACTATTCCGAATCTGAAAGTGAGCTAGTAAGAGGAATTAGAGTGGAATA
TAGG
AGAGTATTATTTTTAGTTATATTTGCTTGCGAGTATTTAATAATGTTTATATTTAGCTGAGTGA
GATTT
ATAGTATTTTGAAGCATAAATGAAATATTGATAGTAATAAACTTGATGTTATTTGTAGTAATG
CGTG GTTCATTTTCTCGACTTCGA

Query 1
GAGTAATGTTATTCATAGCACCTTTAGCTTTTATTGCATGGTTAATAGTGTTATTAAGAG
60
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 383
GAGTAATGTTATTCATAGCACCTTTAGCTTTTATTGCATGGTTAATAGTGTTATTAAGAG
442

Query 61
AGAGTAATCGTACACCATGTGACTATTCCGAATCTGAAAGTGAGCTAGTAAGAGGAATTA
120
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 443
AGAGTAATCGTACACCATGTGACTATTCCGAATCTGAAAGTGAGCTAGTAAGAGGAATTA
502

Query 121
GAGTGGAATATAGGAGAGTATTATTTTTAGTTATATTTGCTTGCGAGTATTTAATAATGT
180
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 503
GAGTGGAATATAGGAGAGTATTATTTTTAGTTATATTTGCTTGCGAGTATTTAATAATGT
562

Query 181
TTATATTTAGCTGAGTGAGATTTATAGTATTTTGAAGCATAAATGAAATATTGATAGTAA
240
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 563
TTATATTTAGCTGAGTGAGATTTATAGTATTTTGAAGCATAAATGAAATATTGATAGTAA
622

Query 241 TAAACTTGATGTTATTTGTAGTAATGCGTGGTTCATTTTCTCGACTTCGA 290
||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 623 TAAACTTGATGTTATTTGTAGTAATGCGTGGTTCATTTTCTCGACTTCGA 672

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COMBINATION
1 &2
Sequence ID: Query_164275Length: 348Number of Matches: 1
Related Information
Range 1: 1 to 334 Graphics Next Match Previous Match First Match
Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand Frame
617 bits (334)	0.0()	334/334(100%)	0/334(0%)	Plus/Plus

Features:

```
Query  54
GAGTCGTGGATTGGGCGAGTGCCTGCCGGCGTGTATACCCGCATATCAACGCGGGTTGCT
113
                                                              ||||||
```

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3 & 6

Sequence ID: Query_206329 Length: 347 Number of Matches: 1 Related Information
Range 1: 1 to 333 Graphics Next Match Previous Match First Match

Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand Frame
616 bits (333)	0.0()	333/333(100%)	0/333(0%)	Plus/Plus Features:

```
Query  44
AGTCGTGGATTGGGCGAGTGCCTGCCGGCGTGTATACCCGCATATCAACGCGGGTTGCTG
103
                ||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  1
AGTCGTGGATTGGGCGAGTGCCTGCCGGCGTGTATACCCGCATATCAACGCGGGTTGCTG
60

Query  104
GTCGAAGGCTCCGTCCTAATAGTCCGGCCACAGCCTAGTCCGGTCTAGATGACTTGATCG
163
                ||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  61
GTCGAAGGCTCCGTCCTAATAGTCCGGCCACAGCCTAGTCCGGTCTAGATGACTTGATCG
120

Query  164
AGATGCTGCGGTGGGTTGTGCTCGAGTCGTGGCTTAATGACATTATACGCGCTCGGGAAG
223
                ||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  121
AGATGCTGCGGTGGGTTGTGCTCGAGTCGTGGCTTAATGACATTATACGCGCTCGGGAAG
180

Query  224
AATCGCACCTATCGTACGCTACGTTGGTCACTTGATCTTGTCTCTATGGTTCGGTCTACG
283
                ||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  181
AATCGCACCTATCGTACGCTACGTTGGTCACTTGATCTTGTCTCTATGGTTCGGTCTACG
240

Query  284
GTTTGTACCGATGGTGTGTGTAATACGCACGAATTGTATAATTGACCCTGACCTCGGATC
343
                ||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  241
GTTTGTACCGATGGTGTGTGTAATACGCACGAATTGTATAATTGACCCTGACCTCGGATC
300

Query  344  AGACGTGATTACCCGCTGAACTTAAGCATATCA  376
                ||||||||||||||||||||||||||||||
Sbjct  301  AGACGTGATTACCCGCTGAACTTAAGCATATCA  333
```

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2&6
Sequence ID: Query_96341 Length: 347 Number of Matches:
1 Related Information
Range 1: 1 to 347 Graphics Next Match Previous Match First Match
Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand Frame
641 bits (347)	0.0()	347/347(100%)	0/347(0%)	Plus/Plus

Features:

```
Query  2
AGTCGTGGATTGGGCGAGTGCCTGCCGGCGTGTATACCCGCATATCAACGCGGGTTGCTG
61
                                                              ||||||
```

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1 & 3

Sequence ID: Query_198955Length: 378Number of Matches: 1
Related Information
Range 1: 1 to 378 Graphics Next Match Previous Match First Match

Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand Frame
699 bits (378)	0.0()	378/378(100%)	0/378(0%)	Plus/Plus

Features:

```
Query  12
GCCTGTCCGAGGGTCGGCTTTTCATCTATCACGGCGCACATTGAGTCGTGGATTGGGCGA
71

Sbjct  1
GCCTGTCCGAGGGTCGGCTTTTCATCTATCACGGCGCACATTGAGTCGTGGATTGGGCGA
60

Query  72
GTGCCTGCCGGCGTGTATACCCGCATATCAACGCGGGTTGCTGGTCGAAGGCTCCGTCCT
131

Sbjct  61
GTGCCTGCCGGCGTGTATACCCGCATATCAACGCGGGTTGCTGGTCGAAGGCTCCGTCCT
120

Query  132
AATAGTCCGGCCACAGCCTAGTCCGGTCTAGATGACTTGATCGAGATGCTGCGGTGGGTT
191

Sbjct  121
AATAGTCCGGCCACAGCCTAGTCCGGTCTAGATGACTTGATCGAGATGCTGCGGTGGGTT
180

Query  192
GTGCTCGAGTCGTGGCTTAATGACATTATACGCGCTCGGGAAGAATCGCACCTATCGTAC
251

Sbjct  181
GTGCTCGAGTCGTGGCTTAATGACATTATACGCGCTCGGGAAGAATCGCACCTATCGTAC
240

Query  252
GCTACGTTGGTCACTTGATCTTGTCTCTATGGTTCGGTCTACGGTTTGTACCGATGGTGT
311

Sbjct  241
GCTACGTTGGTCACTTGATCTTGTCTCTATGGTTCGGTCTACGGTTTGTACCGATGGTGT
300

Query  312
GTGTAATACGCACGAATTGTATAATTGACCCTGACCTCGGATCAGACGTGATTACCCGCT
371

Sbjct  301
GTGTAATACGCACGAATTGTATAATTGACCCTGACCTCGGATCAGACGTGATTACCCGCT
```

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360

Query 372 GAACTTAAGCATATCATA 389
||||||||||||||||||
Sbjct 361 GAACTTAAGCATATCATA 378

2 & 3
Sequence ID: Query_ 178881Length: 378Number of Matches: 1
Related Information
Range 1: 43 to 376 Graphics Next Match Previous Match First Match
Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand Frame
617 bits (334)	0.0()	334/334(100%)	0/334(0%)	Plus/Plus

Features:
Query 1
GAGTCGTGGATTGGGCGAGTGCCTGCCGGCGTGTATACCCGCATATCAACGCGGGTTGCT
60
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 43
GAGTCGTGGATTGGGCGAGTGCCTGCCGGCGTGTATACCCGCATATCAACGCGGGTTGCT
102

Query 61
GGTCGAAGGCTCCGTCCTAATAGTCCGGCCACAGCCTAGTCCGGTCTAGATGACTTGATC
120
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 103
GGTCGAAGGCTCCGTCCTAATAGTCCGGCCACAGCCTAGTCCGGTCTAGATGACTTGATC
162

Query 121
GAGATGCTGCGGTGGGTTGTGCTCGAGTCGTGGCTTAATGACATTATACGCGCTCGGGAA
180
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 163
GAGATGCTGCGGTGGGTTGTGCTCGAGTCGTGGCTTAATGACATTATACGCGCTCGGGAA
222

Query 181
GAATCGCACCTATCGTACGCTACGTTGGTCACTTGATCTTGTCTCTATGGTTCGGTCTAC
240
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 223
GAATCGCACCTATCGTACGCTACGTTGGTCACTTGATCTTGTCTCTATGGTTCGGTCTAC
282

Query 241
GGTTTGTACCGATGGTGTGTGTAATACGCACGAATTGTATAATTGACCCTGACCTCGGAT
300
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 283
GGTTTGTACCGATGGTGTGTGTAATACGCACGAATTGTATAATTGACCCTGACCTCGGAT
342

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```
Query  301  CAGACGTGATTACCCGCTGAACTTAAGCATATCA  334
            ||||||||||||||||||||||||||||||||||
Sbjct  343  CAGACGTGATTACCCGCTGAACTTAAGCATATCA  376
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Appendix VI

University of Ghana

School of Biomedical and Allied Health Sciences

Department of Medical Microbiology

P.O.BOX 4236

Korle-Bu, Accra.

Dear Parent/ Guardian,

INFORMED CONSENT FORM

A study on urinary schistosomiasis (Bilharzia) is being conducted among school children. We would like to seek your permission to include your ward /yourself in this study by collecting urine samples to be analyzed at the laboratory. Drugs will be administered by competent physicians if you are infected with the disease after which urine samples will be collected to monitor treatment progress. The decision to participate is purely voluntary and you will not be penalized in any way if you decide not to consent. Praziquantel has side effects such as vomiting, headache, nausea, anorexia, abdominal pain, epigastric pain, diarrhoea with or without blood, fever myalgia, dizziness, sleeplessness and rarely skin rash with edema in participants with heavy burden of disease. Do not hesitate to report side effects of this medication to the Physicians through whom the drug was administered. Please feel at ease to ask questions in case things are not very clear. The results of this study will be coded and strictly kept confidential. Below is a brief description of the study.

THE STUDY: GENETIC VARIATIONS IN *SCHISTOSOMA HAEMATOBIUM*, RESISTANCE AND REINFECTION IN THE GREATER ACCRA REGION.

Schistosoma haematobium is a parasite that causes urinary schistosomiasis, a disease which presents in most cases as blood in urine. Children especially become easily infected as they fetch, swim, wash and play in contaminated water bodies. The disease has serious effects on their cognitive and physical development. This disease still affects

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millions of people in developing countries, including Ghana. There is no risk involved in this type of study. It will be beneficial to parents after the study has been conducted, to know the status of their wards before and after with regards to *urinary schistosomiasis* so that the needed medical attention can be sought.

Contact

Please direct questions and queries regarding this study to Mr Peter Owadee Forson (0209535142), an MPhil student at the Department of Medical Microbiology, University of Ghana **(**School of Biomedical and Applied Sciences, Korle-Bu).

Supervisors: Dr. Patience Borkor Tetteh-Quarcoo (0244633251).
Rev. Prof Ferdinand Ayeh-Kumi (0244042718).

Participation

Upon reading and understanding the above, I give my full consent to my ward/myself participating in this study.

_____ _____ _____

Name of Parent/ Guardian/StudentSignature/Thumbprint Date

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Appendix VII

QUESTIONNAIRE

BEFORE TREATMENT

I.D of Participant ……...

Age of the Participant …… Sex of the Participant (Male/ Female)

Class of Participant…….……

Occupation of Parent/Guardian ……

Ever visited the dam site? Yes ( ) No ( ). How many times, if yes……?

Ever urinated blood? Yes ( ) No ( ) How long, if yes ……

Duration of residence in the community ……

Distance of house to water body ……...

Ever received treatment? Yes ( ) No ( ) How many times, if yes……

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Appendix VIII

QUESTIONNAIRE

AFTER TREATMENT

I.D of Participant…….

Age of Participant……... Sex of Participant (Male/Female)

Class of Participant…….

Occupation of Parents/Guardian…….

Did You Ever Visit the Dam After Treatment (Yes/No)?

Reason for Visiting the Dam (If Yes) ……...

How Many Times Did You Visit the Dam? ……

Do You Urinate Blood After Treatment? (Yes/No).

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Appendix IX

UNIVERSITY OF GHANA
COLLEGE OF HEALTH SCIENCES
ETHICAL AND PROTOCOL REVIEW COMMITTEE

Ref. No.:.....

28th November, 2016.

Mr. Peter Owadee Forson
Department of Medical Microbiology
School of Biomedical and Allied Health Sciences
University of Ghana
Korle-Bu, Accra

ETHICAL CLEARANCE

Protocol Identification Number: **CHS-Et/M.3 – P 3.5/2016-2017**

The Ethical and Protocol Review Committee of the College of Health Sciences on the 16th November, 2016 unanimously approved your research proposal.

TITLE OF PROTOCOL: **"Genetic variations in *Schistosoma haematobium* and suboptimal treatment, Resistance development and reinfection in the Greater Accra Region"**

PRINCIPAL INVESTIGATOR: **Mr. Peter Owadee Forson**

This approval requires that you submit six-monthly review reports of the protocol to the Committee and a final full review to the Ethical and Protocol Review Committee at the completion of the study. The Committee may observe, or cause to be observed, procedures and records of the study during and after implementation.

Please note that any significant modification of this project must be submitted to the Committee for review and approval before its implementation.

You are required to report all serious adverse events related to this study to the Ethical and Protocol Review Committee within seven (7) days verbally and fourteen (14) days in writing.

As part of the review process, it is the Committee's duty to review the ethical aspects of any manuscript that may be produced from this study. You will therefore be required to furnish the Committee with any manuscript for publication.

This ethical clearance is valid till 30th November, 2017.

Please always quote the protocol identification number in all future correspondence in relation to this protocol.

Signed: .....
PROFESSOR ANDREW A. ADJEI
CHAIRPERSON, ETHICAL AND PROTOCOL REVIEW COMMITTEE

cc: Provost, CHS
Dean, SBAHS
Head of Department

• P. O. Box KB 52, Korle Bu, Accra, Ghana. • Telephone: +233 (0) 306 665103/4 • Fax: +233 (0) 302 660762
• Email: administration@chs.edu.gh / provost@chs.edu.gh • Website: www.chs.ug.edu.gh

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APPENDIX X

UPCOMING PUBLICATIONS

1. Differentiating live and dead eggs of *Schistosoma haematobium* using Hoechst 33258, trypan blue and neutral red on human urine samples.
2. Estimation of resistance or reinfection of *S. haematobium* amongst school children in the Greater Accra region
3. Genetic variations in *Schistosoma haematobium* in the Greater Accra Region.