MALACOLOGICAL SURVEY OF SCHISTOSOMIASIS AND FECAL CONTAMINATION OF PUBLIC WATER SOURCES WITHIN INFORMAL SETTLEMENTS OF KISUMU CITY, KENYA

BY

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ABSTRACT

Human schistosomiasis and soil-transmitted helminthiases (STHs) remain a major public health problem, responsible for significant morbidity and mortality in developing countries, despite availability of effective, safe and cheap drugs for their treatment. Long-term prevention of schistosomiasis and STHs has been difficult to achieve due to persisting conditions of poverty, lack of access to clean water, occupational hazards and poor sanitation. A number of research studies have previously been conducted towards control interventions to ameliorate human suffering from these infections. However, most of the research has focused mainly on controlling of the infections in human populations without considering other targets within the parasite's life cycle that can also aid in disruption of transmission. This study was, therefore, carried out to determine the distribution and prevalence of schistosome infection in intermediate snail vectors, and fecal contamination of public water sources within the informal settlements of Kisumu city, Kenya. Lake Victoria, in Kisumu city is known to be the main source of schistosomiasis in this region, whereas overcrowding and lack of adequate clean water and sanitation within the informal settlements promote STH infections. Water sources were mapped using a Geographical Information System (GIS). Snails were sampled from 81 selected points that had human-water contact activities (25 along the lakeshore and 56 from inland habitats), and transported to the laboratory where they were identified to species level, based on shell morphology and screened for infection. To determine fecal contamination among water sources, the membrane filtration technique was used for enumeration of total and fecal coliform bacteria (*Escherichia coli*) in water samples collected from dams, rivers, springs and wells. Statistical analyses were performed using SAS version 9.2 and a $P < 0.05$ was considered significant. ANOVA and Fisher's exact tests were used to compare differences between variables. Out of 1,059 snails collected, 407 (38.4%) were identified as *Biomphalaria sudanica*, 425 (40.1%) as *Biomphalaria pfeifferi* and 227 (21.5%) as *Bulinus globosus*. The log-transformed mean snail abundance varied significantly across the 6 informal areas ($F_{5, 75} = 4.93$, $P = 0.0006$), with Nyamasaria recording the highest abundance. Overall, 19 (1.8%) of the snails collected shed schistosome cercariae. The infection prevalence in *Bulinus* spp. (3.9%) versus *Biomphalaria* spp. (1.9%) was comparable ($P = 0.0843$). Only water temperature had positive association with snail abundance ($r_s = 0.3$, n = 81, $P = 0.0195$). Out of the 80 water sources sampled, 76 (95%) were highly contaminated with fecal matter The difference in the log-transformed mean fecal coliform density among the informal areas was not significant, indicating similar levels of fecal contamination across the board ($F_{6,68} = 1.46$, $P = 0.2043$). There was a significant negative association between lateral distance from pit latrines and fecal coliform density for wells ($r_s = - 0.34$, n = 53, $P = 0.0142$). The high abundance of *Biomphalaria* and *Bulinus* spp. as well as observation of field-caught snails shedding cercariae confirmed that besides Lake Victoria, the local risk for schistosomiasis transmission exists within the informal areas of Kisumu city. Schistosomiasis control interventions in these areas need to incorporate focal snail control to complement chemotherapy. Treatment of public water sources and improvements in local sanitation and hygiene as well as public health awareness are advocated for in such urban settings. Future studies may benefit from use of more sensitive molecular techniques like PCR for snail identification.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Schistosomiasis (also known as bilharzia) and soil-transmitted helminth (STH) infections are important neglected tropical diseases (NTDs), which cause serious public health problems in sub-Saharan Africa (SSA) (Hotez and Kamath, 2009). It is estimated that 207 million persons are infected with schistosomes, with 93% cases occurring in SSA (Steinmann *et al.*, 2006). In Kenya, prevalence of schistosomiasis ranges from 5% to over 65% in different communities and contributes to significant morbidity in endemic areas (Karanja *et al.*, 1998; 1997; Mwinzi *et al.*, 2004; Ouma *et al.*, 2001). It is estimated that over 9.1 million Kenyans are infected with schistosomiasis (WHO, 2010).

Schistosomiasis is caused by blood flukes from the genus *Schistosoma.* Five species of *Schistosoma* are known to infect human beings: *S. mansoni, S. haematobium S. japonicum, S. mekongi* and *S. intercalatum.* There are two main forms of human schistosomiasis in sub-Saharan Africa; intestinal schistosomiasis, caused by *S. mansoni* and urinary schistosomiasis, caused by *S. haematobium.* Both forms of schistosomiasis, that is, intestinal schistosomiasis and urinary schistosomiasis have focal distribution, meaning that the distribution of the disease is determined by the presence or absence of intermediate host snails, which play a significant role in the transmission of the disease, that is, *Biomphalaria pfeifferi* as an intermediate host for *S. mansoni* and *Bulinus globosus*, as intermediate host for *S. haematobium* (Brooker, 2007). The main schistosome species in western Kenya is *S. mansoni*, while *S.*

haematobium is found mainly at the coast. In western Kenya, Lake Victoria is the primary source of *S. mansoni* infection, with an inverse association between distance to the lake and prevalence of infection (Handzel *et al.*, 2003).

Although mass drug administration (MDA) targeting school-age children is being used to control human schistosomiasis, little success has been achieved in controlling the transmission of the disease which is tied to landscapes where people and snails come together at the same water habitat during their day-to-day activities such as fishing, washing clothes, car wash or swimming (Sturrock, 2001).These activities bring them into contact with river, lake or canal water, contaminated by feces or urine of infected people or animals.

This emphasizes the importance of combining malacological surveys with prevalence spot-checks of the human infection for comprehensive and cost effective control.

Several malacological surveys have been carried out around Lake Victoria (Standley *et al.*, 2010; Steinauer *et al.*, 2009) and around Kisumu city (Kahigi, 2000). Such surveys not only assessed local transmission, but also helped to elucidate associations between disease point prevalences which, in turn, aid in creating more accurate predictive maps of expected schistosome distributions. Most of the surveys around Lake Victoria basin in western Kenya (Kahigi, 2000; Steinauer *et al.*, 2009) have shown *Biomphalaria sudanica* (an intermediate host for *S. mansoni*) to be the most common species. In addition, interactions between natural populations of human (*S. mansoni*) and rodent (*S. rodhaini*) schistosomes have been reported in the Lake Victoria region of Kenya, with both species infecting same species of snail and mammalian hosts (Steinauer *et al.*, 2008). A recent study identified a new rodent

species, *S. kisumuensis* (phylogenetic position within the *S. haematobium* species group) from Nyabera marsh in Kisumu (Hanelt *et al.*, 2009). Another study within the same setting highlighted the role of wild mammals as reservoirs for *S. mansoni* and the potential role for these mammals in renewed transmission (Hanelt *et al.*, 2010). Hybrid schistosome species have also been reported in this area, a phenomenon that could be attributable to co-infection of *S. mansoni* and *S. rodhaini* within the same host species (Hanelt *et al.*, 2010). Currently, no research has been carried out in Kisumu city to determine if there are other possible sources of schistosomiasis transmission other than Lake Victoria. The potential for transmission within the city is supported by numerous freshwater points and dams within the city, which could also provide favorable conditions for survival of intermediate host snail vectors. Identification of active transmission sites for schistosomiasis ensures that resources for disease control such as chemotherapy and molluscicide interventions are directed where they are needed most.

Several gaps still exist towards integrating snail distribution with human infection data. First, fragmentation of infection among human populations versus snail sampling makes it difficult to indicate with certainty the occurrence and distribution of the schistosome snail host in the areas. This is further confounded by the fact that most human populations within endemic areas exhibit high itinerancy (Standley *et al.*, 2010), complicating the pattern for locally acquired versus imported infections. Second, although chemotherapy plays a significant role in reducing morbidity and mortality due to schistosomiasis, the costs and logistical constraints hamper its effectiveness on a wider scale. Parallel preventive measures such as snail control, whose integration requires a thorough understanding of snail distribution, therefore

seem plausible (Kahigi, 2000). Third, the longevity of schistosome infections in the human host makes it difficult to detect when and where transmission actually occurs, without undertaking snail surveillance. Since snails are obligatory hosts for the larval stages of schistosomes, their examination provides important information on active transmission foci. Both the parasite and the vector must be targeted in order to break the cycle of transmission so as to achieve success in controlling schistosomiasis.

On the other hand, increased urbanization characterized by inadequate sanitation and overcrowding may promote the transmission of soil-transmitted helminth (STH) infections that are known to flourish in impoverished areas (United Nations, 2003). One such way through which transmission may occur is through fecal contamination of water sources. STHs are intestinal nematodes (*Ascaris lumbricoides, Trichuris trichiura, Strongyloides stercoralis*) and the hookworms (*Ancylostoma duodenale and Necator americanus*) that infect humans. Using disability adjusted life years (DALYs) as a quantitative measure of disease burden, STHs cause more morbidity than any other parasitic disease, except malaria , an estimated 39 million DALYs may be lost each year due to STH infections (Hotez and Kamath, 2009). Estimates suggest that 1,222 million people are infected with *A. lumbricoides,* 795 million with *T. trichiura* and 740 million with hookworms (de Silva *et al.*, 2003). According to the recently launched Global Atlas of Helminth Infections (GAHI, 2010), over 9.1 million Kenyans are at risk of STH infections, out of which 2.4 million are school-age children (GAHI, 2010). The high prevalence rate is attributed to lack of education, lack of latrines, occurrence of diarrhea, lower socio-economic status, improper disposal of human excreta and the level of sanitation in households (Smith *et al.*, 2001).

Use of Geographical Information Systems (GIS) has provided an opportunity to map the distribution of schistosomiasis and STH infections in various areas at risk of the parasitic infections for the purposes of cost-effective control methods (Brooker and Michael, 2000). Its use can also be applied to consider the spatial patterns of human infection simultaneously with those of intermediate host snails so as to improve efficiency of allocation of available transmission control measures. Although various studies have been carried out in areas around Lake Victoria in western Kenya to determine the prevalence of schistosomiasis (Handzel *et al.*, 2003; Shane *et al.*, 2011), remarkably little research has been done on the distribution and prevalence of infection among intermediate snail hosts within the informal settlements of Kisumu city that borders the lake, which has been shown to be the major source of schistosome infections (Handzel *et al.*, 2003).

This study was, therefore, a follow-up to a recent cross-sectional survey on schistosomiasis and soil-transmitted helminth infections within these informal settlement areas, where prevalence of 21% and 3.6% for *S. mansoni* and *S. haematobium*, respectively, and a prevalence of 16.2% for STH infections was reported (Odiere *et al.*, 2011). The objective of this study was, therefore, to determine the presence and geographical distribution of *Biomphalaria* and *Bulinus* snails, and their infection prevalence among freshwater habitats in informal settlements and along the lakeshore in Kisumu city, Kenya. In addition, the environmental and physico-chemical factors that may influence snail distribution were determined. Given the known association between lack of adequate water and sanitation in impoverished areas and enhanced STH infections, the study also determined the level of fecal contamination in the public water sources, with a view of understanding whether

these water sources may contribute to the reported prevalence of soil-transmitted helminth (STH) infections in the informal settlements of Kisumu city, Kenya.

1.2 Statement of the problem

Despite the fact that praziquantel (PZQ) and albendazole (ALB), the drugs for treating schistosomiasis and STHs infections, respectively, have become so cheap (less than US$ 0.10 per tablet for praziquantel and US$ 0.027 per dose for albendazole), long-term prevention of schistosomiasis and STHs has been difficult to achieve due to persisting conditions of poverty, lack of access to clean water, occupational hazards and poor sanitation. Re-infections in the human population following chemotherapy still pose a challenge for the long term control of schistosomiasis and STHs. It is hoped that a vaccine against the parasite will be the best method to combat the spread of these infections, however, until this becomes available, alternative preventive measures such as snail control (for schistosomiasis) and provision of safe water and improved sanitation, are important in disrupting transmission of these infections. Considering that parasite eggs excreted in human feces or urine, which contaminate the soil or water sources are an important phase in the life cycle for schistosomiasis and STH, re-infection can only occur as a result of new contact with a contaminated environment. The study sites, the informal settlements of Kisumu city, Kenya borders Lake Victoria, which has been shown to be the major source of schistome infections (Handzel *et al.*, 2003). The presence of numerous freshwater points and dams within the city could also provide favorable conditions for survival of intermediate host snail vectors. This study was, therefore, carried out to determine the presence and geographical distribution of *Biomphalaria* and *Bulinus* snails, and their infection prevalence among freshwater habitats in informal settlements and along the lakeshore in Kisumu city, Kenya.

Like many other rapidly growing cities, Kisumu city faces many socio-economic challenges such as overcrowding and lack of adequate water and sanitation that accompany urbanization. For instance, Kisumu is faced with acute water shortage as only 40% of the population have access to piped water (UN Habitat, 2005). A majority of slum dwellers rely on unprotected wells, the lake, and springs that are subject to high degrees of contamination due to the rampant use of pit latrines and to high water tables (UN Habitat, 2005). High water tables, coupled with black cotton soils and rock out-crops in these informal areas, also affect both drainage and latrine construction. Some 11% of slum residents have no latrines and must rely on undignified coping mechanisms such as relying on neighbors' toilets, wrap and throw ("flying toilet") and use of open fields (UN Habitat, 2005).

Therefore, this study also determined the level of fecal contamination in the public water sources, and the association between fecal contamination and prevalence of STH infections (*A. lumbricoides*, *T. trichiura* and hookworms) in the informal settlements of Kisumu city, Kenya.

1.3 Justification of the study

Schistosomiasis and STHs infections continue to be a major public health problem in sub-Saharan Africa due to their high prevalence and associated morbidity, as well as their spread into new areas lacking safe water supplies and sanitary facilities. Although mass drug administration (MDA) targeting school-age children is being used to control human schistosomiasis, little success is being achieved in controlling the transmission due to the costs and logistical constraints hamper its effectiveness on a wider scale resulting to rampant re-infections. Parallel preventive measures such as snail control, whose integration requires a thorough understanding of snail distribution is, therefore, plausible. While it is common knowledge that schistosomiasis tends to be focal in distribution, there is inadequate research on the geographical distribution of infections in the snail vectors in low-income settings of urban areas. In order to disrupt transmission more effectively and achieve prolonged disease control, there is a need to develop more efficient, integrated control programs that are focused in space and time. GIS provides a tool for mapping spatial patterns of snail infection to improve efficiency of allocation for available transmission control measures. The longevity of schistosome infections in the human host makes it difficult to detect when and where transmission actually occurs, without undertaking snail surveillance. Since snails are obligatory hosts for the larval stages of schistosomes, their examination provides important information on active transmission foci. Both the parasite and the vector must be targeted in order to break the cycle of transmission so as to achieve success in controlling schistosomiasis. Identification of active transmission sites for schistosomiasis ensures that resources for disease control such as chemotherapy are directed where they are needed most.

Findings from this study, therefore, provide useful information on distribution of snails and their prevalence of infection in an urban area that informs the design and implementation of cost-effective and targeted control efforts.

Determining fecal contamination of public water sources provides vital information that can be utilized by various stakeholders to design ways of improving the sanitation to avoid contamination and spread of STH infections due to fecal contamination of the water. Unlike many previous studies that have used the multiple-tube fermentation technique to determine fecal contamination in water, the current study employed the membrane filtration (MF) technique which is highly reproducible, can be used to test relatively large volumes of sample, allows isolation and enumeration of discrete colonies of bacteria, and yields numerical results more rapidly than the multiple-tube procedure.

1.4 Overall objective

To determine the distribution and prevalence of infection in intermediate snail vectors for schistosomiasis and fecal contamination of public water sources within the informal settlements of Kisumu city, Kenya.

1.4.1 Specific objectives

1. To determine the presence, distribution and abundance of *S. mansoni* and *S. haematobium* snail intermediate hosts within the informal settlements of Kisumu city using their morphological characteristics.

2. To determine the prevalence of mammalian schistosome infections in *Biomphalaria* and *Bulinus* snails.

3. To evaluate the relationships between environmental and physico-chemical factors and schistosome vector snail abundance.

4. To determine levels of fecal contamination in public water sources present in the informal settlements of Kisumu city, and the association between fecal contamination and prevalence of STH infections (*A. lumbricoides*, *T. trichiura* and hookworms)

1.5 Research Questions

1. What is the distribution and abundance of *S. mansoni* and *S. haematobium* snail intermediate hosts present within the informal settlement of Kisumu city?
2. What is the prevalence of mammalian schistosome infections in *Biomphalaria* and *Bulinus* snails within the informal settlement of Kisumu city?
3. What are the relationships between environmental and physico-chemical factors and schistosome snail vector abundance?
4. What are the levels of fecal contamination in public water sources within the informal settlements of Kisumu city?
5. What is the association between fecal contamination and prevalence of STH infections *(A. lumbricoides*, *T. trichiura* and hookworms)?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Transmission cycle of schistosomiasis

Life cycle of human schistosomes involves snail intermediate hosts, *Biomphalaria pfeifferi* as an intermediate host for *S. mansoni* and *Bulinus globosus*, as intermediate host for *S. haematobium* (Brown, 1994). Human infection occurs when the skin comes in contact with contaminated fresh water in which the intermediate host snail vectors are infected (Figure 1). Infected individuals contaminate the fresh water by releasing eggs into the water either by urinating or defecating. The eggs hatch to release the free-swimming miracidia that infect fresh-water snails by penetrating the snail's foot; the miracidia transform into sporocysts that eventually develop into free-living cercariae that penetrate the skin of a person who comes in contact with the contaminated water through activities such as fishing, washing clothes, car wash or swimming (Sturrock, 2001). The cercariae then penetrate the skin and transform into migrating schistosomulae that enter the blood stream, and then travel to the lungs where they undergo further developmental changes necessary for subsequent migration to the liver. In the liver the mature adult female and male worms grow, pair and mate to produce non-operculated eggs. Worm pairs of *S. mansoni* and *S. japonicum* relocate to the mesenteric veins while *S. haematobium* worm pairs migrate to the perivesical venous plexus of the bladder, ureters, and kidneys (Figure 1). Schistosome transmission occurs when the parasite eggs reach the environment via the excreta of a definitive host, hatch into miracidia on coming into contact with fresh water and infect host snails (Sturrock, 2001). Water contamination with schistosome

eggs and intermediate host snails are key factors in the transmission of schistosomiasis.

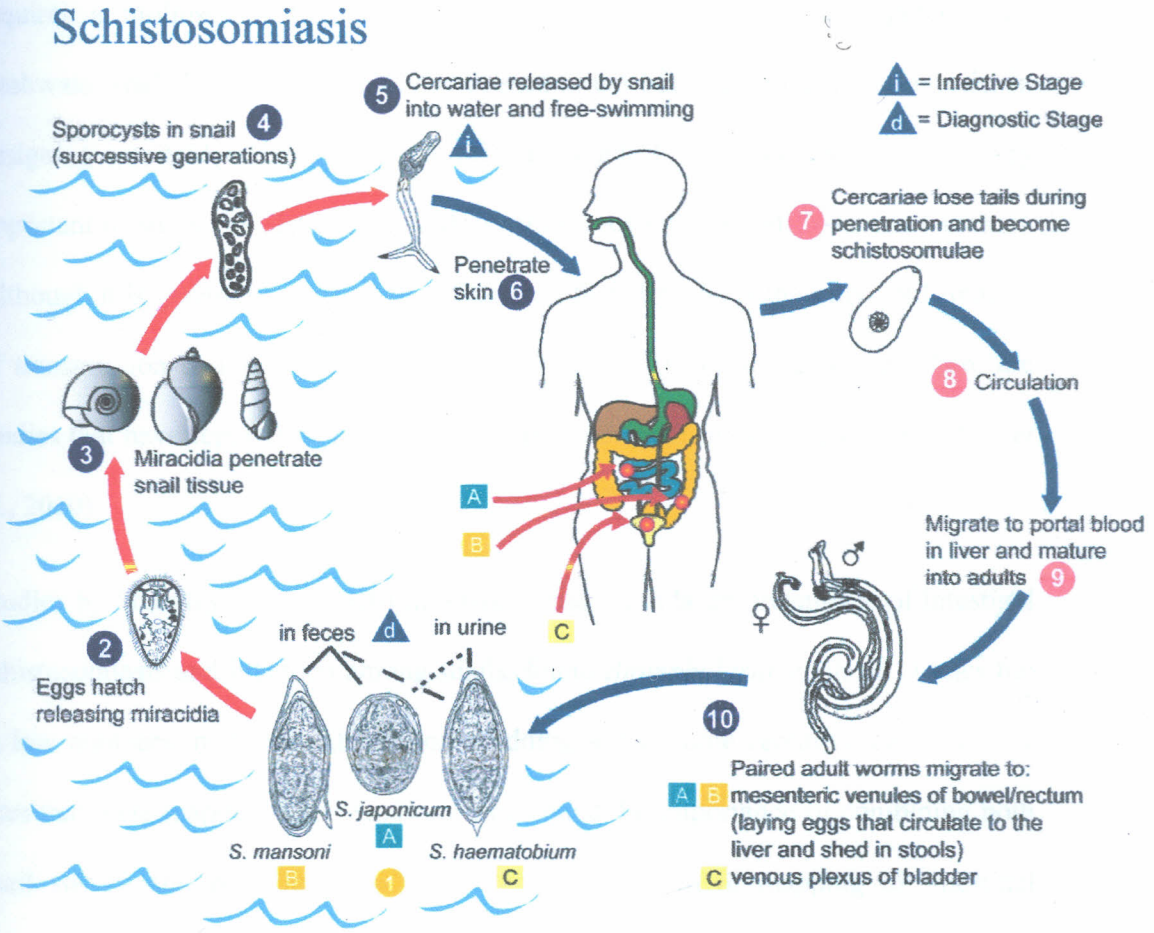

Figure 1. Life cycle of schistosomiasis (Adapted from CDC, available at http://www.dpd.cdc.gov/dpdx/html/schistosomiasis.htm

2.2 Distribution and prevalence of infection of intermediate host snail vectors

The intermediate snail hosts *Biomphalaria pfeifferi; Bulinus globosus* continue to play a significant role in the transmission of schistosomiasis. The schistosome parasite requires a molluscan intermediate host in which to undergo development, and freshwater snails form an essential component in the life cycle of schistosomiasis. In designing a suitable program for cost effective control of schistosomiasis, it is very important to study the distribution and prevalence of intermediate host snail vectors. Although it is generally accepted that finding infected snails is the only confirmation of transmission of the disease, this may not necessarily be the case, and there are studies that have reported none or very low prevalence of infected snails (Standley *et al.*, 2010).

Studies by Standley *et al.*, (2010) in Sesse Island Uganda on prevalence of intestinal schistosomiasis and infection among snails, found *Biomphalaria* snails at 11 sites but in low numbers; none was also found shedding schistosome cercariae out of the 23 sites that were mapped. Their study recommended the importance of combining both snail survey and parasitological survey for comprehensive mapping of intestinal schistosomiasis.

To achieve success in controlling schistosomiasis cost effectively, it will be important to break the cycle of transmission by targeting both the parasite and the vector (King, 2009). Currently, there is large-scale mass drug administration (MDA) of highly effective anthelminthic drug, PZQ, through school-based or community-based programs, which are playing an important role in reducing morbidity and mortality due to schistosomiasis. However, MDAs alone have failed to stop transmission because they target the parasite alone and not the vector (King, 2009). Interruption of

schistosome transmission in high-risk areas, therefore, requires a combination of drug treatment, water management, snail control (through habitat modification, irrigation changes, and the use of molluscicidal sprays), and the control or treatment of sewage (Sturrock, 2001).

Research studies, carried out around L. Victoria, in western Kenya, which is a major source of infection for schistosomiasis, have focused on the prevalence of the infection so as to implement drug treatment (Handzel *et al.*, 2003; Shane *et al.*, 2011). Current research in the area is lacking on the prevalence and distribution of intermediate host snail vectors so as to integrate both the snail control and human infection control. This study was, therefore, carried out to determine the presence and geographical distribution of *Biomphalaria* and *Bulinus* snails, and their infection prevalence among freshwater habitats in informal settlements and along the lakeshore in Kisumu city, Kenya.

2.3 Ecological Factors that influence the abundance of Intermediate snail host vectors.

The snail intermediate host populations are influenced by temperature, food supply, predators, parasites, rainfall and water composition. Sunlight in snail habitats, flowering aquatic weeds, abundance of microflora and high dissolved oxygen content contribute to the abundance of freshwater snails (Thieltges, 2008).

Studies conducted by Yakubu (2003) on distribution and infection rate of schistosome intermediate hosts in river Kubanni and its tributaries located in Zaria, Kaduna State, Nigeria, revealed that distribution and infection rate of schistosome intermediate host

was greatly influenced by rainfall. In the permanent flowing habitat, the swift water currents due to heavy rainfall easily dislodged and flushed away the snails attached to the vegetations and hence accounted for low or lack of snails during the heavy rainy period and the gradual rise long after the stoppage of rains. In the tributary, the heavy rains during this period helped to establish the habitat with the consequent reactivation of snails which had previously passed through aestivation in dry mud. Higher number of infected snails was recorded in dry season which signified the period of intense transmission. It was, therefore, recommended for snail control strategy to be planned towards this period.

Identifying suitable transmission areas for snail-borne diseases in Uganda was done by overlaying the snail distribution maps with a mask of suitable temperature regimes for the intra-molluscan parasite development (Stensgaard *et al.*, 2006). This revealed that, while the snail distributions were restricted in the north and north-eastern parts of Uganda (high temperatures, low precipitation), the distribution of the parasite was instead restricted in the southern and cooler parts of the country (Stensgaard *et al.*, 2006). Their study was in agreement with earlier observations that, while parasites are more sensitive to low temperatures than they are to high temperatures, the opposite is true for many of their snail hosts which do not withstand high temperatures well (Appleton, 1978).

A study carried out on the prevalence of snail vectors of schistosomiasis and their infection rates in two localities within Ahmadu Bello University (A.B.U.) Campus, Zaria, observed that the preference for different environmental conditions such as abundant microflora, oxygen content and other physico-chemical factors could be one reason why the snail populations showed marked differences in each locality. Another

factor could be the natural behavioral mode of adaptation which is different for each species. *B. pfeifferi* is a quiet-water, surface-feeding snail and could be washed down to the dam easily (Utzinger and Tanner, 2000). It finds a resting place when the speed of water current becomes greatly reduced whereas *B. globosus* can cling to or settle to the bottom of the water and later come out to the surface (Ayanda, 2009).

This study also determined the environmental and physico-chemical factors that may influence snail distribution and abundance within the informal settlements of Kisumu, Kenya.

2.4 Transmission dynamics of soil-transmitted helminths (STHs)

STH infections are widely distributed throughout the tropics and subtropics. Climate is an important determinant of transmission of these infections, with adequate moisture and warm temperature essential for larval development in the soil (Brooker and Michael, 2000). Other important determinants are poverty and inadequate water supplies and sanitation where STH infections flourish (Crompton and Savioli, 1993).

The life cycles of STHs (*A. lumbricoides*, *T. trichiura, A. duodenale* and *N. americanus*) infections follow a general pattern ,the adult parasite stages inhabit some part of the host intestine (*A. lumbricoides* and hookworm in the small intestine; *T. trichiura* in the colon), reproduce sexually and produce eggs) (Anderson and May, 1985). Persons infected with STHs have parasite eggs in their feces, in areas where there are poor sanitation facilities and inadequate water, the soil and water around the community become contaminated with feces containing worm eggs (Crompton and Savioli, 1993). In the soil, the eggs mature over 2 to 4 weeks, depending on the type

of worm and environmental conditions, and then infect humans by being ingested or by penetrating the skin (hookworms only) (Anderson and May, 1985).

Since STH infections are transmitted by eggs excreted in human feces, which contaminate the soil or water sources in areas that lack adequate sanitation, re-infection can only occur as a result of new contact with a contaminated environment. Therefore, provision of safe water and improved sanitation are essential for the control of STH infections. Improved sanitation aims at controlling transmission by reducing soil and water contamination. Without a change in sanitation and personal hygiene habits, periodic deworming cannot attain a stable reduction in transmission (Crompton and Savioli, 1993). Studying the prevalence of STHs among the school going children within the informal settlements of Kisumu city, is not sufficient in comprehensive control STHs, therefore, this study, was carried out to determine levels of fecal contamination in public water sources present in the informal settlements of Kisumu city, and the association between fecal contamination and prevalence of STH infections (*A. lumbricoides*, *T. trichiura* and hookworms).

2.5 Impact of urbanization on sanitation

Rapid urbanization has been ongoing in many developing countries, including sub-Saharan Africa, where the urban population is expected to triple between 2010 and 2050 to >1.2 billion people (UN-HABITAT, 2010). Urbanization is often driven by perceived opportunities for improving the family status and for education. However, more often than not, urban areas cannot address the demands of an expanding population with strengthened infrastructure, resulting in extreme poverty (Arnaud, 1998), especially within the informal settlement areas.

In Kenya, rapid urbanization amid economic degradation has resulted in an increased proportion of people living in absolute poverty in the urban areas (CBS, 2000), including slums of cities such as Kisumu where overcrowding and lack of adequate water and sanitation are a major challenge (UN-HABITAT, 2005). Although an adequate supply of safe drinking water is universally recognized as a basic human need, millions of people in the developing world do not have ready access to an adequate and safe water supply. The spread of many infectious diseases, including cholera, typhoid, hepatitis, polio, cryptosporidiosis, soil-transmitted helminths, and schistosomiasis has been associated with human excreta and the lack of adequate personal and domestic hygiene (WHO/UNICEF, 2000). Using the Multiple-tube fermentation technique, a study in Langas, an urban slum in Eldoret municipality, Kenya, found that wells (the main domestic water sources in the study area) were highly contaminated with fecal matter (97% of the samples) (Kimani-Murage and Ngindu, 2007). The study suggested that due to the close proximity between the wells and pit latrines, the pit latrines were a major source of contamination of the wells with fecal matter. Poor sanitation and inadequate water supply in many informal settlements, like Kisumu are ideal conditions for the persistence of STHs infections. Therefore, determining the level of fecal contamination in the public water sources, for instance wells, within the informal settlement of Kisumu city, is important in providing useful information that can be utilized in designing strategies that can be used in improving the sanitation within the informal settlements.

2.6 Morbidities associated with schistosomiasis and soil-transmitted helminths

Helminth infections, caused by soil-transmitted helminths (STHs) and schistosomes, are among the most prevalent afflictions of humans who live in resource-limited areas

of the developing world (Keiser *et al.*, 2002). Schistosomiasis can result in overt clinical disease and contribute to anemia while chronic and intense infections with soil-transmitted helminths can cause malnutrition and iron-deficiency anemia, and also adversely affect physical and mental growth in childhood (Hotez *et al.*, 2004).

A serious acute illness accompanied by fever and lymphadenopathy, known as Katayama Syndrome, can result from heavy schistosome infections. The morbidity commonly associated with *S. mansoni* infection includes lesions of the liver, portal vein, and spleen, leading to periportal fibrosis, portal hypertension, hepatosplenomegaly, splenomegaly, and ascites (Gryseels *et al.*, 2006). Chronic disease is mostly due to perforation of blood vessels and entrapment of eggs by host tissues. The host's reaction to entrapped eggs results in granuloma formation. *S. haematobium* causes bladder wall pathology, leading to ulcer formation, hematuria, and dysuria. Granulomatous changes and ulcers of the bladder wall and ureter can lead to bladder obstruction, dilatation, secondary urinary tract infections and subsequent bladder calcification, renal failure, lesions of the female and male genital tracts, and hydronephrosis (Gryseels *et al.*, 2006). Among the morbidities associated with STH infections include intestinal manifestations (diarrhoea, abdominal pain), general malaise and weakness, that may affect working and learning capacities and impair physical growth leading to stunting. Hookworms cause chronic intestinal blood loss that result in anaemia.

The adverse effects of intestinal parasites among school age children are diverse and alarming since they are groups at high-risk for intestinal parasitic infections. This is especially so because STH infections are transmitted through poor sanitation and

hygiene, and schistosomiasis by contact with infected freshwater streams and lakes where school aged children are more exposed (Savioli *et al.*, 2002).

Following a recent cross-sectional survey on schistosomiasis and soil-transmitted helminth infections within these informal settlement areas of Kisumu, where prevalence of 21% and 3.6% for *S. mansoni* and *S. haematobium*, respectively, and a prevalence of 16.2% for STH infections was reported (Odiere *et al.*, 2011), the current study was a follow up that was carried out within the same informal settlements to determine levels of fecal contamination in public water sources present in the informal settlements of Kisumu city, and the association between fecal contamination and prevalence of STH infections (*A. lumbricoides*, *T. trichiura* and hookworms).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

This study was carried out in the informal settlements of Kisumu city: Manyatta, Kaloleni, Nyalenda, Obunga, Nyamasaria and Bandani, located at latitude (00°05'S) and longitude (34°45'E) and lie close to Lake Victoria in western Kenya, between April to July 2011 (Figure 2). Kisumu is the third largest urban centre in Kenya with an area of 417 sq. km (157 sq. km. of water and 260 sq. km. of land) and a population estimated at 500,000 (UN Habitat, 2005). Majority of the population belong to the Luo community. Occupational hazards associated with the lake such as sand harvesting, fishing and car washing, predispose individuals living close to the Lake shores, to schistosome infections (Handzel *et al.*, 2003). Overcrowding and lack of adequate water and sanitation within the informal settlements promote STH infections among the population (United Nations, 2003).

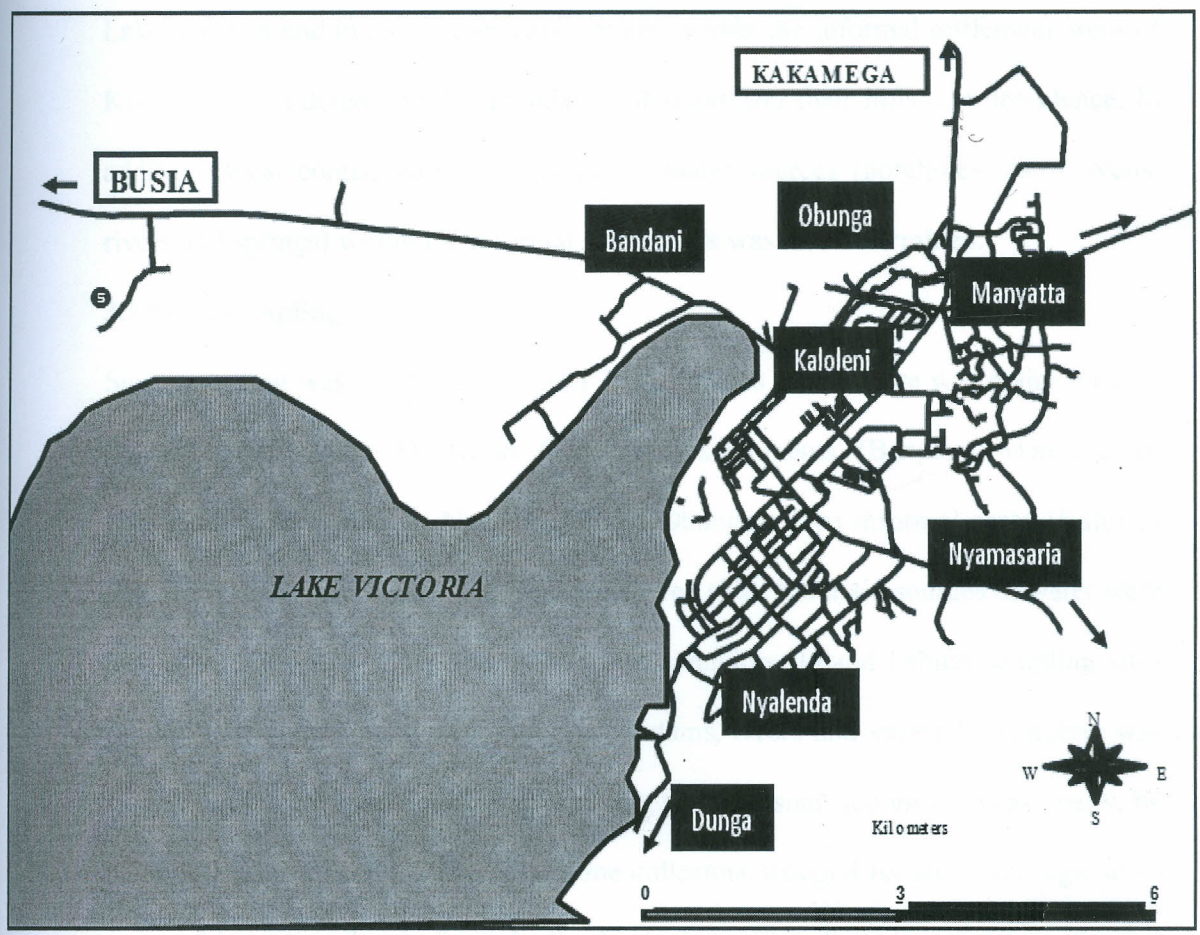

Figure 2. Map showing informal settlements in Kisumu city.

3.2 Study Design

This was a cross-sectional survey involving sampling of snails along the shores of Lake Victoria and in rivers, dams and streams within the informal settlement areas of Kisumu city to determine the abundance of snails and their infection prevalence. In addition, fecal contamination of the public water sources (boreholes, dams, wells, rivers and springs) within the informal settlements was also determined.

3.3 Snail sampling

Snail sampling was conducted in April 2011, in sites where there was major human-water contact (Plate 1) within 6 informal settlements (Bandani, Manyatta B, Nyamasaria, Nyalenda A, Nyalenda B and Obunga). Two informal areas (Kaloleni and Manyatta A) were excluded from the survey since the main sources of water were kiosks selling piped water and mobile water vendors. Inland habitat sampling sites (within the informal settlements) included, dams, rivers and springs). Sampling was carried out by 2 trained field collectors using standard snail scoops or occasionally, by hand collection (Kahigi, 2000). The same collectors scooped for snails throughout so as to achieve some level of standardized sampling effort. Sampling time was fixed at 30 minutes per location and was performed between 0830h and 1030h. Sampling area, per location, was approximately 5 m^2 whereas lengths of 10 metres along streams and lake shoreline were used. At each collection time, snails from each site were appropriately labeled and transported in separate perforated plastic containers to the Ministry of Health's Division of Vector-Borne and Neglected Tropical Diseases (DVBNTD) laboratory, Kisumu. Snails were cleaned, sorted, counted and identified to species level based on shell morphological characteristics using standard keys as shown in Schematic figure 3 and Table 1 respectively (Brown, 1994). Snail

abundance was presented as total snails and also according to species (*Biomphalaria* and *Bulinus*) and categorized as 0, 1-50, 51-100, 101-200 and over 200 snails, based on a modification of Standley *et al.*, 2010.

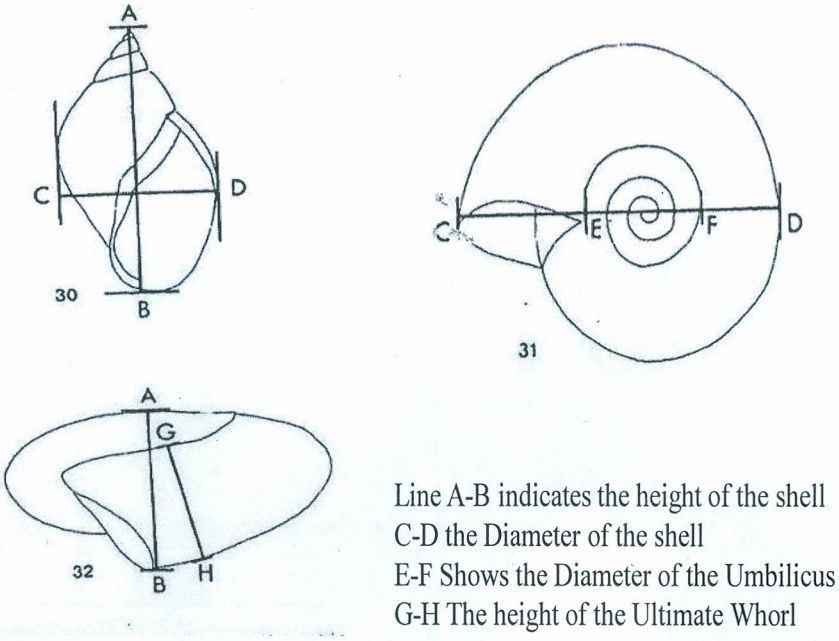

Figure 3 showing morphological identification of intermediate host snails.

Table 1: Morphological differences of the Intermediate snail host vectors

Biomphilaria pfeifferi	*Biomphilaria sudanica*	*Bulinus globossus*
5.2mm in height	4.2 mm in height	16mm in height
Shell discoid in shape	Shell large and flat	Ovate shell
Umbilicus occupies 1/3 of the diameter	Umbilicus occupies 1/2 of the diameter	Umbilicus widely open
13mm diameter	16-17.2mm in diameter	12mm in diameter

Plate 1: Snail sampling at one of the lakeshore sites with human-water contact activities

3.4 Screening of snails for schistosome infections

To determine if the collected snails were infected with mammalian schistosomes, snails were rinsed and placed individually in 24-well culture plates, containing1 ml of clean filtered water (same source as site of collection) and exposed to indirect sunlight for 4 hours to induce cercarial shedding. The time of cercariae shedding was carefully selected to coincide with the early peak shedding time (midday) (Steinauer *et al.*, 2008). The wells of the plates were then examined for the presence of cercariae under a dissecting microscope. Snails that did not shed cercariae on the first exposure were re-exposed on the second day. Bifurcate cercariae were used to indicate that the cercariae were of mammalian origin (Brown, 1994; DBL-WHO, 1998). Snails were killed and stored in 70% alcohol for future prospective analysis and confirmation of species using molecular techniques as previously described (Lotfy *et al.*, 2005).

3.5 Geographical distribution of snails

To determine the geographic distribution of snails, all sampled habitats were mapped using hand-held differential geographic global positioning system (GPS) units (Trimble Navigation Ltd, California, USA) with an estimated accuracy of ± 1 meter (Hightower *et al.*, 1998). Data was downloaded with differential correction into a GPS database (GPS pathfinder office 2.8 Trimble Navigation Ltd, California, USA) and analyses performed using ArcView version 9.2 software (Environmental Systems Research Institute, Inc., Redlands, CA).

3.6 Recording of ecological factors

Ecological factors including pH, temperature and vegetation cover were included in the current study because they are standard key ecological factors that influence the distribution and abundance of snail vectors.

3.6.1 Determination of pH and temperature

The physico-chemical characteristics of the water at each sampling site including pH and temperature were determined using a pH meter with a glass electrode and a temperature probe (CyberScan pH310, model # WAG-WE30220, Wagtech WTD, Palintest Ltd, Gateshead, UK). The meter and the probe were calibrated as per the instructions in the operation manual. The probe was placed in the water that had been collected in the sterile sample bottle. The readings of the pH and temperature were recorded when the pH meter read 'ready'. Buffer solutions of pH 4.0, 7.0 and 10.0 were used to calibrate the pH meter. The electrode was kept in distilled water in between measurements.

3.6.2 Composition of the aquatic vegetation

The presence of vegetation cover and algal cover at the sites of the water body where the snails were sampled from was recorded.

3.7 Identification of water sources and water sampling

Water sources within the informal settlement areas, including boreholes, wells, springs, dams, rivers were identified and provided with unique identification codes. Extra attention was given to wells as they were the most abundant in the areas, and also because they provide information on contamination associated with both surface

run-off and underground seepage (Plate 2). Wells were further categorized as protected or unprotected based on presence or absence of any form of cover/lid. Five wells from each category (protected and unprotected) were randomly chosen from each informal settlement area using a random number generator software (www.randomizer.org/). In cases where there were fewer than 5 wells or an extra of one, all the wells in that area were included in the study. The sample size of 5 wells/category/informal area was determined by financial and logistical feasibility. Water samples were collected aseptically with sterile 250 ml glass sampling bottles. Bottles were uncapped and recapped immediately before and after each sample was taken. The samples were transported within 2 hours of collection in a cool box containing ice packs to the Kenya Government Chemist Laboratory, Kisumu for analysis of fecal contamination. Data on water treatment frequency was also obtained for the different water sources by asking the owners on how frequent they treated their water sources.

Plate 2: Water sampling from one of the unprotected wells within the informal settlements of Kisumu city.

3.8 Water sample analysis for fecal contamination

Fecal contamination of the water was determined through isolation of indicator organisms, total coliforms, and then thermotolerant/fecal (*Escherichia coli*) coliform, using the membrane filtration technique (Plate 3). A sterile, absorbent pad (Wagtech International) was placed in a sterile petri dish using sterilized forceps. M-ColiBlue24® Broth (HACH Company, Loveland, CO, USA, Cat # 26084-42) was dispensed into the sterile petri dish, evenly saturating the absorbent pad. A membrane filter (47 mm diameter, with 0.45 ± 0.02 µm pore size) (Whatman® International, England, Cat # 7184004) was then placed into the funnel assembly (Paqualab® 50, Thelabwarehouse, London) grid side up, and the sample poured into the funnel while

flaming the pouring lip of the sample container. The sample was shaken vigorously to mix, 100 mL of sample was poured into the funnel and vacuum applied to filter the sample. The funnel was rinsed three times with 30 mL of sterile buffered water and turned off. The membrane filter was then transferred to the previously prepared petri dish using sterile forceps. The filter, grid side up, was placed on the absorbent pad and petri dish lid replaced. The petri dish was then inverted and incubated at 35 ± 0.5 °C for 24 hours. The filters were then examined for colony growth using the naked eye. If the colonies were not discrete or if they were too numerous to count (exceeding 200 per membrane), the sample was serially diluted, until final optimal dilutions of 1:10 and 1:100 and then re-analyzed. Coliform density was reported as the number of colonies per 100 mL of sample using the general equation:

$$\text{Coliform colonies per 100 mL} = \left(\frac{\text{Coliform colonies counted}}{\text{mL of original sample filtered}} \right) \text{X } 100$$

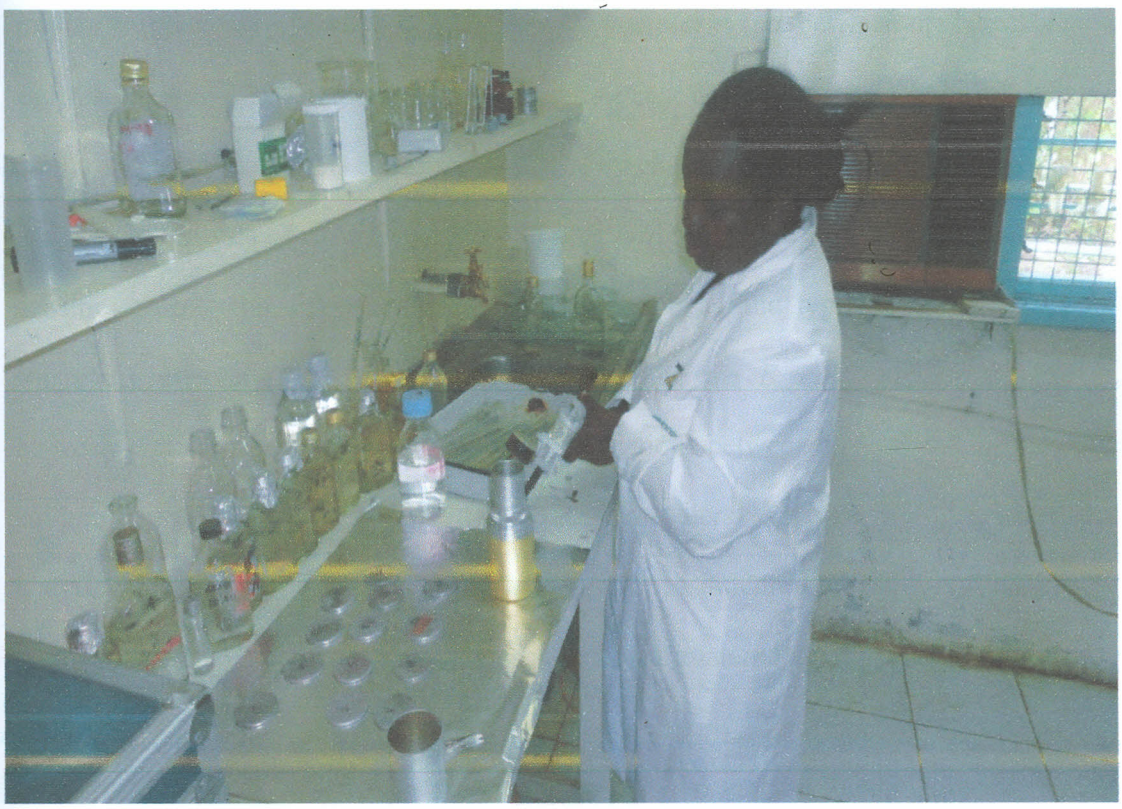

Plate 3: Analyses of water samples using Membrane Filtration Technique

3.9 Data analysis

All data was entered and stored in Microsoft excel. All analyses were performed using statistical analysis software (SAS) (v. 9.2; SAS Institute Inc., Cary, NC, USA) and *P* values < 0.05 were considered statistically significant. Unless otherwise indicated, values are presented as means ± S.D. Data were checked for normality and homogeneity of variance and log-transformed [log (x + 1)] when necessary, but only non-transformed means are reported.

A one-way analysis of variance (ANOVA) was used to compare the difference in snail abundance between the sites along the lakeshore and those in inland habitats. Post-hoc Bonferroni adjustment (where appropriate) was used to account for multiple comparisons. Comparisons for prevalence of infection between *Biomphalaria* and

Bulinus spp. and prevalence of infection for *Biomphalaria* spp. between the lakeshore and inland sites were performed using Fisher's exact test. A one-way ANOVA was used to compare the *E. coli* coliform density between protected and unprotected wells. A two-way analysis of variance (ANOVA) was used to explore variations in coliform densities among the different informal settlement areas and type of water source, whereas a one-way ANOVA was used to compare the *E. coli* coliform density between protected and unprotected wells. Associations between snail abundance and environmental/physico-chemical variables, between lateral distance from pit latrines and *E. coli* coliform density, and between water treatment frequency and *E. coli* coliform density were determined using spearman correlation (r_s).

3.10 Study approvals and ethical considerations

The study was reviewed by the Centre Steering Committee (CSC) at the Center for Global Health Research (CGHR) of KEMRI before it was forwarded for approval to the Institutional Scientific Steering Committee (SSC) of KEMRI, the National/KEMRI Ethical Clearance Committee (ERC)(SSC NO. 2091) (See Appendix 1 for study approval letter). The study was also reviewed by Maseno University. The work reported in this Thesis was part of a larger study on Community-directed interventions for schistosomes and soil-transmitted helminths in an urban setting in western Kenya. The study also sought clearances from other relevant authorities including the Municipal Council of Kisumu, Provincial and local administration, and the Ministry of Public Health and Sanitation (MOPH). In addition, Informed consent was obtained from the owner of the well or person in-charge prior to inclusion of the well into the study (Appendix 2).

CHAPTER FOUR

4.0 RESULTS

4.1 Snail species, distribution and abundance

Table 2 shows a total of 1,059 freshwater snail specimens that were collected from 81 different sampling sites. On the basis of shell morphology, 407 (38.4%) of the snails collected were identified as *Biomphalaria sudanica*, 425 (40.1%) as *Biomphalaria pfeifferi* whereas 227 (21.5%) were identified as *Bulinus globosus*. The log-transformed mean snail abundance varied significantly across the 6 informal areas ($F_{5, 75} = 4.93$, $P = 0.0006$), with Nyamasaria recording the highest abundance.

Table 2. Summary of snail species collected among the 6 informal settlement areas of Kisumu city, Kenya

Informal area	Number of sites	Snail species			Total snail abundance	Mean snail abundance [1]
		B. sudanica	*B. pfeifferi*	*B. globosus*		
Bandani	14	107	33	63	203	15 ± 14
Manyatta B	10	0	0	0	0	0
Nyalenda A	6	0	0	0	0	0
Nyalenda B	27	159	63	91	313	12 ± 13
Nyamasaria	22	141	329	73	543	25 ± 47
Obunga	2	0	0	0	0	0
Total	81	407	425	227	1059	

[1] Values are means ± SD

Table 3 presents data on distribution of snails among water bodies in Kisumu city, Kenya. Out of 81 sites surveyed, 25 were along the shores of Lake Victoria and 56 from inland habitats (dams, rivers and springs) within the informal settlements.

Table 3. Distribution of snails among water bodies in Kisumu city, Kenya

Site type	Number of sites	Snail species			Total snail abundance	Mean snail abundance[1]
		B. sudanica	*B. pfeifferi*	*B. globosus*		
Dams	6	1	9	39	49	8 ± 16^{b}
Lakeshore	25	261	80	112	453	18 ± 12^{a}
Rivers	46	145	336	76	557	12 ± 34^{b}
Springs	4	0	0	0	0	0

[1] Different lowercase letters (represented by superscripts 'a' and 'b') indicate significant difference for site type ($P < 0.05$). Values are means ± SD (rounded off to whole numbers).

As illustrated in Figure 4, the spatial distribution of snails was clustered, with few sites accounting for most of the snails. All the 25 sites sampled along the lakeshore yielded snails, with 17 sites (68%) yielding 11-50 snails. Overall, of the 81 sites surveyed, 39 (all inland sites) did not yield any snail, but notably, the 4 sites with the highest snail densities (> 51 snails) were inland sites. *Biomphalaria* and *Bulinus* snails were found at 16 and 11sites out of the 56 inland sites, respectively. Nyalenda B recorded the highest number of sites with 1-50 snails (18 sites), whereas only Nyamasaria had sites with 51-100 snails (2 sites) and 101-200 snails (2 sites) as shown in Figure 4. Sampled sites within Manyatta B, Nyalenda A and Obunga informal areas did not yield any snail.

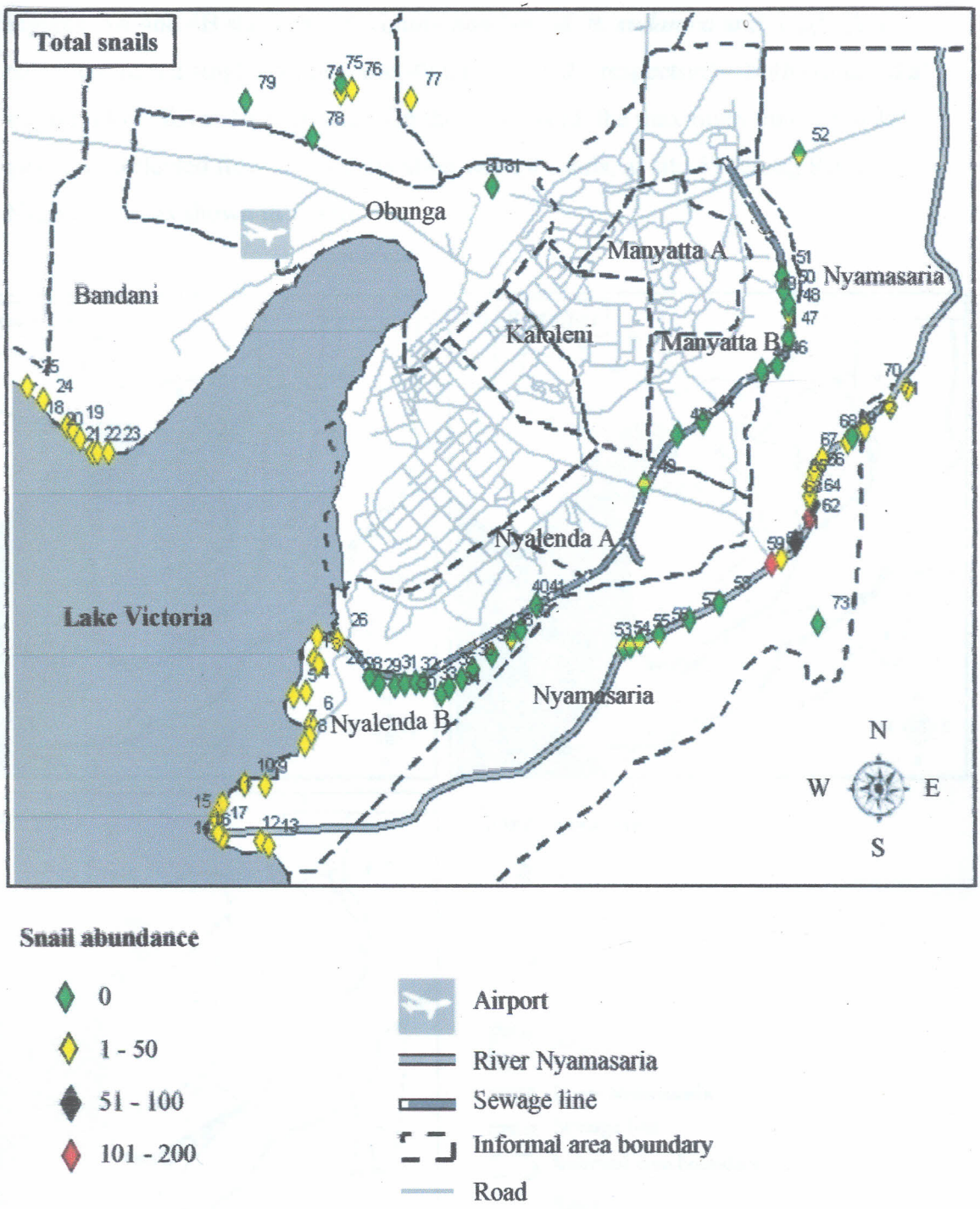

Figure 4. Abundance and distribution of *Biomphalaria* and *Bulinus* snails within the informal settlement areas of Kisumu city, Kenya. Sites are coloured by abundance.

Figures 5A and 4B show the maximum number of *B. sudanica* and *B. pfeifferi* collected from a single location was 49 and 98 snails, respectively, both collected at site 62 (along River Nyamasaria) .On the other hand, the maximum number of *B. globosus* collected from a single location was 58 snails, at site 59 (along River Nyamasaria) as shown in Figure 5C.

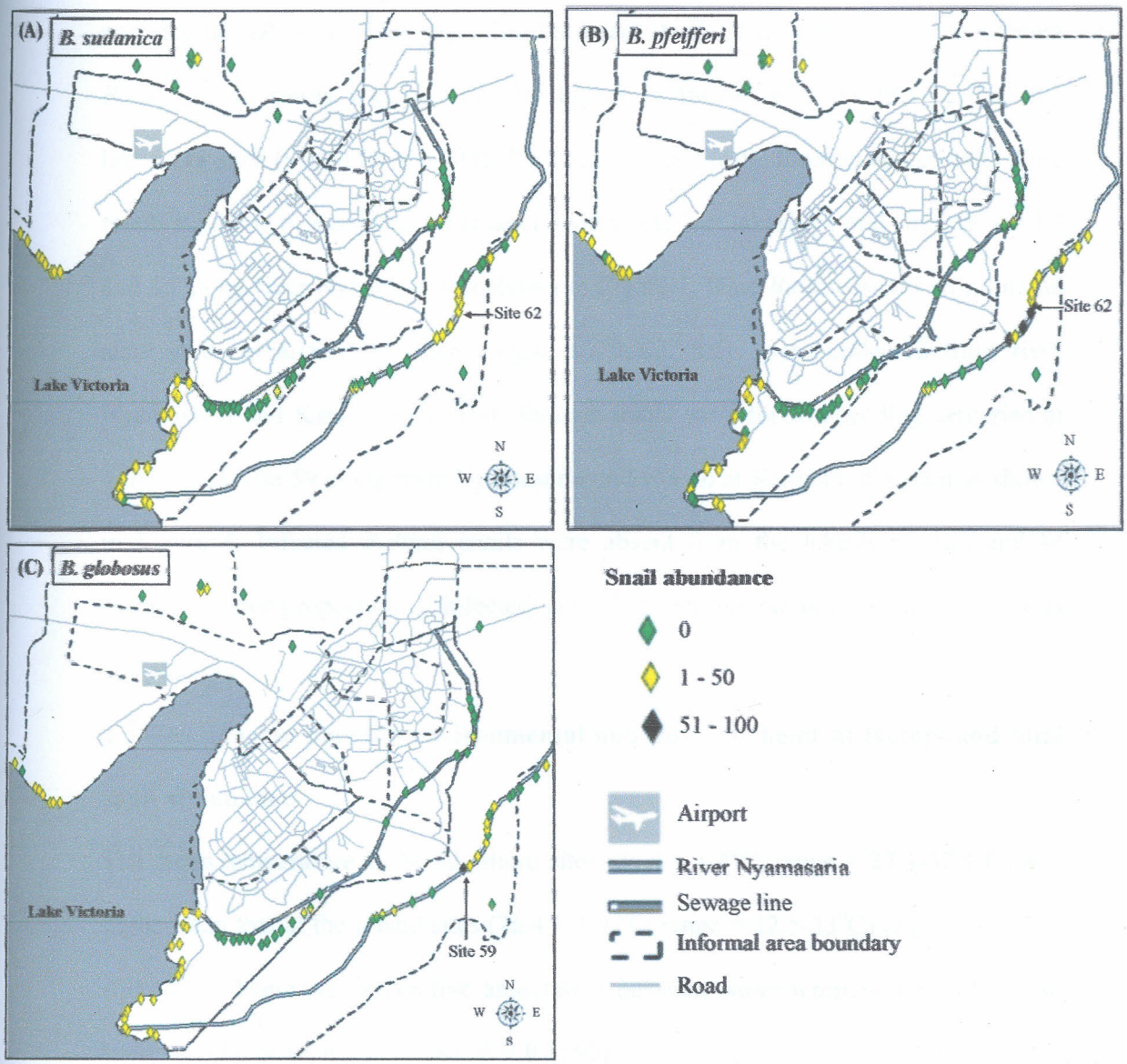

Figure 5. Spatial distribution of snails by species abundance: (A) *Biomphalaria sudanica*, (B) *Biomphalaria pfeifferi*, (C) *Bulinus globosus* within the informal settlement areas of Kisumu city, Kenya, April-May, 2011. Sites are coloured by abundance.

4.2 Infection in snails

Very few of the snails collected during the survey shed cercariae, that is only 19 (1.8%) of the snails were infected. The natural prevalence of shedding schistosome cercariae was 2% in *B. sudanica*, 1.9% in *B. pfeifferi* and 3.9% in *B. globosus*. The infection prevalence in *Bulinus* spp. (3.9%) versus *Biomphalaria* spp. (1.9%) was comparable (P = 0.0843, by Fisher's exact test). The proportion of infected *Biomphalaria* snails was significantly higher in the inland sites (3.1%) than the lakeshore sites (0.3%) (P = 0.0036, by Fisher's exact test). *Biomphalaria* snails were found shedding *S. mansoni* cercariae at 5 sites (site 5 at lakeshore and sites 59, 61, 64 and 65 along river Nyamasaria as shown in Figure 4. Nine *Bulinus* snails from inland sites shed cercariae, and interestingly, all these snails were collected from river Nyamasaria and Kanyamedha dam. *Bulinus* snails were found shedding cercariae at only 2 sites, site 59 along river Nyamasaria and site 76 at Kanyamedha dam as shown in Figure 4. Infected *Bulinus* snails were absent from the lakeshore sites and so comparison for proportion of infected snails between inland and lakeshore sites was not possible.

4.3 Relationship between environmental and physico-chemical factors and total snail abundance

The mean temperature at the lakeshore sites (29.4 ± 1.4^{0}C; range = 27.4-32.4^{0}C) was higher than that at the inland sites (26.4 ± 1.9 ^{0}C; range = 22.5-33^{0}C) ($F_{1,79}$ = 49.47, P < 0.0001). There was a positive association between water temperature and overall snail abundance (r_s = 0.3, n = 81, P = 0.0195).

The mean pH at the lakeshore sites was 8.7 ± 1.1 (range = 6.7-11.2) and 8.5 ± 1.2 (range = 5.9-11.1) at the inland sites, but the difference was not significant. There was

no correlation between pH and snail abundance (r_s = 0.02, n = 81, P = 0.8581). The pH levels varied greatly at the sites. The most common vegetation covers identified were floating macrophytes i.e. water hyacinth (*Eichhornia crassipes*) and water lily (*Nymphaea* spp.). There was no significant correlation between vegetation cover and snail abundance (r_s = 0.15, n = 81, P = 0.1804).

4.4 Distribution of water samples collected and analyzed within the informal settlements of Kisumu city

Eighty (80) water samples were collected from 7 informal settlement areas as shown in Table 4. Kaloleni was excluded from the survey since the main sources of water were kiosks selling piped-water and mobile water vendors. The unprotected wells often had no concrete slab and often the opening was not covered at all or was poorly covered with a loose lid that was not lockable. A total of 7 boreholes, 53 wells, 3 rivers, 6 dams and 11 springs were included in the study.

Table 4. Distribution of water sources surveyed among the 7 informal settlement areas of Kisumu city

Area	Water source					
	Borehole	Dam	Protected well	Unprotected well	River	Spring
Bandani	1	2	2	1	0	2
Manyatta A	2	0	4	6	0	1
Manyatta B	1	2	5	6	0	0
Nyalenda A	0	0	6	5	0	2
Nyalenda B	0	0	4	4	1	3
Nyamasaria	3	2	0	0	2	0
Obunga	0	0	6	4	0	3
Total	**7**	**6**	**27**	**26**	**3**	**11**

4.5 Comparison of fecal contamination among the public water sources by area

There was a significant difference in the total coliform density among the informal settlement areas ($F_{6,67}$ = 2.85, P = 0.0158). The total coliform density was significantly higher in Manyatta B compared to Nyalenda B (P = 0.0306) (Figure 6A). Manyatta B also had a marginally significant higher total coliform density compared to Bandani (P = 0.0523). The highest and lowest total coliform densities were

recorded in Manyatta B (15139 ± 2867 coliforms per 100 ml) and in Nyalenda B (5958 ± 1584 coliforms per 100 ml) areas, respectively (Figure 6A). Similarly, the highest and lowest fecal coliform densities were recorded in Manyatta B (3331 ± 1008 coliforms per 100 ml) and in Nyalenda B (792 ± 187 coliforms per 100 ml) areas, respectively (Figure 6B). However, the difference in the log-transformed mean fecal coliform density among the informal areas was not significant, indicating similar levels of fecal contamination across the board ($F_{6,68} = 1.46$, $P = 0.2043$).

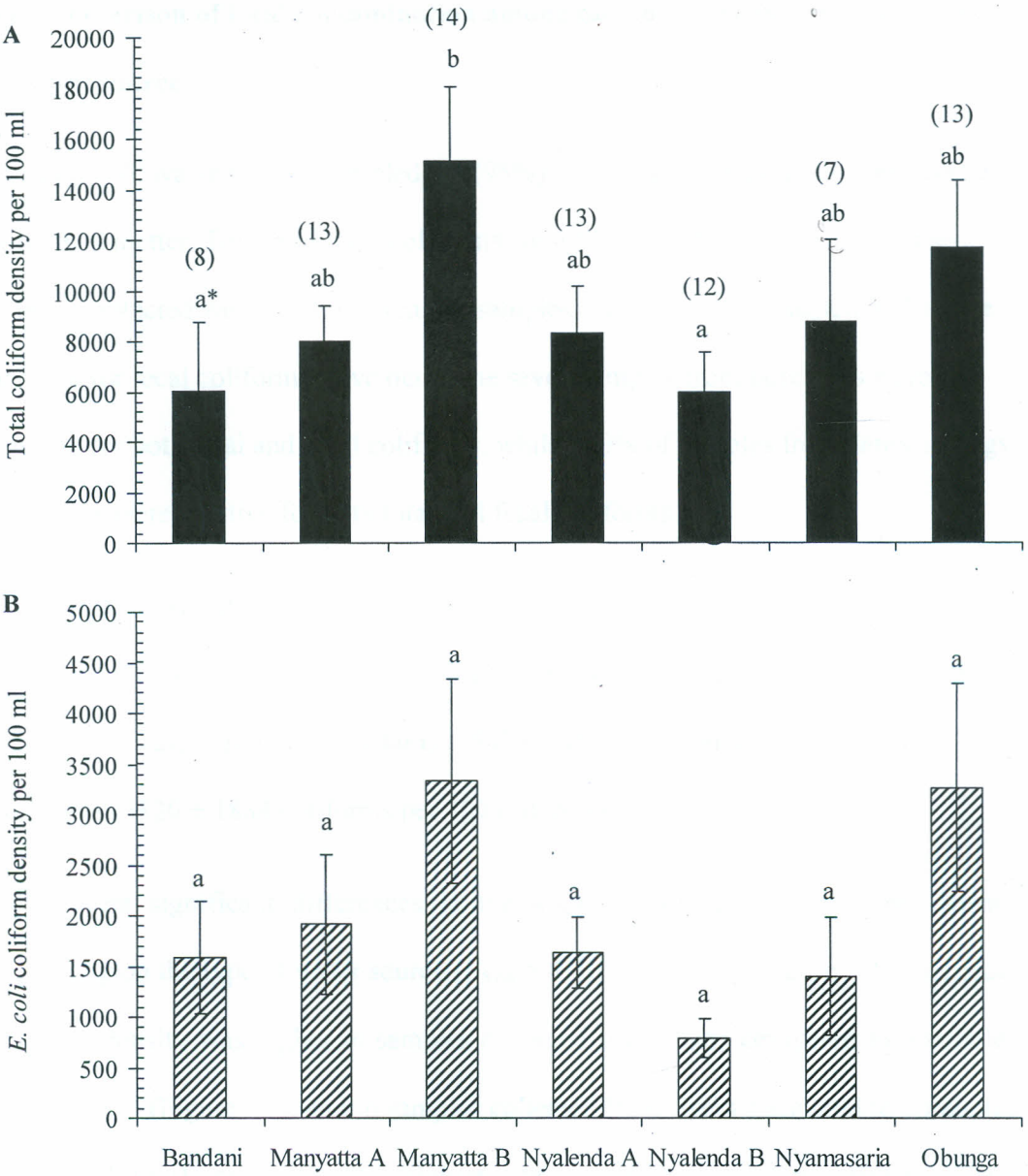

Figure 6. Comparison of Total coliform density (A) and *E.coli* density (B) among the public water sources by area. Different lowercase letters represent significant differences among areas; * indicates marginal significant difference ($P < 0.05$). Sample sizes are in parenthesis.

4.6 Comparison of fecal contamination among the public water sources by type of water source

Out of the 80 water sources sampled, 76 (95%) were found to be highly contaminated with fecal matter. Total and fecal coliforms were found in 100% of water samples from unprotected wells (26), whereas 25 samples (92.6%) from protected wells were positive for fecal coliforms. Five out of the seven samples from boreholes were positive for both total and fecal coliforms, while 100% of samples from dams, springs and rivers were positive for both total and fecal coliforms.

There were no significant differences in the total coliform density depending on the type of water source (Figure 7A). The highest and lowest total coliform densities were observed in water samples from dams (15433 ± 4524 coliforms per 100 ml) and boreholes (3820 ± 1884 coliforms per 100 ml), respectively (Figure 7A).

There were significant differences in the log-transformed fecal coliform density depending on the type of water source ($F_{5,68} = 4.01$, $P = 0.003$) (Figure 7B). The fecal coliform density was higher in samples collected from dams compared to boreholes ($P = 0.0321$) (Figure 7B), and in samples collected from rivers compared to boreholes ($P = 0.0216$) (Figure 7B). The highest and lowest fecal coliform densities were observed in water samples from dams (3800 ± 1807 coliforms per 100 ml) and boreholes (419 ± 223 coliforms per 100 ml), respectively (Figure 7B). The log-transformed fecal coliform density was higher but not significantly different in the unprotected wells (2373 ± 562 coliforms per 100 ml) compared to protected wells (1512 ± 305 coliforms per 100 ml) ($F_{1,51} = 2.32$, $P = 0.1336$).

As expected, water treatment frequency was negatively correlated with fecal coliform density for wells (r_s = - 0.31, n = 53, P = 0.0268).

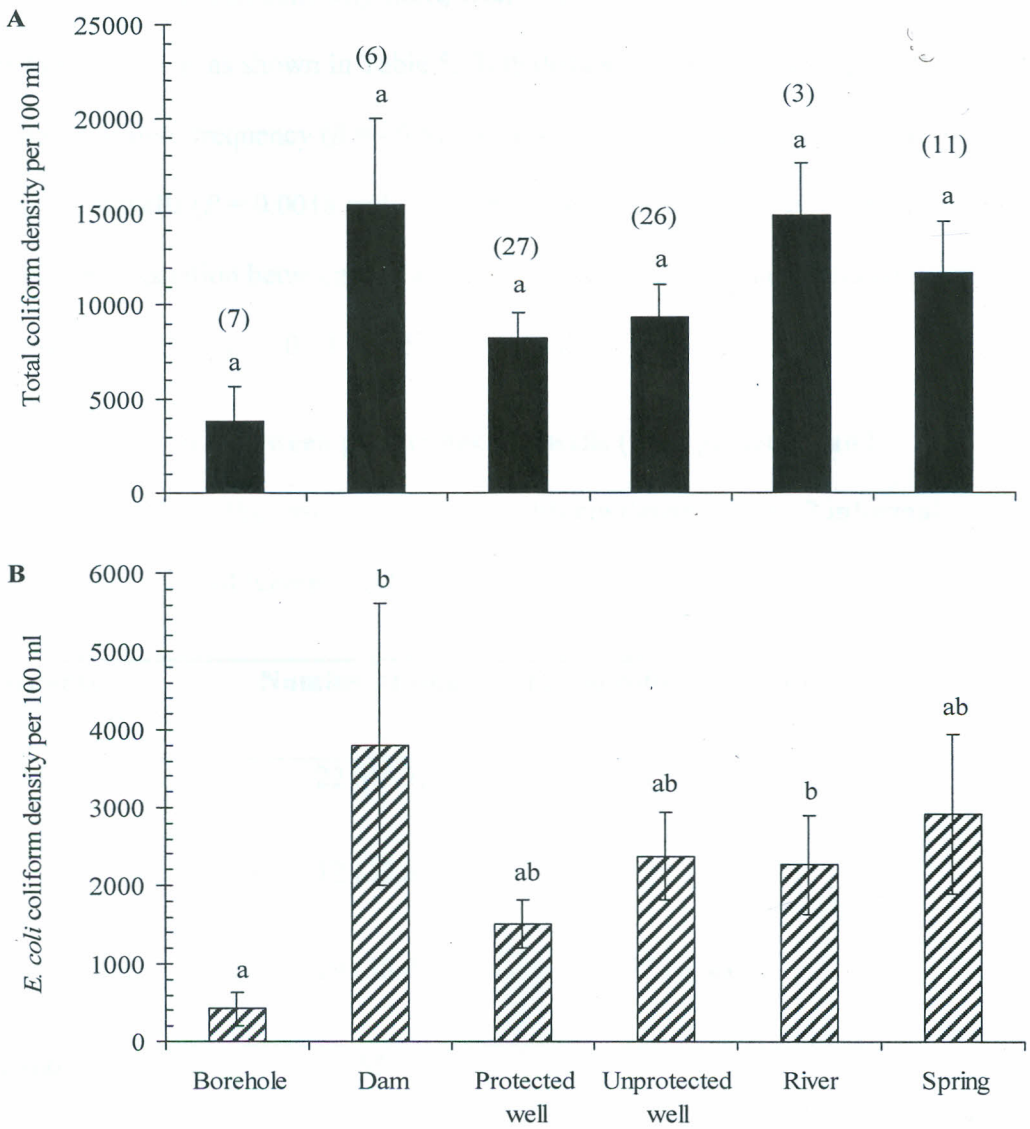

Figure 7: Comparison of Total coliform density (A) and *E.coli* density (B) among the public water sources by type of water source. Different lowercase letters represent significant differences among type of water source (P < 0.05). Sample sizes are in parentheses.

4.7 Distance between pit latrines and wells

The estimated lateral distance between the pit latrines and the wells (both protected and unprotected) was generally short, with 41.5% of the pit latrines being less than 15 m from the wells as shown in Table 5. Both distance from pit latrine (β = - 0.33) and water treatment frequency (β = - 0.61) were strong predictors of *E. coli* coliform density in wells (P = 0.0018 and P < 0. 0001, respectively). There was a significant negative association between lateral distance from pit latrines and fecal coliform density for wells (r_s = - 0.34, n = 53, P = 0.0142).

Table 5. Distance between pit latrines and wells (both protected and unprotected) and the associated *E. coli* coliform density in the 7 informal settlement areas of Kisumu City

Distance	Number of wells[1]	*E. coli* coliform density[2]
1-15 m	22 (41.5)	2646 ± 541
15-30 m	12 (22.6)	1675 ± 660
30 m and above	19 (35.8)	1275 ± 446
Total	**53**	

[1] Values in parenthesis indicate proportion (%)

[2] Values are means ± SEM

CHAPTER FIVE

5.0 DISCUSSION

This study determined the distribution and prevalence of infection in intermediate snail vectors for schistosomiasis and fecal contamination in public water sources within the informal settlements of Kisumu city, Kenya. Screening of over 1,059 snails showed that *B. sudanica* and *B. pfeifferi* were the most abundant snails, approximately 2 times more common than *B. globosus*, but the natural prevalence of shedding schistosome cercariae was similar between *Biomphalaria* and *Bulinus* spp. The spatial distribution of snails was clustered, with few sites accounting for most of the snails. *B. sudanica* and *B. pfeifferi* snails were more abundant along the lakeshore and inland sites, respectively. Lake Victoria has been known to be the main source of transmission for intestinal schistosomiasis in western Kenya (Handzel *et al.*, 2003). However, the high abundance of *Biomphalaria* and *Bulinus* spp. as well as field-caught snails shedding cercariae, together with recent findings of high prevalence of the intestinal schistosomiasis (Odiere *et al.*, 2011) confirms that besides the lake, there is local risk of schistosomiasis infection within Kisumu city. Similarly, high snail abundance and presence of infected *Bulinus* snails along river Nyamasaria (in Nyamasaria) and around Kanyamedha dam (in Bandani) are in congruence with a survey among school children (Odiere *et al.*, 2011) that showed *S. haematobium* prevalence of 3.5% and 6.8% for the two areas, respectively, and suggests that these areas are local hotspots for urogenital schistosomiasis. From the study, there was a geographic stratification for species within the city. Whereas *B. sudanica* was abundant in sites along the lakeshore, *B. pfeifferi* was abundant in inland sites, preferring smaller, man-made habitats such as dams, consistent with previous research

(Sturrock, 2001). *B. globosus* snails were also abundant in the inland sites. *B. pfeifferi* and *B. sudanica* are known to prefer shallow/swampy water, with plant detritus as a substratum (Utzinger and Tanner, 2000), whereas *B. globosus* prefers shallow water, where it may occur on bare substrata, but commonly among aquatic plants (Thomas and Tait, 1984). Preference for different environmental conditions such as abundant microflora, depth of water, oxygen content and other physico-chemical factors and natural behavioral mode of adaptation may explain why the snail species showed marked differences in each locality.

The results of this study showed a very low proportion of snails that shed cercariae. *S. mansoni* cercariae are diurnal and are typically released during daylight hours, peaking around midday and at dawn (Steinauer *et al.*, 2008), emergence times corresponding to times when their putative hosts are present in the water and available for infection. Given that this is a high schistosomiasis-transmission area, it may seem counter-intuitive that very few snails shed cercariae. However, this is not entirely new and findings in this thesis are consistent with other studies from endemic areas with high transmission, where few or none of the snails collected shed any cercariae. In a very early study (McClelland, 1956) it was noted that although 90% of school children were infected with *S. haematobium*, there were difficulties in finding infected snails. Elsewhere, in contrast to the high human prevalence of *S. haematobium* infection in Msambweni, along the Kenyan coast, the proportion of snails shedding *S. haematobium* cercariae was only 1.2% (Kariuki *et al.*, 2004). Still at the Kenyan coast, another study observed that cercarial shedding was either low (range = 0.14–3.4%) or altogether absent (Hamburger *et al.*, 2004). Previous research along Kisumu beach (one of the sites sampled in the present study) observed that cercarial shedding

was lowest during the months of February-April (Kahigi, 2000). In addition, the same study observed that the number of snails shedding cercariae at car-wash beach (another site sampled in this study) was low (between 0 and 5 snails). In the Lake Victoria basin in western Kenya, only 1.04% of snails collected at various sites shed cercariae (Steinauer *et al.,* 2008), while a recent study in Sesse Islands of Lake Victoria, Uganda, reported that none of the snails collected shed any cercariae (Standley *et al.*, 2010). Several explanations may be put forward for the absence or low numbers of snails shedding cercariae. First, it has been suggested that the percentage of infected snails may be very low or cercariae may be shed for only a limited period of time (McClelland, 1956). This, confounded by the focal nature of schistosomiasis and the complexity of sampling vast areas where snails may be dispersed, made it difficult to accurately pin-point which site would contain high numbers of infected snails. Second, snail population and abundance, infection rates and cercarial output are also under seasonal influence (Hamburger *et al.*, 1998; Kahigi, 2000; Kariuki *et al.*, 2004). Perhaps it may not be optimal for snails to shed cercariae around the peak rainy season of April-May (as occurs in Kisumu), when there may be decreased water contact activities associated with swimming and or domestic use. Third, cercarial release from field-collected snails may also be inhibited by a variety of contaminants and invertebrates harbored by the snails. For instance, besides adherence and blockage of the center whorl of the shells of *Biomphalaria*, bdelloid rotifers are known to emit a small molecular weight component that can cause a reversible paralysis of *S. mansoni* cercariae and limit cercarial release from patent snails (Stirewalt and Lewis, 1981). Fourth, it has been suggested that field snails in heavily endemic areas are subjected to pulses of infection rather than to a

continuous flow of miracidia (Sturrock *et al.*, 1979). Considering the fact that pre-patent infection can last for several weeks with only a proportion of snails reaching the stage of cercarial shedding (Joubert *et al.*, 1991), and that pre-patent infection rates can be substantial, and exceed patent infection rates (Woolhouse and Chandiwana, 1989), it is also plausible that majority of snails sampled in the current study may have had pre-patent infections. Clarification of such pre-patent infections may be done using methods such as snail crushing in search of larvae or repeated shedding in the laboratory over time, although such methods are unsuitable for accurate and large-scale monitoring. This may be necessary, especially in light of the observation that as a method, cercarial emergence (which is routinely used), severely under-estimates parasite prevalence (Curtis and Hubbard, 1990). Although it is generally accepted that finding infected snails is the only confirmation of transmission of the disease, findings from the current study suggest that a cautious interpretation of transmission, based on snail infection is necessary. Moreover, a single, brief exposure to cercariae-infested water is sufficient to effect transmission (Vercruysse *et al.*, 1994), even where the number of shedding snails is low (Mubila and Rollinson, 2002).

Environmental factors that influence snail distribution are often overlooked, despite the fact that these can vary considerably from site to site and area to area, even within short distances (Pesigan *et al.*, 1958). Among the physico-chemical variables measured in this study, water temperature appeared to be the key determinant of snail abundance. The positive association between snail abundance and high water temperature observed in the current study is in agreement with observations from Uganda that, snail distributions were restricted in the north and north-eastern parts of the country with high temperatures (Stensgaard *et al.*, 2006). In addition, it has been

demonstrated that *B. pfeifferi* grew and survived better at 25^{0}C than at 19^{0}C (Sturrock, 1966). However, in contrast to findings in this study, Kariuki *et al.* (2004) did not find any association between snail abundance and water temperature, and suggested that this may have been due to the narrow temperatures in their study. In the same study, vegetation type was significantly associated with presence of several snail species. The present study scored for presence or absence of vegetation cover and not by type.

Noteworthy was how widely the pH values varied; snails were found in sites with pH ranging from 6.7 to more than 11.0. It has been suggested that such high pH values may be caused by human contaminants such as cleaning products or may be attributable to the acquisition of H+ ions as occurs during photosynthesis at daylight hours (Standley 2008). The absence of association between pH and snail abundance observed in the study has also been reported previously (Kahigi, 2000), and suggests that pH may not be a key determinant of snail abundance, as is the case with other freshwater organisms (Macan, 1974).

This study found significant levels of *E. coli* contamination in water sources, within informal settlements of Kisumu city. Out of the 80 samples analyzed in the study, only 4 samples (5%) conformed to the WHO drinking water standard of zero thermotolerant colony forming units per 100 ml (WHO, 1997), with 95% of the water sources sampled in this study contaminated with *E. coli* bacteria. The high level of fecal contamination observed in this study is, therefore, not surprising, especially since *E. coli* is a definitive indicator of human or animal fecal pollution (WHO, 1997). These results are consistent with those from previous research in Langas, an urban slum in Eldoret municipality, Kenya, where 97% of the samples were highly

contaminated with fecal matter (Kimani-Murage and Ngindu, 2007) and also from another study in Manyatta and Migosi estates within Kisumu city (Orwa, 2000). A recent study reported *E. coli* contamination in 100% of a sub-sample of fish (*Rastrineobola argentea*) sold in six markets within Kisumu city (Sifuna *et al.*, 2008), further supporting the high level of environmental fecal contamination. Although large quantities of fecal coliform bacteria in water are not necessarily harmful (Doyle and Erickson, 2006), high levels of fecal contamination in this study may serve as a warning to public health officials of the elevated risk of pathogens in the water and subsequently the potential outbreak of water-borne gastroenteritis within these informal areas. Similarly, STH infections often propagate in communities of low socio-economic status secondary to contamination of the soil and water supply with human feces. Recent findings of 16% prevalence for STH infections within these informal settlement areas seem to be in accord with this phenomenon. Comparison of *E. coli* density between protected and unprotected wells revealed a higher density in the latter (albeit non-significant), suggesting that physical barriers such as concrete covers and use of concrete slabs to line the walls of wells may confer some protective benefits from surface run-off contaminated with fecal material. However, such benefits may not be realized in areas where the water table is high and where wells are in close proximity to pit latrines since contamination of wells may likely occur from underground seepage more than from surface run-offs. From this study, it was also shown that the lateral distance from pit latrines was inversely associated with *E. coli* density, consistent with findings from previous research in an urban slum (Kimani-Murage and Ngindu, 2007). Where the use of pit latrines and ground water co-exist, the commonly used guideline is that the well should be located in an area higher than

and, at least, 15 m from the pit latrines and should be at least 2 m above the water table (Kimani-Murage and Ngindu, 2007). Unfortunately, the water table in Kisumu city is quite high, that is, about 2-3 m deep (Orwa, 2000), increasing the chances of contamination through underground seepage. Therefore, close proximity between the wells and pit latrines coupled with the high water table in Kisumu city are a major source of contamination of the wells with fecal matter.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. The findings from this study have indicated that besides Lake Victoria, active transmission of schistosomiasis occurs from inland habitats within the informal areas of Kisumu city and in streams that flow into the Lake, and that transmission patterns are closely related to the abundance and spatial distribution of host snails.

2. The study has shown that there is geographic stratification of species within the city, with *B. sudanica* being abundant in sites along the lakeshore, whereas *B. pfeifferi* predominates in inland sites.

3. Among the physico-chemical variables measured in this study, only water temperature had positive association with snail abundance.

4. This study has also demonstrated that the main water sources were highly contaminated with *E. coli* (an indicator of fecal contamination) and that pit latrines were a major source of this contamination.

6.2 Recommendations

1. The high abundance of *Biomphalaria* and *Bulinus* spp. and confirmation of cercarial shedding in snails within the informal settlements of Kisumu calls for inclusion of focal molluscicide, improvements in local sanitation and hygiene as well as public health awareness in prospective control interventions

to complement chemotherapy in reducing transmission and re-infection in these settings.

2. Well-water may not be suitable for human consumption, and its continued use could increase and constitute a major health risk for the inhabitants of slum areas within Kisumu city because of the high fecal contamination. Strategies are needed to ensure that the massively-increasing number of urban residents have optimal access to adequate and safe water for domestic purposes.

3. A routine system of water-quality control for the wells is, therefore, needed throughout the City, for instance through the Municipal water treatment plants that will monitor drinking and surface water for the presence of fecal contamination.

4. The quality of domestic water may be improved by boiling or by treating with chlorine. In addition, personal hygiene and care such as washing thoroughly with soap after contact with contaminated water may also help prevent infections.

5. Strengthening of water and sewerage infrastructure in the informal settlement areas by the government may improve quality of life, as water quality is closely linked to the level of economic development and overall well being.

6.3 Recommendations for future research

1. In this study snails were sampled on a single day at each site. Given the known local and seasonal variations in snail abundance in the field (Kahigi, 2000; Kariuki *et al.*, 2004), future surveys may be enhanced by sampling snails for,

at least, two consecutive days and at different times of the year for more precise snail abundance determination.

2. Future studies may benefit from use of more sensitive molecular techniques, like PCR, to verify identity of snails and cercariae since in many endemic areas, animal and human schistosomes may appear together in the same transmission sites, necessitating species identification of the schistosome cercariae.

3. Administering questionnaires to obtain information, for instance, on the type of toilet facility used, major source of domestic water, method of human waste disposal, whether drinking water was boiled, and the perceptions of possible sources of water contamination in the area may be of benefit to future studies.

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