2020-02

Ecology of bulinid snail intermediate hosts and transmission of schistosoma haematobium among school aged children in Shinyanga district, Tanzania

Angelo, Teckla

ECOLOGY OF *BULINID* SNAIL INTERMEDIATE HOSTS AND TRANSMISSION OF *SCHISTOSOMA HAEMATOBIUM* AMONG SCHOOL AGED CHILDREN IN SHINYANGA DISTRICT, TANZANIA

Teckla Angelo

A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Life Sciences of the Nelson Mandela African Institution of Science and Technology

Arusha, Tanzania

February, 2020

ABSTRACT

This study investigated transmission of *Schistosoma haematobium* through longitudinal parasitological, malacological and human water contact surveys. Urine samples collected from school children were examined for *S. haematobium* infection using urine filtration method. Snail samples collected were examined for patent schistosome infections by microscopy. Multiplex PCR assessed pre-patent infections and differentiated *S. haematobium* from *S. bovis*. Water contact questionnaire, focus group discussion and semi structured interviews explored community knowledge on schistosomiasis. Pre-treatment prevalence of *S. haematobium* infection among school children was 34.8%. Prevalence of *S. haematobium* infection was higher in older children (12–14 years) compared to younger children (6-11 years) ($p<0.001$) with no significant variation one-year post-treatment. Boys were more infected than girls. No spatial association was observed between children's infection and the distance from child's home to the nearby snail habitats. Integration of malacological surveys linked with GPS data detected spatial association between children living in households next to ponds with high *B. nasutus* having the highest prevalence of *S. haematobium* infection. From 6202 *Bulinus nasutus* collected, 190 (3.06%) had patent infections. Rainfall pattern had significant impact on snail population density. Water conductivity (OR 1.23; 95%CI 1.13-1.34; $p<0.0001$) and vegetation (OR 6.84; 95%CI 2.75-16.99; $P<0.0001$) were significantly associated with snail population abundance. Increase of conductivity in snail habitats with vegetation reduced snail densities significantly (OR 0.76; 95%CI 0.68-0.86; $P<0.0001$). Increase of water temperature was associated with patent infection in pond habitats (OR 0.35; 95%CI 0.45-0.62; $P<0.0001$) but not rivers. Other physico-chemical parameters were not significantly associated with snail abundance. Out of 1898 *B. nasutus* snails for which DNA was extracted, 100 (5.17%), 291 (15.07%) and 16 (0.84%) were *S. haematobium, S. bovis* and *S. haematobium/S. bovis* co-infected, respectively. Water sources shared between humans and livestock had significantly higher *S. haematobium* (OR 2.53; 95%CI 1.59-4.05; $p<0.0001$) and *S. bovis* (OR 2.29; 95%CI 1.53-3.45; $P<0.0001$) infections. Wet season was associated with significant reduction of *S. bovis* infection (OR 0.17; 95%CI 0.09-0.32; $P<0.0001$). Molecular approach, malacological and a parasitological survey when tied together detect specific schistosome species transmitted. Measures for schistosomiasis control should take into account integrated strategies for disease elimination.

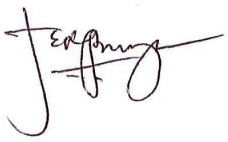
DECLARATION

I, Teckla Angelo do hereby declare to the Senate of Nelson Mandela African Institution of Science and Technology that this dissertation is my own original work and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

Teckla Angelo  **19th February, 2020**

Name and signature of candidate **Date**

The above declaration is confirmed

Prof. Joram Buza (PhD)  **19th February, 2020**

Name and signature of supervisor 1 **Date**

Dr. Safari M. Kinung'hi (PhD)  **19th February, 2020**

Name and signature of supervisor 2 **Date**

COPYRIGHT

CERTIFICATION

The undersigned certify that they have read and hereby recommend for examination of a dissertation entitled, "Ecology of *Bulinid* snail intermediate hosts and transmission of *Schistosomahaematobium* among school aged children in Shinyanga district, Tanzania" in fulfillment of the requirements for the Degree of Doctor of Philosophy in Life Sciences and Bioengineering (LiSBE) at Nelson Mandela African Institution of Science and Technology (NM-AIST).

Prof. Joram Buza (PhD) **19th February, 2020**

Name and signature of supervisor 1 **Date**

Dr. Safari M. Kinung'hi (PhD) **19th February, 2020**

Name and signature of supervisor 2 **Date**

ACKNOWLEDGEMENT

I would like to thank the Almighty God for his grace and blessings in achieving this work successfully.

This research work was carried out within the framework of scientific research partnership between the Department of pathology, University of Cambridge, United Kingdom's, the National Institute for Medical Research (NIMR) Mwanza Centre, Tanzania and the Nelson Mandela African Institution of Science and Technology (NM-AIST) Arusha, Tanzania. Many people were involved in this work in various ways such that their contribution in one way or another lead to the achievement of this work, therefore their support is valued and highly acknowledged.

My sincere thanks are addressed to my supervisors Professor Jorum Buza (School of Life Sciences and Bioengineering, Nelson Mandela African Institution of Science and Technology (NM-AIST) Arusha), Dr. Safari Kinung'hi (Centre Director of Mwanza Medical Research Centre) and to my mentors Dr. Shona Wilson (Project leader Department of Pathology University of Cambridge United Kingdom), Professor Henry Curtis Kariuki (Malacologist, School of Medicine and Health Sciences, Kenya Methodist University) and Dr. Joseph Mwanga (Social scientist, National Institute for Medical Research Mwanza Centre). Obviously these people were responsible for the design of the study and were constantly available for discussion either physically, email or through Skype. They consistently provided the support that was needed to accomplish this work. The friendship and kindness I experienced from Dr. Shona Wilson and her family while in Cambridge was outstanding.

I gratefully acknowledge the support given by Shinyanga district council who allowed this study to be conducted in the district. Special thanks are addressed to Shinyanga education officers Ms. Marry Maka and Ms. Irene Kisweka who visited our study sites regularly during the entire study time. The support they provided is highly appreciated.

This work would have not been possible without the support from laboratory technicians from the National Institute for Medical Research, Mwanza centre. Mr. Philbert Kashangaki and Ms. Paulina Ndaboya who processed over 2500 urine specimen through the filtration method and examination of *S. haematobium* eggs under light microscope. Ms. Happyness Charles for registration of study participants, Mr. James Kubeja, Mr. Revocatus Alphonce, Ms. Yasinta Sylivester, Mr. Sabato Misana for snail collection and examination of patent

infections among *Bulinid* snail intermediate hosts, Mr. Henry Kabogo, Mr. Yusuph Abdulkheri, Mr. Kelvin Chirstopher and Ms. Justina Mosha for administration of questionnaires, Semi structured interview (SSI) and Focus group discussions (FGD), Ms. Ziada Kiwanuka for snail DNA extractions, Mr. Eric Lyimo and Ms. Doris Mangalu for Orientation of Molecular approach on DNA extraction and Mr. Reuben Bugumba who utilized his driving expertise to ride the research team to and from field work. Their support was incredible and I will never forget their tirelessly hard working abilities in different situations even in extra hours.

This work would not have been possible without strong collaboration of school children, teachers and village executive officers Mr. Salehe Manota from Nduguti village and Mr. Castory Salu of Ikingwamanoti village who cordially received us, their outstanding cooperation led to the success of this study.

In the University of Cambridge department of pathology, I would like to thank Mr. Jakub Wawrzyniak and Mr. Jamie Ash Croft who supported household mapping in relation to snail collection sites. Their support is highly appreciated.

Tremendously, I would like to extend my special thanks to my beloved husband Henry Kabogo for his incredible support and encouragement during the entire study period. He was responsible in taking care of our family during my field work and long distance travel. Really this success would have not been possible without him. I am grateful to my sons Mtagaya, Tagata and Mtulwa for their patience and tolerance for my absence during the study period.

Last but not least, I am sincerely grateful to my parents particularly my mother's Susana Bunzali and Tabu Mtagaya for their moral support and taking care of me and my young child. I have nothing to pay back but asking the Almighty God to extremely bless you all.

This study received financial support from The Leverhulme-Royal Society Africa Award - Phase II Round 3, grants number AA130107, awarded jointly to Dr. Shona Wilson and Dr. Safari Kinung'hi and formed part of my PhD training programme.

DEDICATION

To my father the late Angelo Ndaki

My mother Susana Bunzali AND to my husband Henry Kabogo

And our three sons Mtagaya, Tagata and Mtulwa

.....Bright future is nurtured.....

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND SYMBOLS

AIC	Akaike Information Criterion
AIDS	Acquired Immune Deficiency Syndrome
CI	Confidence interval
DNA	Deoxyribonucleic acid
FGD	Focus Group Discussion
GPS	Global Positioning System
HIV	Human Immune Virus
HBREC	University of Cambridge Human Biology Research Ethics Committee
MRCC	Medical Research Coordination Committee
OR	Odds ratio
Rho	Spearman's rank correlation coefficient
NIMR	National Institute for Medical Research
PCR	Polymerase chain reaction
SCI	Schistosomiasis Control Initiatives
SSA	Sub Saharan Africa
SSI	Semi structured Interview
TDS	Total dissolved solids
WASH	Water Sanitation and Hygiene
WHO	World Health Organization

CHAPTER ONE

Introduction

1.1 Background information

1.1.1 Global burden of schistosomiasis

Schistosomiasis is a debilitating disease caused by digenetic trematodes from the genus *Schistosoma* (Gryseels & Polman, 2006a). The disease is estimated to affect more than 230 million people worldwide (Vos *et al*., 2012), while more than 500 million people are at risk of infection (Steinmann, Keiser, Bos, Tanner & Utzinger, 2006a; Weerakoon, Gobert, Cai & Mcmanus, 2015). There are three major schistosome species that affect the human population globally: *S. japonicum* which is distributed in Indonesia, Philippines and in the people's Republic of China, and *S. mansoni* and *S. haematobium* that are mainly distributed throughout Africa, and the middle East, and in some countries of south America. In Brazil, Venezuela, Suriname and some Caribbean islands transmission of *S. haematobium* does not occur (Gryseels & Polman, 2006b). Other schistosomes species that are of local importance to human populations are *S. intercalatum* and *S. mekongi* which respectively are causative of intestinal schistosomiasis in West and Middle Africa and the Southeast of the Mekong river basin in Asia (Gryseels & Polman, 2006) (Fig. 1).

The burden of Schistosomiasis infection concentrates in sub Saharan Africa where there is inadequate sanitation, water supply, lack of health awareness and poor living conditions (WHO, 2014). It is estimated that of the total global burden of schistosomiasis, 93% occurs in Sub Saharan Africa where the disease ranks second amongst those caused by parasitic infections, following malaria (Hotez & Kamath, 2009; Marieke *et al*., 2003; Steinmann *et al.*, 2006b). Based on WHO schistosomiasis reports, it is estimated that of the total world schistosomiasis burden 60% is confined within 10 African countries (WHO, 2012), while in some countries, including Burkina Faso, Cambodia, China, Egypt, Mauritius and Morocco, the disease has been minimized or the transmission cycle completely interrupted through intensive multi-faceted control strategies (Fenwick, Webster, Blair & Fleming, 2009; Muth, Sayasone, Odermatt-biays, Phompida, Duong & Odermatt, 2010; Sinuon *et al*., 2007; WHO, 2010, 2014). Tanzania is one of the 10 African countries that is carrying the major schistosomiasis burden.

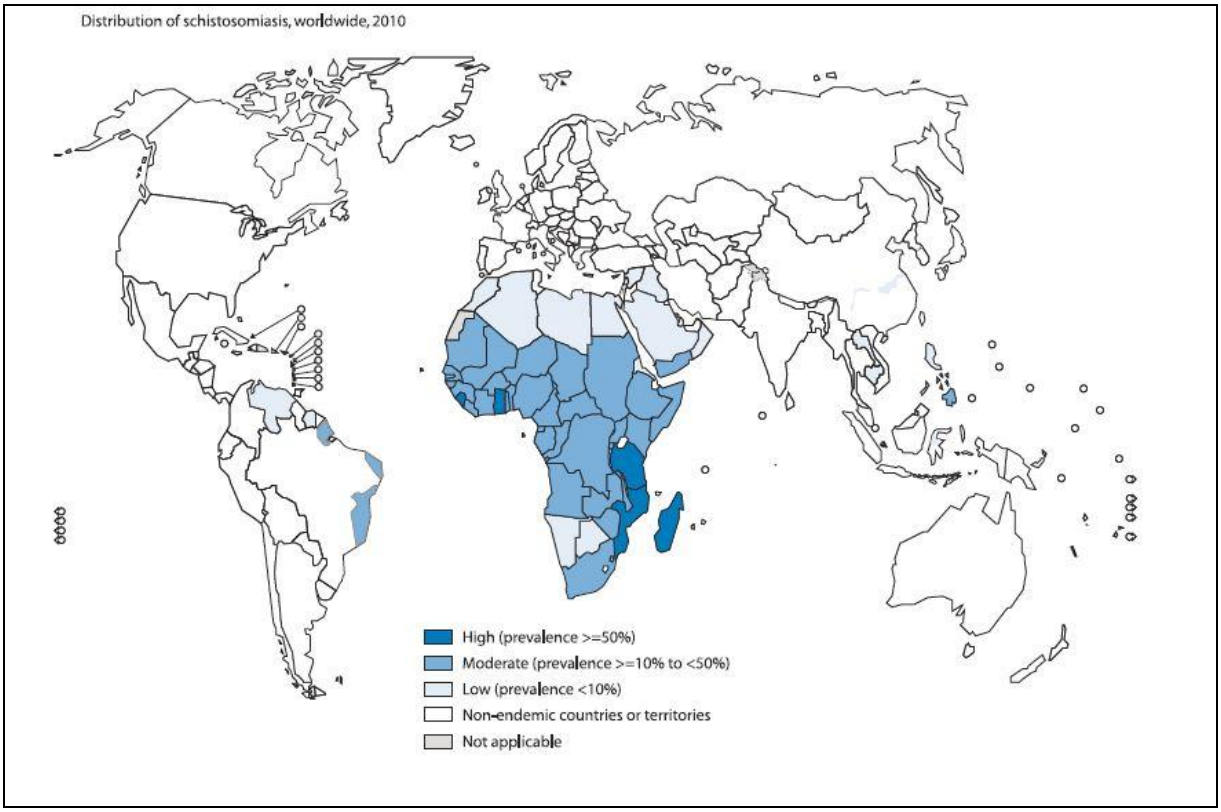


Figure 1: Global distribution of Schistosomiasis transmission foci, displaying high infection levels in Sub Saharan Africa (WHO, 2011)

1.1.2 Life cycle of members of the Schistosoma genus

The genus *Schistosoma* contains digenetic trematodes of the family *Schistosomatidae*. Members of the genus Schistosoma can infect man and a variety of ruminants as well as avians, with different members of the genus displaying different restrictions in their definitive host range. The parasites require two hosts to complete their lifecycle, developing into stages infective to their definitive hosts within different Gastropods. *Schistosoma japonicum* develops in snails of the genus *Onchomelania*, *S. intercalatum* and *S. haematobium* develop in *Bulinus* snail species, *S. mansoni* in the genus *Biomphalaria* and *S. mekongi* in the genus *Tricula* (Sturrock, 2001).

Free living larval stages of the parasite, the miracidia, link the definitive and intermediate hosts. Hatching of the miracidium from eggs released into the environment from the definitive host is stimulated by light and temperature (10-30 °C), and occurs when the egg is

in contact with a conducive fresh water environment. The ciliated miracidia swim to locate compatible snail hosts, utilising a behaviour stimulated by macromolecular glycoproteins (Kömer, Haas & Haberl, 1995) and chemicals secreted by potential snail hosts (Donnell, 2016a). Miracidia enter the snail body via the snail's foot, in a process aided by lytic enzymes, and form non motile mother sporocysts. Soon after penetration, these mother sporocysts produce mobile daughter sporocysts that migrate to the digestive glands and ovotestis of the snail (Donnell, 2016b). While in the snail body, daughter (or secondary) sporocysts reproduce asexually, resulting in numerous cercariae. Cercariae gain maturity in a period of 5 to 6 weeks after penetration of the snail by the miracidium (Donnell, 2016) and leave the snail body through the body edges and mantle.

Free-living in fresh water, the schistosome cercariae have a bifurcated tail to aid swimming, are non-feeding and are short lived. Within a period of 36-48 hours of release from the intermediate host, cercariae need to locate a suitable definitive host. When human-infective cercariae locate their definitive host they penetrate the epidermis of the skin very quickly, in just 2 minutes they start their transformation from freshwater cercariae into intra-host schistosomula.

This sudden morphological change of the schistosome into schistosomula consists of loss of the parasite's tail and series of structural modifications that allow establishment of the parasite within its new intra-host environment. The schistosomula is transferred to the heart through the lymphatic system and then to the lungs after 3-4 days of infection. From the lungs schistosomula migrate to the hepatic sinusoid of the liver where they develop, and attain maturity after 31 days (Fig. 2). Adult schistosomes vary in size and width, with the female schistosome being 1.4 cm long, and she is thin, with a width of 0.016 cm, compared to a dorsoventrally flatted male schistosome whose length is 1cm and width 0.11 cm. Schistosomes are dioecious. In the liver schistosome worm's pair up, the female worm becoming partially but permanently enclosed by a male schistosome in his gynaecophoric canal. From the liver, the paired schistosome couple moves to their definitive site, this varies between species. For *S. haematobium*, causative agent of urogenital schistosomiasis, this is the Venus plexus. Here the male attaches to the vascular wall via his suckers, resulting in a niche where there is plentiful access to the oxygen and food required for copulation and egg production. The female worm can lay 20-300 eggs per day (Utzinger, 1999). The eggs leave the blood stream, migrating into the walls of the urinary bladder by mechanical or enzymatic

means and are voided in urine by the host to start life cycle (WHO, 2012). However, it is estimated that half of the eggs fail to escape the urinary bladder, becoming lodged in the host tissues, where they are responsible for inflammation, haemorrhages and pseudopolyposis. In advanced stages, calcification of the eggs and associated fibrosis of the bladder wall may result in occlusion of the ureter, which in turn can lead to hydroureter, hydronephrosis, kidney failure and stasis. The long-term inflammation of the bladder wall can also lead, in severe cases, to squamous cell cancer of the bladder (Barsoum, Esmat & El-Baz, 2013; Gryseels & Polman, 2006). The process of successful cercariae penetration to commencement of egg laying takes between 30 and 40 days.

Schistosome egg are species specific, allowing differentiation between infecting species based on the egg size, the position of spines on the eggs produced by the female schistosomes worms and by the final location within their definitive hosts, determined by the excreta in which they are present. *Schistosoma intercalatum* and *S. haematobium* have oval shaped eggs with the size range between 140-170 µm and have terminal spines, *S. japonicum* and *S. mekongi* lay diminutive eggs with vestigial spines with 50-90 µm size range, while *S. mansoni* eggs have lateral spined eggs (Donnell, 2016; Utzinger, 1999; WHO, 1994; Loker, 1981). Using this morphological discrimination of infecting schistosomes, only *S. haematobium* is considered a widely distributed parasite in Tanzania, with *S. mansoni* displaying a more restricted geographical range (Brooker *et al.*, 2011; Clements, Barnett, Nyandindi, Lwambo, Kihamia & Blair, 2008).

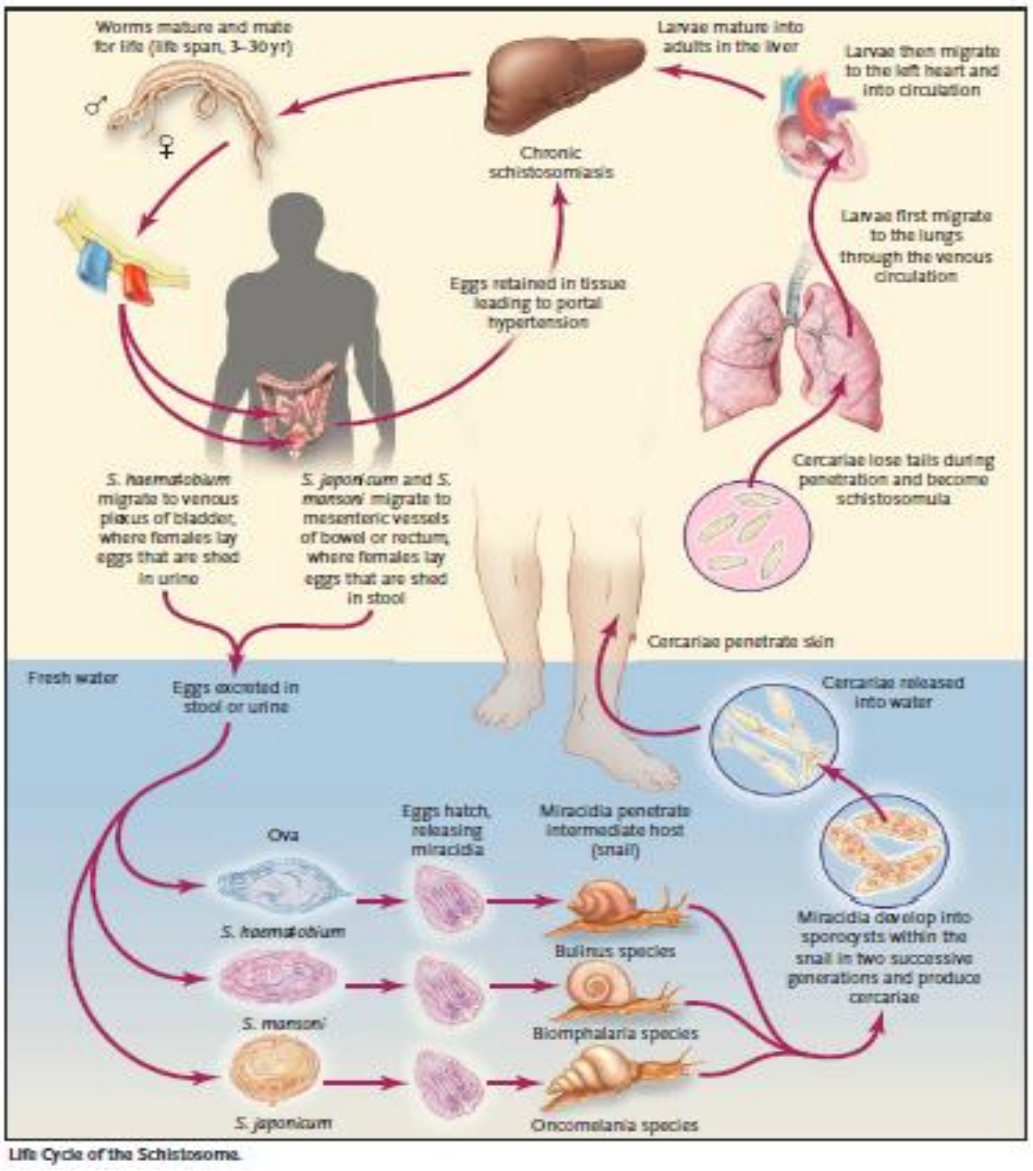


Figure 2: Life cycle of members of the *Schistosoma* genus: (adapted from Charles King, 2009)

1.1.3 Transmission of human schistosomiasis

Due to nature of the schistosome life-cycle, schistosomiasis transmission occurs mainly in poor communities with poor sanitation and where access to safe and clean water is limited, such that frequent search of water by the human population is inevitable (Secor, 2014; WHO, 2014). Transmission takes place when schistosome infected people defecate or urinate in or close to freshwater sources that provide a niche for the intermediate snail host, contaminating the water with schistosome eggs, from which hatch the miracidia (Donnell, 2016). In turn, people contract the disease when the schistosome larval stage (cercariae) released by snail intermediate hosts penetrate the skin of people who are in direct contact with the infested

water during activities such as fishing, irrigation, swimming, bathing, playing, laundry and water collection for their domestic use (Anto *et al.*, 2013).

Transmission is also significantly associated with the population numbers of the intermediate host (Mas-coma, Valero & Bargues, 2009). Population dynamics of snail intermediate hosts are affected by different environmental conditions that exist in particular environmental settings. The population of *Bulinid* snails is effected by season. A state of being wet or dry of the water sources contributes significantly to the determination of snail population densities (McCullough, 1962). Therefore within micro-geographical settings the distribution of *Bulinus* snails is determined by rainfall patterns (Kariuki *et al*., 2004). However, during high waters the rainy season, snails can be washed away by running water thus reducing their population density (Abe, Ombugadu, Oluwole & Mogaji, 2017). The nature of the waterbody can therefore interact with observed seasonality of snail population numbers.

Some environmental factors may favour snail distribution while others do not. Previous studies have documented that vegetation cover has an impact on snail population densities (Dazo, Hairston & Dawood, 1966). Snails are able to accept different ranges of chemical factors; pH, total dissolved solutes (TDS), conductivity and salinity of water sources have been reported to not have considerable impact on presence or absence of snail intermediate hosts (Allan *et al.*, 2017; Knopp *et al*., 2013). Water turbidity has no direct impact on snail density but may affect development of algae food sources for snails if high turbidity persists (Hubendick, 1958). The transmission patterns of *Schistosoma haematobium* therefore tend to be focal, and significantly influenced by water body vegetation and by season (Klumpp & Chu, 1977a).

High temperatures cause snail mortality while at low temperatures snails become inactive. The range of temperatures at which *Bulinus* snails survive and reproduce is between 10°C and 31°C (Kalinda, Chimbari & Mukaratirwa, 2017a). In addition, a temperature range of 22°C -27°C is an important factor for cercarial release by snail intermediate hosts (Rollinson *et al*., 2001a; Shiff, 2017).

1.1.4 Diagnosis of human schistosomiasis

In order to quantify the disease burden in a particular geographical settings sensitive and specific diagnosis is crucial. Different parasitological diagnostic approaches that are robust and simple are available to diagnose schistosome infections. In addition to parasitological

assays, immunodiagnostic for people infected with schistosomiasis by detection of antibody or antigen has been developed (Steinmann, 2008). Furthermore detection of schistosomiasis pathology through ultrasound imaging is available especially in medical settings to clearly specify the impact of schistosomiasis infection (WHO, 2014). The diagnostic methods applied in this study were based on human parasitology investigation via urine filtration.

For examination of terminally spined *S. haematobium* eggs, urine samples need to be collected between 10 hours in the morning to 14 hours in the afternoon, the time with the highest egg counts of the day. Ten (10) milliliters of urine sample is filtered in a polycarbon or polyamide (Nytrel) membrane stained by 5% stock solution of lugol's Iodine. The filter membrane is examined by microscopy for presence of terminally spined eggs. Quantification of eggs is based on the 10 ml of urine examined (WHO, 1999, 2011).

1.1.5 Schistosomiasis control and prevention

Currently, the major strategy for schistosomiasis control adopted by the international community is preventative chemotherapy using the safe and efficacious drug praziquantel. The recent World Health Organization 2020 Roadmap on Neglected Tropical Diseases (NTDs) recommends implementation of policies and strategies, setting out the global plans to combat Neglected Tropical Diseases and encourages the community of partners to maintain and expand their commitments to overcome schistosomiasis (WHO, 2012). The WHO prioritized programme of annual chemotherapy has indicated positive results in many countries, with high country-level response rates of chemotherapy administration for schistosomiasis control. In 2017, 88.3% of all praziquantel treatment delivered worldwide was received within African countries while globally 98.7 million people from reporting countries received praziquantel treatment (WHO, 2018). The treatment coverage among school aged children has increased from 54.3% in 2016 to 68.0% in 2017, representative of an increase of 9.4 million treatments (WHO, 2018).

School-aged children constitute the group most at risk, with infection levels peaking in early adolescence (Colley, Bustinduy, Secor & King, 2014), along with adults with occupational activities that involve prolonged or regular water contact (Black, Steinauer, Mwinzi, Secor, Diana & Colley, 2010). Control programmes that are currently being implemented, mostly provide treatment through schools (Burnim, Ivy & King, 2017), but are extended into the community when heavy transmission among adults also occurs (Burnim *et al*., 2017; Leslie *et al*., 2011).

Despite the successes in providing treatment and the reduction in infection intensities, persistence in disease prevalence in the majority of the endemic countries has been observed (French *et al.,* 2015). Maintenance of high infection levels is particularly true from countries within Africa who do not reach the 75% coverage of treatment recommended in the WHO roadmap to control schistosomiasis (WHO, 2012). Some countries do not even have control pogrammes and reaching the people most at risk is a great challenge such as Chad and Syria where political conflicts affects control program (Sokolow *et al*., 2016). Furthermore non-compliance and missing drug treatment phases of targeted groups complicates the success of preventive chemotherapy (Secor, 2016). While exclusion of pre-school children and adults from treatment maintains schistosomiasis transmission (Secor, 2016), the expansion of snail intermediate host populations introduces the disease into new areas (Yang *et al*., 2018). Due to this, there are discrepancies in disease control and burden reduction between Sub Saharan Africa countries, and non-African countries (Engels, Chitsulo, Montresor & Savioli, 2002). Learning from the countries who have managed to reduce the burden of schistosomiasis or interrupt transmission, most African countries need to adopt intensive, multifaceted strategies of controlling schistosomiasis such as provision of altenative sources of clean and safe water, healtn education, behaviour change in relation to water contact practices that place communities at risk of infection and snail control (WHO, 2012).

1.2 Problem statement

In Tanzania urogenital schistosomiasis is one of the most important public health problems. Control measures has been in place by rounds of praziquantel treatment resulting in a reduction of infection into a half (French *et al*., 2015). Exclusion in reduction has been reported in Tanzania following low coverage of praziquantel treatment (French *et al*., 2015). It has been observed that praziquantel alone rarely removes transmission. In Tanzania, the disease is widely distributed due to the widespread nature of the *Bulinid* snail hosts (Fig. 3). Three main snail species are responsible for the transmission of *S. haematobium* in Tanzania, *Bulinus africanus, Bulinus globosus and Bulinus nasutus* (Webbe & Msangi, 1958). *Bulinus globosus* colonises permanent water sources and is the major intermediate host along coastal areas of Tanzania including the islands of Zanzibar (Rollinson, Stothard & Southgate, 2001a), while *B. nasutus* colonises variety temporary water sources and is the principal host transmitting *S. haematobium* and *S. bovis* in north-western Tanzania (Lwambo, Siza, Brooker, Bundy & Guyatt, 1999a; Webbe, 1962; Zumstein, 1983). Therefore, although the

disease is distributed throughout the country, the transmission is highly focal, due to the nature of the snail habitats (Lwambo *et al*., 1999b).

1.3 Rationale of the stuudy

Transmission patterns of *S. haematobium* in Tanzania is seasonal, with high peaks soon after the rainy season (Webbe, 1962) and tends to fluctuate in line with snail population and rainfall patterns. This, along with the ability of *Bulinus* snails to aestivate during the long dry season, means high transmission occurs during the short dry season of February and March and in the months of July to September immediately after the rainy season (Lwambo, 1988). In the southern vicinity of Lake Victoria, the greatest transmission has been documented in Ukirigiru, Usagara and Misungwi areas of the *Sukuma land* (Lwambo, 1988), near Mwanza (Loker, 1981) and in Shinyanga region (Angelo *et al.*, 2018; Clements *et al*., 2006). These hot-spot of transmission is facilitated by the availability of compatible *Bulinus* snail intermediate hosts. It is therefore important to understand the combination of geographical distribution of susceptible snail intermediate hosts, poor sanitation and human water contact activities with infested water which are the critical determinants of transmission of schistosomiasis in any particular area (Chadeka *et al.*, 2017; Kloos *et al*., 2001; Woolhouse & Chandiwana, 1988).

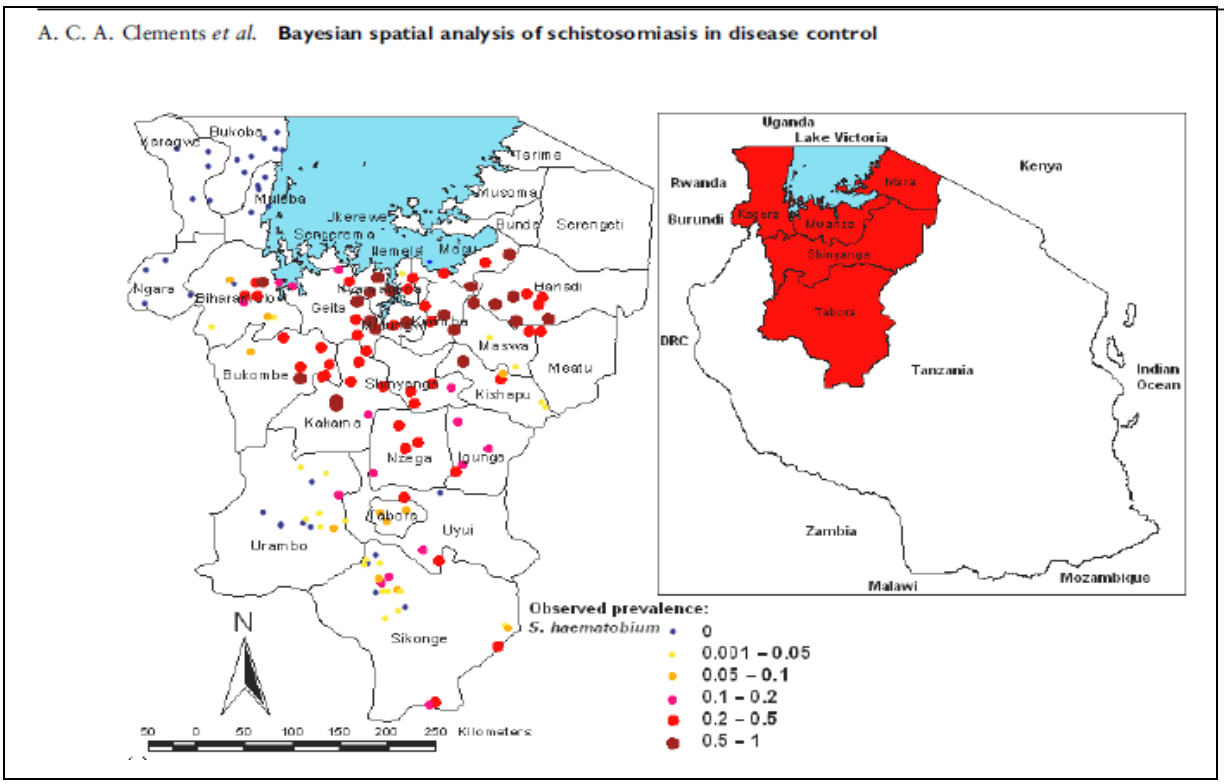


Figure 3: Distribution of urinary schistosomiasis in Tanzania the map is adapted from Clement *et al*…2006.

This study therefore aimed to assess transmission of *Schistosoma haematobium* and investigate the associated ecological factors and to understand the dynamics of the snail intermediate host populations. The study also assessed community knowldege, perceptions and practices on schistosomiasis and water contact behaviour.

1.4 Objectives

1.4.1 General objective

To gain an in-depth understanding of *S. haematobium* transmission in the study area with a focus on the role played by snail intermediate hosts and human water contacts.

1.4.2 Specific objectives

(i) To investigate human water contact behavior in relation to *S. haematobium* transmission in the study area by questionnaire and observation.
(ii) To determine patent and pre-patent schistosome infections in *Bulinid snails*, including differentiation of *S. haematobium* from *S. bovis* using molecular methods
(iii) To examine the ecological factors determining *Bulinid* snail population dynamics in the study area.

1.5 Study hypotheses and research questions

(i) There is an association between Schistosome infection in Bulinid snails and Schistosome infection observed in the human population in a given geographical area.
(ii) Schistosome infection of Bulinid snails in transmission hotspots is associated with high human water contact activities
(iii) The population dynamics of Bulinid snails and their infectivity is determined by different ecological conditions.

1.6 Significance of the study

Bulinid snails are necessary intermediate hosts for schistosome species to complete the intramolluscan part of their life cycle. In Tanzania the snails are focally distributed occupying a mosaic of water sources, especially in the southern vicinity of Lake Victoria, including Shinyanga district. Currently there is limited information on the role of *Bulinid* snail population dynamics in transmission and maintenance of *S. haematobium* infections. Previous studies reported prevalence of schistosome infections in snails based on cercariae shedding a method which may be misleading due to failure to differentiate schistosome

species transmitted morphologically. Therefore assessment of *Bulinid* population dynamics and screening of schistosome species infecting *Bulinid* snails by molecular approaches can provide accurate quantification of snail infection prevalence; a useful assessment of schistosomiasis transmission dynamics for designation of targeted control interventions to interrupt transmission.

This study produced important findings from longitudinal parasitological, malacological and medical anthropological studies. The findings provide an indepth understaning of the transmission patterns of urinary schistosomiasis, ecological, environmental and behavioral factors that influence transmission of *S. haematobium* infection in Shinyanga district. The findings point out to the need for design and implementation of integrated schistosomiasis control interventions which take into acccount the local setting in order to accelerate progress towards schistosomiasis control and elimination.

1.7 Delineation of the study

Molecular identification of morphologically identical snails mainly classified as *B. nasutus* in this study could be considered for further studies and confirms the snail species responsible in transmitting *S. bovis* or *S. haematobium* in Shinyanga district. This study identified only schistosome species by molecular methods through PCR and did not ascertain the species of snail intermediate hosts.

CHAPTER TWO

Geographical and behavioral risks associated with *Schistosoma haematobium* infection in an area of complex transmission[1]

Teckla Angelo[1, 2]*, Joram Buza[1], Safari M. Kinung'hi[2], Henry Curtis Kariuki[3], Joseph Rogathe Mwanga[2], David Zadock Munisi[1] and Shona Wilson[4]

1. Department of Global Health, School of Life Sciences and Bioengineering, Nelson Mandela African Institution of Science and Technology, P. O. Box 447, Arusha, Tanzania.
2. National Institute for Medical Research, Mwanza Centre, P. O. Box 1462, Mwanza, Tanzania.
3. Kenya Methodist University, School of Medicine and Health Sciences, P.O. Box 267, Meru, Kenya.
4. Department of Pathology, University of Cambridge, Tennis Court Road CB2 1QP, United Kingdom.

*Correspondence: tecklaangelo@yahoo.com

[1]Parasites & Vectors August (2018) 11:481-9

Abstract

Background: *Schistosoma haematobium* infection in endemic areas varies depending on the nature and complexity of the transmission networks present. Studies of micro-geographical transmission of *S. haematobium* infection indicate that discrepancy in prevalence between households is associated with diverse water contact behaviors and transmission that is restricted to particular sites harboring snail intermediate hosts. Detection of variations in the transmission sources with complex transmission networks of water bodies is required for optimization of malacological control. Longitudinal parasitological and malacological surveys were conducted to investigate geographical variations in transmission of urogenital schistosomiasis in Ikingwamanoti village, Shinyanga District, Tanzania.

Methods: Urine samples were collected at baseline and follow-up time points from 282 school-aged children and examined microscopically for the presence of *S. haematobium* eggs. Malacological surveys involved collection of *Bulinus nasutus* every month from 30 sites. Snails were examined for patent infections. Global positioning system was used to map household distances from *S. haematobium* transmission sites, while water contact behavior was assessed using a questionnaire.

Results: *Schistosoma haematobium* infection was observed to be prevalent among older children (12–14 years) 49.37% compared to younger groups 19.54% prior to treatment, but no significant difference in infection prevalence was observed at one-year. Boys were highly infected than girls at both time points $P>0.0001$. No spatial influence was observed between children's infection and the distance from child's residence to the nearby snail habitats. Nor was any significant association observed between children's reported water contact behavior with *S. haematobium* infection. However, malacological surveys with cercarial shedding combined with GPS data detected significant variation among different water sources in the transmission of S. *haematobium* with children living in households near to ponds with high *B. nasutus* populations having the highest prevalence of infection.

Conclusions: Interaction between malacological surveys with cercarial shedding combined with GPS mapping in endemic settings can help detection of transmission sources even in areas with complex transmission networks. Subsequent studies are needed to determine whether the combination of GPS mapping and parasitology screens can aid the detection of

transmission hotspots across varied transmission settings to enhance schistosomiasis control programmes.

Keywords: Urogenital schistosomiasis, Malacological surveys, complex transmission, Water contact behavior.

2.1 Background

Schistosomiasis is a debilitating disease of humans caused by digenetic trematodes of the genus *Schistosoma* (Gryseels & Polman, 2006; Steinmann *et al.*, 2006b). Globally schistosomiasis affects more than 230 million people (Vos *et al*., 2012). The disease has an extensive geographical distribution and is highly infective to people living in areas with limited access to safe water, sufficient sanitation and hygiene (Colley *et al*., 2014a; King *et al*., 2005) and adequate levels of appropriate health education (Kloos, 1995). Implementation of different schistosomiasis targeted controls strategies should mainly be determined from the local geographical diversities that enhance the nature of schistosomiasis transmission (Clennon, Mungai, Muchiri, King & Kitron, 2006; Rudge *et al*., 2008; Simoonga *et al*., 2008; Woolhouse, Etard, Dietz, Ndhlovu & Chandiwana, 1998). Human infection tends to vary with host immunity, water contact patterns, and geographical location, due to the presence and distribution of suitable snail intermediate hosts in water sources.

In most schistosomiasis endemic areas, design of targeted control measures and facilitating progress towards schistosomiasis elimination by adjustment of the ongoing schistosomiasis control interventions is necessary. By considering the focal geographical distribution and transmission of the disease, evaluation of the current schistosomiasis control interventions can occur.

In Tanzania, urinary schistosomiasis is recognized to be endemic in Sukumaland in the south and southeast part of the Lake Victoria (Lwambo, 1988). The disease is transmitted by *Schistosoma haematobium* a digenetic trematode that develops into the human infective stage within *Bulinus* snails that act as the intermediate hosts (Mandahl-Barth, 1965). The infection is highly prevalent among school aged children as reported in previous studies (Gryseels &Polman, 2006; Siza *et al*., 2015), but infection is highly focally distributed, and the risk of acquiring infection by humans is determined by the presence of the compatible snail intermediate hosts that are responsible for parasite transmission (Brooker *et al.*, 2001; Mandahl-Barth, 1965; Mazigo *et al*., 2012; sturrock, 1965). Studies on micro-geographical schistosomiasis transmission patterns reveal that there is discrepancy in infection intensities among households that is mainly associated with diverse water contact behavior which interacts with the limitation of transmission to particular sites due to the distribution of snail hosts (Clennon *et al.*, 2004; Clennon *et al*., 2006; Kloos *et al.*, 1998). In Shinyanga transmission of urinary schistosomiasis is complex, occurring within networks of temporary

ponds, and streams**.** The water sources where snail intermediate hosts reside are mainly temporary pools and ponds that are created from dry river beds after the rainy season. They are the major water sources for domestic and animal use by the local population.

Here, we examine micro-geographical patterns in transmission of urinary schistosomiasis, in this area with a complex network of potential transmission water bodies (ponds), by combining school-aged parasitological surveys with malacology surveys, behavioral questionnaires and GPS mapping of household, with the intention of determining their usefulness as tools for identifying transmission hotspots.

2.2 Methods

2.2.1 Study area and population

This study was carried out from October 2015 to July 2017 as longitudinal study of school children and snail populations in Ikingwamanoti Village, Shinyanga Region. No prior preventative chemotherapy mass drug administration had been implemented in the area. Ikingwamanoti Village lies 03. 92064°South and 33.12066°East and the altitude of the area is approximately 1000m above sea level. The region has two rainy seasons, the long rainy season from March through May/June and the short rainy season from November to December each year. *Wasukuma* is the pre-dominant ethnic group in the area.

Shinyanga is an area with black cotton soil with non-permanent streams that occasionally flow after rain. The local population gets water mainly from pools and ponds along dry river beds or man-made pools. Boreholes are not common. Irrigation, especially along water sources is practiced, particularly during the dry season.

Economically, most of the inhabitants earn their living from small scale agricultural and livestock farming (mainly cattle, goats and sheep). Livestock farming goes together with crop production (mainly maize, sorghum and rice), but relative contribution of livestock and crop production varies between individual households. Therefore land and labor constitute the main components of the economic system, the basis of which is founded on family labor.

All children, aged 6-14 years, attending Ikingwamanoti Primary School, a total of 282, were enrolled in the study. Children were followed-up three-weeks and one-year after treatment for schistosomiasis. A total of 30 water contact points in the village were surveyed for presence of *Bulinus* snails on monthly basis.

2.2.2 Parasitological investigation and treatment

Three consecutive urine samples were collected from all participating children pre-treatment, which took place during the dry season and again three weeks later to assess treatment efficacy, and one-year later to assess re-infection. Ten (10 ml) of each urine sample were agitated and filtered through a 10 micron Nucleopore polycarbonate filter membrane (Whatman) for counting of *S. haematobium* eggs. Immediately after the pre-treatment survey and after the one-year follow-up survey, all children were treated with a single dose of praziquantel (~40 mg/kg), using the standardized praziquantal dose pole (Montresor *et al.*, 2001).

2.2.3 Water contact questionnaire surveys

A total of 250 school children attending Ikingwamanoti Primary School who were recruited into the study were interviewed by trained research assistants using a structured questionnaire in *Kiswahili* to record demographic information, water contact activities, water contact sites, frequency of water contact, time of day when water contact occurred and duration of water contact. The questionnaire was administered during the parasitological follow up survey.

2.2.4 Malacological surveys

All water contact points in Ikingwamanoti village were identified in 2015 through observations and unstructured interviews with members of the community. Sites were selected on the basis of availability of water and observation of human or animal water contact activities. A total of 30 snail sampling sites for schistosomiasis transmission hot spots were determined and followed monthly from March 2016 to July 2017 to assess for the dynamics of snail intermediate hosts and their ability to transmit *S. haematobium*. Sites were grouped into snail habitats, each composed of one large pool or series of small pools. A handheld Garmin GPSMAP 64s GPS was used to record the geographical co-ordinates for each habitat.

During the surveys, snails were collected by a single person using a hand held scoop made of 2mm wire mesh for a period of 10 minutes per site to quantify snail abundance. All snails collected were classified based on shell morphology to genus level, with provisional identification to species level. Snails were individually placed in wells filled with distilled water and then exposed under natural light for 2 hours to initiate shedding. Using a dissecting microscope each snail was examined to determine presence of schistosome cercariae

potentially infective to humans. *Schistosoma bovis* was transmitted in the area, and cannot be differentiated from *S. haematobium* at the morphological level.

2.2.5 Household Mapping

The households of each child attending Ikingwamanoti Primary School was mapped using a hand held Garmin GPSMAP64s GPS. Household co-ordinates, along with snail sampling site co-ordinates, were imported in ArcView software. For each habitat, a central co-ordinate was assigned from those of the associated snail sampling sites, using visualization from satellite images to guide this assignment. The distances of school-childrens' residence from these snail habitats were calculated and t

he children assigned to the nearest habitat (Fig. 4).

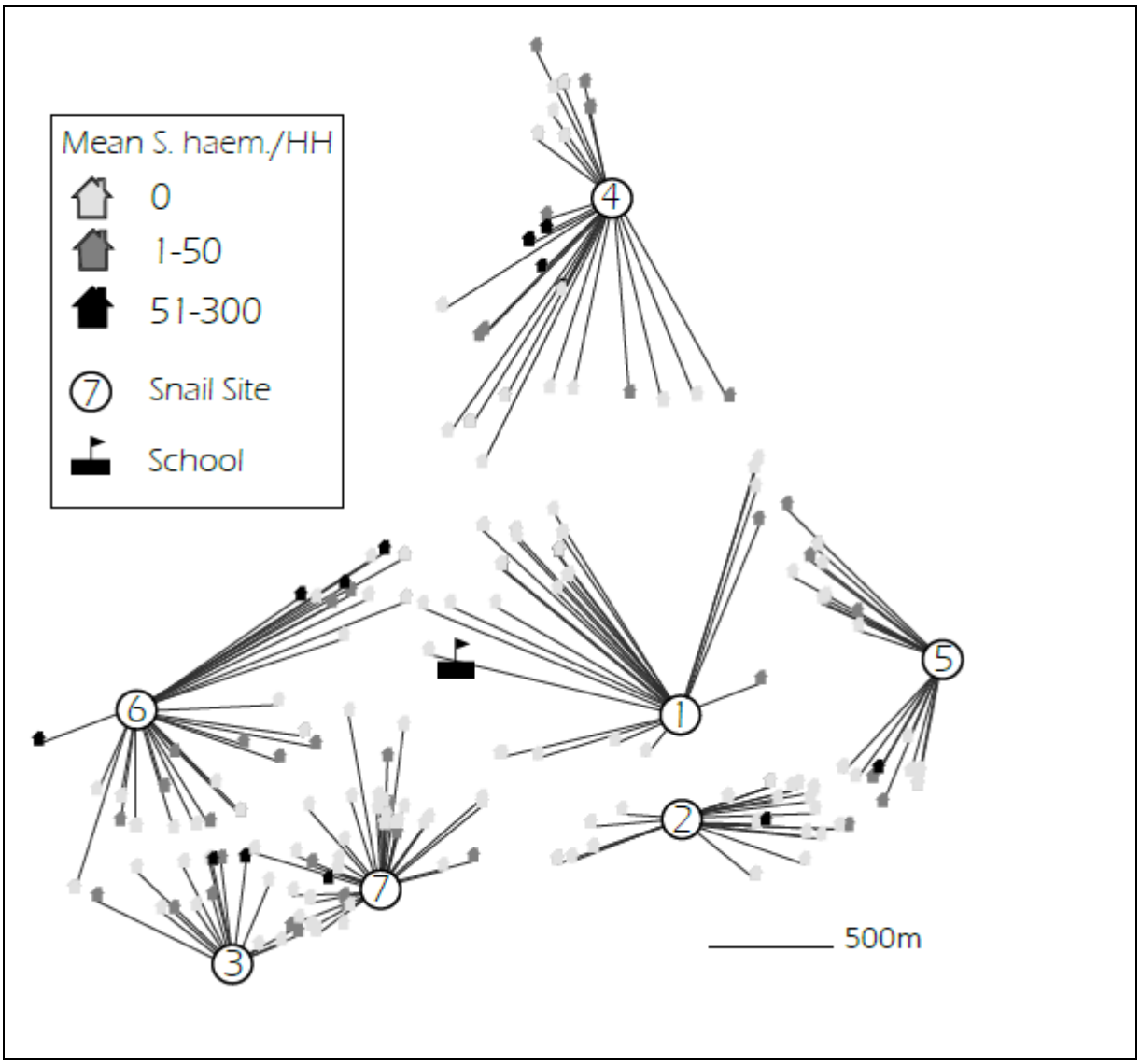


Figure 4: Household allocation to snail site and mean household intensity of *S. haematobium* infection

The assignment of households to central coordinates of the nearest habitat is shown, as are the mean pre-treatment infection intensities, grouped according to no infection, light infection (1–49 eggs/10 ml of urine) or high infection intensity (≥ 50 eggs/10 ml of urine), recorded for each household

2.3 Ethical considerations

This study was approved by the Medical Research Coordination Committee (MRCC) of the National Institute for Medical Research (NIMR), Tanzania (ethics clearance certificate no. NIMR/HQ/R.8a/Vol.IX/2107) and the University of Cambridge Human Biology Research Ethics Committee (HBREC.2015.28). The purpose of the study and procedures for sample collection were explained to the school children, adults in the communities, local leaders, school administrators, education personnel, teachers and health personnel before requesting for urine samples. Inclusion criteria for school children were based on the provision of assent for participation. Parents/guardians of school children attending Ikingwamanoti primary school were asked to provide signed/marked consent for their children to be included in the study. Only children who assented to participate and had parental or guardian's consent were eligible for inclusion. Each child recruited into the study was assigned with a unique identification number that linked samples and results. All data relating to the study participants were entered into a confidential file to which only members of the research team had access.

2.4 Data analysis

The study data was analyzed using R Statistical package version 3.2.1 (2015-06-18). Study participants were classified into three groups based on age (6-8 years, 9-11year and 12-14 years). The binomial regression model was used to calculate the odds ratio (OR) of infection and confidence intervals for age and sex during baseline and in re-infection prevalence. The re-infection model was controlled for infections not cleared by treatment by inclusion of the children's infection status three weeks post treatment (5.6%). To determine demographic and geographical predictors of *S. haematobium* infection a logistic regression model was generated by including all parameters in a multivariate analysis controlled a priori for age and sex. In a step wise manner, non-significant variables were removed to improve the model on the bases of Akaike Information Criterion (AIC) value resulting in the final model.

Spearman's Rank correlation was used to correlate the prevalence of infection within children and number of snails shedding potential human-infective cercariae. Each water contact activity was analysed independently in logistic regression models adjusted a prior for sex and age group. A p-value of 0.05 was considered statistically significant.

2.5 Results

2.5.1 Prevalence of *S. haematobium* infections by demographic characteristics

The prevalence of *S. haematobium* infection before treatment and one-year post treatment was 34.8% and 16.8%, respectively. This represents a 51.7% reduction in the prevalence of *S. haematobium* infection after one round of single dose praziquantel treatment. Children aged 12-14years had a significantly higher prevalence of *S. haematobium* infection at pre treatment compared to younger age groups, but at one year post praziquantel treatment, there was no significant differences in the prevalence of *S. haematobium* infection among age groups (Table 1). At the assessment of treatment efficacy, three weeks post treatment, a considerable reduction of *S. haematobium* prevalence from 34.8% to 5.6% was observed.

The sex- specific infection prevalence of *S. haematobium* at pre-treatment and at one –year re-infection is shown in Table 1. Pre-treatment, boys had a higher prevalence of *S. haematobium* infection (48.08%) compared to girls (25.34%). One-year post treatment, the prevalence of *S. haematobium* infection in boys decreased to 33.65% but remained significantly higher compared to girls (4.79%) (Table1).

Table 1: Demographic characteristics of school-aged children in relation to pre-treatment infection and one-year post-treatment re-infection prevalence of *S. haematobium*

		Pre-treatment			Re-infection[a]		
	N	**Number (%)**	**OR (95% CI)**	***P-value***	**Number (%)**	**OR (95% CI)**	***P*-value**
Sex							
Girls	146	37 (25.34)	1		7(4.79)	1	
Boys	104	50 (48.08)	2.57 (1.49–4.50)	0.0001	35 (33.65)	8.95 (3.94–23.15)	0.0001
Age (years)							
6–8	87	17 (19.54)	1		12 (13.79)	1	
9–11	84	31 (36.90)	2.38 (1.19–4.91)	0.01	15 (17.85)	1.24 (0.49–3.11)	0.648
12–14	79	39 (49.37)	3.75 (1.88–7.73)	0.0001	15 (18.98)	1.17 (0.47–2.93)	0.734

[a]Model of re-infection prevalence was controlled for presence or absence of detectable infection 3-weeks post-praziquantel treatment
Abbreviations: n, number enrolled, CI, confidence interval

2.5.2 Malacological surveys

A total of four thousand eight hundred ninty nine (4899) snails were collected from seven habitats sampled within the village. Overall, 132 snails (2.7%) shed schistosome cercariae. Although, the highest snail density was observed in habitat 3 (Mwakasela) where 1678 snails were collected, representing 34.25% of the total *Bulinus nasutus* collected in the village, this habitat had neither the highest number nor the highest prevalence of snails with patent infection, with only 22 (1.31%) shedding schistosome cercariae. The highest number and prevalence of snails with patent infection was observed from habitat 5 (Jumanne), where 691 snails were collected, of which 48 (6.95%) shed schistosome cercariae. Habitat 4 (Mwamunonge) had the second highest number of snails collected (1450) and the second highest number of snails (45) with patent infections (3.1%). The habitat with the lowest transmission potential was habitat 1 (Mwachumi) from which only one snail without patent infection was collected throughout the whole survey period (Table 2).

Table 2: Relative snail population numbers and patent infection with mammalian schistosomes by habitat

Habitat no.	Habitat name	No. of snails collected (%)	No. of snails infected (%)
1	Mwachumi	1(0.02)	0 (0)
2	Miyu	49 (1.00)	1 (2.04)
3	Mwakasela	1678(34.25)	22 (1.31)
4	Mwamunonge	1450(29.60)	45 (3.1)
5	Jumanne	691 (14.10)	48 (6.95)
6	Mwamalago	149 (3.04)	1 (0.67)
7	Mwakangota	881(17.98)	15(1.70)
	Total	4899 (100)	132 (2.69)

2.5.3 *Schistosoma haematobium* pre-treatment infection and snail habitats

Geographically, infection intensity (Fig. 5a) and prevalence (Fig. 5b) amongst school-children tended to be greatest near habitats with high numbers of snails shedding schistosome cercariae, indicating that the population size of the compatible snail intermediate hosts

influences transmission levels to children living near a particular habitat. The prevalence of *S. haematobium* pretreatment within school children was relatively highly correlated with the number of snails collected in the particular habitat (Fig. 5a) but not significantly so due to the low number of habitats (Rho= 0.643, p=0.139). A moderate correlation was also observed between children's infection prevalence and the number of *Bulinus* snails shedding mammalian cercariae in the nearest snail habitats (Fig. 5b) but again this was not significant (Rho=0.523, p=0.229).

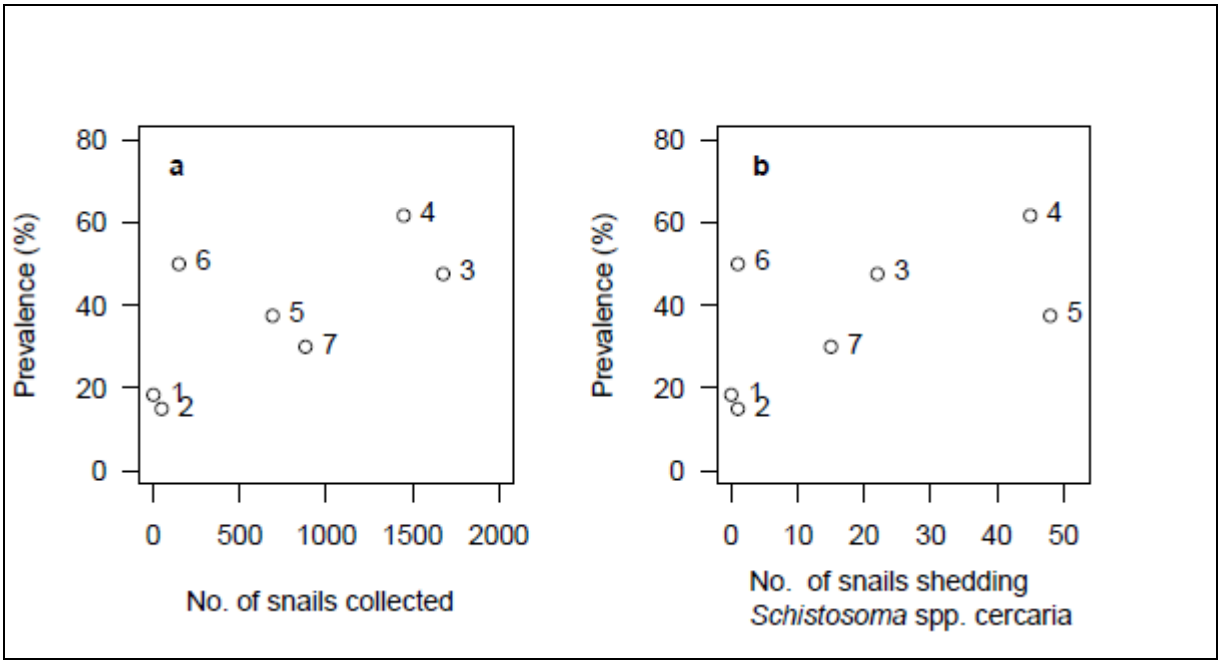


Figure 5: Prevalence of *S. haematobium* in school children in relation to intermediate host site, numbers and patent infection

Scatter plots of the prevalence of *S. haematobium* infection within school children assigned to their nearest snail habitat, indicated numerically, against the accumulated snail count for that habitat (a), or the accumulated number of snails that shed *Schistosoma* species. cercariae (b)

To investigate whether the observed differences in snail numbers and *S. haematobium* infection rates between habitats were associated with differing infection prevalence among school children, a logistic regression model was built (controlling for age and sex)that included the nearest habitat to the child's household. Habitat 1, for which only one uninfected snail was collected, and was the most centrally situated habitat (Fig. 4), was used as the reference. Although habitat 3 had the highest observed number of snails, and high patent infection in snails, residence close to habitat 3 was not significantly associated with a

higher pre-treatment prevalence of *S. haematobium* infection in school children (OR 3.16; 95%CI 0.84 -12.63; P=0.093), compared to those whose households were closest to habitat 1. However, residence close to habitat 4, the habitat with the second highest snail population numbers, and patent infection, was significantly associated with higher pre-treatment *S. haematobium* infection prevalence (OR 6.46; 95% CI 2.14-21.88; P<0.001) followed by residence close to snail habitat 6, which was of borderline significance (P=0.055) (Table 3). Classification as resident closest to habitat 6 is the greatest anomaly amongst the trend between snail habitat and prevalence of infection in school children (Fig. 5).

Residence close to snail habitats 2, 5 and 7 was not significantly associated with a higher infection prevalence of *S. haematobium* than residence closest to habitat 1 (Table 3). There was no detectable spatial influence on prevalence of re-infection among school children living in Ikingwamanoti village.

Table 3: Pre-treatment logistic regression model of predictors of *S. haematobium* infection among school-aged children in Ikingwamanoti village

Variable[a]	**OR (95%CI)**	***P*-value**
Sex		
Boys	2.64 (1.49–4.88)	0.001
Age(years)		
9–11	2.99 (1.39–6.68)	0.006
12–14	4.29 (2.00–9.55)	0.0001
Snail habitat		
Habitat 2	0.38 (0.08–1.57)	0.188
Habitat 3	3.16 (0.84–12.63)	0.093
Habitat 4	6.46 (2.14–21.88)	0.001
Habitat 5	2.89 (0.88–10.29)	0.087
Habitat 6	3.07 (1.01–10.29)	0.055
Habitat 7	1.34 (0.44–4.41)	0.609

[a]Female sex, aged 6–8years and Habitat 1 were used as reference groups

2.5.4 Water contact activities

From a water contact questionnaire, the most reported water contact activity conducted by children was fetching water, which was reported by 84% of the children; however, this involved collecting water from different water sources for their domestic use. No permanent water sources were available in the community, thus people depended on temporary water sources that were shared with livestock. Children responses to livestock watering were high (81.2%). Since the majority of the community members are pastoralists and tend to move from one place to another searching for water, 71.6% children reported having to crossed water in their daily activities. The frequency of paddy farming and other ways of water contact were 51.6% and 40% respectively. The least reported water contact activities were swimming and fishing with frequencies of 24.4 % and 14 % respectively.

A logistic regression model was built to assess various water contact activities performed by school children to confirm the risk of infection and re-infection with *S. haematobium* among school children. The model assessed different individual activities conducted by school children, controlling a priori for age and sex. No specific activity was observed to be a significant risk activity for S. *haematobium* infection (Table 4), and being aged 12-14 years (OR 3.29 95%CI 1.57-7.16, P>0.001) and being a boy (OR 3.54 95%CI 1.57-7.74, P =0.002) remained significant predictors of *S. haematobium* infection.

Logistic regression models were adjusted *a priori* for age-group and sex. For each activity, the children who reported not participating in it were used as the reference

Table 4: Assessment of individual water contact activities in relation to *S. haematobium* prevalence amongst school-aged children in Ikingwamanoti village.

Water contact activity	**No. of individuals (%)**	**OR (95% CI)**	***P*-value**
Livestock watering	203 (81.2)	1.22 (0.60–2.58)	0.596
Fetching water	209 (83.6)	1.16(0.52–2.69)	0.725
Swimming	60 (24)	0.85(0.43–1.63)	0.627
Irrigation	95 (38)	0.99 (0.56–1.76)	0.979
Crossing water	179 (71.6)	1.13 (0.60–2.14)	0.715
Paddy farming	129 (51.6)	1.28 (0.72–2.26)	0.397
Other	99 (39.6)	1.33 (0.75–2.35)	0.325

Logistic regression models were also used to assess *where, when, frequency and time spent* in water contact activities as risk factors for the transmission of *S. haematobium* among school aged children. All models were adjusted a priori for age and sex and reduced in a step wise manner removing insignificant variables. No individual activity was found to be a significant risk factor for S. *haematobium* infection (P>0.05). Domestic activities, such as laundry and dish washing, along with bathing were mostly reported to be conducted at home and were not analysed (Supplementary Table 1). Although majority of the water contact activities were observed to be performed by the school children at different frequencies and time, no specific reported activity was demonstrated to be a significant risk factor for transmission of *S. haematobium* in school children in the village (Supplementary Table 1).

2.6 Discussion

Schistosomiasis control approaches that are well adapted to epidemiological settings need to address the foremost risks associated with local transmission, in addition to applying the essential recommended Schistosomiasis control with praziquantel mass drug administration as a foundation (WHO, 1993, 1998). Therefore understanding the predictors for local *S. haematobium* transmission is critical. This study presents findings on the risk factors that are significantly associated with infection and re-infection among school aged children over duration of 12 months. Our findings indicated that sex, age and proximity of residence to habitats with high population numbers of the intermediate snail host were independent determinant factors for *S. haematobium* infection among school age children. Reported water contact activities were not significantly associated with infection with *S. haematobium* in this study.

Male sex was a significant predictor of pre-treatment *S. haematobium* infection and re-infection in our study. It is expected that sex role differences had impact on the level of exposure to *S. haematobium* infection among boys than girls. It is possible that boys were more exposed to *S. haematobium* infection compared to girls due to their routine livestock caring, in which much time is spent searching for different pastures and water sources for watering, and since boys spent most of their time in the field, they utilize that time to swim after cattle watering (supplementary table 2). However, we found that implementation of a simple questionnaire was insufficient to significantly attribute this to infection prevalence. A higher prevalence of infection amongst boys is consistent with previous studies carried out

elsewhere (Clements *et al*., 2008; Guyatt, Brooker, Lwambo, Siza & Bund, 1999; Liao *et al*., 2011).

Pre-treatment, children aged 12-14 years had higher risk of *S. haematobium* infection than the youngest age group. This could be due to increased duration of water contact activities that expose them to a higher risk of contracting schistosomiasis, or their greater lifetime of accumulating the parasite without the development of resistance to infection. After 12 months of praziquantel treatment there was no significant differences in infection levels between age groups, as has been reported by previous studies (Amazigo, Anago-amanze & Okeibunor, 1997; Barbour, 1985; N'Goran *et al.*, 2001; Klumpp & Webbe, 1982; Saathoff, Olsen, Magnussen, Kvalsvig, Becker & Appleton, 2004). This indicates that protective immunity has not developed and that changes in behavior are also not as significant factor as time for accumulation of infection. Regular treatment is therefore necessary to maintain low levels of schistosomiasis infection and prevent development of disease morbidity within this community.

In this study, the children's reported involvement in any particular water contact activity was not directly associated with *S. haematobium* infection. In the assessment of water contact activities among school aged children as predictors of *S. haematobium* infection, our study demonstrated that only sex and age were significant risk factors for infection and not type of water contact performed by children. This reflects that a simple knowledge, attitude and practice questionnaire approach is insufficient to provide the necessary information required to identify status of water contact performed by children in relation to schistosomiasis transmission. Engagement of in-depth water contact discussions is necessary to clearly determine time, where and when water contact activities are performed by children. Previous studies also observed that only age and sex (i.e. universal variables) were predictors of *S. haematobium i*nfection and not water contact activities (Klumpp & Webbe, 1982).

Snail habitats containing infected snails have an impact on the magnitude of exposure as a risk factor for transmission of schistosomiasis (Clennon *et al.*, 2004). The distance between households and snail habitats in the village were determined by the use of GPS. The use of GPS proved to be reliable for determination of specific locations as the source of transmission in this area, as confirmed by malacology surveys. The closest snail habitat to child's household was a significant predictor of pre-treatment infection. However, there was no spatial influence on re-infection among the school children. Although, previous studies

have indicated that distance from homestead to an open water source is a determinant risk for *S. haematobium* infection (Amazigo *et al*., 1997), in our study it was the actual habitat that was closest that predicted infection prior to treatment, rather than the measured distance. School children mostly at risk of *S. haematobium* infection in the village were those whose households were located closer to snail habitats harboring the most snail intermediate hosts, though not the highest prevalence of patently infected snail hosts. This may reflect that we were unable to identify *S. haemtobium* from *S. bovis* cercariae at the morphological level; future studies need to employ molecular techniques to specifically differentiate *S. bovis* from *S. haematobium* cercariae, and to critically elucidate the transmission pattern of schistosomes species existing within particular geographical settings. Other anomalies included assignment to habitat 1, where only one uninfected snail was collected throughout the duration of the study, but infection prevalence amongst the children was almost 20%, and assignment to habitat 6, for which 50% of the children were infected but only 149 snails, one patently infected, were collected. This may reflect mis-assignment of children to habitats, as distance to habitat may not capture fully actual sites of water contact. As, our observation has confirmed that use of questionnaire alone in identifying predictors of *S. haematobium* transmission can be insufficient to reveal the reality of where transmission takes place, or the riskiest behaviors for infection, linkage of GPS with in-depth water contact discussions may correct for the types of anomaly observed. However, the GPS tool was strong compared to the use of questionnaires, though it should be noted that not all recruited participants participated in the questionnaire exercise. Those who missed the questionnaire surveys were removed during data analysis.

Although, the concurrent use of pre-treatment parasitological surveys with malacological surveys, and GPS enabled the detection of specific micro-geographical sites responsible for *S. haematobium* transmission in this area with a complex network of water sources, this was not apparent in the analysis of the one-year re-infection data. At one-year the majority of children were not infected. Therefore, under the current force of infection of urinary schistosomiasis occurring in the village observation of the spatial influence on re-infection was not possible, and it depended on collective exposure to *S. haematobium* infection over several years. Therefore, in endemic areas where transmission is low, or that have received several rounds of chemotherapy with praziquantel, parasitological and GPS surveys alone may be insufficient to detect on-going transmission sites, and detailed malacological surveys may be required.

2.7 Conclusion

Our study shows that the interaction between humans, snail hosts and the parasite can be tied together with GPS in an endemic setting and can help determine specific transmission sources that exist within a complex transmission network for enhancement of targeted control strategies. As there commended schistosomiasis control initiatives, which are currently adapted in many endemic countries as per WHO guidelines (WHO, 1998), rarely breaks the transmission of schistosome parasites within the endemic human communities, new and integrated interventions are required and predictors of transmission in local settings are required to best target these integrated interventions (Colley *et al.*, 2014b; Grimes, Croll, Harrison, Utzinger, Freeman & Templeton, 2015; Rollinson *et al.*, 2013; WHO, 2012; Utzinger, Raso, Brooker, Savigny & Tanner, 2009). Subsequent studies are needed, combining the use of GPS in identification of specific transmission sources across areas with differing transmission patterns, in order to validate the breadth of applicability of this approach. Additionally, upon identification, in-depth malacological studies may be required to determine further key aspects of transmission such as seasonality, if targeted malacological control interventions, in combination with the current preventative chemotherapy interventions, are to help the move towards schistosomiasis elimination.

CHAPTER THREE

Community knowledge, perceptions and water contact practices associated with transmission of urinary schistosomiasis in an endemic region. A qualitative cross-sectional study[2]

Teckla Angelo[1,2*], Safari M. Kinung'hi[2], Joram Buza[1,], Joseph R. Mwanga[2], Curtis Kariuki[3], Shona Wilson[4]

1. Department of Global Health and Bio-Medical Sciences, School of Life Sciences and Bioengineering, Nelson Mandela African Institution of Science and Technology (NM-AIST), P. O. Box 447, Arusha, Tanzania.

2. National Institute for Medical Research (NIMR), Mwanza Centre P.O. Box 1462 Mwanza, Tanzania.

3. Kenya Methodist University, School of Medicine and Health Sciences, P. O. Box 267, Meru, Kenya.

4. Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge, UK.

*Corresponding author. E-mail: tecklaangelo@yahoo.com

[2] BMC Public Health (2019) 19:703

Abstract

Background: In an effort to complement the current chemotherapy based schistosomiasis control interventions in Shinyanga district, community knowledge, perceptions and water contact practices were qualitatively assessed using focus group discussions and semi structured interviews involving 271 participants in one *S. haematobium* prevalent community of Ikingwamanoti village, Shinyanga district, Northwestern, Tanzania.

Methods: In October, 2016 we conducted 29 parent semi structured interviews and 16 focus group discussions with a total of 168 parent informants. Adult participants were conveniently selected from three sub-villages of Butini, Miyu, and Bomani of Ikingwamanoti village, Shinyanga district. In March, 2017, a total of 103 children informants participated in 10 focus group discussions and 20 semi structured interviews, administered to children from standard four, five, six and seven attending Ikingwamanoti Primary School. Note taking and digital recorders were used to collect narrative data for thematic analysis of emergent themes.

Results: Among participants, 75% parents and 50% children considered urinary schistosomiasis as a low priority health problem. Of the informants, 70% children and 48.3% parents had misconceptions about the cause, modes of transmission and control of schistosomiasis demonstrating gaps in their biomedical knowledge of the disease. Assessment of treatment seeking behavior for urinary schistosomiasis revealed a combination of traditional and modern health care sectors. However, modern medicines were considered effective in the treatment of urinary schistosomiasis. Lack of alternative sources of water for domestic and recreational activities and unhygienic water use habits exposed community members to high risk of acquiring urinary schistosomiasis.

Conclusion: Use of *Schistosoma haematobium* contaminated water sources for daily domestic and recreational use facilitated contraction of urinary schistosomiasis among community members in Shinyanga district. People's perceptions of urinary schistosomiasis as a less priority health problem promoted persistence of the disease. Future efforts to control urinary schistosomiasis should take into account integrated approaches combining water, sanitation and hygiene, health education, alternative sources of clean and safe water to facilitate behavior change.

Key words; Perceptions, Urinary schistosomiasis, water contact, community knowledge

3.1 Background

Schistosomiasis is an infectious disease affecting more than 230 million people worldwide (Colley *et al*., 2014). An estimated 95% of cases occur in Africa (Knopp *et al*., 2012; Maseko, Mkhonta, Masuku, Dlamini & Fan, 2018; Steinmann *et al*., 2006; Utzinger *et al*., 2009). It is caused by six blood fluke species of the genus *Schistosoma* (Rollinson *et al*., 2013). Transmission of the parasite depends on unhygienic conditions, in which human excreta directly contaminate freshwater bodies that are habitats for specific snail intermediate hosts (Grimes *et al*., 2016). The disease is mainly transmitted in rural settings where there is insufficient sanitation, poor health awareness and poverty (Kabatereine, Fleming, Thuo, Tinkitina, Tukahebwa & Fenwick, 2014). Measures to control and eventually eliminate the disease through preventive chemotherapy have been in place for several years in most African endemic countries using praziquantel as the drug of choice. However the strategy has proved to have limitations where there is persistent re-infection due to praziquantel's inability to kill immature worms and prevent re-infection (Colley *et al*., 2014b; Yepes, Varela-m, López-abán, Rojas-caraballo, Muro & Mollinedo, 2015). In Tanzania, schistosomiasis remains a serious public health problem. Despite of ongoing control interventions, high prevalence of the disease still persists (Mazigo *et al*., 2012). The sixty five (65) World Health Assembly recommendation states that endemic countries must strengthen their local control programmes and enable provision of at least a single annual round of praziquantel treatment to school age children (WHO, 2012). However, the chemotherapy approach alone in most endemic areas like Tanzania has not yielded a sustainable impact as people continue to enter water containing infective cercariae, re-infection following praziquantel treatment has been common. Therefore additional interventions are necessary to facilitate progress towards control and elimination (Knopp *et al*., 2013; Olliaro, Vaillant, Diawara & Coulibaly, 2015; Raso, Essé, Dongo, Ouattara & Zouzou, 2018; Sokolow *et al*., 2016). These include, water based interventions such as water sanitation and hygiene (WASH) and molluscicide (Secor, 2014; Grimes *et al*., 2015). Further, correct knowledge, attitude and practices of community members in endemic areas have a significant role to play in achieving success of the planned control interventions and achieve sustainable control of the disease (Mwanga & Lwambo, 2013). Health education and encouragement of behavioral change, in addition to a chemotherapy approach, are likely to become key elements in future elimination efforts (WHO, 2010; Manderson, Aagaard-Hansen, Allotey, Gyapong & Sommerfeld, 2009; Mwanga *et al*., 2015; Tchuenté, Garba, Mohammed & Schur, 2012). Even though introduction of alternative water supply in resource

limited settings is expensive and sometimes lacks priority (Secor, 2014), reduction of contact with infectious water as one of the schistosomiasis control strategies is even more complicated in these settings, requiring a community adapted approach to facilitate control and elimination. To generate effective behavioral change interventions and encourage acceptance of protective behaviors and reduction of risk behaviors, understanding the community knowledge is critical (Mwai, Njenga & Barasa, 2016). Here we present results of a qualitative study implemented in adults and school children to facilitate the design of community based disease control interventions.

3.2 Methods

3.2.1 Study area and population

This study was conducted among community members (parents/guardians and school children) living in Ikingwamanoti village, Shinyanga District in Northwestern Tanzania between October 2016 and March 2017. The village has one school, Ikingwamanoti Primary School with 517 enrolled school children. The village is located along the highway to Tabora region, connecting Mwanza and Dar Es Salaam and lies between 02°65283' South of the Equator and 32°64063' East of the Greenwich Meridian. The population size of Butini sub village was 783, Bomani sub village 753 and Miyu sub village 563, totaling to 2099 inhabitants for the whole Ikingwamanoti village (NBS, 2013). The district is predominantly inhabited by *Wasukuma* ethnic group (Bantu-speaking people) who are the natives of the *Sukumaland* located to the west and south of the Lake Victoria. The district receives two phases of rainfall, the short rainy season from November to December and a long rainy season from March through May with rain almost every day. The district is endemic to *S. haematobium* infection with transmission occurring in temporary pools throughout the region (Angelo *et al.*, 2018; Mazigo *et al.*, 2012). The major income generating activities are largely based on subsistence farming and livestock–keeping. Crops that are cultivated include cassava, millet, yams and paddy which constitutes the food crops whereas cotton is the main cash crop. Crop production and livestock-keeping are undertaken within the framework of individual households. More details of the study area and quantitative analysis of school-children's *S. haematobium* infection levels and their infection-risk behaviours is described in (Angelo *et al*., 2018).

3.2.2 Study design and sampling procedure

The study was a descriptive qualitative inquiry that was designed to complement quantitative findings of a larger parasitological and malacological study conducted in the same area**.** For adult community participants, a local community leader of each sub village (Butini, Bomani and Miyu) was asked to invite community members to participate in the study by blowing a whistle one day before the event. During the next day, participants who responded the previous day's call and were willing to participate, gathered in open grounds from which convenient sampling was used to select participants to be recruited into the study. For school children, the research team with the assistance of class teachers selected participants from standard four to seven and included them into the study. Sample size determination was mainly based on salience of ideas and themes for thematic analysis of revealed outcomes rather than reaching saturated recording of all beliefs within the community as described by Weller and others (Weller *et al*., 2018) whereby only 10 interviews and further probing are sufficient to capture on average 95% of salient ideas. To capture salient perceptions from individual experiences with urinary schistosomiasis, semi structured interviews were conducted with 29 adult community members and 20 primary school children. Sixteen focus group discussions with adult community members, totaling 168 participants and ten FGDs, with a total of 103 school children were administered to enable generation of community ideas on urinary schistosomiasis.

3.2.3 Data collection methods

To enable comparison of experiences and creation of new ideas formed from a socio context about urinary schistosomiasis transmission, focus group discussions (FGD) with pre determined questions (additional information 1) were administered to homogenous groups with similar experiences of urinary schistosomiasis practices. Discussions were conducted in quiet places and comprised of circular seating groups guided by moderators (Breen, 2007; Krueger & Krueger, 2002). To investigate individual experiences of school aged children and adult community members a semi structured interviews (SSI) (additional information 2) with standardized predetermined list of questions were used to solicit information on schistosomiasis knowledge, attitude and practices among community members (adults and school aged children). The rationale of using two different strategies in data collection in this study (focus group discussion and semi structured interviews) was to apply methodological triangulation in order to improve the capture of knowledge, attitudes, perceptions and

practices on schistosomiasis within the community and compare the results generated from the two strategies (Guest *et al.*, 2017). Semi structured interviews explore more on individual views, perceptions and practices on urinary schistosomiasis, while focus group discussions complement SSI by providing in-depth exploration about community knowledge, attitude, perceptions and practices on urinary schistosomiasis. Either approach is limited in terms of their ability to generalize findings to the whole population mainly because of the small numbers of people participating and the likelihood that the participants will not be a representative sample.

The size of focus group discussion ranged from 8-12 participants composed of individuals of the same sex and other aspects emphasized in FGD literature (Breen, 2007; Dawson, Manderson & Tallo 1993; Mwanga, Mugashe, Magnussen, Gabone & Aagaard-Hansen, 1998). Male FGDs were conducted by two trained males one being a moderator and the second being the recorder. In the female focus group discussions, two trained females facilitated the discussion, one being a moderator while the other recorded the information. The interviews and discussions were conducted in Kiswahili, a national language, using a simple topic guide with open ended questions and probes to allow in-depth investigation of the topic. Permission to record the discussions was sought prior to the session. In focus group discussions, participants were assigned unique numbers instead of names to use when contributing their views. Participants were encouraged to speak their views freely and spontaneously. Discussions were held in natural settings outside, under the shade of trees and in quiet places. Data was documented by both note taking and digital recordings.

3.3. Data management and analysis

All textual data were categorized into selected topics, thematic analysis was conducted by classifying all the FGD and SSI responses into specific themes, where cases did not fit the emergent theories, the theories were revised. Digitally recorded information was not typed but used to improve field notes prepared from interviews and focus group discussions in the form of hand written reports. Content analysis was executed by comparing raw data and summaries using the principles of grounded theory (Strauss & Corbin, 1990.). To support the findings, quotes were taken from recorded materials (Sunday, 2012). Investigators assigned more or less the same meaning to the data. Inter-investigator reliability was high as data from FGDs and SSIs validated each other. The study employed explicit, systematic and reproducible methods to increase validation and reliability of the findings (Greenhalgh &

Taylor, 1997). Percentages given are of reported perceptions from the SSIs only, with FGD data used to provide further in-depth exploration. When a perception was reported by 20% or more of the respondents, it was considered salient.

3.4. Ethical considerations

In 2015, the Medical Research Coordination Committee (MRCC) of the National Institute for Medical Research (NIMR), Tanzania received and approved the study (ethics clearance certificate no. NIMR/HQ/R.8a/Vol.IX/2107). Additional approval was made by the University of Cambridge Human Biology Research Ethics Committee (HBREC.2015.28) to conduct this study in Shinyanga district, Tanzania. The study objectives, data collection procedures, potential risks and benefits were explained to participants, local leaders, school administrations before administering interviews and discussions. Adequate time was given to participants to ask questions about the study and it was highlighted that participation was an individual's choice, to participate in the study or deny participation without losing their rights to health care. Participants were informed on their right to withdraw in the study at any time when they wish to do so. Informed consent was sought through signature or thumb print on consent forms. Due to inability of some study participants to read and write, informed consent was read clearly to participants by a research assistant, participants provided a verbal consent to participate in the study. Grade and provision of assent to participate in the study were the inclusion criteria for school children. Consent for children to participate in the study was sought from parents and guardians. The parents/guardians were invited by a local community health worker one day before the interview, the purpose of the study, procedures, harms and benefits were explained to parents and their children i.e what study participation involved, including their right to withdraw without losing their rights to health services. Children who volunteered to participate in the study had to assent to be eligible. Only assenting children with parental or guardians' consent were eligible to participate in the study. Participants were given a unique study identification number to ensure confidentiality of the data collected. All adult participants consented to participate into the study. Information obtained from the study was stored in a locked cabinet and a password protected digital database that only the research team was capable of accessing.

3.5 Results

3.5.1 Socio-demographic characteristics of the study participants

A total of 103 children, of whom 49.5% were female and 168 parents of whom 51.2% were female, participated in focus group discussions (FGDs). Of the children FGD participants, 48.5% belonged to Butini sub village, 26. 2% belonged to Bomani subvillage and 25.2% belonged to Miyu sub village. For parents, 26.8% belonged to Butini sub villages, 35.1% belonged to Bomani subvillage and 38.1% belonged to Miyu subvillage. Ten and 16 FGDs were conducted with children and parents, respectively (Table 5).

Table 5: Distribution of focus group discussion participants by sex and sub-village

Sex	**Children FGD (n=10)**			**Parents FGD (n=16)**		
	Male No. (%)	Female No.(%)	Total No. (%)	Male No.(%)	Female No.(%)	Total No (%)
Sub village						
Miyu	13(12.6)	13(12.6)	26(25.2)	40 (23.8)	24(14.3)	64 (38.1)
Bomani	14(13.6)	13(12.6)	27(26.2)	21(12.5)	38(22.6)	59(35.1)
Butini	25(24.3)	25(24.3)	50(48.5)	21(12.5)	24(14.3)	45(26.8)
Total	52 (50.5)	51(49.5)	103(100)	82 (48.8)	86(51.2)	168 (100)

Twenty nine parents and 20 children were involved in semi-structured interviews. Fifty percent (50%) of children participating in the semi-structured interviews were females while 14 (48.3%) of parent participants were females. All children were primary school pupils with age ranging from 12 to 15 years. Median age was 13 years. The parent's age ranged from 25 to 80 years. Nineteen (66%) of the parents had primary school education, 7 (24.1%) were illiterate while only 3 (10%) had secondary school education. All participants belonged to the *Wasukuma* ethnic group and majority of the parents (93.1%) were subsistence farmers (Table 6).

Table 6: Socio-demographic characteristics of interviewees in the semi structured interviews (SSI).

Variable	Children (n=20) Frequency (%)	Parents (n=29) Frequency (%)
Sex		
Males	10 (50)	15 (51.7)
Females	10 (50)	14 (48.3)
Age (yrs)		
12-13	15 (75)	-
14-15	5 (25)	-
≤ 25	-	5 (17.2)
25-34	-	11 (37.9)
35-44	-	4 (13.8)
45-54	-	5 (17.2)
≥55	-	4 (13.8)
Education		
Standard four	4 (20)	-
Standard five	6 (30)	-
Standard six	6 (30)	-
Standard seven	4 (20)	-
No formal	-	7 (24.1)
Primary	-	19 (65.5)
Secondary	-	3 (10.3)
Occupation		
Pupil	20 (100)	-
Subsistence farmer	-	27 (93.1)
Other	-	2 (6.9)

3.5.2 Priority health problems in the community

Parents and children participants identified a number of diseases such as malaria, sexually transmitted infections including HIV/AIDS, gonorrhea and syphilis, fever, typhoid, sickle cell anemia, dehydration, pneumonia, coughs, headaches, tuberculosis, stomachaches, skin diseases, epidemics such as cholera, dysentery, and diarrhea, gut helminths, ulcers, pains (including joints, waist, back); swollen legs, eye and heart diseases as common health problems affecting the community. Very few participants mentioned schistosomiasis as a common health problem in the community.

The majority (78.5%) of parent respondents and 50% of children respondents revealed that schistosomiasis is a disease of no health priority in the community compared to other acute health problems. Disease priority at family level was given to diseases that are acute and life threatening such as malaria and cholera. Urinary schistosomiasis is given priority only when patients are seriously sick (Table 7). This was demonstrated by participants in interviews. One child reported: *"when we compare this with other diseases like malaria, the malaria patient must be treated first because the disease is more severe than urinary schistosomiasis. The person with urinary schistosomiasis will be treated when the condition is advanced"* [Children SSI]. Similarly a parent participant reported:

> *Our community does not grant schistosomiasis the priority it deserves, unlike cholera. There are those who go to hospitals but others visit traditional healers. Even myself I am suffering from urinary schistosomiasis but I am only using herbs to treat it. It is difficult to be open to others, therefore a patient treats it as a secret and sometimes even family members are not informed*. [Parent SSI_male].

Likewise, one parent said:

> *People do not take urinary schistosomiasis seriously because it is not an acute condition. It starts slowly and children between 5 and 16 years old get infected and healed after treatment. Some people think that the disease is hereditary and one can grow up with it. The disease affects mostly school children* [Parents FGD_male].

Generally, adult participants were more likely to perceive urinary schistosomiasis as a disease of low priority compared to children.

3.5.3 Perceptions on transmission of urinary schistosomiasis

Discrepancies in schistosomiasis knowledge were revealed among both parents and children, with misconceptions on the real cause of schistosomiasis. Majority (65%) of child respondents and 27% of parent informants had incorrect knowledge on the transmission of urinary schistosomiasis. Informants associated urinary schistosomiasis with diet related infection by eating too much tamarind fruit, eating too much table salt, eating raw food, drinking dirty water or as a hereditary disease where children are born with schistosomiasis. Of the respondents interviewed, 70% of children and 48.3% of parent participants had no clear knowledge to distinguish between schistosomiasis and soil transmitted helminths. Participants pointed out that going to the toilet and bath rooms with bare feet as well as walking bare feet is a means of transmission of urinary schistosomiasis a perception which is incorrect (Table 7). One participant reported: *"Children in our community normally attend cattle. They walk long distances bare footed and therefore they are likely to get infected with urinary schistosomiasis"* [Children SSI _male]. On the same note, one participant said: "*We normally get urinary schistosomiasis because we walk bare feet, we don't like walking like this but we don't have shoes"* [children FGD females].

However, a few adult participants wrongly described urinary schistosomiasis as a sexually transmitted disease. As one parent reported: "Urinary *schistosomiasis is transmitted through having sex with the affected person and drinking dirty water*" [Parent SSI_male]. Other participants reported to know nothing about the transmission of the disease.

Of the total informants in the study, the majority of child participants (75%) did know that urinary schistosomiasis is transmitted through swimming in ponds and wells infested with schistosome cercariae. Of the adult informants, only 44.8% reported to have the correct knowledge of urinary schistosomiasis transmission through water contact (Table 7).

In case of knowledge about transmission of the disease, only 40% of children participants reported that the disease is transmitted by snails. Of the parent informants, very few (31%) knew that snails present in water sources are responsible for the transmission of urinary schistosomiasis (Table 7). One standard six female pupil said: *"We children normally play in ponds and wells, the water in these sources is not safe, it is full of micro organisms and snails that made us get urinary schistomiasis".* In one focus group discussion, a participant mentioned: *"The presence of snails, children eat a lot of tamarind fruit, drink stagnant water and take baths in infested water pools."*[Parents FGD_male].

Most (80%) of the child respondents perceived urinary schistosomiasis as a man made disease. Fewer (37.8%) adult respondents agreed that urinary schistosomiasis is a result of human behavior due to indiscriminate disposal of human waste products. Participants argued that had it not been for human insanitary behavior (indiscriminate excreta disposal) schistosome eggs would not have been introduced in water and the whole schistosomiasis life-cycle would not have begun (Table 7). One participant remarked: "*Indeed schistosomiasis is a man made disease because of non-use of latrines for defecating and urinating. Excreta disposal in or near water is responsible for the infection when people get into contact with infected water."* [Parent SSI_female]. Children mentioned the issue of urinating in open spaces as the factor for the spread of urinary schistosomiasis from one person to another. One child reported: *"When an infected person urinates or defecates around or inside the water sources, this transmits urinary schistosomiasis because people use the same water for different uses like drinking, cooking, washing clothes and taking baths"* [Children FGD_female]. However, some participants disagreed with the disease being man made, one parent said, "*It is because of the environment we live in, and as from time immemorial we have been using the same water from shallow wells and ponds. And it is not 'man-made' because snails are the cause."* [Parents FGD_male].

3.5.4 Water contact practices

About three-quarters of the children participating in the study knew that water contact practices had impact on urinary schistosomiasis transmission, they highlighted that children have a habit of playing in water as part of recreational activities. In contrast, only 44.8% of parent informants reported knowing that swimming, playing and fishing in water ponds, is a risk behavior for transmission of urinary schistosomiasis among children (Table 7). The study participants reported that children prefer playing in water during the evenings. One pupil reported;

> *Children play in the infested water during the evening because most of the time the rain comes in evening so we play in settled rain water, also in the evening we go to swim in the ponds as the owners of the ponds will have left already.* [Children FGD _female].

3.5.5 Symptoms of urinary schistosomiasis

Of the respondents interviewed, 65.5% of parents had correct knowledge of urinary schistosomiasis symptoms. They reported lower abdominal pain and passing blood in urine as

the common symptoms for the disease. For children informants, the majority had adequate knowledge of the symptoms of urinary schistosomiasis (Table 7). However, symptoms like irritation and frequent urination were infrequently mentioned by respondents. Very few participants had no clear understanding of the disease symptoms. One adult participant said: "*Among the symptoms of urinary schistosomiasis include lower abdominal pains, lesions along urinary tract and then passing blood in urine and more often experiencing pain in the genitals, yellowish urine and painful urination"* [Parents FGD_male].

3.5.6 Measures to prevent and control urinary schistosomiasis

Study participants agreed that schistosomiasis is preventable. They mentioned a number of preventive measures of which 30% of school children and 24.1% of parent participants stated that avoiding random excreta disposal in or near water bodies, environmental hygiene and observing general cleanliness is an important strategy for overcoming transmission of urinary schistosomiasis. Seventy five percent of child participants and 31% of parents interviewed stated that avoiding playing in water and swimming in ponds and water pools will reduce transmission of urinary schistosomiasis. However, very few (5%) of child participants and 6.8% of parent participants mentioned snail control in the water sources through the use of chemicals (molluscicide) as a means of reducing transmission (Table 7).

Over 27% of parents who participated in the study noted that health education among community members is the most important preventive measures against schistosomiasis. Twenty five percent of school children also reported that health education is necessary means of prevention against schistosomiasis (Table 7).

Participants went further by categorizing the measures to minimize contact with infested water in two ways, government and community efforts. For the government, participants suggested provision of deep protected wells and water taps as the most important and permanent alternative source of safe water in their local context. One child said "*The government has been trying to treat water sources in our communities but that is not enough, it has to be done frequently"* [Children FGD_female]. Another participant said *"The Government can even treat the same water that we are using, we are requesting the government to do that"* [Children FGD_male]. At community level, participants suggested construction and proper use of latrines and bathrooms as efforts to minimize contamination of water sources. It was suggested that for proper human excreta disposal, each household should construct and use latrines and bathrooms instead of using open spaces for urination.

To prevent urinary schistosomiasis, parents and children expressed a need for mutual efforts among community members. Most of the parents and children suggested that children should not be allowed to play, swim and fish in water bodies contaminated with human excreta, wearing protective shoes while working in water environments, boiling water and health education as measures to control urinary schistosomiasis among children. One child said: "*We are advising to have people who will act as watchmen in those ponds where children go to swim. This will scare children from going there to swim"* [Chldren FGD_male]. Furthermore, some of the study participants suggested the use of molluscicides for snail control so as to make their water sources safe. The majority of study participants agreed that urinary schistosomiasis is curable by modern medicines, by provision of praziquantel as an anti-schistosomal drug, whilst traditional sector of health care was considered to have insignificant role in the treatment of schistosomiasis. However, community health seeking behavior differs from one family to another and from one person to another. Some participants reported that they attended hospitals only when the disease was advanced. One child said *"A schistosomiasis patient is only taken to the hospital when his/her condition becomes severe and fails to walk alone, otherwise he/she will not be taken to the hospital"* [Children FGD_ male]. One of the children FGD participant reported, "*There is a traditional medicine which is known by local name as (Ntangala). Patients have to boil its roots mixing with water then drink"* [Children FGD_ female].

Table 7: Comparison of responses between children and parents on semi structured interview

Variable	**Children response (%) n=20**	**Parents/guardians response (%) n=29**
Sex		
Female	10 (50%)	14 (48.3%)
Males	10 (50%)	15 (51.7%)
Age	12-15 years	25- 80years
Schistosomiasis not perceived to be a major health problem	10 (50%)	22 (75.8%)
Transmission		
Completely wrong (hereditary, diet related)	13 (65%)	8 (27%)
Mixed up with gut helminth (eg bare foot around toilets)	14 (70%)	14 (48.3)
Correct that it involves water contact	15 (75%)	13 (44.8%)
Knowledge that snails transmit	8 (40)	9 (31%)
Perception that is man made through excrete disposal	16 (80%)	11 (37.9%)
Water contact through playing	19 (95%)	13 (44.8%)
Symptoms		
Abdominal pains	16 (80%)	19 (65.5%)
Passing of blood	16 (80%)	19 (65.5%)
Measures to prevent control		
Avoiding random excreta disposal in or near water water bodies (environmental hygiene)	6 (30%)	7 (24.1%)
Avoid playing/swimming in water	15 (75%)	9 (31%)
Snail control	1 (5%)	2 (6.8%)
Health education	5 (25%)	8 (27%)

3.6 Discussion

For the successful and sustainable control of urinary schistosomiasis, the disease must be considered as a major public health problem in the community. To achieve this, community members needs to have the relevant knowledge, correct perceptions and to apply the correct preventive and control measures. This study was conducted with the intention to complement quantitative findings of a parasitological and malacological study conducted in Shinyanga district (Angelo *et al.*, 2018), by exploring individual and community social contexts of urinary schistosomiasis. The study used descriptive qualitative research inquiry design to solicit information from community members on knowledge, perceptions and practices of schistosomiasis transmission.

The study findings revealed that in Shinyanga, community members considered schistosomiasis as a disease of low priority. Participants reported acute diseases such as malaria and cholera as high priority diseases compared to schistosomiasis (Goran *et al*., 2010). In Shinyanga district, this was attributed to the chronic nature of schistosomiasis where many years of infection often occurring before experiencing severe symptoms. A similar observation was reported by Kloos who argued that "*except for heavy infections, schistosomiasis is considered a relatively unimportant disease in many endemic areas. Affected communities tend to consider haematuria as a normal growth stage during childhood* (Kloos, 1995). This suggests that both parents and children in the community were not well informed about the disease, consistent with findings from Kenya (Odhiambo *et al*., 2014). Control and eventual elimination of the disease could be very difficult if the affected community does not perceive the disease as a major public health problem (Dawaki *et al*., 2015; Kapito-tembo *et al*., 2009; Maseko *et al.*, 2018; Rollinson *et al.*, 2013). There is therefore a strong need to supplement parents and children's knowledge on schistosomiasis to sufficiently raise disease awareness to a level that influences practices with an emphasis on behavioral change.

Most of the child respondents did, however, have correct knowledge on the transmission of urinary schistosomiasis, knowing that it occurs through water contact. Parents also had fair knowledge about true transmission modes of the disease. These findings were consistent with an observation from Ghana where over 60% of the community members knew that the disease was transmitted through water contact (Yirenya-tawiah *et al.*, 2011). However, this knowledge was often combined with misconceptions whereby some respondents had

misconceptions about the disease aetiology perceiving the disease to be hereditary, sexually transmited or attributing it to diet related infections. Similar misconceptions were reported by Mwanga *et al* in Magu district, Tanzania and by Bruun et et al in the North-west part of the country (Bruun & Aagaard-Hansen, 2008; Mwanga *et al*., 2004). In Mozambique, 22% of participants reported that schistosomiasis is a sexually transmitted disease, while 12% thought that the disease is hereditary indicating that similar misconceptions can occur over wide geographical and cultural settings (Rassi *et al.*, 2016). Amongst, child participants, misconceptions went further by associating the disease with eating too much tamarind fruit, too much table salt and drinking of dirty water. A similar observation where drinking of contaminated water was perceived to be among the cause of schistosomiasis infection has previously been reported (Odhiambo *et al.*, 2014; Rassi *et al*., 2016). Our study participants, particularly children also confused urinary schistosomiasis transmission with soil-transmitted helminth infections and reported that going to the toilet or bathrooms with bare feet as well as walking bare feet could lead to transmission of urinary schistosomiasis. However, overall, the majority of the study participants were more informed about the disease symptoms of urinary schistosomiasis. This observation is in line with other studies conducted elsewhere, where high knowledge of urinary schistosomiasis was reported (Mwai *et al.,* 2016; Rassi *et al.*, 2016; Sacolo, Chimbari & Kalinda, 2018; Sady *et al.*, 2015; Yirenya-tawiah *et al.,* 2011).

Despite clear understanding amongst school children that water contact practices relate to transmission of urinary schistosomiasis, study respondents revealed that children were more exposed to schistosomiasis infection due to their active water contact to activities such as swimming, fishing and playing in infested water in line with findings of other studies (Traore, Karthe, Ali, Coulibaly & Martin, 2016; Gbalégba *et al.*, 2017; Goran *et al*., 2010; King, 2012; Lothe, Zulu, Øyhus, Kjetland & Taylor, 2018; Mwanga & Lwambo, 2013). Some study participants did suggest refraining from infested water as a preventive measure against schistosomiasis. However, without investment in water and sanitation infrastructure, this is not easy to achieve, as people have no other alternative sources of water to use (Rollinson *et al.,* 2013). It should be born in mind that improved access to safe water such as deep and protected wells; water taps and sufficient sanitation are effective in reducing schistosomiasis infection (Freeman, Grimes, Croll, Harrison & Templeton, 2014).

Parents and children health seeking behavior relied on both traditional and modern medicines. Based on the knowledge of disease symptoms and observable treatment outcome

(Bruun & Aagaard-Hansen, 2008), modern treatment for urinary schistosomiasis was the preferred option over traditional medicine among most participants. This was influenced by increased effectiveness of modern medicines in curing the disease over herbal remedies. Similar observations were reported in Ghana and elsewhere in Tanzania (Danso-Appiah, Bosompem & Habbema, 2004; Mwanga, 2005). However, some participants opted for traditional medicine due to perceptions that the disease lacks serious impact and were assumed to be part of their normal life. Self-treatment was practiced by participants as they considered the disease to be a secret of an infected individual and shameful to speak about, even with other family members. This is consistent with findings reported in Ghana and elsewhere (Cronin, Sheppard & de Wildt, 2013; Danso-Appiah *et al.,* 2004; Rassi *et al.*, 2016). The use of combined therapies (traditional and modern) for schistosomiasis treatment was also reported in other studies (Midzi *et al.*, 2011; Mwanga *et al*., 2004; Person *et al*., 2016).

3.7 Conclusion

A meaningful schistosomiasis control programme should take into account people's knowledge, perceptions, attitudes and practices on the disease. Despite good knowledge, perceptions, attitudes and practices on schistosomiasis by a substantial segment of the study participants, this study revealed misconceptions and inadequate knowledge on the disease among participants in Shinyanga district which suggest that participants had limited information about the disease which in turn calls for health education campaigns as part of disease control interventions. Collaborative efforts between the government and the community are required to reduce water contact behaviors that results in the transmission of urinary schistosomiasis. To make this feasible, health education and availability of alternative sources of clean and safe water are recommended to complement ongoing efforts to control schistosomiasis in Shinyanga district and other endemic areas.

CHAPTER FOUR

Patent and pre-patent infection trends of *Schistosoma haematobium* and *Schistosoma bovis* in *Bulinus nasutus* snails in a schistosomiasis endemic setting of Shinyanga district, Tanzania[3]

Teckla Angelo[1, 2]*, Henry Curtis Kariuki[3], Safari M. Kinung'hi[2], Joram Buza[1,] Shona Wilson[4]

1. Department of Global Health, School of Life Sciences and Bioengineering, Nelson Mandela African Institution of Science and Technology, P. O. Box 447, Arusha, Tanzania.
2. National Institute for Medical Research, Mwanza Centre, P. O. Box 1462, Mwanza, Tanzania.
3. Kenya Methodist University, School of Medicine and Health Sciences, P.O. Box 267, Meru, Kenya.
4. Department of Pathology, University of Cambridge, Tennis Court Road CB2 1QP, United Kingdom.

*Corresponding author (tecklaangelo@yahoo.com)

[3] Manuscript not yet submitted

Abstract

Background

Urogenital schistosomiasis, a snail water borne disease caused by *Schistosoma haematobium* is of medical importance in schistosomiasis endemic areas. The transmission of *S. haematobium* is determined by the distribution of *Bulinid* snails and overlaps with the transmission patterns of the closely related schistosome *S. bovis.* In some transmission areas the two parasites may infect the same snail hosts, resulting in competition between parasites. Therefore, patterns of sympatric transmission between *S. haematobium* and *S. bovis* and species identification need to be assessed to identify if transmission of *S. haematobium* is modified by the presence of *S. bovis.*

Methodology

A longitudinal malacology survey was conducted over 46 sites in two neighbouring villages of Shinyanga District, Northwestern Tanzania, for 16-months. DNA was extracted from all snails found to shed cercaria, and a subset of non-schistosome shedding snails, to a maximum of 30 snail samples per time point and site, totaling 1898 snails across all sites and time points. A multiplex PCR of the Cox1 repeat, that differentiates *S. haematobium* from *S. bovis* infections, was used to identify species of patent infection and prevalence of pre-patent infection within *B. nasutus*. Seasonal and spatial trends in transmission of the two parasites were analysed.

Results

Out of 1898 *Bulinus nasutus* snails extracted, 100 (5.17%) were infected with *Schistosoma haematobium,* 291 (15.07%) were infected with *Schistosoma bovis* while 16 (0.84%) were *S. haematobium* and *S. bovis* coinfected snails. *S. bovis* was highly transmitted throughout the different habitats sampled, ranging in prevalence from 5.44% to 40%. Among habitats, Lyandu river had the highest prevalence of *S. bovis* (40%), while amongst seasonal ponds, Miyu ponds, had the highest prevalence at 30%. For *Schistosoma haematobium* infection, the prevalence between habitats varied but less so than *S. bovis.* A high prevalence of 8.7% was found amongst snails from Miyu and Mwakasela pond habitats. Other snail habitats had a prevalence of *S. haematobium* ranging from 0% to 5%. Water sources shared between humans and domestic animals had significantly higher *S. haematobium* (OR 2.53; 95%CI 1.59-4.05; *p<0.0001)* and *S. bovis* (OR 2.29; 95%CI 1.53-3.45; P<0.0001) infection rates in

snails. The prevalence of *S. bovis* infection in snail intermediate hosts was significantly reduced, during the wet season (OR 0.17; 95%CI 0.09-0.32; P<0.0001).

Conclusion

The current study findings demonstrated that both *S. haematobium* and *S. bovis* are transmitted by morphologically identical snails mostly *B. nasutus* in a sympatric situation. Infection prevalence pattern varied by season and mainly occurred in water sources shared between humans and animals. *Schistosoma bovis* was more dominant over *S. haematobium* which suggests that parasite competition for snail intermediate hosts may occur in this area. Further experimental and genomic studies are required to investigate *B. nasutus*-parasite compatibility and the potential for host competition between *S. haematobium* and *S. bovis* in this area where sympatric transmission occurs.

Key word: *Bulinid snail, S. haematobium, S. bovis* schistosomiasis, pre-patent infection, Sympatric transmission.

4.1 Background

Urinary schistosomiasis caused by *Schistosoma haematobium* (Rollinson *et al.*, 2001) and bovine schistosomiasis caused by *S. bovis* (Malek, 2017) are both water borne diseases transmitted by snail intermediate hosts from *Bulinus africanus* group (Kinoti, 1964). *Schistosoma bovis* infects cattle, goats, sheep and other ruminants causing intestinal schistosomiasis that can lead to intense farm production losses (Abbasi, Webster, King, Rollinson & Hamburger, 2017; Bont & Vercruysse, 1997; Christensen, 1983; Kinoti,1964). *Schistosoma bovis* very rarely infects humans (Christensen, 1983), unlike the closely related *S. haematobium* which is mainly transmitted among humans and estimated to infect more than 110 million individuals in sub Saharan Africa, causing an estimated 70 million cases of haematuria, 18 million cases of bladder morbidity, and10 million cases of complicated kidney disease (Boissier *et al*., 2016; King, Dickman & Tisch, 2005; Marieke, Werf, Vlas, Brooker, Looman, Nagelkerke & Engels, 2003). The parasite rarely infects other primates like monkeys, chimpanzees, and baboons (Pitchford, 1977).

Schistosomes require a snail intermediate host to complete the asexual part of their lifecycle (Webster, Diaw, Seye, Webster & Rollinson, 2013), with variation in parasite-snail compatibility. Different schistosomes may exploit a similar susceptible snail intermediate host concurrently, for instance *S. haematobium* often occurs sympatrically with *S. bovis* and naturally infect common snail hosts from *Bulinus* genus (Christensen, 1983). *Schistosoma bovis* is reported to have a range of snail vectors from *Bulinus* genus (Mutani, Christensen & Frandsen, 1983), and its transmission overlaps with that of *S. haematobium* (Abbasi *et al*., 2017) and occurs in similar habitats (Abbasi *et al.*, 2017; Barber, Mkoji & Loker, 2000; Kinoti, 1964). However, any potential impact of sympatric transmission of *S. bovis* in the area over *S. haematobium* transmission is yet not well understood, requiring thorough investigation of the transmission patterns of the two schistosome species transmitted in the same habitats by common snail hosts.

In Tanzania, the *B. africanus* group which serve as intermediate hosts for *S. haematobium* and *S. bovis* consists of *Bulinus africanus, B. globosus* and *B. nasutus* (Webbe & Msangi, 1958). In coastal areas of Tanzania *B. globosus* is the major host for disease transmission (Rollinson *et al.*, 2001) while *B. nasutus* is less important; an effect enhanced by the majority of its distribution being outside of the schistosomiasis transmission zone (Allan, Rollinson, Smith & Dunn, 2009; Stothard *et al*., 2002). In Northwestern Tanzania, *B. nasutus* is a

principle host for transmission of urinary schistosomiasis (Webbe, 1962). It is widely spread in seasonal pools occupying different types of habitats and capable of withstanding prolonged dry periods by aestivation (Lwambo *et al.*, 1999). It is also the intermediate host of *S. bovis* (Jelnes, Thiongó, Lwambo & Ouma, 2003; Kariuki *et al.*, 2004).

Schistosoma haematobium and *S. bovis* are closely related parasites making it practically impossible to differentiate their cercariae by morphological means (Abbasi *et al.*, 2017; Barber *et al.*, 2000; Kaukas, Johnston, Simpson, Reseurch & Prudente, 1997; Kinoti, 1964; Webster *et al.*, 2010). This necessitates the use of molecular methods to clearly identify specific schistosome species infecting *Bulinus* snail hosts. In this study, we elucidated patent and pre-patent infection trends of *B. nasutus* snails by *S. haematobium* and *S. bovis* in Shinyanga district, Northwestern Tanzania and discriminate the two schistosome parasites using multiplex PCR.

4.2 Methods

4.2.1 Study area and population

The study was conducted in Ikingwamanoti and Nduguti villages, Itwangi ward in Shinyanga district, which is situated in the vicinity of the southern part of Lake Victoria. The district lies between 03. 846002° South of the Equator and 33.107313° East of the Greenwich Meridian. Shinyanga receives two rainy seasons, the short rainy season that occurs from November to December, with intermittent rain, and the long rainy season from March to May. The district is semi arid with black cotton soil. Inhabitants of the area are the *Sukuma* ethnic group whose major income generation activities are crop farming and livestock keeping. Major crops cultivated in the area include maize, cotton, millet, sorghum and sweet potatoes, and cultivation of rice in paddy farms, Water supply in Shinyanga is scant, especially in rural areas where the limited water sources are often demarcated for livestock watering. During the dry season the major source of water in the village is primarily from temporary water pools and series of ponds created from dry river beds after the rains. These ponds and streams harbour the snail intermediate hosts for schistosomiasis.

4.2.2 Snail collection and cercarial shedding

Snail surveys were conducted on monthly basis from 10 habitats with 46 transmission sites from March 2016 to July 2017, from different water sources available in Ikingwamanoti and Nduguti villages. To quantify the snail abundance, snails were collected by one person

searching for 10 minutes per site using hand held scoop made up of pole covered by 2mm wire mesh. Snails were searched for the entire part and edges of the site and in the underside of water lilies, floating fragments in the site were picked using forceps and checked for snails. Snails collected were provisionally identified to genus level based on shell morphology using the Danish Bilharziasis Laboratory field guide for African Freshwater snails (Danish identification key for snails). Snails were placed in labeled aerated plastic containers covered by wet cotton wool and transferred to the laboratory. In the laboratory, snails were cleaned, separated into size categories based on shell size, and categorized into eight groups: size 1 (1-3 mm), size 2 (4-6 mm), size3 (7-9 mm), size 4 (10-12 mm), size 5 (13-15 mm), size 6 (16-18 mm), size 7 (19-21 mm) and size 8 (> 21 mm). Snails were counted and then placed individually in welled plates filled with bottled mineral water and exposed to sunlight for 2-hours to stimulate cercarial shedding, after which the wells were examined for cercariae using a Stereomicroscope. Emerging schistosome cercariae were identified based on morphology. The number and size of snails shedding schistosome cercariae were recorded and immediately stored into a labeled glass vials filled with absolute ethanol. Snails shedding non mammalian cercariae were also recorded. On the third day of shedding, all snails were preserved in absolute ethanol. The list of habitats surveyed during the entire study period is shown in (Table 8).

Table 8: Snail habitats and sampling sites identified

Village name	Habitat number	Habitat name	Habitat type	No. of sites
Ikingwamanoti	1	Mwachumi	Pond	5
Ikingwamanoti	2	Miyu	Pond	2
Ikingwamanoti	3	Mwakasela	Pond	7
Ikingwamanoti	4	Mwamunonge	Pond	6
Ikingwamanoti	5	Jumanne	Pond	5
Ikingwamanoti	6	Mwamalago	Pond	3
Ikingwamanoti	7	Mwakangota	Pond	2
Nduguti	8	Kitongo	Stream	4
Nduguti	9	Bulaya	Pond	5
Nduguti	10	Lyandu	River	7
Total				**46**

4.2.3 Snail DNA extraction and Polymerase Chain Reaction (PCR)

From each snail habitat, up to a maximum of 30 preserved snails, including patent infected snails, were tested individually by PCR to detect pre-patent and speciate schistosome infections into *S. haematobium* and/or *S. bovis*. The genomic DNA was extracted from the whole snail tissue using Qiagen DNeasy blood and tissue kit (Qiagen Crawley, UK). A multiplex PCR amplification was conducted using primers specific to the mitochondrial cytochrome oxidase sub unit 1 (*Cox1*), with one universal forward primer and two species specific reverse primers as described in (Webster *et al*., 2010b). A total reaction volume of 25µl was used for PCR amplification, including 100 µM GE Healthcare 'Ready-To-Go' PCR beads, 10pmol of each primer and 3µl of genomic DNA. In a conventional PCR machine (GeneAmp® PCR System 9700 from Applied Biosystems) the thermal cycler programme was set at 95°C for 5 minutes for the initial denaturation, 95°C for 30 seconds for 40 cycles, 58°C for 30 seconds for annealing of primers, 72°C for 1 minute and 72°C for 10 minutes for final elongation period. The PCR amplicons were visualized on 2 percent (%) agarose gels stained with Gel Red, along with a DNA ladder. *S. haematobium* amplicons were 543bp in length while *S. bovis* amplicons were 306 bp in length.

4.3 Ethics

The study was part of a larger study linking human *S. haematobium* infection to intermediate host population dynamics that received approval from the Medical Research Coordination Committee (MRCC) of the National Institute for Medical Research (NIMR), Tanzania (ethics clearance certificate no. NIMR/HQ/R.8a/Vol.IX/2107 and the University Of Cambridge Human Biology Research Ethics Committee (HBREC.2015.28).

4.4 Statistical analysis

Data were analyzed using R version 3.5.1 statistical software. Snail habitat Mwachumi was removed from the analysis as only one snail was collected throughout the study time. Kitongo Stream is also not included in the analysis as no snail was found to be infected from this habitat for the entire study period. Binomial logistic regression models were built to assess significant variables contributing to the infection of snails on a particular site. Model fit were assessed using the Akaike Information Criterion (AIC). Two-way interactions were explored but none were significant and they were removed from the model. Odds ratios were calculated with 95% confidence intervals (CI). A *P*- value with 0.05 was considered statistically significant.

4.5 Results

4.5.1 Detection of pre-patent schistosome infections

In total, genomic DNA was extracted from 1898 *B. nasutus* snails collected from Miyu, Mwakasela, Jumanne, Mwamunonge, Mwakangota, Mwamalago habitats in Ikingwamanoti village, all of which are ponds and Bulaya pond and Lyandu River habitats from Nduguti village. One hundred snails (5.17%) were found to be infected with *S. haematobium,* while 291(15.07%) were infected with *S. bovis* (Table 9). The prevalence of *S. haematobium* infection of snails varied from one habitat to another. The highest *S. haematobium* infection prevalence was observed in Mwakasela habitat with 51 (8.67%) and Miyu 2 (8.70%) snails been infected, followed by Mwamunonge habitat where 24 (5.58%) of snails were infected by *S. haematobium*. A low number of the tested snails were found to be infected at other sites. The snail site with the lowest *S. haematobium* prevalence was Jumanne habitat, where 5 (1.67%) snails were found infected with *S. haematobium*.

The prevalence of *S. bovis* infection within the intermediate host snails was observed to be higher compared to *S. haematobium* at all habitats surveyed. Among snail habitats, Lyandu River had the highest prevalence of snails infected by *S. bovis* (40%), followed by Miyu pond (30.43%) and Jumanne Pond (27.09%).All other snail habitats had a moderate prevalence of *S. bovis* infection, ranging from 5.44% to 15.81%. The *S. bovis* snail habitat with the lowest prevalence was Mwakasela habitat where 32 (5.44%) of the snails extracted from this site were infected *by S. bovis.*

Co-infection of *S. bovis* and *S. haematobium* among snails extracted varied between habitats, high prevalence of snails co-infected were found in Lyandu river (n=7(5%)), while no co-infection of snails were observed at several habitats, and low prevalence at others for example, co-infection was observed in only one snail collected from Mwamunonge snail habitat (0.23%) (Table 9).

Of those shedding cercaria, 18.02% were snails shedding *S. haematobium* while 66.67% were shedding *S. bovis* cercariae. Between habitats, Mwakasela had highest proportion of *S. haematobium* shedders compared to other snail habitats; the habitat with the lowest proportion of *S. haematobium* shedders was Mwamalago habitat with only 1%.

Table 9: Snail Infection by *S. haematobium* and *S. bovis* by snail habitats

Village	Habitat	Number Snails collected (%)	Number Snails tested (%)	Number infected		
				S. h (%)	*S. b* (%)	Co (%)
Ikingwamanoti	Miyu	49 (0.79)	23 (46.93)	2 (8.70)	7 (30.43)	2 (8.69)
Ikingwamanoti	Mwakasela	1677 (27.35)	588 (35.06)	51 (8.67)	32 (5.44)	4 (0.68)
Ikingwamanoti	Mwamunonge	1450 (23.65)	430 (29.65)	24 (5.58)	68 (15.81)	1 (0.23)
Ikingwamanoti	Jumanne	691 (11.27)	299 (43.27)	5 (1.67)	81 (27.09)	2 (0.67)
Ikingwamanoti	Mwamalago	150 (2.44)	49 (32.67)	1 (2.04)	7 (14.28)	0
Ikingwamanoti	Mwakangota	881 (14.36)	216 (24.51)	10 (4.63)	26 (12.03)	0
Nduguti	Bulaya	879 (14.33)	153 (17.41)	0	14 (9.15)	0
Nduguti	Lyandu	354 (5.57)	140 (39.54)	7 (5)	56 (40)	7 (5)
Total		6131 (100)	1898 (30.96)	100 (5.17)	291 (15.07)	16 (0.84)

S.h: *S. haematobium*; S.b: *S. bovis;* Co: Co-infected.

4.5.2 Detection of other factors contributing to *S. haematobium* and *S. bovis* infection

A binomial logistic regression model with robust standard errors to account for multiple sampling from the same habitats was used to assess the contribution of different factors on the prevalence of *S. haematobium* and *S. bovis* infection amongst the sampled intermediate snail hosts collected within the area. The model demonstrated that in water sources shared by

humans and domestic livestock, prevalence of both *S. haematobium* (OR 2.53; 95%CI 1.59-4.05; *P*<0.0001) and *S. bovis* (OR 2.29; 95%CI 1.53-3.45; P<0.0001) infection was significantly greater, compared to those where only human based activities were observed.

Season was classified into wet and dry based on whether or not 75% of snail sites contained water at the time of sampling. Using this criteria, the model indicated that season was a significant factor determining the prevalence of infection in snail intermediate hosts for the ruminant pathogen *S. bovis,* with prevalence being significantly reduced during the wet season (OR 0.17; 95%CI 0.09-0.32; P<0.0001) compared to dry season (Table 10). The same was not true for *haematobium*, for which season was insignificant. However, the number of snails found to be infected with *S. haematobium* was higher during the wet season, reflective of the expanded snail population size, with 1492 snails being tested during the wet season compared with 406 in the dry season (Table 10). Snail size, was not significant in predicting infection of either schistosome species and was removed from the model. As flow of water has previously been associated with snail populations, nature of habitat and its interaction with season were retained in the model, but were not significant predictors of infection for either parasite.

Table 10: Robust standard error regression model depicting different aspects contributing to the infection of *S. haematobium* and *S. bovis*.

Variable	No. of snails tested	No. (%) *S. haematobium* infected	OR (95%CI)	*P*-value	*No. (%) S. bovis* Infected	OR (95%CI)	*P*-value
Water contact							
Human based	562	15 (2.7)	1		41 (7.29)	1	
Human and Animals	1336	85 (6.36)	2.53 (1.59-4.05)	0.0001	250(18.71)	2.29 (1.53-3.45)	0.0001
Nature of habitat							
Pond	1758	93 (5.29)	1		235 (13.4)	1	
River	140	7 (5)	0.79 (0.43-1.46)	0.455	56 (40)	1.77 (0.61-5.16)	0.279
Season							
Dry	406	23 (5.6)	1		162 (39.5)	1	
Wet	1492	77 (5.1)	0.95 (0.65-1.42)	0.829	129 (8.5)	0.17 (0.09-0.32)	0.0001
Season and nature of habitat							
Dry season: pond	314	18 (5.73)	1		5.67)	1	
Wet season: river	48	2 (4.16)	0.79 (0.53-1.17)	0.226	6 (12.5)	0.69 (0.36-1.30)	0.248

4.6 Discussion

The study observed that both *S. haematobium* (human urinary schistosomiasis) and *S. bovis* (ruminant intestinal schistosomiasis) are transmitted in Shinyanga district from common habitats by morphologically identical *B. nasutus* snail intermediate hosts. Shared water sources between humans and domestic livestock are the chief source of infection of both *S. haematobium* and *S. bovis. A* significant reduction in the prevalence of *S. bovis* infection was observed during the wet season. *Schistosoma bovis* infection dominated the infection of *S. haematobium* in terms of the prevalence of infection observed amongst the intermediate hosts. Among other aspects, snail size had no significant impact in the transmission of *S. bovis* and *S. haematobium* parasites.

Amongst those habitats where infected snails were found, the prevalence of infection in snails was higher for the ruminant parasite S. *bovis* than for the human pathogen *S. haematobium*. Interestingly, Mwakasela snail habitat which had the highest detected prevalence of *S. haematobium* had the lowest prevalence of *S. bovis*, while Lyandu snail habitat which had the highest prevalence of *S. bovis* infected snails had the smallest number of *S. haematobium* infected snails. This could indicate: a) variations in type of mammalian host contact with the snail habitats, or b) equivalent water contact takes place in both habitats but the intermediate snail hosts complementary to the two parasites differ in ways not identifiable morphologically, and the conditions that support these snail populations differs or c) differing conditions for mammal-snail parasite transmission occurs for the two parasites. However, the provisional species identification of *Bulinus s*nail intermediate hosts by morphological means did not clearly elucidate the snail species responsible for transmission of both *S. haematobium* and *S. bovis* in this area. To determine which of the above scenarios is at play in Shinyanga region, molecular approaches to snail species identification, combined with snail - parasite compatibility experiments are required.

The infection patterns of schistosomes varied between seasons, low infection of *S. bovis* was observed during the wet season compared to dry season. This may be explained by floods and high flowing speed of rain water which sweep away snails. However, neither habitat type nor the interaction between habitat type and season had any significant relationship with prevalence of *S. bovis* observed amongst those snails tested for infection. The reduction in the prevalence of infection of snails with S. *bovis* may instead reflect greater availability of water, reducing contact by domestic livestock at any individual habitat during the period of

high level of water, thereby reducing the force transmission of the miracidia to the snail population. The duration of pre-patent period in relation to life expectancy of *Bulinus* snail intermediate hosts may also have an impact, as the cyclical change of environmental conditions with the season can alter the probability of schistosome infection development within individual snails (Woolhouse & Chandiwana, 1988). Similar observations of disparity of *S. bovis* infection by season, whereby high peak of *S. bovis* infection was observed in summer and remained low until the next summer period have previously been reported (Shiff, *Coutts*, Yiannakis & Holmes, 1979; Sturrock, 2001).

In schistosomiasis endemic areas with limited supply of clean and safe water for inhabitants, the available water sources are mainly shared by both human and their domestic livestock (Huyse *et al*., 2009). This is the case with schistosomiasis transmission; in Shinyanga district especially in rural settings where bore holes are not common and people use their source of water for domestic purposes, as well as for watering their domestic livestock. It remains to be determined whether the finding that both *S. haematobium* and *S. bovis* are more prevalent within the habitats where humans and animals have shared water contact is due purely to behavioural reasons or whether complex interactions between definitive host behaviour and intermediate snail host and parasite ecology are at play in this increased transmission between definitive and intermediate host. However, reduction of contact with water providing a niche for *Bulinus* snail species, by provision of clean and safe water to community members, is likely to reduce transmission. Whether demarcation of water resources for livestock could also impact transmission of the disease requires further investigation.

4.7 Conclusion

Findings of this study reveal that morphologically identical snails, typical of *B. nasutus*, transmit both *S. haematobium* and *S. bovis* in Shinyanga district. Water sources accessed by both humans and domestic livestock harbor obligate *Bulinus* snail intermediate hosts and foster transmission of both *S. haematobium* and *S. bovis* in this area, but only *S. bovis* infection among snail intermediate hosts showed a seasonal effect, being significantly lower when water sources were more readily available. *Schistosoma bovis* did though dominate infection of intermediate hosts over *S. haematobium*, suggesting that host competition between the two schistosome species may occur, but this requires further examination. Further studies should investigate in detail the species of *Bulinid* snails responsible in transmission of *S. haemtobium* and *S. bovis*, validate compatibility of *B. nasutus* with *S.*

haematobium and *S. bovis* using molecular techniques and assess the sympatric impact of *S. bovis* over *S. haematobium* in schistosomiasis endemic settings in order to ascertain any influence on the transmission of the human parasite.

CHAPTER FIVE

Ecological determinants of *Bulinus nasutus* snail intermediate hosts population and transmission patterns of urogenital schistosomiasis in endemic setting of Shinyanga district, Tanzania[4]

Teckla Angelo[1,2]., Curtis Kariuki[3]., Safari M. Kinung'hi[2]., Joram Buza[1]., Shona Wilson[4]

1. Department of Global Health, School of Life Sciences and Bioengineering, Nelson Mandela African Institution of Science and Technology, P. O. Box 447, Arusha, Tanzania.
2. National Institute for Medical Research, Mwanza Centre, P. O. Box 1462, Mwanza, Tanzania.
3. Kenya Methodist University, School of Medicine and Health Sciences, P. O. Box 267, Meru, Kenya.
4. Department of Pathology, University of Cambridge, Tennis Court Road CB2 1QP, United Kingdom.

Corresponding author: tecklaangelo@yahoo.com

[4] Manuscript not submitted

Abstract

Background: Transmission of urogenital schistosomiasis in Tanzania is determined by the presence of *Bulinus* group of snails as a major host for *Schistosoma haematobium*. However, population abundance and transmission patterns of *B. nasutus* tends to vary requiring thorough understanding of *the* ecological factors determining this variations in order to interrupt schistosomiasis transmission.

Methods: Snail habitats were identified from interviews with local community leaders and monitored for the duration of 17 months. Snails were collected on monthly basis for 10 minutes per site using hand held scoops. Snail sizes were measured using a standard height sheet. Patent infection of snails was determined by exposure to sunlight and microscope. On every snail site physicochemical parameters of water conditions were measured and recorded using a hand held water meter.

Results: Out of 6202 *Bulinus nasutus* snails collected 190 (3.06%) were found to shed schistosome cercariae. Between snail habitats the prevalence of cercarial shedding ranged from 0% to 13.84%. The population abundance of *B. nasutus* was determined in relation to rainfall patterns. Water conductivity and presence of vegetation were significantly associated with snail population abundances (OR 1.23; 95%CI 1.13-1.34; P<0.0001) and (OR 6.84; 95%CI 2.75-16.99; P<0.0001) respectively. In addition, increase of conductivity within snail habitats with no vegetation was associated with high snail population density (OR 0.76; 95%CI 0.68-0.86; P<0.0001) than in snail habitats with vegetations. Patent infection was, significantly associated with increased temperature in pond habitats (OR 0.35; 95%CI 0.45-0.62; P<0.0001), but not rivers. Other physico-chemical parameters had no effect on snail abundance and patent infections (P>0.05).

Conclusion: The study findings indicate that *B. nasutus* population densities were determined by patterns of rainfall, conductivity and vegetation. For *B. nasutus* to shed schistosomes cercariae increase of water temperatures in pond habitats is important. Considering the trend of *B. nasutus* population density and temporal variations of snail infectivity control measures should consider timing of snail control measures in relation to season in order to interrupt transmission of urogenital schistosomiasis in endemic areas.

Key words*:* Ecological determinants, *B. nasutus*, snail population abundance, schistosomiasis transmission patterns, Shinyanga district, Tanzania.

5.1 Background

Schistosoma haematobium is a parasitic digenetic trematode that is widely distributed in different geographical areas of Tanzania (Clements *et al*., 2008; Rollinson *et al.*, 2001b). It is transmitted by snail intermediate hosts of the genus *Bulinus* (Stothard *et al*., 2002). In Northwestern Tanzania including Shinyanga region, *Bulinus nasutus* is the principal host for the transmission of urogenital schistosomiasis (Brooker *et al*., 2001; Lwambo *et al*., 1999a; Webbe, 1962). *Bulinus nasutus* occurs in different geographical settings due to its ability to colonize various temporary water sources that develop after the rainy season, allowing rapid growth of the snail population (Labbo, 1995).

In Shinyanga region, observed prevalence of *S. haematobium* among school age children decreased after treatment. On a microgeographical scale, prevalence of *S. haematobium* also varied spatially and was linked with snail numbers (Angelo *et al.*, 2018). Taking into account that schistosome transmission is affected by temporal fluctuations of snail intermediate host abundance and exposure of these snail hosts to miracidia (Woolhouse & Chandiwana, 1988), the transmission of schistosome cercariae varies not only by geographical location but also by season (Klumpp & Chu, 1977b). The seasonal transmission pattern of urogenital schistosomiasis varies geographically. In Zimbabwe more snails shed cercariae during the dry season, at the time where water sources are limited and people have high water contact (Chandiwana, 1987). In contrast, in Kenya, shedding patterns of *B. nasutus* were found to follow the temporal variation of snail population densities (Kariuki *et al*., 2004).

The growth of snail populations can be affected by several factors including water turbidity, with algae development acting as food for snails, while water pollution by sewage and industrial wastes has an adverse impact on snail population (Dazo, Hairston & Dawood, 1966; De meillon, Frank & Allanson, 1958). As rainfall patterns impact on snail abundance, in addition to other biotic and abiotic factors (Fashuyi, 1981; Kariuki *et al.*, 2004), it is self evident that any effort to control the disease would require a thorough understanding of the snail population dynamics, bionomics and transmission patterns displayed by *Bulinid* snails.

This study investigated the fluctuation of *B. nasutus* snail population densities, the ecology of *B. nasutus* snails and the relationship between environmental factors and transmission patterns of urogenital schistosomiasis in Shinyanga district, Tanzania. The intention was to determine the ecological factors underlying seasonal *B. nasutus* snail population dynamics over a micro-geographical scale.

5.2 Materials and methods

5.2.1 Study area and population

Shinyanga District, which is located in northwestern Tanzania, has been shown historically to have high endemicity and recent ongoing *S. haematobium* transmission in areas with water bodies that provide a niche for the snail intermediate hosts (*Bulinid).* An anthropological study conducted in the community verified that the community relies on traditional wells, pools, ponds, streams and rivers available for water (manuscript submitted) for daily domestic needs. As a result occupational activities such as irrigation, fishing, collection of water for domestic uses, cattle watering and recreational activities are mostly conducted in these water sources, exposing individuals to *S. haematobium* infection. Agriculture (crop production such as rice farming and livestock keeping) is the primary activity practiced by inhabitants of Shinyanga region. Paddy farming provides the required conditions for breeding of snail intermediate hosts and transmission of infection.

5.2.2 Study design and sampling

The study was a longitudinal malacological survey with monthly assessments of snail abundance and patent (cercarial shedding) infections of *Bulinus* snails conducted from March, 2016 to July, 2017 in Ikingwamanoti and Nduguti villages. Forty six sampling sites were selected from streams, rivers and ponds (Fig. 6). Of these, 30 snail collection sites were from ponds, 9 sites were from streams and 7 were selected from a river (Table 11). A hand held Global Positioning System (GPS) was used to record the geographical coordinates of each sampling site during the survey. Snail sampling sites were selected based on observed/reported human water contact with a specific water body. The sites were categorized into habitats based on their geographical location, in which several snail collection sites were included. The size of each site ranged from 15 to 20 meters long based on the WHO snail sampling guideline (Operational & Programme). Snails were sampled by one person for ten minutes using handheld scoops and hand picking where necessary. Snails were searched for the entire part and edges of the site and in the underside of water lilies, floating fragments in the site were checked for snails and picked using forceps. Snails collected were immediately identified to species level based on shell morphology using Danish Bilharziasis fresh water identification key.

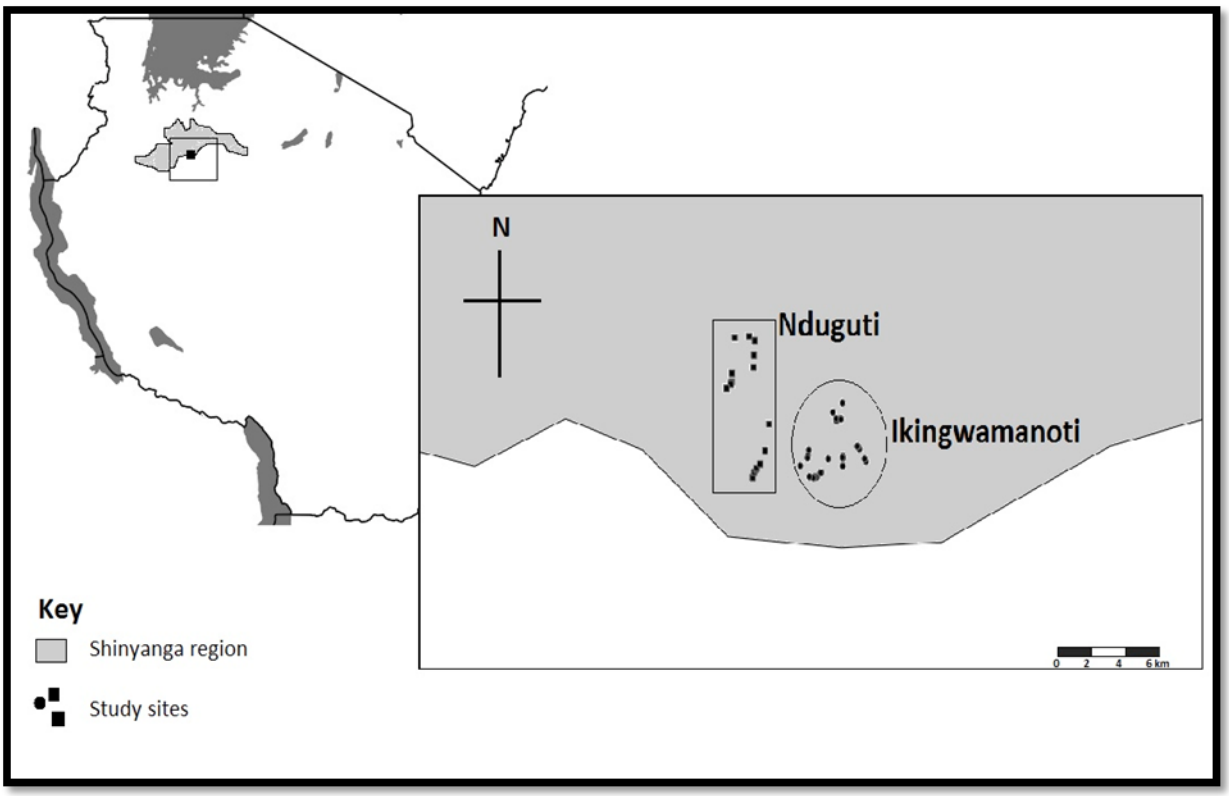


Figure 6: Map of 46 snail sampling sites of Ikingwamanoti and Nduguti villages, Shinyanga district.

Table 11: Snail habitats and snail transmission sites studied (n=46)

Village name	Habitat name	No.of sites	Site type
Ikingwamanoti	Mwachumi	5	Pond
Ikingwamanoti	Miyu	2	Pond
Ikingwamanoti	Mwakasela	7	Pond
Ikingwamanoti	Mwamunonge	6	Pond
Ikingwamanoti	Jumanne	5	Stream
Ikingwamanoti	Mwamalago	3	Pond
Ikingwamanoti	Mwakangota	2	Pond
Nduguti	Bulaya	5	Pond
Nduguti	Kitongo	4	Stream
Nduguti	Lyandu	7	River
Total		**46**	

5.2.3 Physico-chemical conditions and water contact observations

During the monthly snail collections for sites with a depth of >0.5 meters the pH, conductivity, salinity, total dissolved solids (TDS), and temperature of the water were recorded using hand held water meter (multi-parameter-PCSTestr 35, Eutech PCSTEST35-01X441506/Oakton 35425-10). Observations were made on the pace of water, whether flooded, normal, and low or dry, the nearby vegetation coverage and the substrate of water body floor. Types of livestock (cow, goat, sheep, donkey, dog) attending the sites and human activities around the collection site were observed and recorded.

5.2.4 Snail size and detection of patent infections

Snails were cleaned using cotton wool and the height of each snail from apex to the end of aperture was measured using a standard height sheet. The height of snails were categorized into eight groups depending on their size as 1 (1-3 mm), 2 (4-6 mm), 3 (7-9 mm), 4 (10-12 mm), 5 (13-15 mm), 6 (16-18 mm), 7 (19-21 mm) and those with the height 8 (greater than 21 mm). The eight snail groups were further set into three main categories comprising of young snails with the height of (1-9 mm), mature (10 mm to 15 mm) and adult (>16 mm). To examine for patent schistosome infections, snails were placed individually in 12 welled plates filled with bottled mineral water and exposed to sunlight for 2 hour to stimulate cercarial shedding. Light exposed snails were examined using a dissecting microscope. The shed cercariae were identified based on morphological characteristics and distinctive movement using standard identification guidelines (Christensen, 2017). The number and size of snails shedding mammalian cercariae were recorded. Snails shedding non mammalian cercariae were also recorded. The cercarial shedding exercise was repeated for three consecutive days.

5.3 Statistical analysis

The study data was analyzed using R Statistical package version 3.5.1 (2018-07-02). Negative binomial regression models were built to assess the role of water temperature, vegetation and abiotic parameters (pH, total dissolved solids (TDS), Salinity, Conductivity) to *Bulinus* snails abundance and numbers shedding schistosome cercariae. Snail sites grouped into the 10 habitats were used in the analysis. Season, with wet season classified as more than 75% of snails sites containing water, was included a priori. The models were improved based on the Akaike Information Criterion (AIC), by removing insignificant parameters in a step wise manner to get a final best fit model. Odds ratio (OR) were calculated with 95% confidence interval (CI). A *P*-value <0.05 was considered statistically significant.

5.4 Ethical considerations

The study protocol was reviewed and approved by the Medical Research Coordination Committee (MRCC) of the National Institute for Medical Research (NIMR), Tanzania with ethics clearance certificate no. NIMR/HQ/R.8a/Vol.IX/2107. The study also received further approval from the University Of Cambridge Human Biology Research Ethics Committee (HBREC.2015.28) to conduct this study in Shinyanga, Tanzania.

5.5 Results

5.5.1 Seasonal patterns and association between environmental factors and snail population density

The *Bulinid* snail population trends is compared in Fig. 7, with rainfall, conductivity and temperature, these indicated some similarity with the patterns of peaks in snail numbers. High snail counts were observed during the months with high rainfall (March through May), and low snail number during the dry period from June to November. The population number of snails started to build up after the onset of rainfall and exhibited a fluctuating pattern in abundance following the rainfall trends. The snail populations declined with the commencement of dry period where the water sources in ponds and rivers were very low and drying up making conditions unsuitable for snails to survive. The dry conditions of water sources necessitate snails to undergo aestivation until conducive conditions become available for their survival (Fig. 7).

Physical, biological and chemical factors are significantly important for the distribution, survival and reproduction of fresh water snails. In this context vegetation cover and water chemistry were recorded. The snail habitats contained differing vegetation types which varied from one snail collection site to the other. The most common vegetation covers were different species of grasses, reeds and water lilies (*Nymphaea spp*.). However, vegetation cover such as water hyacinth and duck weeds did not dominate most of the snail habitats. A negative regression model built to assess the role of biotic factors on snail abundances revealed that presence of vegetation in snail habitats had significant association with increased snail population densities (OR 6.84; 95%CI 2.75-16.99; P<0.0001) (Table 12).

Water conductivity demonstrated an irregular relationship with snail population densities; a negative binomial regression model, adjusted a priori to season, demonstrated that water conductivity contributed significantly to snail population abundances (OR 1.23; 95% CI 1.13-1.34; P<0.0001). However, this relationship between conductivity and snail counts was

modified when vegetation was present, with those sites with vegetation having less snails predicted for a particular conductivity than would be present if no vegetation was present (Table 12), Other physico-chemical properties, including temperature, were not associated with snail abundance and were removed from the model.

Representation of snail size within *B. nasutus* snail population varied between seasons, the snail population size increased when snail sites were more than 75% water coverage. The snail populations decreased with decrease of water coverage in snail sites (Fig. 7). At the start of the survey in March 2016, snails 13-15 mm were most abundant, followed by snail size 10-12 mm which was more slightly similar in counts to 7-9 mm. These three snail sizes remained the most frequently observed throughout the study period. Snails sized 16-18 mm were very few, and those 19-21 mm and 4-6 mm were the groups least present during the rainy season of the first year (2016) of study. Although the most commonly observed, sizes a considerable decrease in the number of snails sized 7-9 mm, 10-12 mm, 13-15 mm and 16-18 mm) was still observed during the dry season with eventual disappearance of snails size (4-6 mm,16-18 mm and 19-21 mm). Only snails sized (7-9 mm, 10-12 mm) survived to the next rain season, indicating that these are the ones observed to tolerate aestivation, and take the generation of *B. nasutus* snails through to the next rainy season.

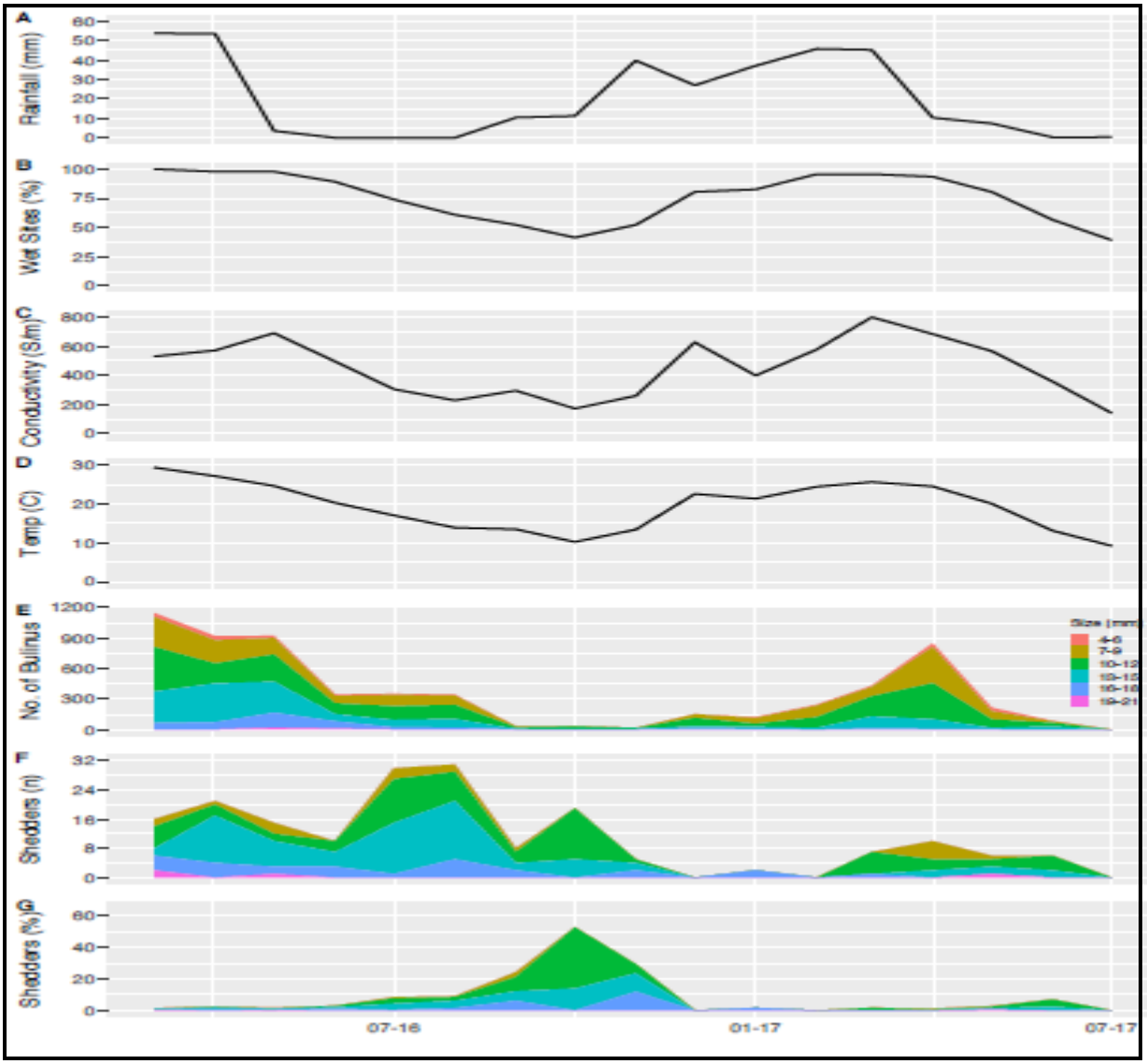


Figure 7: Temporal correlation of *B. nasutus* population density and cercarial shedding with Abiotic factors.

Table 12: Negative binomial regression model Indicating factors predicting the abundance of *B. nasutus*

Variable	OR (95%CI)	P-value
Season		
Dry season	1	
Wet season	1.62 (0.86-3.06)	0.135
Conductivity	1.23 (1.13-1.34)	0.0001
Vegetation		
Absence of vegetation	1	
Presence of vegetation	6.84 (2.75-16.99)	0.0001
Conductivity and vegetation		
Conductivity : Absence of vegetation	1	
Conductivity : Presence of vegetation	0.76 (0.68-0.86)	0.0001

Dry season, absence of vegetation and conductivity in absence of vegetation were used as reference group.

5.5.2 Association of environmental factors with patent snail infections

The shedding patterns of *B. nasutus* snails also had a fluctuating trend; in the wet season few snails were shedding schistosome cercariae. The high peak in snails shedding schistosome cercariae was observed in the dry season in the month of July. Patterns of snail shedding schistosomes continued to vary with time and were determined by reduction of water in the transmission sites (Fig. 7).

A negative binomial regression model, controlled a priori for season, was constructed to determine the factors affecting the numbers of snails collected that shed schistosome cercariae. The model demonstrated that as water temperatures increased patent infections of snails in the field were less likely to be observed in the river than in the pond habitats (OR 0.53; 95% CI 0.45-0.62; P<0.0001). However, during the wet season, the model indicated that rivers may contain less shedding snails than ponds, but this just failed to reach

significance, and the confidence intervals of this association were large (OR 0.46; 95%CI 0.22-1.01; P=0.053). Conversely the model showed that there was no significant interaction between season and water temperature in predicting the number of snails shedding schistosome cercariae (P>0.05) (Table 13).

The highest proportion of snails shedding cercariae were those sized 10-12 mm, the same size that survived the dry season, accounting for 50% of snails found to shed cercariae. All other snail size had low shedding ability throughout the study period (Fig. 7). However, snail size did not significantly contribute to the binomial regression model of whether or not snails were found to shed cercariae.

Table 13: Negative binomial model indicating predictors of snail infections

Variable	**OR (95%CI)**	***P*-value**
Season		
Dry season	1	
Wet season	0.83 (0.38-1.84)	0.654
Temperature	0.99 (0.83-1.18)	0.951
Water body		
Pond	1	
River	1.98 (0.86-4.58)	0.105
Season and Temperature		
Dry season : Temperature	1	
Wet season : Temperature	1.10 (0.98-1.25)	0.099
Season and Water body		
Dry season : Pond	1	
Wet season : River	0.46 (0.22-1.01)	0.053
Temperature: water body		
Temperature : Pond	1	
Temperature : River	0.53 (0.45-0.62)	0.0001

The model was adjusted priori to season.

5.6 Discussion

Tanzania is implementing countrywide schistosomiasis control intervention and moving from control to elimination of schistosomiasis as a public health problem by 2025 (WHO, 2010, and 2012). For this to be achieved, an understanding of the fluctuation patterns of *Bulinus* snail population densities and transmission trends of *S. haematobium* is vital. The current study examines environmental factors that are significantly associated with fluctuation of *Bulinus* snail population densities and the mode of schistosomiasis transmission over a period of 17 months. Our study findings indicate that rainfall pattern, conductivity and vegetation cover are potential determinants of snail population densities. Patent infections of *Bulinus* snails occurred at high water temperature in pond habitats than in river habitats. Other abiotic factors did not show any significant contribution to *Bulinus* snail population abundance and transmission patterns.

Bulinus nasutus snail population density followed rainfall patterns. High number of *B. nasutus* was observed from 2016 March through May, 2016 which is the rain season in the study area, with the onset of dry season in June, the snail population decreased, even more so in the mid to late dry season in September to November. Snail populations started to increase in December with onset of the rains. Similar observations are reported in other studies where snail populations were high during the rainy season (Kariuki *et al.*, 2004; Labbo, 1995; Teesdale, 1962). This may indicate that *B. nasutus* have short reproduction cycles that facilitate the quick increase of their population after the onset of the rainy season. However, it should be noted that season itself was not significant in a negative binomial model built to assess snail numbers. This indicates that, although undoubtedly the increased presence of water is essential for providing a habitat for these populations, it is other factors related to those water bodies that permit the greatest expansion of these populations to occur.

Within the snail habitats, it was observed that *B. nasutus* population density showed temporal variations between habitats, high number of snails was found residing in all temporary ponds and seasonal rivers, comparable to previous studies (Clennon *et al*., 2006; Kariuki *et al.*, 2004; Labbo, 1995; Sturrock *et al*., 2001; Senghor, Diaw, Doucoure, Seye, Talla & Diallo, 2015). In river habitats snail densities were very low throughout the survey suggesting that the speed of water flow may dislodge the snails thus reducing their population density.

For physical and chemical factors of snail habitats tested, conductivity which is composed of ions derived from dissolved salts and inorganic materials was an important determinant factor for snail population abundances; this observation was consistent with other studies (Perez-saez, Mande, Ceperley, Bertuzzo, Mari & Gatto, 2016). This is due to the abundance of inorganic materials favouring development of algae, an important food for snails population growth (Dida *et al.*, 2014; Ofoezie, 1999). Other studies reported that optimal ions in snail habitats increased snail numbers weekly (Fashuyi, 1981; Teesdale, 1962). An increase of conductivity in snail habitats without vegetation had little impact on snail's population densities but reduced snail densities in habitats covered with vegetation. This reflects that snail intermediate hosts have an optimal tolerance to ions within their natural habitats, such that variations of conductivity based on the nature of the habitat may alter snail population densities.

Presence of vegetation in snail habitats strongly affected snail population abundance; this was comparable with previous reports (Boelee & Laamrani, 2004). Availability of vegetation in snail habitats would have facilitated provision of plenty food for survival, reproduction and protection of snail vectors against predators (Kariuki *et al.*, 2004). This indicates that vegetation has important role to play in flourishing of snail population densities by creating conducive environment necessary for snail's survival.

Conversely, water temperature had no significant impact on snail abundance, as previously reported (Kariuki *et al.*, 2004; Mccreesh, Arinaitwe, Arineitwe, Tukahebwa & Booth, 2014), but is in contrast with the findings of others (Kalinda *et al.*, 2017a; Opisa, Odiere, Jura, Karanja & Mwinzi, 2011) who observed significant contributions of increased temperature to snail abundance by altering breeding conditions, growth and survival. Since snail intermediate hosts thrive within limited range of temperature, temperature remains potential factor in disease transmission (Paull & Johnson, 2011). However in this study, increase of water temperature contributed significantly in snail infection in ponds habitats, but less so in river habitats. Other abiotic conditions did not have a significant impact on snail population numbers which confirms findings of other studies where the abiotic factors were insignificant to snail population growth (Ebenezer, Nwadiuto & Blessing, 2018).

Throughout the study period snail sizes 7 mm to 12 mm were the only group managed to survive during dry period, this reflect that the snail group is most capable of withstanding changes from optimal environmental conditions and managed to survive the adverse

conditions of the dry period. Since this size group is capable of maintaining the snail population by taking the population from adverse conditions to more favourable situations, it should be noted that during dry season patent infections of snail intermediate hosts is high. This may indicate that snails sized 7 mm to 12 mm, which are more tolerant to dry conditions, were confined to limited water sources during the dry season. This in turn allows the efficient spread of schistoisome larvae to this snail group as they were only available hosts for schistosome larvae to infect. However this observation is in contrast to other studies reported previously where small sized snails had lower survival in dry conditions compared to the large snails who survived by burrowing deep in the mud (Watson & Sc, 1958). It is likely, therefore, that the survival of snail intermediate hosts is determined by their local ecological conditions (Ernould, Ba & Sellin, 1999).

Infection of snails increased more as water temperature increased in pond habitats than in river habitats. An increase in water temperature within pond habitats may therefore facilitate development of the infection within snail intermediate hosts, having a significant role in the initiation of cercarial shedding by stimulating transformation of early schistosome parasite stage within the snail intermediate hosts to infective stage (cercariae). Comparable observations were documented by others where temperature affected transmission of schistosomes (Rollinson *et al.,* 2001c). Temperature therefore has a vital role in inducing the development and release of intra molluscan parasites, as previously reported (Sturrock *et al.*, 2001). However different snail intermediate hosts demonstrate diverse lethal temperatures influencing infection and development of intramolluscan parasite (Kalinda *et al.*, 2017b). The infection trend of snail intermediate hosts throughout study period varied significantly among snail habitats. This reflected a need to understand the optimal temperature and other abiotic factors necessary for snail intermediate hosts infections in different microgeographical settings.

5.7 Conclusion

The current study findings revealed that rainfall patterns, conductivity and vegetation cover were important determinants of *B. nasutus* snail population density. The patent infections of *B. nasutus* were affected by increased water temperature in pond habitats than in the rivers. Therefore, for effective and sustainable schistosomiasis control interventions control strategies should focus on temporal variations of snail densities and infections to reduce and interrupt transmissions of schistosomiasis in endemic settings.

CHAPTER SIX

General Discussion, Conclusion and Recommendations

6.1 General discussion

The aim of this research work was to explore in-depth understanding of *S. haematobium* transmission with focus on snail ecology and human water contact activities in Shinyanga district, Tanzania. The study estimated patent and pre-patent infections of *Bulinid* snails and differentiated the closely related schistosomes *S. bovis* from *S. haematobium* through a multiplex PCR. In addition the study investigated the ecological determinants of *B. nasutus* population dynamics and assessed community knowledge, perceptions, practices and water contact behaviour in relation to *S. haematobium* transmission.

In chapter one, current global schistosomiasis burden, ecological conditions and transmission modes of urogenital schistosomiasis by *Bulinid* snails was reviewed. Along with evidence that despite differences in disease control and burden reduction between countries, significant reduction of schistosomiasis has been achieved through increased utilization of praziquantel (Engels *et al*., 2002; WHO 2018). Finally, how the parasite's life history in the context of Tanzania was described and how this shaped the research questions that this thesis addressed.

Chapter two examined pre and post-treatment infection levels of *S. haematobium* among school aged children. The study revealed that sex, age, and proximity of residence to habitats with large numbers of snail intermediate hosts were independent determinant factors for *S. haematobium* infection. At both time points, boys had higher *S. haematobium* infection compared to girls. This suggests that boys are more exposed to infection of *S. haematobium* during implementation of their day to day activities particularly caring of livestock which involves searching for pastures and water for their livestock. This observation that boys are more infected than girls has been documented in several studies (Clements *et al*., 2008; Guyatt *et al.*, 1999; Liao *et al*., 2011). Children aged 12-14 years were more infected than younger age group before treatment but no difference in infection between ages was observed one year after treatment. Other studies reported similar observations among school aged children (Amazigo *et al.*, 1997; Barbour, 1985; N'Goran *et al.*, 2001; Klumpp & Webbe, 1982; Saathoff *et al*., 2004). This points towards infection accumulating with time and is not necessarily due to changes in behaviour within this age group. Therefore regular treatment with praziquantel is essential to keep infections at minimum level and prevent adverse impact of the schistosomiasis infection.

However, in the context of this thesis and its research questions, a very important finding was that integration of malacological surveys linked with GPS data detected spatial association between children living in households next to ponds with high *B. nasutus* having the highest prevalence of *S. haematobium* infection, was an important determinant of *S. haematobium* infection among school children during the pre-treatment period. This indicated that the behaviour of the children and the ecology of the snail intermediate hosts were intrinsically linked in the transmission of the parasite within the study village. However after one year post treatment no spatial influence on *S. haematobium* infection was observed. Other studies have documented distance from homestead to unprotected water sources as a major predictor of *S. haematobium* infection (Amazigo *et al*., 1997).

However, patent prevalence of infected snail hosts was low, reflecting a need for molecular application to illuminate infectivity rate of snails within this micro-geographical networks of complex transmission. Since the use of a questionnaire in determining the potential risk predictors for *S. haematobium* infection was inadequate in revealing the reality of where transmission occurs, linkage of GPS, malacological surveys and parasitological screening was concluded to be a potential method for determining hotspots of *S. haematobium* transmission even in complex networks on a micro geographical scale. However, anomalies between childhood infection and snail populations in the nearest water contact sites, were apparent, most notably in habitat 6, Mwamalago Pond. This highlights the need for detailed assessment of where water contact actually occurs.

In reviewing the community knowledge, perceptions, attitudes and practices on *S. haematobium* transmission, it was observed that community members (children and parents) perceive that urinary schistosomiasis is a low priority disease in their community. This perception could reflect the long-term infection required prior to the appearance of adverse conditions resulting from contraction of schistosome parasites, particularly in comparison to other infectious disease such as malaria. The signs of urinary schistosomiasis among community members can be perceived as a sign of maturity (Amazigo *et al.,* 1997). Therefore, treatment for schistosomiasis patients takes place only when the situation is critical enabling development of advanced morbidities. Previous studies have documented that urinary schistosomiasis is perceived as a less important disease within differing communities (Gazzinelli *et al.*, 1998; Odhiambo *et al.,* 2014). Most of the respondents had partially correct knowledge on transmission modes of urinary schistosomiasis but had

misconceptions about the cause of the disease and mixed urinary schistosomiasis transmission with that of soil transmitted helminthes infections (STH). Treatment seeking behaviour between parents and children relied on combined therapies of modern medicines and traditional herbs based on symptoms. However modern therapy was more preferred compared to tradition remedies, indicating that in this community fear of side-effects from praziquantel treatment may not have inverse effects on the success of preventive chemotherapy based intervention. The frequent contact with infested water did though remain due to unavoidable practices among children in the community due to the lack of clean and safe water supply in their community, facilitating exposure to schistosomes, Therefore development of health education interventions to raise community awareness about risk behaviours on disease contraction, causes and misconceptions that people have about the disease will improve the knowledge of the disease among community members. In addition, health education programmes must be tied with the education system to ensure that knowledge about the disease is constantly being provided and that it is delivered in an effective manner that promotes understanding and removes confusion that appears to arise from co-delivery of education on helminth infections. This will boost awareness of disease impact and reduce risk behaviours for disease transmission.

In view of patent and pre–patent schistosome infection of snail intermediate hosts in Shinyanga district, the study demonstrated that morphologically identical snails *Bulinus nasutus* transmit both S. *haematobium* and *S. bovis*. Both schistosomes are transmitted from common water sources highlighting transmission of schistosomes to both ruminant animals and humans. This shows that snail intermediate hosts from similar water habitats are capable of facilitating transmission of different species of schistosomes to different definitive hosts. Identification of these infections to snail intermediate hosts require molecular assays that specify different schistosome species being harboured by similar snail intermediate hosts within common water sources and determine schistosome species infecting snail intermediate hosts in a particular geographical location. A molecular study conducted in Zanzibar on *B. globosus* snail intermediate hosts have revealed that *B. globosus* not only transmits *S. haematobium* but also transmits *S. bovis* (Pennance *et al.*, 2018). Water sources used by both humans and domestic animals that harboured snail vectors, mainly *B. nasutus,* facilitated the co-transmission of *S. haematobium* and *S. bovis* schistosomes to the snail hosts. The lack of distinct use of water sources for humans and domestic livestock in Shinyanga district likely is to foster the transmission of *S. haematobium* and *S. bovis*, although local

livestock were not surveyed. In African context, where supply of clean and safe water for human and domestic animals consumption is scarse, the available water sources are mainly shared by animals and humans a condition which facilitates

Co-transmission of these closely related schistosomes *S. bovis* and *S. haematobium* (Huyse, *et al*., 2009). This situation necessitates provision of sustainable supply of clean and safe water to minimize infection taking place in snail intermediate hosts and eventually secure both ruminants and humans from contracting the schistosome parasites.

However in view of pre-patent infection among snail intermediate hosts in this study, infection of *B. nasutus* by *S. bovis* dominated that of *S. haematobium* throughout sampling sites with high infection during the dry season when water sources is in short supply in the environment confining majority of ruminants to only utilize the available water sources whereby the frequency of water contact and supply of *S. bovis* eggs from ruminant feaces is maximized. However it was not known whether infection of *B. nasutus* by *S. bovis* has impact on the *S. haemtobium* infection of snails inhabiting similar water habitats. The impact of season on schistosome infection was previously noted elsewhere (Shiff *et al*., 1979; Sturrock, 2001). This indicates that the higher rates of cercarial shedding observed in chapter 5 during the months that constitute early to mid dry season, may reflect increased transmission of the veterinary parasite, rather than transmission of the human parasite, for which prevalence of infection within the snails, remained at a constant level. The study recommends further molecular studies on the morphologically identical *B. nasutus* to investigate the compatibility of *S. bovis* and *S. haematobium* with the snail host. In view of the finding that Mwakasela habitat 4, had the highest prevalence of snail infected with *S. haematobium* but the lowered prevalence of snails infected with *S. bovis* it is recommended that assessment of whether infection of *B. nasutus* by *S. bovis* inhibits infection of *B. nasutus* by *S. haematobium* be carried out to rule out the possibility that such findings reflect differing host water contact patterns between humans and their livestock.

The study revealed that rainfall patterns, conductivity and vegetation cover were important ecological determinants of *B. nasutus* population abundances. The trend of rainfall determined the density of *B. nasutus* snails by configuring time for desiccation which in turn affected *Bulinid* snail densities through fluctuation of water level in snail habitats and expansion of snail habitats which become conducive for snails to proliferate (Fashuyi, 1981; Kariuki, *et al.*, 2004). It should be however noted that snail population densities is regulated

by several factors which may be acting individually or in a complex interaction which naturally depend on each other and their combined effects affects snail population densities (Hubendick, 1958). In the current study temperature was a potential ecological factor for patent infection of *B. nasutus* snail intermediate hosts by enabling development of schistosome parasites. However, the irregularity of snail population densities observed among snail habitats indicates that population density of *B. nasutus* snail intermediate hosts is determined by specific local biotic and abiotic factors acting on specific snail habitats. Therefore, control measures of snail intermediate hosts should take into account the temporal variation of snail population densities prior to targeting control interventions. It is of note however that, the ecological determinants of snail population numbers versus that of patent infections did differ, and hence further understanding of the factors underlying this and whether targeting snail control should be timed according patterns of snail populations, evidence of infection or both.

6.2 Conclusion

The current study has demonstrated that water sources shared by both domestic ruminants and humans were responsible for transmission of both *S. haematobium* and *S. bovis* infections in Shinyanga district. *Bulinus nasutus* snails co-transmitted *S. haematobium* and *S. bovis* in this area.

Further, the study revealed that rainfall patterns, conductivity and vegetation cover were important determinants of *B. nasutus* population densities. Both factors either singly or in a complex interaction contributed significantly to *B. nasutus* population densities by ensuring availability of needs for *Bulinid* snails to thrive. Patent infections of *Bulinid* snails was influenced by increases in water temperature in pond habitats, but less so in river habitats.

The study further demonstrated that integration of malacological surveys linked with GPS data detected spatial association between children living in households next to ponds with high *B. nasutus* having the highest prevalence of *S. haematobium* infection during pre-treatment period. However one year after praziquantel treatment of school children, there was no spatial influence on *S. haematobium* infection observed which indicates the need for comprehensive assessment of where water contact takes place. Linkage of GPS, malacological and parasitological screenings are important in determining hotspots of *S. haematobium* transmission.

6.3 Recommendations

The infection of *S. bovis* among *B. nasutus* snail intermediate hosts dominated the infection of *S. haematobium*, further studies should be conducted to investigate whether infection of *S. bovis* in *B. nasutus* snail intermediate hosts prevents infection of *S. haematobium* in the same snail intermediate host and whether there is compatibility of *B. nasutus* to *S. bovis* and *S. haematobium.*

Further studies are recommended to address the different factors facilitating *Bulinid* population densities and infection patterns in different micro-geographical habitats.

Health education interventions are recommended to enhance awareness on schistosomiasis and reduce behaviours that promote transmission of the disease in these and many other similar settings to accelarate progress towards schistosomiasis control and elimination.

Policy makers should consider integration of snail control in Negelected tropical diseases control programmes as part of schistosomiasis control intervention to interrupt schistosomiasis transmission and move towards elimination of the disease in endemic settings.

Further studies should be done in nomadic communities where boys search water and pastures for domestic livestock as an exposure to urinary schistosomiasis transmission.

REFERENCES

Abbasi, I., Webster, B. L., King, C. H., Rollinson, D., & Hamburger, J. (2017). The substructure of three repetitive DNA regions of Schistosoma haematobium group species as a potential marker for species recognition and interbreeding detection. *Parasites and Vectors*, 1–9. https://doi.org/10.1186/s13071-017-2281-7.

Abe, E. M., Ombugadu, A., Oluwole, A. S., & Mogaji, H. (2017). Population Abundance and Bionomics of Snail Intermediate Hosts of Trematode Parasites in Nasarawa State, Nigeria. *Research Journal of Parasitology, 12: 8-18* (December 2016). https:// doi.org/ 10.3923/ jp. 2017 .8.18.

Allan, F., Rollinson, D., Smith, J. E., & Dunn, A. M. (2009). Host choice and penetration by Schistosoma haematobium miracidia. *Journal of Helminthology*, 83, 33–38. https://doi.org/10.1017/S0022149X08073 628.

Allan, F., Sousa-figueiredo, J. C., Emery, A. M., Paulo, R., Mirante, C., Sebastião, A., … Rollinson, D. (2017). Mapping freshwater snails in north-western Angola : distribution , identity and molecular diversity of medically important taxa. *Parasites and Vectors1*–10. https://doi.org/10.1186/s13071-017-2395-y.

Amazigo, U. O., Anago-amanze, C. I., & Okeibunor, J. C. (1997). Urinary Schistosomiasis Among School Children in Nigeria: Consequences of Indigenous Beliefs and Water Contact Activities. *Journal of Biosocial Science*, *29*(1), S0021932097000096. https:// doi.org/ 10.1017/S0021932097000096.

Angelo, T., Buza, J., Methusela, S., Kariuki, H. C., Mwanga, J. R., Munisi, D. Z., & Wilson, S. (2018). Geographical and behavioral risks associated with Schistosoma haematobium infection in an area of complex transmission. *Parasites and Vectors*, 1–9.

Anto, F., Victor, A., Martin, A., Thomas, A., Abraham, O., James, A., ... Philip, A. (2015). Water Contact Activities and Prevalence of Schistosomiasis Infection among School-age Children in Communities along an Irrigation Scheme in Rural Northern Ghana. *Journal of Bacteriol Parasitol*, *4*(4). https://doi.org/10.4172/2155- 9597.1000177.

Barber, K. E., Mkoji, G. M., & Loker, E. S. (2000). PCR-RFLP Analysis of the ITS2 Region to identify *Schistosoma haematobium* and *S. bovis* from Kenya. *American Journal Tropical Medicine Hygiene, 62*(4), 434–440.

Barbour, A. D. (1985). The importance of age and water contact patterns in relation to Schistosoma haematobium infection. *Transaction of the Royal Society Tropical Medicine Hygiene*, *79*, 151–153.

Barsoum, R. S., Esmat, G., & El-Baz, T. (2013). Human Schistosomiasis: Clinical Perspective Review. *Journal of Advanced Research, 4*(5), 433–444. https://doi.org/10. 1016 /j.jare.2013.01.005.

Black, C. L., Steinauer, M. L., Mwinzi, P. N. M., Secor, W. E., Diana, M. S., & Colley, D. G. (2010). Impact of intense, longitudinal retreatment with praziquantel on cure rates of schistosomiasis mansoni in a cohort of occupationally exposed adults in western Kenya. *NIH Public Access*, *14*(4), 450–457. https://doi.org/10.1111/j.1365-3156. 2009 .02234 . x.

Boelee, E., & Laamrani, H. (2004). Environmental control of schistosomiasis through community participation in a Moroccan oasis. *Tropical Medicine and International Health, 9*(9), 997–1004.

Boissier, J., Grech-Angelini, S., Webster, B. L., Allienne, J. F., Huyse, T., Mas-Coma, S., … Mitta, G. (2016). Outbreak of urogenital schistosomiasis in Corsica (France): an epidemiological case study. *The Lancet Infectious Diseases, 16*(8), 971–979. https://doi.org/10.1016/S1473-3099(16)00175-4.

Bont, J. De., & Vercruysse, J. (1997). The Epidemiology and Control of Cattle Schistosomiasis. *Parasitology Today*,13(7), 255-262.

Breen, R. L. (2007). A Practical Guide to Focus-Group Research, *8265*(2006). https://doi.org/10.1080/03098260600927575.

Brooker, S., Hay, S. I., Issae, W., Hall, A, Kihamia, C. M., Lwambo, N. J., … Bundy, D. A. (2001). Predicting the distribution of urinary schistosomiasis in Tanzania using satellite sensor data. *Tropical Medicine and International Health : TM & IH*, *6* (12), 998–1007.

Brooker, S., Hay, S. I., Issae, W., Hall, A., Kihamia, C. M., Lwambo, N. J. S., … Bundy, D. A. P. (2001). Predicting the distribution of urinary schistosomiasis in Tanzania using satellite sensor data. *Tropical Medicine and International Health*, *6*(12), 998-1007.

Bruun, B., & Aagaard-Hansen, J. (2008). The social context of schistosomiasis and its control: an introduction and annotated bibliography. *Special Program for Research and Training Tropical Diseases*.

Burnim, M., Ivy, J. A., & King, C. H. (2017). Systematic review of community-based , school-based , and combined delivery modes for reaching school-aged children in mass drug administration programs for schistosomiasis. *PloS Neglected Tropical Diseases* 1–16. https://doi.org/10.1371/journal.pntd.0006043.

Chadeka, E. A., Nagi, S., Sunahara, T., Cheruiyot, B., Bahati, F., Ozeki, Y., … Ichinose, Y. (2017). Spatial distribution and risk factors of Schistosoma haematobium and hookworm infections among schoolchildren in Kwale, Kenya. *PloS Negleced Tropical Diseases*,1–17. https://doi.org/10.1371 /journal.pntd.0005872.

Chandiwana, S. K. C. (1987). Seasonal Patterns in Water Contact and the Influence of Water Availability on Contact Activities in Two Schistosomiasis- Endemic Areas in Zimbabwe. *Central African Journal of Medicine*, *33*(I).

Christensen, N. O., Mutani, A., & Frandsen, F, T (1983). A Review of the Biology and Transmission Ecology African Bovine Species of the Genus Schistosoma. *Z. Parasitenkd.* 69, 551–570.

Clements, A. C. A., Brooker, S., Nyandindi, U., Fenwick, A., & Blair, L. (2008). Bayesian spatial analysis of a national urinary schistosomiasis questionnaire to assist geographic targeting of schistosomiasis control in Tanzania, East Africa. *International Journal for Parasitology*, *38*(3–4), 401–15. https://doi.org/10.1016/j.ijpara.2007.08.001.

Clements, A. C. A., Barnett, A. G., Nyandindi, U., Lwambo, N. J. S., Kihamia, C. M., & Blair, L. (2008). Age and gender effects in self-reported urinary schistosomiasis in Tanzania. *Tropical Medicine and International Health*, *13*(5), 713–721. https:// doi.org/10.1111/j.1365-3156.2008.02048.x.

Clements, A. C. A., Lwambo, N. J. S., Blair, L., Nyandindi, U., Kaatano, G., Kinung, S., … Brooker, S. (2006). Bayesian spatial analysis and disease mapping : tools to enhance planning and implementation of a schistosomiasis control programme in Tanzania. *Tropical Medicine and International Health 11*(4), 490–503. https:// doi.org/ 10.1111/j. 1365-3156.2006.01594.x.

Clennon, J. A., Mungai, P. L., Muchiri, E. M., King, C. H., & Kitron, U. (2006). Spatial and Temporal Variations in Local Transmission of Schistosoma Haematobium. *American Journal of Tropical Medicine Hygiene,* 75(6), 1034–1041.

Clennon, J. A., King, C. H., Muchiri, E. M., Kariuki, H. C., Ouma, J. H., Mungai, P., & Kitron, U. (2004). Spatial patterns of urinary schistosomiasis infection in a highly endemic area of coastal Kenya. *The American Journal of Tropical Medicine and Hygiene*, *70*(4), 443–8.

Colley, D. G., Bustinduy, A. L., Secor, W. E., & King, C. H. (2014). Human schistosomiasis. *The Lancet*, *383*(9936), 2253–2264. https://doi.org/10.1016/S0140-6736(13)61949-2.

Cronin,T., Sheppard, J., & de Wildt, G. (2013). Health-seeking behaviour for schistosomiasis: A systematic review of qualitative and quantitative literature. *Pan African Medical Journal*, *16*, 1–9. https://doi.org/10.11604/pamj.2013.16.130.3078.

Dalton, P. R., & Pole, D. (1978). Water-contact patterns in relation to Schistosoma haematobium infection. *Bulletin of the World Health Organization*, *56*(3), 417–426.

Danish identification key for snails (1980.) A practical guide to the identification of African Freshwater snails.

Danso-Appiah A., Bosompem, K. & Habbema J. D. (2004). Determinants of health-seeking behaviour for schistosomiasis related sympotms in the context of intergrating schistosomiasis control within the regular health services in Ghana. *Tropical Medicine and International Health*, *9*(7), 784–794.

Dawaki, S., Al-Mekhlafi, H. M., Ithoi, I., Ibrahim, J., Abdulsalam, A. M., Ahmed, A., … Atroosh, W. M. (2015). The menace of schistosomiasis in Nigeria: Knowledge, attitude, and practices regarding schistosomiasis among rural communities in Kano State. *PLoS ONE*, *10*(11), 1–14. https://doi.org/10.1371/journal.pone.0143667.

Dawson, S, Manderson, L., & Tallo V. (1993). A Manual for the Use of Focus Groups. Boston: International Nutrition Foundation for Developing Countries.

Dazo, B. C., Hairston, N. G., & Dawood, I. K. (1966). The Ecology of Bulinus truncatus and Biomphalaria alexandrina and its Implications for the Control of Bilharziasis in the Egypt-49 Project Area. *Bulletin of the World Health organization* (1951), 339–356.

DE meillon, B., Frank, G. H., & Allanson, B. R. (1958). Some aspects of snail ecology in South Africa; a preliminary report. *Bulletin of the World Health Organization*, *18*(5–6), 771–783.

Dida, G. O., Gelder, F. B., Anyona, D. N., Matano, A. S., Abuom, P. O., Adoka, S. O., … Ofulla, A. V. O. (2014). Distribution and abundance of schistosomiasis and fascioliasis host snails along the Mara River in Kenya and Tanzania. *Infection Ecology and Epidemiology*, *4*(1), 1–7. https://doi.org/10.3402/iee.v4.24281.

Donnell, A. O. (2016a). Immunology of Schistosomiasis. *Parasitology*, (*51)*, 1–11.

Donnell, A. O. (2016b). Immunology of Schistosomiasis. *Parasitology*, (*51)*, 1–11.

Ebenezer, A., Nwadiuto, E. C., & Blessing, O. B. (2018). Spatial distribution of fresh water snail intermediate host in Yenagoa Metropolis Bayelsa State Nigeria. *Journal of Parasitology and Vector Biology*, *10* (June), 73–78. https:// doi.org/ 10. 5897/ JPVB 2018. 0320.

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

Ernould, J. C., Ba, K., & Sellin, B. (1999). The impact of the local water-development programme on the abundance of the intermediate hosts of schistosomiasis in three villages of the Senegal River delta. *Annals of Tropical Medicine and Parasitology*, *93*(2), 135–145. https://doi.org/10.1080/0003498995861.

Secor, E. (2014). Water-based interventions for schistosomiasis control. *Pathogens and Global Health*, *108*(5), 246–54. https://doi.org/10.1179/2047773214Y.0000000149.

Fashuyi, S. A. (1981). Observations on the ecology of *Bulinus* physopsis globosus morelet, B *Bulinus* forskalii ehrenberg and lymnaea natalensis krauss in rice swamps in sierra leone. *Journal of mollusicas Stud*y, 47, 328–333. .

Freeman, M. C., Grimes, J. E. T., Croll, D., Harrison, W. E., & Templeton, M. R. (2014). The Relationship between Water, Sanitation and Schistosomiasis A Systematic Review and Meta-analysis. *PloS Neglected Tropical Diseases*, *8*(12). https:// doi.org/ 10.1371/ journal. pntd.0003296.

French, M. D., Churcher, T. S., Webster, J. P., Fleming, F. M., Fenwick, A., Kabatereine, N. B., ... Basáñez, M. (2015). Estimation of changes in the force of infection for intestinal and urogenital schistosomiasis in countries with schistosomiasis control initiative-assisted programmes. *Parasites & Vectors*, 1–18. https://doi.org/10.1186/s13071-015-1138-1.

Fenwick, A., Webster, J. P., Blair, L., & Fleming, F. M. (2009). The Schistosomiasis Control Initiative (SCI) : rationale , development and implementation from 2002 – 200. *Parasitology*,1719–1730. https://doi.org/10.1017/S0031182009990400.

Goran, K. N., Tschannen, A. B., Bogoch, I. I., Acka, C. A., Raso, G., Tanner, M., ... Obrist, B. (2010). Parasitic Worms : Knowledge Attitudes and Practices in ˆ te d ' Ivoire with Implications for Integrated Western Co Control. *PloS Neglected Tropical Diseases 4*(12). https://doi.org/10.1371/journal. pntd.0000910.

Gbalégba, N. G. G. C., Silué, K. D., Ba, O., Ba, H., Tian-bi, N. T. Y., Yapi, G. Y., … Koudou, B. G. (2017). Prevalence and seasonal transmission of Schistosoma haematobium infection among school-aged children in Kaedi town, southern Mauritania. *Parasites & Vectors* 1–12. https://doi.org/10.1186/s13071-017-2284-4.

Greenhalgh, T. & Taylor, R. (1997). Papers that go beyond numbers (qualitative research) - ProQuest. *British Medical Journal*, *315*(September), 740. https://doi.org/10.1136/BMJ. 315.7110.740.

Grimes, J. E., Croll, D., Harrison, W. E., Utzinger, J., Freeman, M. C., & Templeton, M. R. (2015). The roles of water, sanitation and hygiene in reducing schistosomiasis: A review. *Parasites and Vectors*, *8*(1), 1–16. https://doi.org/10.1186/s13071-015-0766-9.

Grimes, J. E. T., Tadesse, G., Mekete, K., Wuletaw, Y., Gebretsadik, A., French, M. D., … Templeton, M. R. (2016). Transmitted Helminths and Schistosomes : National Mapping in Ethiopia. *PLoS Neglected Tropical Diseases* 1–21. https:// doi.org/ 10.1371/ journal. pntd.0004515.

Guest, G., Namey, E., Taylor, J., Eley, N., Mckenna, K., Guest, G., … Eley, N. (2017). Comparing focus groups and individual interviews : findings from a randomized study. *International Journal of Social Research Methodology*, *5579*(December), 1–16.

Gryseels, B., & Polman, K. (2006a). Human schistosomiasis. *The Lancet*, *368*(9541), 1106–1118.

Gryseels, B., & Polman, K. (2006b). Human schistosomiasis. *The Lancet*, *368*(9541), 1106–1118.

Guyatt, H., Brooker, S., Lwambo, N. J. S., Siza, J. E., & Bundy, D. A. P. (1999). The performance of school-based questionnaires of reported blood in urine in diagnosing Schistosoma haematobium infection: Patterns by age and sex. *Tropical Medicine and International Health*, *4*(11), 751–757. https://doi.org/10.1046/j.1365-3156 .1999. 00483. x.

Hamburger, J., Hoffman, O., Kariuki, H. C., Muchiri, E. M., Ouma, J. H., Koech, D. K., … King, C. H. (2004). Large-scale, polymerase chain reaction-based surveillance of Schistosoma haematobium DNA in snails from transmission sites in coastal Kenya: A new tool for studying the dynamics of snail infection. *American Journal of Tropical Medicine and Hygiene*, *71*(6), 765–773. https://doi.org/71/6/765.

Hotez, P. J., & Kamath, A. (2009). Neglected tropical diseases in sub-saharan Africa: review of their prevalence, distribution, and disease burden. *PLoS Neglected Tropical Diseases*, *3*(8), e412. https://doi.org/10.1371/journal.pntd.0000412.

Hubendick, B. (1958). Factors conditioning the habitat of freshwater snails. *Bulletin of the World Health Organization*, *18*(5–6), 1072–80.

Huyse, T., Webster, B. L., Geldof, S., Stothard, J. R., Diaw, O. T., Polman, K., ... Rollinson, D. (2009). Bidirectional introgressive hybridization between a cattle and human schistosome species. *PLoS Pathogens*, *5*(9), e1000571. https://doi.org/10.1371/journal. ppat.1000571.

Israel, B. A., Schulz, A. J., Parker, E. A., & Becker, A. B. (1998). Review of community-based research: Assessing Partnership Approaches to Improve Public Health. *Annual Review of Public Health*, *19*(1), 173–202. https://doi.org/10.1146/annurev. publhealth. 19.1.173.

Jelnes, J. E., Thiongó, F. W., Lwambo, N. J. S., & Ouma, J. H. (2003). The Bulinus nasutus complex (Bulinus nasutus (Martens, 1879) and Bulinus productus Mandahl-Barth, 1960) (Gastropoda: Planorbidae) in the Lake Victoria area elucidated by enzyme-profile electrophoresis and natural infections with Schistosoma species (Trematoda: Schistosomatidae). *Steenstrupia* 27(2): 257–262.

Kabatereine, N., Fleming, F., Thuo, W., Tinkitina, B., Tukahebwa, E. M., & Fenwick, A. (2014). Community perceptions, attitude, practices and treatment seeking behaviour for schistosomiasis in Lake Victoria islands in Uganda. *BMC Research Notes*, 7:900 http://www.biomedcentral.com/1756-0500/7/900 1–11.

Kalinda, C., Chimbari, M. J., & Mukaratirwa, S. (2017a). Effect of temperature on the Bulinus globosus Schistosoma haematobium system. *Infectious Diseases of Poverty* (4–10. https://doi.org/10.1186 /s40249-017-0260-z.

Kalinda, C., Chimbari, M., & Mukaratirwa, S. (2017b). Implications of Changing Temperatures on the Growth, Fecundity and Survival of Intermediate Host Snails of Schistosomiasis : A Systematic Review. *International Journal of Environmental Research Public Health*, 14, 80; https://doi.org/10.3390/ijerph14010080.

Kapito-tembo, A. P., Mwapasa, V., Meshnick, S. R., Samanyika, Y., Banda, D., Bowie, C., ... Radke, S. (2009). Prevalence Distribution and Risk Factors for Schistosomahematobium Infection among School Children in Blantyre, Malawi. *PloS Neglected Tropical Diseases*, *3*(1). https://doi.org/10.1371/journal.pntd.0000361.

Kariuki, H. C., Clennon, J. A., Brady, M. S., Kitron, U., Sturrock, R. F., Ouma, J. H., … King, C. H. (2004). Distribution patterns and cercarial shedding of bulinus nasutus and other snails in the msambweni area, coast province, Kenya. *The American Society of Tropical Medicine and Hygiene,70*(4), 449–456.

Kariuki, H. C., Madsen, H., Ouma, J. H., Butterworth, A. E., Dunne, D. W., Booth, M., … Vennervald, B. J. (2013). Long term study on the effect of molluscicide with niclosamide in stream habitats on the transmission of schistosomiasis mansoni after community-based chemotherapy in Makueni District, Kenya. *Parasites and Vectors*, *6*(1), 1–11. https://doi.org/10.1186/1756-3305-6-107.

Kaukas, A., Johnston, D. A., Simpson, A. J. G., Reseurch, C., & Prudente, R. P. A. (1997). Some Molecular Insights into Schistosome Evolution. *hrwfxiriod Jorrmrrl /or Puru.~itoh*. *27*(I). I I X IY97.

King, C. H. (2009). Toward the Elimination of Schistosomiasis. *Global Health* 106-109.

King, C. H., Sudtida A. S., Muchiri, E. M., Ouma, J. H., Whalen., & C. C. (2012). Factors Affecting Infection or Reinfection with schistosoma haematobium in coastal Kenya: survival analysis during a nine-year, school-based treatment program. *Journal of Tropical Medicine Hygiene*; 75 (1): 83–92.

King, C. H., Dickman, K., & Tisch, D. J. (2005). Reassessment of the cost of chronic helmintic infection: A meta-analysis of disability-related outcomes in endemic schistosomiasis. *The Lancet*, *365*(9470), 1561–1569. https://doi.org/10.1016/S0140-6736(05) 66457-4.

King, C. H., Sturrock, R. F., Kariuki, H. C., & Hamburger, J. (2006). Transmission control for schistosomiasis - why it matters now. *Trends in Parasitology*, *22*(12), 575–582. https://doi.org/10.1016/j.pt.2006.09.006.

Kinoti, G. (1964). Observations on the Transmission of Schistosoma haematobium and Schistosoma bovis in the Lake Region of Tanganyika. *Bulletin of the World Health Organization,* 31, 815–823.

Kinoti, G. (1983). A Note on the Susceptibility of Some Gastropod Molluscs to Schistosoma Bovis and S. Mattheei. *Annals of Tropical Medicine & Parasitology 4983*(August). https://doi.org/10.1080/00034983.1964.11686243.

Kloos, H. (1995). Human Behavior, Health Education and Schistosomiasis Control: a Review. *Social Science Medicine 40*(11), 1511.

Kloos, H., de Souza, C., Gazzinelli, Soares Filho, B. S., da Costa Temba, Bethony, J., … Oliveira, R. C. (2001). The distribution of Biomphalaria species in different habitats in relation to physical, biological, water contact and cognitive factors in a rural area in Minas Gerais, Brazil. *Memórias Do Instituto Oswaldo Cruz*, *96 Suppl* (Freitas 1968), 57–66.

Kloos, H., Francisco, S., Road, T. C., England, U. K., England, U. K., District, M., … Zuyle, V. (1997). Spatial Patterns of Human Water Contact and Schistosoma mansoni Transmi ssion and Infection in four rural areas in Machakos District, Kenya. *Social Science Medicine*, 44(7), 949–968.

Kloos, H., Gazzinelli, A., & Van Zuyle, P. (1998). Microgeographical Patterns of Schistosomiasis and Water Contact behavior; Examples from Africa and Brazil. *Memorias Do Instituto Oswaldo Cruz*, *93 SUPP.*, 37–50. https://doi.org/10.1590/S0074-02761998000700006.

Klumpp, R. K., & Chu, K. Y. (1977a). Ecological studies of Bulinus rohlfsi, the intermediate host of Schistosoma haematobium in the Volta Lake. *Bulletin of the World Health Organization*, *55*(6), 715–730.

Klumpp, R. K., & Webbe, G. (1982). Focal, seasonal and behavioural patterns of infection and transmission of Schistosoma haematobium in a farming village at the Volta lake, Ghana. *Tropical Medicine and Hygiene*, *865*(8), 2233–2234.

Knopp, S., Mohammed, K. A., Ali, S. M., Khamis, I. S., Ame, S. M., Albonico, M., … Rollinson, D. (2012a). Study and implementation of urogenital schistosomiasis elimination in Zanzibar (Unguja and Pemba islands) using an integrated multidisciplinary approach. *BMC Public Health*, *12*(1). https://doi.org/10.1186/1471-2458-12-930.

Knopp, S., Person, B., Ame, S. M., Mohammed, K. A., Ali, S. M., Khamis, S., … Fenwick, A. (2013). Elimination of Schistosomiasis Transmission in Zanzibar : Baseline Findings before the Onset of a Randomized Intervention Trial. *PloS Neglected Tropical Diseases 7*(10), 2474. https://doi.org/10.1371/journal.pntd.0002474.

Knopp, S., Stothard, J. R., Rollinson, D., Mohammed, K. A., Khamis, I. S., Marti, H., ... Utzinger, J. (2013). From morbidity control to transmission control: Time to change tactics against helminths on Unguja Island, Zanzibar. *Acta Tropica*, *128*(2), 412–422. https://doi.org/10.1016/j.actatropica.2011.04.010.

Kömer, M., Haas, W., & Haberl, B. (1995). Snail-host-finding by Miracidia and Cercariae. *Trends in Parasitology*, 11(12), 468-472.

Krueger, R. A., & Krueger, R. (2002). Designing and Conducting Focus Group Interviews.

Labbo, R. (1995). Of Bulinus Senegalensis and B . truncatus (planorbidae) in Temporary Pools in a Focus of Schistosoma haematobium in Niger: implications Pools Villages. *Journal of Molluscan,* 79–88.

Liao, C. W., Sukati, H., Nara, T., Tsubouchi, A., Chou, C. M., Jian, J. Y., … Fan, C. K. (2011). Prevalence of schistosoma haematobium infection among schoolchildren in remote areas devoid of sanitation in Northwestern Swaziland, Southern Africa. *Japanese Journal of Infectious Diseases*, *64*(4), 322–326.

Leslie, J., Garba, A., Oliva, E. B., Barkire, A., Aboubacar, A., Djibo, A., … Fenwick, A. (2011). Schistosomiais and Soil-Transmitted Helminth Control in Niger : Cost Effectiveness of School Based and Community Distributed Mass Drug Administration. *PloS Neglected Tropical Diseases 5*(10). https://doi.org/10.1371/journal.pntd.0001326.

Lothe, A., Zulu, N., Øyhus, A. O., Kjetland, E. F., & Taylor, M. (2018). Treating schistosomiasis among South African high school pupils in an endemic area, a qualitative study. *BMC Infectious Diseases*,1–10.

Lwambo, N. J. (1988). Transmission of urinary schistosomiasis in Sukumaland, Tanzania. 1. Snail infection rates and incidence of infection in school children. *Journal of Helminthology*, *62*(3), 213–7. https://doi.org/10.1017/S0022149X00011536.

Lwambo, N. J. S., Siza, J. E., Brooker, S., Bundy, D. A. P., & Guyatt, H. (1999a). Patterns of concurrent hookworm infection and schistosomiasis in schoolchildren in Tanzania. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *93*(5), 497–502. https://doi.org/10.1016/S0035-9203(99)90349-8.

Lwambo, N. J. S., Siza, J. E., Brooker, S., Bundy, D. A. P., & Guyatt, H. (1999b). Patterns of concurrent hookworm infection and schistosomiasis in schoolchildren in Tanzania. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *93*(5), 497–502. https://doi.org/10.1016/S0035-9203(99)90349-8.

Malek, E. A. (2017). Studies on bovine schistosomiasis in the Sudan. *Annals of Tropical Medicine & Parasitology 4983*. https://doi.org/10.1080/00034983.1969. 11686654.

Mandahl-Barth, G. (1965). The species of the genus Bulinus, intermediate hosts of Schistosoma. *Bulletin of the World Health Organization*, *33*(1), 33–44.

Manderson, L., Aagaard-Hansen, J., Allotey, P., Gyapong, M., & Sommerfeld, J. (2009). Social research on neglected diseases of poverty: Continuing and emerging themes. *PLoS Neglected Tropical Diseases*, *3*(2), 1–6. https://doi.org/10.1371/journal.pntd. 0000332.

Marieke, J., Werf, D., Vlas, S. J. De, Brooker, S., Looman, W. N., Nagelkerke, N. J. D., … Engels, D. (2003). Quantification of clinical morbidity associated with schistosome infection in sub-Saharan Africa. *Acta Tropica*, *86*,125–139.https://doi.org/10.1016/ S0001-706X(03)00029-9.

Maseko, T. S. B., Mkhonta, N. R., Masuku, S. K. S., Dlamini, S. V., & Fan, C. K. (2018). Schistosomiasis knowledge, attitude, practices, and associated factors among primary school children in the Siphofaneni area in the Lowveld of Swaziland. *Journal of Microbiology Immunology and Infection*, *51*(1),103–109. https:// doi.org/10. 1016/j.jmii.2015.12.003.

Mas-coma, S., Valero, M. A., & Bargues, M. D. (2009). Author ' s personal copy Veterinary Parasitology Climate change effects on trematodiases , with emphasis on zoonotic fascioliasis and schistosomiasis. *Veterinary Parasitology*, *1*63, 264–280 https: //doi. org/ 10.1016/j.vetpar.2009.03.024

Mazigo, H. D., Nuwaha, F., Kinung'hi, S. M., Morona, D., Pinot de Moira, A., Wilson, S., … Dunne, D. W. (2012). Epidemiology and control of human schistosomiasis in Tanzania. *Parasites & Vectors*, *5*(1), 274. https://doi.org/10.1186/1756-3305-5-274.

Mccreesh, N., Arinaitwe, M., Arineitwe, W., Tukahebwa, E. M., & Booth, M. (2014). Effect of water temperature and population density on the population dynamics of Schistosoma mansoni intermediate host snails. *Parasites and Vectors*,*7*(1),1–9. https://doi.org/ 10.1186/s13071-014-0503-9.

McCullough, F. S. (1962). Observations on bulinus (bulinus) truncatus rohlfsi (clessin) in ghana. *Annals of Tropical Medicine and Parasitology*, *56*(1), 53–60. https:// doi.org/ 10.1080/00034983.1962.11686089.

Mclafferty, I., & Mclafferty, I. (2004). Focus group interviews as a data collecting strategy.

Midzi, N., Mtapuri-zinyowera, S., Mapingure, M. P., Paul, N. H., Sangweme, D., Hlerema, G., Mduluza, T. (2011). Knowledge attitudes and practices of grade three primary schoolchildren in relation to schistosomiasis, soil transmitted helminthiasis and malaria in Zimbabwe. *BMC Infectious Diseases*, 11:169 https://doi.org/10.1186/1471-2334-11-169.

Midzi, N., Sangweme, D., Zinyowera, S., Mapingure, M. P., Brouwer, K. C., Kumar, N., … Mduluza, T. (2008). Efficacy and side effects of praziquantel treatment against Schistosoma haematobium infection among primary school children in Zimbabwe. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 102, 759—766 available https://doi.org/10.1016/j.trstmh.2008.03.010.

Mkoji, G. M., Hofkin, B. V., Kuris, A. M., Stewart-Oaten, A., Mungai, B. N., Kihara, J. H., … Loker, E. S. (1999). Impact of the Crayfish Procambarus Clarkii on Schistosoma Haematobium Transmission in Kenya. *American Journal of Tropical Medicine Hygiene*, *61*(5), 751–759.

Mojtahed, R., Nunes, M. B., Martins, J. T., & Peng, A. (2014). Equipping the Constructivist Researcher : The Combined use of Semi-Structured Interviews and Decision-Making maps. *Electronic Journal of Business Research Methods*,*12*(2), 87–95.

Montresor, A., Crompton, D. W. T., Hall, A., Bundy, D. A. P., Savioli, L. (n1998). Guideline for the evaluation of Soil-transmitted helminthiasis and schistosomiasis at community level. *World Health Organization Geneva*.

Montresor, A., Engels, D., Chitsulo, L., Bundy, D. A. P., Brooker, S., & Savioli, L. (2001). Development and validation of a "tablet pole" for the administration of praziquantel in sub-Saharan Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *95*(5), 542–544. https://doi.org/10.1016/S0035-9203(01)90034-3.

Mutani, G., Christensen, N. O., & Frandsen, F. (1983). The genus Bulinus, *Parasitenkunde* 483–484.

Muth, S., Sayasone, S., Odermatt-biays, S., Phompida, S., Duong, S., & Odermatt, P. (2010). *7 - Schistosoma mekongi in Cambodia and Lao People's Democratic Republic*. *Important Helminth Infections in Southeast Asia* (Vol. 72).Elsevier Ltd. https:// doi.org/10.1016/S0065-308X(10)72007-8.

Mwai, J., Njenga, S., & Barasa, M. (2016). Knowledge, attitude and practices in relation to prevention and control of schistosomiasis infection in Mwea Kirinyaga county, Kenya. *BMC Public Health*, *16*(1), 1–11. https://doi.org/10.1186/s12889-016-3494-y.

Mwanga, J. R., Magnussen, P., Mugashe, C. L., Gabone, R. M., Aagaard-Hansen, J. (2004). Schistosomiasis-related perceptions, attitudes and treatment-seeking practices in Magu District, Tanzania: Public health implications. *Journal.of Biosocial Sciences*, 36, 63–81.

Mwanga, J. R. (2005). Perception and practices on Schistosomiasis among communities inUkerewe district, Tanzania. *Tanzania Health Research Bulletin*.

Mwanga, J. R., & Lwambo, N. J. S. (2013). Pre- and post-intervention perceptions and water contact behaviour related to schistosomiasis in north-western Tanzania. *Acta Tropica*, *128*(2), 391–8. https://doi.org/10.1016/j.actatropica.2012.09.017.

Mwanga, J. R., Mugashe, C. L., Magnussen, P., Gabone, R. M., & Aagaard-Hansen, J. (1998). Experience from video-recorded focus group discussion on schistosomiases in Magu, Tanzania. *Qualitative Health Research*, *8*(5), 707–717. https://doi.org/10.1177 /104973239800800510.

Mwanga, J. R., Kaatano, G. M., Siza, J. E., Chang, S. Y., Ko, Y., Kullaya, C. M., … Changalucha, J. M. (2015). Improved perceptions and practices related to schistosomiasis and intestinal worm infections following PHAST intervention on Kome Island, North-Western Tanzania. *Korean Journal of Parasitology*, *53*(5), 561–569. https://doi.org/10.3347/kjp.2015.53.5.561.

NBS (2013). 2012 Population and housing census Population Distribution by administrative areas.

N'Goran, E. K., Utzinger, J., N'Guessan, A. N., Müller, I., Zamblé, K., Lohourignon, K. L., … Tanner, M. (2001). Reinfection with Schistosoma haematobium following school-based chemotherapy with praziquantel in four highly endemic villages in Côte d'Ivoire. *Tropical Medicine and International Health*, *6*(10), 817–825. https://doi.org/10.1046 /j.1365-3156.2001.00785.x.

Odhiambo, G. O., Musuva, R. M., Atuncha, V. O., Mutete, E. T., Odiere, M. R., Onyango, R. O., … Mwinzi, P. N. M. (2014). Low Levels of Awareness Despite High Prevalence of Schistosomiasis among Communities in Nyalenda Informal Settlement, Kisumu City, Western Kenya. *PLoS Neglected Tropical Diseases*, *8*(4). https://doi.org/10.1371 /journal.pntd.0002784.

Ofoezie, I. E. (1999). Distribution of freshwater snails in the man-made Oyan Reservoir, Ogun State, Nigeria. *Hydrobiologia, 416*, 181–191. https://doi.org/10.1023/A: 1003 87 5706638.

Ohmae, H., Iwanaga, Y., Nara, T., Matsuda, H., & Yasuraoka, K. (2003). Biological characteristics and control of intermediate snail host of Schistosoma japonicum. *Parasitology International, 52, 409–417. https://doi.org/10.1016/S1383-5769*.

Olliaro, P. L., Vaillant, M., Diawara, A., & Coulibaly, J. T. (2015). Toward Measuring Schistosoma Response to Praziquantel Treatment with Appropriate Descriptors of Egg Excretion. *Journal of Biosocial Science*, 1–20. https://doi. org/ 10.1371/ journal. pntd.0003821.

Olsen, A & Samuelsen, H. (2001). A study of Risk Factors for Intestinal Helminth Infections Using Epidemiological and Anthropological Approaches. *Journal of Biosocial Science,* 569–584.

Opisa, S., Odiere, M. R., Jura, W. G. Z. O., Karanja, D. M. S., & Mwinzi, P. N. M. (2011). Malacological survey and geographical distribution of vector snails for schistosomiasis within informal settlements of Kisumu City, western Kenya. *Parasites & Vectors*, *4*(1), 226. https://doi.org/10.1186/1756-3305-4-226.

Paull, S. H., & Johnson, P. T. J. (2011). High temperature enhances host pathology in a snail-trematode system: Possible consequences of climate change for the emergence of disease. *Freshwater Biology*, *56*(4), 767–778. https://doi.org/10.1111/j.1365-2427.2010.02547.x.

Pennance, T., Ame, S. M., & Amour, A. K. (2018). Occurrence of Schistosoma bovis on Pemba Island, Zanzibar : implications for urogenital schistosomiasis transmission monitoring occurrence of Schistosoma bovis on Pemba Island, Zanzibar: implications for urogenital schistosomiasis transmission monitori. *Parasitology (August).* https://doi.org/10.1017 /S00311820180011.

Perez-saez, J., Mande, T., Ceperley, N., Bertuzzo, E., Mari, L., & Gatto, M. (2016). Hydrology and density feedbacks control the ecology of intermediate hosts of schistosomiasis across habitats in seasonal climates. *PNAS* 1–6. https://doi. org/ 10. 1073 /pnas .1602251113.

Person, B., Ali, S. M., A'Kadir, F. M., Ali, J. N., Mohammed, U. A., Mohammed, K. A., … Knopp, S. (2016a). Community Knowledge, Perceptions, and Practices Associated with Urogenital Schistosomiasis among School-Aged Children in Zanzibar, United Republic of Tanzania. *PLoS Neglected Tropical Diseases*, *10*(7), 1–19. https://doi.org/10.1371 /journal.pntd.0004814.

Pitchford, R. J. (1977). Supplementary Review Article A check list of definitive hosts exhibiting evidence of the genus Schistosoma Weinland, 1858 acquired naturally in Africa and the Middle East. *Journal of Helminthology*, 51,229-252.

Raso, G., Essé, C., Dongo, K., Ouattara, M., & Zouzou, F. (2018). An Integrated Approach to Control Soil-Transmitted Helminthiasis Schistosomiasis Intestinal Protozoa Infection and Diarrhea : Protocol for a Cluster Randomized Trial. *JMIR Research Protocol,* 7(6) e145 https://doi.org/10.2196/resprot.9166.

Rassi, C., Kajungu, D., Martin, S., Arroz, J., Tallant, J., Zegers de Beyl, C., … Graham, K. (2016). Have You Heard of Schistosomiasis? Knowledge, Attitudes and Practices in Nampula Province, Mozambique. *PLoS Neglected Tropical Diseases*, *10*(3), 1–19. https://doi.org/10.1371/journal.pntd.0004504.

Rollinson, D., Knopp, S., Levitz, S., Stothard, J. R., Tchuem Tchuenté, L.A., Garba, A., … Utzinger, J. (2013). Time to set the agenda for schistosomiasis elimination. *Acta Tropica*, *128*(2), 423–40. https://doi.org/10.1016/j.actatropica.2012.04.013.

Rollinson, D., Stothard, J. R., & Southgate, V. R. (2001a). Interactions between intermediate snail hosts of the genus Bulinus and schistosomes of the Schistosoma haematobium group. *Parasitology*, *123 Supplemntary*, S245–S260. https://doi.org/10.1017 /S003 1182001008046.

Rollinson, D., Stothard, J. R., & Southgate, V. R. (2001b). Interactions between intermediate snail hosts of the genus Bulinus and schistosomes of the Schistosoma haematobium group. *Parasitology*, *123*(07). https://doi.org/10.1017/S0031182001008046.

Rollinson, D., Webster, J. P., Webster, B., Nyakaana, S., Jrgensen, A., & Stothard, J. R. (2009). Genetic diversity of schistosomes and snails: Implications for control. *Parasitology*, *136*(13), 1801–1811. https://doi.org/10.1017/S0031182009990412.

Rudge, J. W., Stothard, J. R., Basáñez, M. G., Mgeni, A. F., Khamis, I. S., Khamis, A. N., & Rollinson, D. (2008). Micro-epidemiology of urinary schistosomiasis in Zanzibar: Local risk factors associated with distribution of infections among schoolchildren and relevance for control. *Acta Tropica*, *105*(1), 45–54. https://doi.org/10.1016 /j.actatropica. 2007.09.006.

Saathoff, E., Olsen, A., Magnussen, P., Kvalsvig, J. D., Becker, W., & Appleton, C. C. (2004). Patterns of Schistosoma haematobium infection , impact of praziquantel treatment and re-infection after treatment in a cohort of schoolchildren from rural KwaZulu-Natal / South Africa, *BMC Infectious Diseases, 10*,1–10. https:// doi.org/ 10.1186/1471-2334-4-40.

Sacolo, H., Chimbari, M., & Kalinda, C. (2018). Knowledge, attitudes and practices on Schistosomiasis in sub-Saharan Africa: A systematic review. *BMC Infectious Diseases*, *18*(1). https://doi.org/10.1186/s12879-017-2923-6.

Sady, H., Al-Mekhlafi, H. M., Atroosh, W. M., Al-Delaimy, A. K., Nasr, N. A., Dawaki, S., Surin, J. (2015). Knowledge, attitude, and practices towards schistosomiasis among rural population in Yemen. *Parasites and Vectors*, *8*(1), 1–13. https:// doi.org/ 10.1186/ s13071-015-1050-8.

Sokolow, S. H., Wood, C. L., Jones, I. J., Swartz, S. J., Lopez, M., Hsieh, M. H., … Leo, G. A. De. (2016). Global Assessment of Schistosomiasis Control Over the Past Century Shows Targeting the Snail Intermediate Host Works Best. *PloS Neglected Tropical Diseases*, 1–19. https://doi.org/10.1371/journal.pntd.000479.

Schmidt, R. H. (1994). University of Nebraska - Lincoln. *The Handbook: Prevention and Control of Wildlife Damage*, *4*, 87–91. https://doi.org/10.1146/DQQXUHY.

SCI (2012). Widespread Schistosomiasis and organizations spearheading control of schistosomiasis in Tanzania.

Senghor, B., Diaw, O. T., Doucoure, S., Seye, M., Talla, I., & Diallo, A. (2015). Study of the snail intermediate hosts of urogenital schistosomiasis in Niakhar, region of Fatick, West central Senegal. *Parasites & Vectors*, 1–8. https://doi.org/10.1186/s13071-015-1030-z.

Shiff, C. J., Coutts, W. C. C., Yiannakis, C., & Holmes, R. W. (1979). Seasonal patterns in the transmiss'on of Schistosoma haematobium Rhodesia, and its control by winter application of molluscicide. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 375–380.

Simoonga, C., Kazembe, L. N., Kristensen, T. K., Olsen, A., Appleton, C. C., Mubita, P., & Mubila, L. (2008). The epidemiology and small-scale spatial heterogeneity of urinary schistosomiasis in Lusaka province, Zambia. *Geospatial Health*, *3*(1), 57–67. https://doi.org/10.4081/gh.2008.232.

Sinuon, M., Tsuyuoka, R., Socheat, D., Odermatt, P., Ohmae, H., Matsuda, H., … Palmer, K. (2007). Control of Schistosoma mekongi in Cambodia : results of eight years of control activities in the two endemic provinces. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 34–39. https://doi.org/10.1016/j.trstmh.2006. 04.011.

Siza, J. E., Kaatano, G. M., Chai, J. Y., Eom, K. S., Rim, H. J., Yong, T. S., … Changalucha, J. M. (2015). Prevalence of schistosomes and soil-transmitted helminths and morbidity associated with schistosomiasis among adult population in Lake Victoria basin, Tanzania. *Korean Journal of Parasitology*, *53*(5), 525–533. https://doi.org/10.3347 /kjp.2015.53.5. 525.

Sokolow, S. H., Lafferty, K. D., & Kuris, A. M. (2014). Regulation of laboratory populations of snails (biomphalaria and bulinus spp.) by river prawns, macrobrachium spp. (decapoda, palaemonidae): Implications for control of schistosomiasis. *Acta Tropica*, *132*(1), 64–74. https://doi.org/10.1016/j.actatropica.2013.12.013.

Steinmann, P., Keiser, J., Bos, R., Tanner, M., & Utzinger, J. (2006a). Schistosomiasis and water resources development : systematic review meta-analysis and estimates of people at risk. *The Lancet,*411–425.

Steinmann, P., Keiser, J., Bos, R., Tanner, M., & Utzinger, J. (2006b). Schistosomiasis and water resources development : systematic review meta-analysis and estimates of people at risk. *The Lancet,*411–425.

Steinmann, P. (2008). Epidemiology and diagnosis of Schistosoma japonicum , other helminth infections and multiparasitism in Yunnan province , People ’ s Republic of China

Stothard, J. R., Llewellyn-Hughes, J., Griffin, C. E., Hubbard, S. J., Kristensen, T. K., & Rollinson, D. (2002). Identification of snails within the Bulinus africanus group from East Africa by multiplex SnaPshot™ analysis of single nucleotide polymorphisms within the cytochrome oxidase subunit I. *Memorias Do Instituto Oswaldo Cruz*, *97*(SUPPL. 1), 31–36. https://doi.org/10.1590/S0074-02762002000900008.

Stothard, J. R., Mgeni, A. F., Khamis, S., Seto, E., Ramsan, M., Hubbard, S. J., & Kristensen, T. K. (2002). Fresh from the field - New insights into the transmission biology of urinary schistosomiasis in Zanzibar. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *96*(5), 470–475. https://doi.org/10.1016 /S0035-9203(02)90406-2.

Sturrock, R. F. (2001). Schistosomiasis Epidemiology and Control : How Did We Get Here and Where Should We Go ? *Mem Inst Oswaldo Cruz, Rio de Janeiro*, (*96)*, 17–27.

Sturrock, R. F. (1965). The Development of Irrigation and Its Influence on the Transmission of Bilharziasis in Tanganyika. *Bulletin of the World Health Organization*, *32*, 225–236.

Sturrock, R. F., Diaw, O. T., Talla, I., Niang, M., Piau, J. P., Capron, A. (2001). Seasonality in the transmission of schistosomiasis and in populations of its snail intermediate hosts in and around a sugar irrigation scheme at Richard Toll, Senegal. *Parasitology*, 123, 77-89.

Sunday, C. E. (2012). Qualitative Data Analysis Qualitative Data. https://doi.org/10.1136 /ebnurs.2011.100352.

Strauss, A. L & Corbin, J. (1990). Grounded theory methodology: An overview. *Handbook of Qualitative Research.* Sage, London, New Delhi. 1994; p 27-258.

Teesdale, C. (1962). Ecological Observations on the Molluscs of Significance in the Transmission of Bilharziasis in Kenya. *Bulletin of the World Health Organization*, (1956), 759–782.

Tchuenté, L. T., Garba, A., Mohammed, K. A., & Schur, N. (2012). Time to set the agenda for schistosomiasis elimination. *Acta Tropica*. https://doi.org/10.1016 /j *Acta tropica.* 2012.04. 013.

Traore, I., Karthe, D., Ali, A., Coulibaly V., & Martin K. M. (2016). Influence of air temperature on children water contacts with respect to schistosomiasis transmission risk in the Sourou. *Stochastic Environmental Research and Risk Assessment*, *30*(8), 2097–2107.https://doi.org/10.1007/s00477-016-1214-x.

Utzinger, J., Bergquist, R., Shu-Hua, X., Singer, B. H., & Tanner, M. (2003). Sustainable schistosomiasis control--the way forward. *The Lancet*, *362*, 1932–1934. https:// doi .org /10.1016/S0140-6736(03)14968-9.

Utzinger, J., Raso, G., Brooker, S., Savigny, D. D. E., & Tanner, M. (2009). Schistosomiasis and neglected tropical diseases : towards integrated and sustainable control and word of caution, *Europe PMC Funders Group 136*(13), 18591874. https://doi. org/10. 1017 /S0 031182009991600.

Utzinger, J. (1999). Novel approaches in the control of schistosomiasis : from rapid identification to chemoprophylaxis. *edoc.unibas.ch.*

Vlas, S. J. De, Bosompem, K. M., & Habbema, J. D. F. (2004). Determinants of health-seeking behaviour for schistosomiasis- related symptoms in the context of integrating schistosomiasis control within the regular health services in Ghana. *Tropical Medical International Health 9*(7), 784–794.

Vos, T., Flaxman, A. D., Naghavi, M., Lozano, R., Michaud, C., Ezzati, M., … Moradi-Lakeh, M. (2012). Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990-2010: A systematic analysis for the Global Burden of Disease Study 2010. *The Lancet*, *380*(9859), 2163–2196. https://doi.org/10.1016 /S01406736 (12)61729-2.

Watson, J. M., Sc, D., & Sc, A. R. C. (1958). Bulinus truncatus in the Middle East With Comments on the Effect of Some Human Activities in their Relationship to the Snail Host on the Incidence of Bilharziasis Haematobia in the Middle East and Africa. *The Bulletin of the World Health Organization*, 833–894.

Webbe, G.(1962). The transmission of Schistosoma haematobium in an area of Lake Province, Tanganyika. *Bulletin of the World Health Organization*, *27*, 59–85.

Webbe, G., & Msangi, A. S. (2017). Observations on Three Species of Bulinus on the East Coast of Africa. Annals of Tropical Medicine & Parasitology. https://doi. org/ 10. 1080 /00034983.1958.11685870.

Webster, B. L., Diaw, O. T., Seye, M. M., Webster, J. P., & Rollinson, D. (2013). Introgressive Hybridization of Schistosoma haematobium Group Species in Senegal : Species Barrier Break Down between Ruminant and Human Schistosomes. *PloS Neglected Tropical Diseases, 7*(4). https://doi.org/10.1371/journal.pntd.0002110.

Webster, B. L., Rollinson, D., Stothard, J. R., & Huyse, T. (2010a). Rapid diagnostic multiplex PCR (RD-PCR) to discriminate Schistosoma haematobium and *S . bovis*. *Journal of Helminthology*, 84, 107–114. https://doi.org/10.1017/S0022149X09990447.

Webster, B. L., Rollinson, D., Stothard, J. R., & Huyse, T. (2010b). Rapid diagnostic multiplex PCR (RD-PCR) to discriminate Schistosoma haematobium and *S . bovis*. *Journal of Helminthology*, 84, 107–114. https://doi.org/10.1017/S0022149X09990447

Weerakoon, K. G. A. D., Gobert, G. N., Cai, P., & Mcmanus, P. (2015). Advances in the Diagnosis of Human Schistosomiasis. *American Social Microbiology, 28*(4), 939–967. https://doi.org/10.1128/CMR. 00137-14.

Weller, S. C., Vickers, B., Bernard, H. R., Blackburn, A. M., Borgatti, S., Gravlee, C. C., … Johnson, J. C. (2018). Open-ended interview questions and saturation. PloS one, 1–18. https://doi.org/10.1371/journal.pone.0198606.

Woolhouse, M. E. J., & Chandiwana, S. K. (1988). Spatial and temporal heterogeneity in the population dynamics of Bulinus globosus and Biomphalaria pfeifferi and in the epidemiology of their infection with schistosomes, *Parasitology*, 21–34.

Woolhouse, M. E.. J., Etard, J. F., Dietz, K., Ndhlovu, P. D., & Chandiwana, S. K. (1998). Heterogeneities in schistosome transmission dynamics and control. *Parasitology*, *117*(5), S003118209800331X. https://doi.org/10.1017/S003118209800331X.

World Health Organization. (1993). The Control of Schistosomiasis.WHO technical report series 830, Geneva.

World Health Organization. (2012). Sixty-Fifth World Health Assembly. *Wha65 /2012 /Rec/ 1*, (May), 21–26.

World Health Organization. (2014). World Health Organization. Investing to overcome the global impact of neglected tropical diseases Third WHO report on neglected tropical diseases, Geneva.

World Health Organization. (1994). Bench Aids for the diagnostic of Intestinal Parasites.

World Health Organization. (2010). Working to overcome the global impact of neglected tropical diseases First WHO report on neglected tropical diseases.

World Health Organization. (2010). Schistosomiasis: Progress report 2001–2011 and strategic plan 2012–2020.

World Health Organization. (1998). Report of the WHO Informal Consultation on Schistosomiasis Control Planning and Technical Guidance.

World Health Organization. (1991). Basic laboratory methods for Medical Parasitology.

World Health Organization. (2015). Draft Field Use of Molluscicides in Schistosomiasis Control Programmes,an operational mannual for the programme managers 1–20.

World Health Oganization. (2010). Progress report 2001–2011 and strategic plan for preventive Chemotherapy 2012–2020.

World Health Organization. (2014). Weekly epidemiological record (2), 21–28.

Yang, Y., Cheng, W., Wu, X., Huang, S., Deng, Z., Zeng, X., … Jiang, Q. (2018). Prediction of the potential global distribution for Biomphalaria straminea , an intermediate host for Schistosoma mansoni. *PloS Neglected Tropical Diseases*, 1–16. https://doi .org/10. 1371/ journal.pntd.0006548.

Yepes, E., Varela-m, R. E., López-abán, J., Rojas-caraballo, J., Muro, A., & Mollinedo, F. (2015). Inhibition of Granulomatous Inflammation and Prophylactic Treatment of Schistosomiasis with a Combination of Edelfosine and Praziquantel. *PloS Neglected Tropical Diseases* 1–22. https://doi.org/10.1371/journal.pntd.0003893.

Yirenya-tawiah, D. R., Annang, T., Otchere, J., Bentum, D., Edoh, D., Amoah, C., & Bosompem, K. M. (2011). Urinary Schistosomiasis among Adults in the Volta Basin of Ghana: Prevalence, Knowledge and Practices, *Journal of Tropical Medicine Parasitol. 2011;34:1-16.*

Zumstein, A. (1983). A study of some factors influencing the epidemiology of urinary schistosomiasis at Ifakara (Kilombero District, Morogoro Region, Tanzania). *Acta Tropica*, *40*(3), 187–204.

APPENDICES

Appendix 1: Assessment within activity to determine WHERE, WHEN, FREQUENCY and duration of water contact activities in relation *to S. haematobium* infection in school children in Ikingwamanoti village.

ACTIVITY	Categories	N (%)	OR(95% CI)	P-VALUE
Livestock	**Having livestock**	143 (57.2)	0.22 (0.60- 2.58)	0.596
	Where livestock watering occurs			
	Home		3.19 (0.61 -18.48)	0.168
	Modern wells		1.22 (0.22 -5.40)	0.802
	Traditional wells		0.66 (0.29- 1.50)	0.316
	Pond		1.71 (0.82 - 3.65)	0.161
Dish washing	**Dishwashing**	159 (63.6)		
	Where dish washing is done			
	Home		0.77 (0.29 -2.02)	0.587
	Pond		1.60 (0.05 - 46.62)	0.756
Water collection	**Collecting water**	209 (83.6)	1.15 (0.52 -2.67)	0.734
	Where water is collected			
	Traditional well		0.20 (0.66 -2.21)	0.551
	Pond		0.99 (0.41 -2.33)	0.980
Swimming	**Swimming**	60 (24)	3.69 (1.85 -7.62)	0.627
Laundry	**Laundry**	46 (18.4)		
	Where laundry is done			
	Pond		0.97 (0.47 – 1.97)	0.936
	Frequency of laundry			
	Daily		1.17 (0.63 - 2.20)	0.650
	Time of a day for laundry			
	After lunch to sunset		0.96 (0.50- 1.89)	0.918
Fishing	**Fishing**	35 (14)		
	Time spent in fishing			
	Morning to lunch		1.65 (0.69- 4.02)	0.263
	After lunch to sunset		0.72 (0.09 -4.19)	0.720

Irrigation	**Doing irrigation**	95 (38)	3.74(0.27 -106.73)	0.346
	Frequency of irrigation			
	1-2 times per week		1.59 (0.15 – 18.09)	0.694
	Daily		1.44 (0.19- 13.92)	0.728
	Time spent for irrigation			
	Morning to lunch		0.64(0.07 - 4.88)	0.671
	After lunch to sunset		0.49(0.05 - 4.13)	0.524
Bathing	**Place of bath**			
	Pond		1.10 (0.31 3.94)	0.876
	Frequency of bathing			
	Twice per day		1.12 (0.61 -2.09)	0.718
Crossing water	**Crossing water**	179 (71.6)	0.46 (0.05- 4.24)	0.466
	Place of water contact			
	Home		3.37 (0.33- 35.89)	0.284
	Traditional wells		2.07(0.21- 21..07)	0.516
	Paddy farms		2.84 (0.28- 29.56)	0.354
Other ways of contacting water	**Time of a day for water contact**			
	Evening		1.30(0.40 3.93)	0.645
	Morning		1.35 (0.70- 2.60)	0.368
	Afternoon		1.03(0.34- 2.94)	0.958
Paddy farming	**Working in paddy farms**	129 (51.6)	1.28(0.72 -2.26)	0.397

Appendix 2: Breakdown of reported water contact activities by sex and age

	Male(n = 104)			Female (n = 146)		
Water contact activity	**6-8yrs**	**9-11yrs**	**12-14yrs**	**6-8yrs**	**9-11yrs**	**12-14**
	n (%)	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**
Livestock watering	23 (22.1)	27 (25.9)	34 (32.7)	43 (29.5)	44 (30.1)	32 (21.9)
Domestic activities*	34 (32.7	29 (27.8)	40 (38.5)	101(69.2)	93 (63.7)	65 (44.5)
Fetching water	26 (25)	27 (25.9)	35 (33.7)	39 (26.7)	44 (30.1)	38 (26)
Swimming	5 (4.8)	9 (8.6)	10 (9.6)	16 (10.9)	15 (10.3)	5 (3.4)
Irrigation	10 (9.6)	10 (9.6)	15 (14.4)	18 (12.3)	25 (17.1)	17 (11.6)
Crossing water	24 (23.1)	21 (20.2)	34 (32.7)	32 (21.9)	38 (26)	30 (20.5)
Paddy farming	9 (8.6)	17 (16.3)	24 (23.1)	24 (16.4)	30 (20.5)	25 (17.1)
Other	10 (9.6)	12 (11.5)	18 (17.3)	23 (15.7)	19 (13)	17 (11.6)

***** Includes both laundry and dish washing.

Appendix 3: Water contact questionnaire

Dodoso juu ya matumizi ya maji

Kwa muhojaji:

Tafadhali andika jibu ulilopewa na mshiriki kwa kuzungushia jibu sahihi au kujaza nafasi zilizoachwa wazi. Kumbuka kuwa swali moja laweza kuwa na jibu sahihi zaidi ya moja . Majibu yatakayotolewa na mshiriki ni siri.

Tarehe	DD/MM/YYYY
Jina la muhojaji	

1.Taarifa za mshiriki

Na.	**Habari za mshiriki**	**Majibu**
1	Nambari ya utambulisho	☐☐☐☐☐☐☐
2	Jina la shule	
3	Kijiji	
4	Jina la Kitongoji	
5	Wilaya	
6.	Jinsi	1= ME 2 =KE
7.	Umri (miaka tu)	
8	Kabila	1=Msuukuma 2=Mkerewe 3=Mjita 4=Mkurya 55=Nyingine (taja).....

2. Taarifa za kimazingira na tabia za watu katika matumizi ya maji na maambukizi ya kichocho cha Mkojo

9.	Ni kwa muda gani umeishi katika kijiji hiki?	1= chini ya mwaka 1 2= mwaka 1-2 3= miaka 3-5 4= miaka 6-10 5= zaidi ya miaka 10

10	Je kuna mifugo kwenye familia yenu?	1=Ndiyo 2=Hapana ***Kama jibu ni hapana nenda swali na.15***
11.	Kama jibu ni ndiyo huwa mnanyweshea wapi mifugo yenu?	1= Bwawani 2= mtoni 3= mferejini 4=Dimbwini 5= maji ya bomba 6=kisima cha kienyeji 7=kisima cha kisasa 55= nyingine (taja) ……
12.	Ni kwa muda upi huwa unaenda kunywesha mifugo kwa siku?	1= Asubuhi kabla ya mapumziko 2= Baada ya chakula cha mchana 3= Jioni kabla ya jua kuzama 55=Nyingine (taja)…….
13.	Ni kwa muda gani huwa unakaa huko ?	1= asubuhi mpaka wakati wa chakula cha mchana 2= baada ya chakula cha mchana mpaka jua kuzama 3= siku nzima 55= Nyingine (taja)……
14.	Huwa mnapata wapi maji ya kunywa na kupikia katika familia yenu?	1= Bwawani 2= mtoni 3= mfereji 4=Dimbwini 5= maji ya bomba 6=kisima cha kienyeji 7=kisima cha kisasa 55= nyingine (taja) ……
15.	Je huwa unachota maji katika familia yenu?	1= Ndiyo 2= Hapana ***Kama jibu ni hapana nenda swali la 18***
16.	Ni mara ngapi huwa unachota maji ya kunywa na kupikia?	1 = Kila siku 2 = Mara 1-2 kwa wiki 3 = Mara 3-5 kwa wiki 4 = Mara chache kwa mwezi 55 =Nyingine(taja).....
17.	Ni muda gapi huwa mnachota maji ?	1= Asubuhi kabla ya jua kuchomoza 2= Baada ya kuchomoza jua mpaka wakati wa mapumuziko 3= Mchana 4= Jioni 55=Nyingine (taja)…….
18.	Huwa unaogea wapi?	1= Bwawani 2= mtoni

		3= mfereji 4=Dimbwini 5= maji ya bomba 6=kisima cha kienyeji 7=kisima cha kisasa 8=Nyumbani 55= nyingine (taja) ……..
19.	Huwa unaoga mara ngapi kwa siku?	1 = Mara 1 kwa siku 2 = Mara 2 kwa siku 3 = Mara 3-5 kwa wiki 55=Nyingine (taja).....
20	Je huwa unaogelea?	1=Ndiyo 2=Hapana
21.	Huwa unaoshea wapi vyombo?	1= Bwawani 2= mtoni 3= mfereji 4=Dimbwini 5= maji ya bomba 6= kisima cha kienyeji 7= kisima cha kisasa 8= Nyumbani 55= nyingine (taja) ……..
22.	Ni mara ngapi huwa unaosha vyombo?	1 = Kila siku 2 = Mara 1-2 kwa wiki 3 = Mara 3-5 kwa wiki 4 = Mara chache kwa mwezi 55=Nyingine (taja).....
23.	Ni wakati gani kwa siku unatumia kuosha vyombo?	1= Asubuhi 2= Mchana 3= Jioni 55=Nyingine (taja)……
24	Huwa unafulia wapi nguo?	1= Bwawani 2= mtoni 3= mfereji 4=Dimbwini 5= maji ya bomba 6=kisima cha kienyeji 7=kisima cha kisasa 8=Nyumbani 55= nyingine (taja) ……..
25.	Ni mara ngapi huwa unafua nguo?	1 = Kila siku 2 = Mara 1-2 kwa wiki 3 = Mara 3-5 kwa wiki

		4 = Mara chache kwa mwezi 55=Nyingine(taja).....
26.	Kama jibu ni kila siku, ni wakati gani Huwa unafua nguo?	1= asubuhi mpaka wakati wa chakula cha mchana 2= baada ya chakula cha mchana mpaka jua kuzama 3= siku nzima 55= Nyingine (taja)……
27.	Je huwa unavua samaki ?	1=Ndiyo 2=Hapana ***Kama jibu ni hapana nenda swali na. 29***
28.	Kama wewe ni mvuvi unaweza kukadilia muda unaotumia kuvua samaki ?	1= Hapana 2= asubuhi mpaka wakati wa chakula cha mchana 3= baada ya chakula cha mchana mpaka jua kuzama 4= siku nzima 55= Nyingine (taja)…….
29.	Je huwa unafanya kazi ya umwagiliaji?	1=Ndiyo 2=Hapana ***Kama jibu ni hapana nenda swali na. 32.***
30.	Kama jibu ni ndiyo ni mara ngapi unamwagilia?	1 = Kila siku 2 = Mara 1-2 kwa wiki 3 = Mara 3-5 kwa wiki 4 = Mara chache kwa mwezi 55=Nyingine (taja).....
31.	Kama jibu ni ndiyo ni muda gani huwa unafanya umwagiliaji?	1= asubuhi mpaka wakati wa chakula cha mchana 2= baada ya chakula cha mchana mpaka jua kuzama 3= siku nzima 55= Nyingine (taja)……
32.	Je katika kazi mnazofanya katika familia yenu huwa Unakanyaga maji yoyote?	1=Ndiyo 2=Hapana ***Kama jibu ni hapana nenda swali na. 36.***
33.	Kama jibu ni ndiyo ni mara ngapi huwa unakanyaga maji?	1 = Kila siku 2 = Mara 1-2 kwa wiki 3 = Mara 3-5 kwa wiki 4 = Mara chache kwa mwezi 55=Nyingine(taja).....
34.	Kama jibu ni ndiyo je ni wapi huwa unakanyaga maji hayo?	1…… 2…… 3……
35.	Kama jibu ni ndiyo ni kwa muda upi kwa siku unafanya kazi hizo?	1= asubuhi mpaka wakati wa chakula cha mchana 2= baada ya chakula cha mchana

		mpaka jua kuzama 3= siku nzima 55= Nyingine (taja)……
36.	Je una njia zingine ambazo unakanyaga maji?	1=Ndiyo 2=Hapana ***Kama jibu ni hapana nenda swali na. 42.***
37.	Kama jibu ni ndiyo ni wapi huwa unakanyaga maji?	1…… 2…… 3……
38.	Kama jibu ni ndiyo huwa unakanyaga maji hayo kwa sababu zipi?	1…… 2…… 3……
39.	Kama jibu ni ndiyo ni mara ngapi unakanyaga maji hayo?	1 = Kila siku 2 = Mara 1-2 kwa wiki 3 = Mara 3-5 kwa wiki 4 = Mara chache kwa mwezi 55 =Nyingine (taja).....
40.	Kama jibu ni ndiyo, ni kwa muda upi unakanyaga maji hayo?	1= Asubuhi 2= Mchana 3= Jioni 55=Nyingine (taja)……
41.	Je huwa unafanya kazi majarubani?	1=Ndiyo 2=Hapana
42.	Je huwa unatumia choo wakati wote unapohitaji kujisaidia?	1=Ndiyo 2=Hapana
43.	Kama jibu ni hapana ni kwa nini?	1…… 2…… 3……

3. Madhara ya kukanyaga maji kwa binadamu

44.	Je huwa unakojoa mkojo wenye damu?	1=Ndiyo 2=Hapana
45.	Kama jibu ni ndiyo ni kwa muda gani umekuwa ukikojoa damu? ***Mshukuru mshiriki kwa ushirikiano wake na katisha Mahojiano***	1= wiki moja 2= mwezi mmoja 3= Zaidi ya mwezi 55= Nyingine (taja)……

Appendix 4: Semi structure Interview guide

Kwa ajili ya wazazi/walezi wa watoto.

(Semi structured Interview (SSI) Schedule)

FOMU YA MAHOJIANO NA MTOA TAARIFA (SSI)

1. Namba ya mtoa taarifa:
2. Tarehe: DD/MM/YYYY
3. Jina la mhojaji:
4. Mfumo wa unakilishaji:

5. Taarifa binafsi za mhojiwa

S/N	Swali	Majibu
1	Namba ya mshiriki	
2	Umri (miaka tu)	
3	Kabila	
4	Kiwango cha juu cha elimu	
5	Shughuli kuu inayompatia kipato	
6	Dini(dhehebu)	
7	Kata	
8	Kijiji	
9	Kitongoji	

6. MUONGOZO WA MAJADILIANO KATI YA MHOJAJI NA MHOJIWA

1. Je ni matatizo gani ya kiafya yanayoathiri watu mara kwa mara katika jamii yenu ? (**Dadisi:** ni matatizo yapi yanayoathiri watoto wa umri wa kwenda shule ? ni kwa namna gani?, na kwa nini?)

2. Je unafikiri ugonjwa wa kichocho cha mkojo ni tatizo linalopewa kipaumbele katika jamii yenu? (**Dadisi**: ni kundi lipi linaloathirika zaidi na tatizo hili na kwa nini?)
3. Je ni nini chanzo cha ugonjwa wa kichocho cha mkojo? (**Dadisi:** uhusiano kati ya tabia za binadamu, mazingira, konokono na utumiaji wa maji?)
4. Dalili za ugonjwa wa kichocho cha mkojo (kisambale) ni zipi? (**Dadisi**: kukojoa damu? maumivu wakati wa kukojoa?)
5. Je ugonjwa wa kichocho cha mkojo huenezwa kwa njia zipi? (**Dadisi**: nafasi ya tabia na vitendo vya binadamu vya kujisaidia ovyo katika kusambaza maambukizi ya ugonjwa wa kichocho?)
6. Je unafikiri ni kwa nini watoto wemye umri wa kwenda shule katika jamii yenu wanamaabukizi ya ugonjwa wa kichocho cha mkojo? (**Dadisi:** ni kwa vipi na kwa nini watoto wanachezea maji yenye vijidudu vya ugonjwa wa kichocho?)
7. Je nini kifanyike ili kupunguza tatizo la maambukizi ya kichocho cha mkojo kwa watoto wenye umri wa kwenda shule? (**Dadisi :** Wewe kama mwanakijiji unafanya nini ili kuthibiti tabia ya watoto kucheza kwenye maji yenye vijidudu vya ugonjwa wa kichocho cha mkojo? Je unafikiri serikali ifanye nini katika kutatua tatizo hili?|)
8. Je unafikiri ni jitihada zipi zifanyike kupunguza utumiaji wa maji yenye vijidudu/vimelea vya kichocho ? (**Dadisi** kwa kutoa mbinu mbadala ya maji safi, sehemu salama ya kufulia nguo, sehemu salama ya kuogea, vyoo na mifereji?
9. Je kuna jitihada gani za kitaifa za kupambana na kuthibiti maambukizi ya kichocho?(**Dadisi** jamii ina mchango gani katika jitihada hizo?)
10. Inaaminika kuwa ugonjwa wa kichocho ni ugonjwa wa kujitakia kwa sababu chanzo chake na kuenea kwake kunasababishwa na tabia ya binadamu ya kujisaidia ovyo haja ndogo na haja kubwa karibu kabisa ama kwenye maji. Je unakubaliana ama unakataa hoja hiyo? (**Toa sababu).**

Appendix 5: Focused Group Discussion guide

Iulizwe kwa wazazi/walezi wa wanafunzi

Fomu ya majadiliano katika vikundi

FGD_namba:	
Tarehe:	(dd/mm/yyyy)
Anuani:	
Mahala pa mjadala:	

Mratibu:	Jina	Jinsi
Mnakilishi:		

Mfumo wa unakilishaji:	

Muda:	Kuanza:	Kumaliza:	muda:

Taarifa za wanakikundi

S/N	Jinsi	Umri (miaka)	Kiwango cha juu cha elimu	Kabila	Shughuli kuu inayokupatia kipato	Dini (dhehebu)	Kata	Kijiji	Kitongoji
1.									
2.									
3.									
4.									
5.									
6.									
7.									
8.									
9.									
10.									
11.									
12.									

MUONGOZO WA MAJADILIANO KATIKA VIKUNDI

1. Je ni matatizo gani ya kiafya yanayoathiri watu mara kwa mara katika jamii yenu ? (**Dadisi:** ni matatizo yapi yanayoathiri watoto wa umri wa kwenda shule ? ni kwa namna gani?, na kwa nini?)

2. Je munafikiri ugonjwa wa kichocho cha mkojo ni tatizo linalopewa kipaumbele katika jamii yenu? (**Dadisi**: ni kundi lipi linaloathirika zaidi na tatizo hili na kwa nini?)

3. Je ni nini chanzo cha ugonjwa wa kichocho cha mkojo? (**Dadisi:** vijidudu/vimelea vya kichocho)

4. Dalili za ugonjwa wa kichocho cha mkojo (kisambale) ni zipi? (**Dadisi**: kukojoa damu? maumivu wakati wa kukojoa?)

5. Je ugonjwa wa kichocho cha mkojo huenezwa kwa njia zipi? (**Dadisi**: nafasi ya tabia na vitendo vya binadamu vya kujisaidia ovyo katika kusambaza maambukizi ya ugonjwa wa kichocho?)

6. Je munafikiri ni kwa nini watoto wemye umri wa kwenda shule katika jamii yenu wanamaabukizi ya ugonjwa wa kichocho cha mkojo? (**Dadisi:** ni kwa vipi na kwa nini watoto wanachezea maji yenye vijidudu vya ugonjwa wa kichocho?)

7. Je nini kifanyike ili kupunguza tatizo la maambukizi ya kichocho cha mkojo kwa watoto wenye umri wa kwenda shule? (**Dadisi :** kama wanakijiji munafanya nini ili kuthibiti tabia ya watoto kucheza kwenye maji yenye vijidudu vya ugonjwa wa kichocho cha mkojo? Je munafikiri serikali ifanye nini katika kutatua tatizo hili?|)

8. Je munafikiri ni jitihada zipi zifanyike kupunguza utumiaji wa maji yenye vijidudu/vimelea vya kichocho ? (**Dadisi:** kwa kutoa mbinu mbadala ya maji safi, sehemu salama ya kufulia nguo, sehemu salama ya kuogea, vyoo na mifereji?)

9. Je kuna jitihada gani za kitaifa za kupambana na kuthibiti maambukizi ya kichocho? (**Dadisi:** jamii ina mchango gani katika jitihada hizo?)

10. Inaaminika kuwa ugonjwa wa kichocho ni ugonjwa wa kujitakia kwa sababu chanzo chake na kuenea kwake kunasababishwa na tabia ya binadamu ya kujisaidia ovyo haja ndogo na haja kubwa karibu ama kwenye maji. Je munakubaliana ama munakataa hoja hiyo? (**Toeni sababu).**

POSTER PRESENTATION

ECOLOGY OF *BULINID* SNAIL INTERMEDIATE HOSTS AND TRANSMISSION OF *SCHISTOSOMA HAEMATOBIUM* AMONG SCHOOL AGED CHILDREN IN SHINYANGA DISTRICT, TANZANIA

Teckla Angelo,* Joram Buza, Safari Kinung'hi, Curtis Kariuki, Joseph Mwanga and Shona Wilson

Nelson Mandela African Institution of Science and Technology, P. O. Box 447 Arusha, Tanzania.

Corresponding author: angelot@nm-aist.ac.tz

Introduction: *Schistosoma haematobium*, is the species of schistosome parasites that causes urogenital schistosomiasis in humans and transmitted by *Bulinus* snails. Infection with *S. haematobium* leads to damage of bladder tissues, kidney damage, infertility, bladder cancer and death. This study was conducted in Shinyanga district to investigate the ecology of *Bulinid* snails intermediate hosts in relation to transmission of *S. haematobium* among school aged children.

General objective: To gain an in-depth understanding of *S. haematobium* transmission with focus on snail dynamics and human water contact behavior in Shinyanga

Specific Objectives:

- To investigate human water contact behavior in relation to *S. haematobium* transmission in the study area
- To determine the patent and pre-patent schistosome infections in *Bulinid* snails including differentiation of *S. haematobium* from *S. bovis* using molecular methods
- To examine the ecological factors determining *Bulinid* snail population dynamics in the study area

Methods

Water contact behaviour


Urine filtration

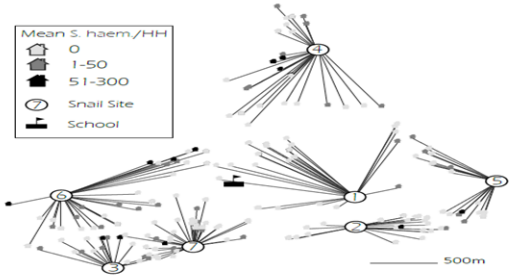


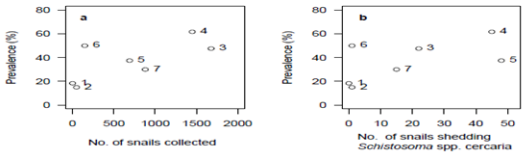

Examination of patent infection of snails




Results

Household location in relation to snail sites and mean intensity of *S. haematobium* infection





Conclusion

The study findings indicated that Conductivity and vegetation cover were significant determinants for *B. nasutus* population density. Proximity of the children's households to snail habitats harbouring high population of snais shedding cercariae was a significant determinant factor for *S. haematobium* infection. Similarly, Water sources shared by both domestic ruminants and humans were more likely to transmit *S. haematobium* and *S. bovis* infections than unshared water sources

Recommendations

- *S. bovis* infection dominated *S. haematobium* infection of *B. nasutus* snails. Further studies are needed to investigate if infection of *S. bovis* in *B. nasutus* snails prevents infections with *S. haematobium* parasites and assess compatibility of *B. nasutus* to *S. bovis* and *S. haematobium*.
- Determination of geographical distribution of urinary schistosomiasis should take into account integration of (GPS) data coupled with malacology surveys to enhance designing of targeted snail control interventions.
- Further malacological studies are needed to fully understand the role of different snail species and ecological factors in the transmission of urinary schistosomiasis to complement ongoing schistosomiasis control interventions.

Scientific contribution of the study

- Ecological determinants of *B. nasutus* population density
- Detection of schistosomiasis transmission sites by integration of malacological surveys linked with GPS data
- The importance of water sources shared between humans and livestock as a risk factors for *S. haematobium* and *S. bovis* infection
- Baseline information for targeted snail control interventions in complex transmission networks

Reference

1. Angelo, T., Buza, J., Methusela, S., Kariuki, H. C., Mwanga, J. R., Munisi, D. Z., & Wilson, S. (2018). Geographical and behavioral risks associated with Schistosoma haematobium infection in an area of complex transmission, 1–9.
2. Angelo, T., Kinung'hi S. M., Buza, J., Mwanga, J. R., Kariuki, H. C & Wilson, S. (2019). Community knowledge , perceptions and water contact practices associated with transmission of urinary schistosomiasis in an endemic region : a qualitative cross- sectional study, 1–10.
3. Angelo, T., Kinung, S. M., Buza, J., & Kariuki, H.C & Wilson, S. (2020). Patent and pre-patent infection trends of *Schistosoma haematobium* and *Schistosoma bovis* in *Bulinus nasutus* snails in a schistosomiasis endemic setting of Shinyanga district, Tanzania (In press)
4. Angelo, T., Kinung, S. M., Buza, J & Kariuki, H. C. & Wilson, S.(2020). Ecological determinants of *Bulinus nasutus* snail intermediate hosts population and transmission patterns of urogenital schistosomiasis in endemic setting of Shinyanga district, Tanzania (In press)